45th Annual Meeting of The Society for Leukocyte Biology

INFLAMMATION IN INNATE AND ADAPTIVE IMMUNE MECHANISMS

October 28 – 30, 2012

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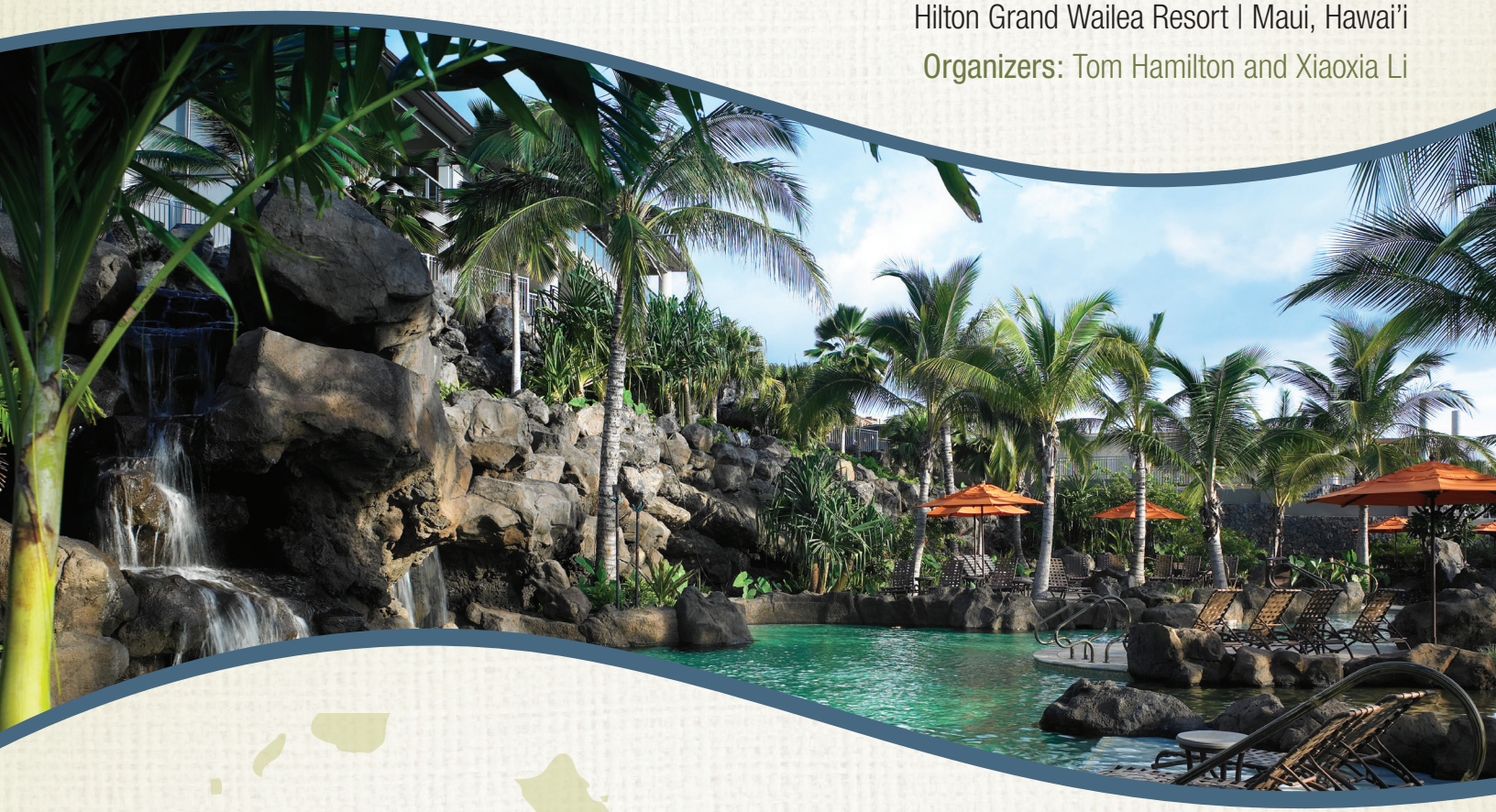

ABSTRACTS

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45th ANNUAL MEETING OF THE SOCIETY FOR LEUKOCYTE BIOLOGY

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ABSTRACTS

1

Alcohol and Drugs of Abuse Interaction with HIV/AIDS: Systems Biology Approach in the SIV-Infected Macaque

Patricia E. Molina
Comprehensive Alcohol Research Center, Louisiana State University Health Sciences Center, NOLA

The two most commonly used and abused drugs are alcohol and the cannabinoids. Alcohol and drugs of abuse have been demonstrated to alter host response to human immunodeficiency (HIV) infection; by affecting progression of infection, tissue injury, and time to death. Several factors can be involved in this, those pertaining to the host response, as well as those related to the ability of the virus to integrate itself into the host genome. Alcohol use disorders frequently coexist with infection. Cannabinoid use is frequent in HIV-infected individuals, both as a recreational agent and a therapeutic adjuvant, in its synthetic form of Δ9-tetrahydrocannabinol. The biomedical consequences of alcohol abuse are multi-systemic. While considerable advances have been made in our understanding of alcohol-induced injury of hepatic, behavioral, and neural substrates; the metabolic consequences of chronic alcohol abuse have been previously neglected. Cannabinoids, the principal chemical constituents of marijuana, exert neurobehavioral effects and in addition have the potential of affecting the immune system. Viral entry, integration and replication, and cell injury involve numerous cellular signaling and effector mechanisms determining the overall systemic response to the infection. The multiplicity and interconnectedness of factors cannot be effectively reconciled using isolated organ, cellular, or molecular approaches. Using an integrated systems biology analysis our studies have identified salient cellular and molecular signatures prevailing during the infection and their modulation by chronic alcohol and cannabinoid administration in the Simian Immunodeficiency Virus (SIV)-infected rhesus macaque. Our findings suggest that chronic alcohol and cannabinoid administration differentially modulate key interconnected and tissue-specific mechanisms responsible for control of disease progression. The systemic pathophysiological effects of alcohol have been identified to adversely affect the course and progression of HIV infection. The significant multi-systemic pathophysiological mechanisms including; but not limited, to nutritional, metabolic, oxidative, and disruption of neuroendocrine pathways have all been implicated as modulators of disease progression. In contrast, our data suggests that cannabinoids may exert an overall protective effect. Although these effects are multisystemic, specific organ systems have been identified to be central to disease progression; including the central nervous system, the immune system, and gut mucosa. We hypothesize that the contrasting disease phenotype resulting from chronic alcohol and cannabinoid administration to SIV-infected non-human primates provides a unique opportunity for systems biology analysis of the epigenetic, genetic, and proteomic profiles prevailing during SIV infection that are translatable to HIV disease progression. Supported by NIDA-020419, NIDA030053, AA-09803, AA-07577, AA-11290.

2

Chronic Alcohol Consumption Increases Mortality in Sepsis

Benyam P. Yoseph, Zhe Liang, Elise Breed, Kevin McConnell, David M. Guidot, Michael Koval, Craig M. Coopersmith
Emory University School of Medicine

Introduction: Excessive alcohol abuse is a problem of particular concern in the intensive care unit (ICU), as the rate of morbidity and mortality in all patients admitted to ICU is 2-4 times greater than in non-alcoholics. Sepsis is the leading cause of death in ICU. The purpose of this study was to examine the pathophysiology of chronic alcohol abuse in sepsis.

Methods: FVB/N mice were given liquid ethanol diet (20% w/v) or water for 12 weeks. Twelve weeks later all mice underwent 2X25 cecal ligation and puncture (CLP). Mice were sacrificed seven-days or 24 h after CLP. Gut apoptosis was quantified in 100 crypts/mouse by caspase-3 staining. Villus length was measured from the crypt neck to villus tip in 12 villi/mouse. Permeability was measured by gavaging mice with 22mg/ml FITC-dextran 19hrs post-CLP. The FITC concentration in the plasma was determined at 24hrs. Blood, peritoneal fluid (PF), and bronchoalveolar lavage (BAL) levels of TNF-α, IL-6, IL-10, IL-1β, IL-13, MCP-1, G-CSF, IFN-γ, and TGF- β concentration were evaluated using a multiplex assay. Data were compared using Mann-Whitney tests.

Results: Septic alcoholic (SA) mice had a significant higher mortality than septic non-alcoholic (SNA) mice (74% vs 41%, p=0.01). SA mice had increased in gut apoptosis compared with SNA mice (25 ± 1 vs 14 ± 1cells, p=0.0001, n=9-10/group). SA mice had shorter villi than SNA mice (178 ± 8 vs 246 ± 7μm, p=0.0002, n=9/group). SA mice had higher permeability than SNA mice (632 ± 57 vs 372 ± 38 pg/ml, p=0.0009, n=13-15/group). SA mice had significantly lower levels of IL-6 in serum, significantly higher levels of MCP-1 in PF, and significantly higher levels of G-CSF and TGF- β in BAL compared to SNA mice (Table 1).

Table 1. Cytokine levels in serum, peritoneal fluid, and bronchoalveolar lavage

	Serum, pg/ml		Peritoneal, pg/ml		BAL, pg/ml	
	H20	EtOH	H20	EtOH	H20	EtOH
IL-1β	9096 ± 1947	7630 ± 1262	3253 ± 1467	1351 ± 724	1280 ± 302	1768 ± 602
IL-6	22421 ± 3752	11872 ± 2220[a]	16502 ± 4876	10774 ± 1899	99 ± 23	213 ± 93
IL-10	2203 ± 521	3660 ± 849	816 ± 264	501 ± 85	232 ± 41	186 ± 41
IL-13	1415 ± 204	1600 ± 529	1207 ± 552	619 ± 126	911 ± 407	1729 ± 999
G-CSF	306011 ± 6606	312684 ± 15801	280086 ± 26961	250883 ± 21230	17280 ± 3114	33976 ± 8226[a]
IFN-g	22 ± 3	17 ± 1	9 ± 3	11 ± 4	35 ± 11	39 ± 5
MCP-1	42164 ± 7906	29871 ± 5586	24145 ± 5434	55975 ± 7518[a]	808 ± 268	1065 ± 281
TNF-a	667 ± 98	984 ± 194	524 ± 362	119 ± 19	159 ± 41	308 ± 76
TGF-β					376 ± 81	778 ± 71[a]

Data are expressed as means ± SE. [a]$P < 0.05$ vs no-alcohol.

Conclusions: These results indicate that chronic alcohol abuse causes higher mortality in septic mice compared to previously healthy mice subject to the same insult. This was associated with increased gut apoptosis, decreased villus length, and increased gut permeability. Among the cytokines, systemic IL-6, local MCP-1, and BAL G-CSF and TFG- β were the predominant cytokines affected by chronic use of alcohol in sepsis.

3

Inhibition of Myosin Light Chain Kinase Prevents Gut Inflammation and Alterations in Tight Junction Proteins Following Binge Ethanol Exposure and Burn Injury

Anita Zahs[1], Luis Ramirez[1], Jerrold R. Turner[2], Mashkoor A. Choudhry[1], Elizabeth J. Kovacs[1]
[1]Loyola University Medical Center Stritch School of Medicine; [2]Department of Pathology University of Chicago

Elevated myosin light chain kinase (MLCK) activity has been attributed to intestinal permeability following numerous diseases and insults. Burn injury or ethanol exposure alone induces greater MLCK activity, specifically in the intestine. As nearly 50% of the adult burn patient population has a positive blood alcohol content at the time of admission, we examined the role of MLCK in intestinal barrier dysfunction in a mouse model of ethanol exposure and burn injury. Two hours after the combined insult both tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), two major activators of MLCK, are 10 times higher in the serum of mice exposed to burn injury alone or the combined insult as compared to either sham group. By 3 hours post injury, mice exposed to binge ethanol and burn injury have twice as much MLCK and significantly more phospho-myosin light chain (pMLC) in intestinal epithelial cells than all other groups. This increase in pMLC was associated with elevated villus blunting, bacterial translocation, and ileum levels of IL-1β at 6 hours post insult. Furthermore, tight junction proteins, ZO-1 and occludin, were no longer localized with actin and IL-6 had doubled in mice exposed to the combined insult by 24 hours. To attempt to alleviate these detrimental responses, we treated mice with membrane permeant inhibitor of MLCK (PIK) 30 min after insult. PIK treatment significantly reduced pMLC in intestinal epithelial cells of binge ethanol-exposed and burn-injured mice and decreased intestinal tissue damage as compared to mice not receiving PIK treatment. Moreover, mice subjected to ethanol and burn and given PIK had no alterations in ZO-1 or occludin localization and 33% less IL-1β and 50% less IL-6 at 6 and 24 hours respectively as compared to the non-PIK treated group. These data suggest that early activation of MLCK after the combined insult causes intestinal tissue damage as well as alterations to the intestinal epithelial layer possibly leading to severe systemic consequences. [This work was supported by NIH R01AA012034 (EJK), T32 AA013527 (EJK), F31 AA019913 (AZ), R01DK06271 (JRT), Dr. Ralph and Marian C. Falk Medical Research Trust (EJK).]

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Chronic Alcohol Effects on Oxidative Stress Markers in the Rat Heart

Robin Walker, George Haddad
Howard University

Cardiovascular disease is among the major causes for increased morbidity and mortality rates. High alcohol consumption may lead to cardiomyopathy, cardiac arrhythmias, and a suite of other disorders. Previous studies in our lab have linked the AKT/PI3K pathway to cardiovascular disease. Alcohol consumption induces oxidative stress and increases the risk of liver disease, heart disease, certain cancers, brain damage and several other complications involving metabolic disturbances and organ damage. Clinical features of the consequences of prolonged and excessive ethanol consumption encompass defects in myocardial contractility and derangement of cellular architecture, including disarray of the contractile elements. Although the incidence of heart muscle abnormalities in alcohol misusers is generally higher than previously considered, the mechanisms are only just being elucidated. Oxidative stress has been reported to be the major contributor for these alcohol induced pathologies. In the present study, our aim is to investigate the effects of chronic alcohol treatments on expression of NRF2, a core regulator of antioxidant machinery on oxidative stress markers of alcohol induced cardiomyopathy; as well as other phase II oxidative stress genes. Our preliminary results show that with increasing concentrations of alcohol from low (5mM Ethanol) to high (100mM Ethanol) we increase *NRF2* and *SOD-3* gene expression. Our data suggest that NRF2 may act as a molecular switch to control the oxidative balance during chronic alcohol consumption in the heart. We will use this data to continue to examine the link between NRF2 signaling and the AKT/PI3K pathway in alcohol-induced cardiomyopathy. This work was supported in part by grants 1R15AA19816-01A1 NIH/NIAAA, GM08016-38 from NIH/NIGMS and 2G12 RR003048 RCMI Program, Division of Research Infrastructure, NIH/NCRR.

5

Cold Shock Proteins and Alcohol-Induced Neurosuppression

Ping Wang, Asha Jacob
The Feinstein Institute for Medical Research, Manhasset, NY

Among substance abuse, alcohol is one of the most wildly used substances. Alcohol abuse affects 18 million people with a direct cost of $27 billion annually for alcohol-related illnesses in the United States alone. Excessive alcohol ingestion is associated with the impairment of cognitive function and even structural deterioration in the brain. The brain imaging technique has significantly contributed to our understanding of the effect of alcohol abuse and its correlation to functional and structural changes in the human brain. The assessment of brain glucose metabolism is a reliable measure of cerebral function. Studies using positron emission tomography (PET) have indicated that acute alcohol exposure results in lower brain glucose utilization, reflecting a decrease in brain activities. However, the molecular factor responsible for such neurosuppression has not yet been elucidated. In this regard, we have proposed to test a novel concept that a

newly-identified inflammatory mediator, cold-inducible RNA-binding protein (CIRP), is responsible for the reported decrease in neuronal activities in alcoholics. We have established the use of the advanced, non-invasive, and real-time microPET imaging technology to monitor brain activities in the mouse. Wild-type and CIRP knockout mice were exposed to ethanol at a dose of 1.75 g/kg BW followed by continuous infusion at a rate of 200-300 mg/kg/h for 15 h and brain glucose metabolism was analyzed using 18F-flurodeoxyglucose (FDG) and a microPET scanner. Our results showed that alcohol intoxication in wild-type mice produced significant decrease in brain glucose metabolism. In contrast, CIRP knockout mice were more resistant to the decrease in brain glucose metabolism induced by acute alcohol exposure, suggesting CIRP may mediate central neurosuppression. In addition, CIRP protein levels increased in the hypothalamus of the brain and cerebral spinal fluid in alcohol-exposed wild-type animals. In murine microglia (brain macrophages) BV2 cells, alcohol exposure increased CIRP's mRNA and protein expression as well as its release into the extracellular matrix in a dose-dependent manner. Moreover, recombinant murine CIRP (rmCIRP) caused inflammation and tissue injury. Thus, it appears that alcohol intoxication increases brain CIRP expression and release, which cause the reduction of brain activities.

6

Diminished Vaccine Responses and Cytokine Production Are Associated with Increased Expression of Multiple microRNAs in a Nonhuman Primate Model of Alcohol Self-Administration

Mark J . Asquith[1], Flora Engelmann[1], Christine Meyer[1], Kathleen Grant[2, 3], Ilhem Messaoudi[1, 3, 4]

[1]Vaccine and Gene Therapy Institute, Oregon National Primate Research Center; [2]Behavioral Neuroscience Oregon Health and Science University, Portland, OR, USA ; [3]Division of Neurosciences, Oregon National Primate Research Center; [4]Division of Pathobiology and Immunology, Oregon National Primate Research Center, Beaverton, OR, USA

Using a rhesus macaque model of alcohol self-administration, we have previously shown that chronic alcohol consumption significantly modulates the serum cytokine profile. In this study, we extended these observations by investigating the impact of chronic exposure on both peripheral and mucosal immune homeostasis. All animals were vaccinated modified vaccinia ankara (MVA) before ethanol induction and then again after 6 months of self-administration. Animals were euthanized after 12 months of ethanol consumption. At necropsy, blood and gut biopsies were collected to determine the impact of chronic ethanol consumption on peripheral and mucosal cytokine production. Strikingly, we found ethanol exposure inhibited both CD4 and CD8 T cell responses, but not B cell-mediated immunity to MVA during the booster vaccination. CD4 and CD8 cytokine responses in PBMC, lung, small and large intestine (duodenum, jejunum, ileum and colon) and their draining lymph nodes were also analyzed. Increasing ethanol dose correlated with diminished PBMC growth factor production and diminished Th1 and Th17 responses in the intestinal mucosa. These changes were associated with the upregulation of multiple microRNAs implicated in the regulation of cytokine expression in both PBMC and the colonic mucosa.

7

Circulating microRNAs in Exosomes Indicate Hepatocyte Injury and Inflammation in Acute and Chronic Liver Disease

Shashi Bala, Jan Petrasek, Donna Catalano, Karen Kodys, Gyongyi Szabo

University of Massachusetts Medical School, Worcester, MA-01605

Background: MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. The levels of miRs not only change in diseased tissues but also in circulation. Further, miRs are stable in frozen samples and in extreme conditions that make them attractive potential biomarker. Circulating miRNAs are either associated with exosomes or proteins and are shown to involve in cell-to-cell communication. MiRNA-122 is highly abundant in hepatocytes where it regulates various metabolic pathways while miR-155 is a central regulator of inflammation. The aim of this study was to evaluate circulating miRNAs as potential markers of hepatocyte damage and/or inflammation in liver diseases. **Methods:** Serum/plasma and liver samples were collected from C57/BL6 mice after: 1 Chronic alcohol feeding with Lieber-deCarli diet (induces steatosis and inflammation) 2 Acetaminophen (APAP, 500mg/kg) administration (induces hepatocyte necrosis) 3 TLR9+4 administration (induces inflammation and massive recruitment of lympocytes) 4 CCl_4 administration for 2-6 weeks (induces inflammation and fibrosis). Serum/plasma ALT was evaluated and total RNA was analyzed for miRNAs and mRNAs expression. Non-parametric Mann-Whitney test was used for statistics. **Results:** The alcohol, APAP, TLR9+TLR4 and CCl_4, -induced liver injury models all resulted in ALT increase and more important, in increased serum/plasma miR-122 levels compared to control mice. There was a linear correlation between miR-122 and ALT levels. After CCl_4 treatment, serum miR-122 was upregulated as early as two weeks over controls and it remained elevated. Furthermore, there was no increase in serum miR-122 in TLR4 or NADPH oxidase–deficient mice (KO) after alcohol feeding as these KO mice were protected from alcohol-induced liver injury, steatosis and inflammation. Alcohol-, APAP, TLR9+TLR4 and CCl_4 -induced liver damage are all involved in activation of the inflammatory cascade and consistent with this, we found increased serum miR-155 levels. Additionally, miR-125b was increased only in APAP-induced liver injury whereas miR-146a was upregulated both in APAP- and CpG+LPS-induced liver necrosis and inflammation respectively. Importantly, in ALD and in CpG+LPS-induced liver inflammation, serum/plasma miR-122 and miR-155 were predominantly associated with the exosome-rich fraction while in APAP-induced liver necrosis these miRNAs were prevalent in the protein-rich fraction. The hepatic expression of these miRNAs was also differentially regulated among different liver injury models. **Conclusion:** In conclusion, our results suggest that circulating miRNAs may serve as biomarkers to differentiate between hepatocyte injury and inflammation and the exosome versus protein association of miRNAs may provide further specificity to mechanisms of liver pathology. The utility of circulating miRNAs and their association with exosome versus proteins deserves further investigation in human disease states.

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ABSTRACTS

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Alcohol Modulates Airway Hyperresponsiveness and Inflammation in Allergic Asthma

Peter J. Oldenburg, Joe H. Sisson

Pulmonary, Critical Care, Sleep and Allergy Division, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198-5910

Historically, alcohol has been utilized as a bronchodilator and has been used as an asthma treatment. Our laboratory and others have demonstrated that many lung functions can be directly altered by alcohol. We previously reported that alcohol exposure decreases airway hyperresponsiveness (AHR) through a nitric oxide/PKG mediated pathway in non-allergic mice. In allergic asthma, allergen exposure leads to increased bronchial constriction, increased mucus secretion, and increases in the localization of inflammatory cells to the airways, but it is unknown how ethanol affects these allergic asthma parameters. We hypothesized that ethanol exposure will decrease AHR and decrease airway inflammation. To test these hypotheses, BALB/c mice were exposed to either water or 18% alcohol in their drinking water for 6 weeks. Following 6 weeks of drinking, mice were sensitized with i.p. injections of ovalbumin (20 μg/mouse) on days 1 and 14, then received aerosol challenges of a 1% ovalbumin solution on days 23-29. Alcohol-consuming mice continued drinking 18% ethanol throughout the entire experiment. Control mice received i.p. injections of vehicle and were exposed to aerosol challenges of 0.9% saline. Airway responsiveness was assessed via *in vivo* (Penh, total lung resistance (RL)), *ex vivo* (precision cut lung slices), and *in vitro* (airway smooth muscle) methods. In all methods tested, AHR to methacholine was blocked with alcohol exposure. In sensitized mice exposed to ethanol, AHR was no different than non-ethanol controls. Bronchoalveolar lung lavage fluid (BALF) from the non-sensitized control and non-sensitized ethanol-fed mice contained no inflammatory cell increases and consisted primarily of macrophage cells. However, BALF from the sensitized ethanol-fed group contained inflammatory cell infiltrates and consisted of approximately 65% fewer eosinophils than the sensitized water-fed mice. Concurrent with this, we observed an approximate 50% increase in the ratio of macrophage cells present in the BALF of the ethanol-fed mice when compared to the sensitized water-fed group. These data, for the first time, strongly support a positive role for ethanol's influence on the airways by suggesting novel roles for alcohol in the modulation of bronchial motor tone and the airway inflammatory response. In addition, the data suggests a mechanical and cellular basis for clinical observations in humans thereby substantiating the bronchodilator properties of alcohol.

9

Donor Chronic Alcohol Abuse Increases the Risk for Primary Graft Dysfunction in Lung Transplant Recipients

Erin M. Lowery[1, 2], Elizabeth J. Kovacs[1, 3, 4]

[1]Alcohol Research Program, Loyola University Health Sciences Division, Maywood, IL 60153, USA.; [2]Department of Medicine, Loyola University Health Sciences Division, Maywood, IL 60153, USA.; [3]Department of Surgery, Loyola University Health Sciences Division, Maywood, IL 60153, USA.; [4]Department of Microbiology and Immunology, Loyola University Health Sciences Division, Maywood, IL 60153, USA.; [5]Burn & Shock Trauma Institute, Loyola University Health Sciences Division, Maywood, IL 60153, USA.

Compared to all other solid organ recipients, lung transplant recipients have the highest morbidity and mortality following lung transplantation, with a five year survival of only 50%. Early mortality is highest from primary graft dysfunction (PGD), affecting 15-55% of recipients. Severe PGD is the leading cause of death in the early perioperative period. Beyond the early perioperative period, development of chronic rejection is the most common cause of early and late mortality following transplant. PGD following lung transplant is a major risk factor for the development of chronic rejection. Additional research into improvement ofpatient and lung allograft survival is needed. The effects of alcohol abuse in donors of lung allografts on transplant outcomes is unknown. Alcohol abuse has known pulmonary consequences including an increased risk of acute lung injury. Therefore, we hypothesize that donor alcohol abuse will lead to an increased risk of graft dysfunction following lung transplantation. We conducted a retrospective cohort study of lung transplant recipients who received transplantation at Loyola University Medical Center from 2007-2010, reviewing patient charts for donor information and lung transplant outcome data. Statistical analysis performed included Mann-Whitney U and Odds Ratio tests. Recipients of allografts from donors with reported alcohol abuse clearly required prolonged mechanical ventilation when comparing recipients whose donors had reported alcohol abuse to recipients with no reported donor alcohol abuse, [mean 43.5±28.7 days versus 6.01±1.6 days on the ventilator], p=0.001 (Fig. 1A). Subsequently, the duration of hospitalization following transplant was prolonged in those recipients who received organs from reported alcohol abusers compared to recipients with no reported donor alcohol abuse, [mean 29.3±7.8 days versus 18.8±1.5 days hospitalized], p=0.04 (Fig. 1B). 100% of the recipients whose donors reported chronic alcohol abuse suffered from PGD. The risk of severe PGD in recipients of donor alcoholic lung allografts was 5.47 [95% CI 1.08-24.75], p=0.04. Lung transplant recipients who received allografts from alcoholic donors had an increased risk of PGD. Development of PGD leads to prolonged need for mechanical ventilation and longer hospitalizations following lung transplantation. This ultimately places the recipients at risk for hospital associated infections and pneumonia. We speculate that donor allografts from alcoholics leads to an increase in the pro-inflammatory environment in the lung, which is worsened by the oxidative stresses of transplantation, resulting in an increased rate of PGD.

ABSTRACTS

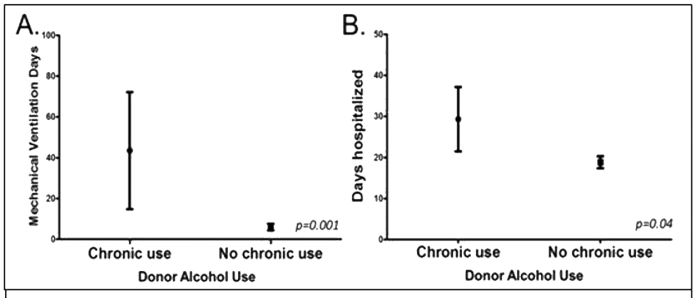

Fig. 1. Lung transplant recipients who receive allografts from chronic alcohol abusing donors have prolonged need for mechanical ventilation (**Panel A**) and longer hospitalizations (**Panel B**) post-transplant than those receiving lungs from non-alcohol abusers.

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Differential Modulation of Chronic Alcohol Consumption on NKT Cells in Non-tumor Injected and Melanoma-Bearing Mice

Hui Zhang, Zhaohui Zhu, Gary G. Meadows
Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Pullman, WA 99164-6534

NKT cells, with the dual functions of innate and adaptive immune cells, are important immune regulatory cells. They play important roles in shaping the immune response and outcome in autoimmune diseases and in modulating anti-tumor immunity. Chronic alcohol consumption increases the incidence of cancer and decreases the survival of cancer patients. How chronic alcohol consumption affects NKT cells in hosts with and without cancer is not known. Using a well-established chronic alcohol consumption and subcutaneous B16BL6 melanoma inoculation model in mice we found that alcohol consumption increases NKT cells in the thymus and liver without affecting these cells in the spleen and blood of non-tumor injected mice. The portion of $NK1.1^-$ NKT cells is decreased in these mice compared to their water-drinking counterparts. Upon activation, the percentage of IFN-γ-producing NKT cells is higher in alcohol-consuming mice than in water-drinking mice. However, there is a lower rate of proliferating NKT cells in the liver of alcohol-consuming mice than in the water-drinking mice. Alcohol consumption not only increases NKT cells in the thymus and liver of melanoma-bearing mice, but also dramatically increases NKT cells in the blood. The ratio of $NK1.1^-$ to $NK1.1^+$ NKT cells decreases further as compared to the non-tumor injected mice. More importantly, alcohol consumption interacting with melanoma reverses the cytokine profile from IFN-γ (Th1) to IL-4 (Th2) dominance. Taken together these results suggest that alcohol consumption induces a signal (Signal I) to activate NKT cells in non-tumor-bearing mice. In the tumor-bearing mice, alcohol interacting with melanoma induces another signal (Signal II) that further activates NKT cells. We hypothesize that the continuous stimulation of NKT cells in the presence of the alcohol/melanoma interaction induces NKT cell anergy, which results in NKT cells producing predominately Th2 cytokines. This leads to inhibition of antitumor immunity in alcohol-consuming, melanoma-bearing mice.(Supported by grants R01AA07293 and K05AA017149 and by funds provided for medical and biological research by the State of Washington Initiative Measure No. 171).

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Increase in Gut Bacterial Load and Nod-1 Expression in the Intestinal Epithelial Cells Following Ethanol and Burn Injury

Suhail Akhtar, Xiaoling Li, Mashkoor A. Choudhry
Alcohol Research Program, Burn & Shock Trauma Institute, Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153.

Nucleotide oligomerization domain (Nod)-1 and Nod-2 are cytosolic sensor molecules which play an important role in sensing and mediating the effects of bacteria. While Nod-1 ligand γ-D-glutamyl-meso-diaminopimelic acid is primarily found in Gram-negative bacteria, Nod-2 senses muramyl dipeptide which is found in the peptidoglycan of nearly all Gram-positive and Gram-negative bacteria. Nod proteins also play a critical role in regulation of the host innate immune response by activating caspase-1 leading to the formation of inflammasome, a multi-protein complex, subsequently releasing proinflammatory cytokine IL-18. We have shown previously a significant increase in IL-18 in the gut following alcohol/ethanol (EtOH) and burn injury. In the present study, we evaluated the effect of EtOH intoxication and burn injury on gut bacterial load and the expression of Nod-1 and Nod-2 by intestinal epithelial cells. To accomplish this, male C57BL/6 mice were divided into four groups: Sham Vehicle, Sham EtOH, Burn Vehicle and Burn EtOH. Mice were gavaged with water or EtOH (2.9 mg/Kg) to achieve a blood EtOH level of ~100 mg/dL prior to receiving a ~12.5 % total body surface area sham or burn injury. One day after injury, mice were sacrificed and intestinal luminal content and intestinal tissue were harvested and homogenized. The luminal content and tissue homogenates were plated on MacConkey agar plates and bacterial colony forming units (CFU) were counted. Intestinal epithelial cells were isolated, sonicated and the supernatants were used for western blot analysis of Nod-1 and Nod-2 expression. As compared to sham vehicle, EtOH or burn injury alone does not cause a significant change in gut bacterial load. However, EtOH combined with burn injury resulted in a several fold increase in the Gram-negative bacterial CFU both in intestinal tissue and the luminal content compared to sham or burn injury alone. There was no change in Nod-1 level in the intestinal epithelial cells of sham animals, regardless of ethanol exposure, as well as in animals receiving burn injury alone. However, approximately 50% increase in Nod-1 expression was observed in the intestine of animals receiving a combined insult of ethanol and burn injury as compared to shams ($p<0.05$). We could not detect Nod-2 protein expression in the intestinal epithelial cells in any of the treatment groups. These findings indicate that an increase in Gram-negative bacterial load in the gut can activate Nod-1-dependent signaling cascade leading to the production of inflammatory cytokines such as IL-18. (NIH R01AA015731 and the Dr. Ralph and Marian C. Falk Medical Research Trust)

ABSTRACTS

12

Chronic Binge Alcohol Administration Alters Murine IL-22 Production and the Microbiota Following Cutaneous Wounding

Jennifer K. Plichta[1], Tina Griffin[1], Sasha Shafikhani[2], Katherine A. Radek[1]

[1]Loyola University Chicago, Health Sciences Campus, Department of Surgery; [2]Rush University Chicago, Department of Immunology and Microbiology

Interleukin (IL)-22 is a member of the IL-10 superfamily that stimulates pro- and anti-inflammatory responses through its interaction with cell surface receptor complexes expressed by epithelial cells. IL-22 is produced by activated Dendritic and T cells to facilitate the control of bacterial infection and tissue repair via the production of antimicrobial peptides (AMPs) and pro-inflammatory molecules. Elevated levels of IL-22 in the skin can be detrimental by blocking keratinocyte differentiation and increasing their mobility, while stimulating the production of dermal proteases. We previously found that ethanol exposure dampens AMP and chemokine production, delays closure, and increases protease activity in mouse wounds. Here, we sought to evaluate whether the suppression of inflammatory responses during early tissue repair via chronic binge ethanol administration would impact the microbiota and dissemination of bacteria, potentially via alterations in IL-22 production. Male C57Bl/6 mice were administered intraperitoneal injections of either sterile saline (controls) or ethanol (2g/kg) for four consecutive days per week for a total of six weeks. At week five, mice underwent excisional wounding (six 3mm wounds per mouse) followed by topical application of either sterile saline, or 10^6 CFU of *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Saline or ethanol injections were continued as indicated above for 10 days post-wounding. Day 10 skin, lung, spleen, and kidney were processed to determine the number of surviving bacteria and IL-22 levels by ELISA. Chronic binge ethanol administration significantly elevated total bacteria counts in wounds, unwounded skin, lung, and spleen, regardless of topical saline or bacterial inoculated wounds, as compared to controls ($p<0.05$). For mice subjected chronic binge ethanol, topical inoculation with *P. aeruginosa* was associated with over two-fold higher bacteria counts in wounds ($p<0.05$) compared to *S. aureus*. A similar trend for spleen tissue homogenate ($p=0.0588$). No significant differences were noted in kidney homogenates. Chronic ethanol administration resulted in a >50% increase in total IL-22 levels in wounds ($p<0.05$) compared to controls. Furthermore, regardless of topical saline or bacterial inoculated wounds, chronic binge ethanol administration significantly increased IL-22 production in excisional wounds, and promoted a 25% reduction in IL-22 in lung homogenates ($p<0.05$) compared to controls. Chronic binge ethanol administration alters the total microbiota in several tissues and increases bacterial dissemination, regardless of topical saline or bacterial inoculation. In lung, the decreased IL-22 levels and its associated antimicrobial properties may contribute to the increased bacteria counts observed. In contrast, the elevated IL-22 in wounds may be associated with defective keratinocyte barrier properties and increased protease activity to alter the local microbiota and tissue repair process.

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Hepatic Killing of Lymphocytes Is Decreased Following Inhibition of the Hepatocyte-Specific Asialoglycoprotein Receptor: Role in Alcoholic Liver Injury

Benita McVicker[1, 2], Dean Tuma[2], Natalia Osna[1, 2], Geoffrey Thiele[1, 2], Carol Casey[1, 2]

[1]VA Nebraska-Western Iowa Health Care System, Omaha, NE; [2]Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE

It is known that the liver is a site of lymphocyte trapping and killing used in the control of peripheral immune responses. Recently it has been shown that hepatocytes may play a significant role in the homeostatic regulation of lymphocytes within the liver via interactions mediated by the hepatocyte-specific asialoglycoprotein receptor (ASGPR). During alcohol-induced liver injury the presence of intrahepatic lymphocytes is enhanced leading to T cell-mediated hepatitis. Additionally, marked ethanol-induced impairments to the ASGPR occur. Here, we investigated whether the inhibition of ASGPR function via blocking agents or ethanol treatment affects the hepatocyte killing of target T cells in vitro. Methods: Hepatocytes (HCs), intrahepatic lymphocytes (IHLs) and splenocytes (SPCs) were isolated from rats chronically-fed Lieber-DeCarli control or ethanol containing diets. SPC populations (CD8+ and CD4+ T cells) were isolated by magnetic bead separation and SPCs and IHLs were treated with or without T cell activators, phorbol myristate acetate (PMA) or anti-CD3/CD28 antibodies. Confirmation of T cell phenotype and activation state was obtained by flow cytometric and cytokine analysis. ASGPR-mediated cytotoxicity of radiolabeled target IHLs or SPCs was analyzed in HC-lymphocyte co-cultures containing specific inhibitors of ASGPR. Results: Both IHLs and SPCs bound to hepatic ASGPRs with high specificity. Activation of IHL and SPC cells resulted in a 30-40% increase of lymphocyte killing by HCs that was blocked in the presence of competitive ligand (asialofetuin) or anti-ASGPR antibody (ASGPR Ab). In addition, the inclusion of colchicine, an inhibitor of perforin/granzyme-mediated cytotoxic mechanisms, reduced the killing of the target SPCs and IHLs to baseline levels. When HCs from ethanol-fed animals were incubated with activated SPCs, a significant reduction ($P<0.05$) in SPC killing was observed compared to the T cell death measured in the cultures containing hepatocytes from normal control-fed rats. Also, HCs killed all subtypes of SPCs tested (CD8 and CD4 enriched). However, more CD8+ T cells were killed compared to CD4+ T cells and more SPCs derived from the spleens of ethanol animals were killed compared to controls. Conclusions: We present evidence that impaired ASGPR function results in altered T cell regulation, in part by reducing perforin/granzyme induced killing of T cells by hepatocytes. Overall, the loss of hepatic ASGPR-mediated T cell regulation, particularly the regulation of cytotoxic CD8+ T cells, could significantly impact T cell homeostasis in the liver and may be related to the accumulation of IHLs that is observed in alcoholic hepatitis.

ABSTRACTS

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Acute Ethanol Intoxication and Burn Injury Differentially Affects Th17 Effector Cytokines IL-17 and IL-22

Juan L. Rendon[1, 5, 6], Xiaoling Li[1, 2, 6], Aleah L. Brubaker[2, 5, 6], Elizabeth J. Kovacs[2, 3, 6], Richard L. Gamelli[1, 2, 6], Mashkoor A. Choudhry[1, 3, 6]

[1]Alcohol Research Program; [2]Burn & Shock Trauma Institute; [3]Departments of Surgery and Microbiology & Immunology; [5]Cell Biology, Neurobiology & Anatomy Program; [6]Loyola University Chicago Health Sciences Campus Stritch School of Medicine

T helper (Th)-17 lymphocytes play a crucial role in the maintenance and regulation of gut immunity. Our laboratory has demonstrated that acute alcohol/ethanol (EtOH) exposure prior to burn injury results in intestinal T cell suppression and enhanced bacterial translocation. Clinically, adult burn patients with a measureable blood EtOH level at the time of hospital admission demonstrate increased susceptibility to infection, require more surgical interventions and exhibit higher mortality than burn victims without EtOH intoxication at the time of injury. In our current study, we examined the effects of EtOH and burn injury on Th17 effector responses within intestinal lymphoid Peyer's patches. Peyer's patches are important intestinal secondary lymphoid organs that play a critical role in T cell immunity, as well as containment of gut bacterial translocation. IL-23, released by mucosal dendritic cells, is indispensable in the expression of IL-22; thus, we further investigated whether restitution of IL-23 enhances Peyer's patch cell IL-17 and IL-22 following EtOH and burn injury. Male mice, ~25g, were gavaged with EtOH (2.9mg/kg) prior to receiving a ~12.5% total body surface area full thickness burn. One day post injury, Peyer's patch mixed cells were cultured in the presence of plate bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (1 μg/ml) in presence or absence of IL-23 (10 ng/ml) for 48 h. Supernatants were harvested and tested for IL-17 and IL-22 levels. When combined with EtOH intoxication, burn injury suppressed IL-17 and IL-22, as compared to sham injury (90% and 65% respectively, $p<0.01$). IL-23 treatment successfully increased levels of IL-22 in the burn EtOH group (5.8 fold, $p<0.001$, as compared to anti-CD3/anti-CD28 treatment alone). IL-23 treatment did not affect IL-17. To further delineate the mechanism of differential IL-17 and IL-22 suppression, Peyer's patch cells were treated with phorbol 12-myristate 13-acetate (PMA) (10 ng/ml) and ionomycin (50 ng/ml), which signal via protein kinase C (PKC) and calcium flux. Treatment with PMA and ionomycin induced IL-17 (4.7 fold, $p<0.01$, as compared to anti-CD3/anti-CD28 alone) following EtOH and burn injury, but did not influence IL-22. These findings suggest that IL-23 mediates restoration of IL-22, whereas IL-17 requires activation of PKC and intracellular calcium signaling. (Supported by NIH R01AA015731(MAC), F30AA020167(JLR), T32AA013527(EJK), the Loyola SSOM MD/PhD Program and the Dr. Ralph and Marian C. Falk Trust)

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miRNA155-Mediated IL-2 Production in T Cells Following Acute Alcohol Intoxication Combined with Burn Injury

Xiaoling Li, Juan L. Rendon, Suhail Akhtar, Mashkoor A. Choudhry

Alcohol Research Program, Burn & Shock Trauma Institute, Dept. of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL 60153.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that are recognized as important regulators of gene expression at the post-transcriptional level. Studies have indicated that miRNAs regulate innate and adaptive immunity, through modulation of immune cell differentiation, proliferation, apoptosis and signaling transduction. MiRNA155 is required for the normal function of immune cells, including T cells, B cells, macrophages and dendritic cells. Previous studies from our laboratory show that acute alcohol (EtOH) intoxication combined with burn injury suppresses T cell IL-2 and IFN-γ production. In this study, we determined whether acute alcohol intoxication combined with burn injury alters the expression of miRNA-155 in T cells. Male C57BL/6 mice were divided into four groups: Sham Vehicle, Sham EtOH, Burn Vehicle and Burn EtOH. Animals were gavaged with H2O or EtOH (2.9 mg/Kg) to achieve a blood EtOH level of ~100 mg/dL prior to receiving a ~12.5 % total body surface area sham or burn injury. One day after injury, mice were sacrificed and spleen T cells were isolated. T cells were cultured with plate bound anti-CD3 (2μg/ml) for 24 h and cells were harvested to determine miRNA155 expression by qRT-PCR. No significant difference was observed in miRNA155 expression in T cells obtained from sham vehicle and sham EtOH animals. A tendency towards a decrease in T cell miRNA-155 expression was observed in T cells obtained from burn injury alone animals (burn vehicle), but this did not reach statistical significance, as compared with sham animals. However, acute EtOH combined with burn injury resulted in 40% decrease in expression of miRNA155 as compared with shams ($p<0.05$). In next experiment, we treated T cells with anti-CD3 plus Phorbol 12-myristate 13-acetate (PMA, 10ng/ml) and ionomycin (50 ng/ml) which directly stimulates T cell activation and cytokine production, by regulation of intracellular [Ca+2] and Protein Kinase C (PKC) pathway for 48h. Supernatants were collected to measure IL-2 and IFN-γ production by ELISA and cells were harvested to determine miRNA155 expression. We observed that treatment of T cells with anti-CD3 plus PMA/Ionomycin significantly increased IL-2 production, but not IFN-γ production in both sham vehicle and burn EtOH animals, as well as prevented the decrease in miRNA 155 expression in T cells obtained from burn EtOH animals. These findings suggest that miRNA155 may play an important role in IL-2 production following EtOH intoxication combined with burn injury. (NIH R01AA015731 (MAC), F30AA020167 (JLR) and the Dr. Ralph and Marian C. Falk Medical Research Trust).

ABSTRACTS

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Identification of an E3 Ubiquitin Ligase Required for IRF3 K63-Ubiquitination and Activation

Hui Sun, Yukun Guan, Fuping You, Hong Tang, Jianli Tao, Chen Huihui, Jiahuai Han, Zhengfan Jiang
State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing 100871, China

Virus infection induces the production of type I IFNs. Transcription factor IFN regulatory factor 3 (IRF3) plays an essential role and is tightly regulated in this process. It was demonstrated that TBK1 phosphorylates IRF3 at key serine residues, leading to its dimerization and translocation to the nucleus, where it functions together with NF-κB to regulate gene expression. Recent studies have showed that several ubiquitination and deubiquitination enzymes regulate this process. TRIM25, an E3 ubiquitin ligase, was shown to catalyze K63 polyubiquitination of RIG-I, in this way led to the activation of RIG-I. TRIM21 was found to be essential to sustain IRF3 activation by preventing IRF3 ubiquitination and degradation. More recently, it was reported that K63 poly-ubiquitination is essential for IRF3 activation by viral infection. However, direct evidence that K63 poly-ubiquitination is required for viral activation of IRF3 is still lacking.

Here we report a protein, IUK63, that is required for IRF3 K63 ubiquitination. Over-expression of IUK 63 greatly promoted IRF3 activation, and caused strong K63-polyubiquitination of IRF3. MEFs deficient of IUK63 showed no type I interferon production in response to viral infection, suggesting that this protein is required for anti-viral innate immunity. IUK63 protein contains a Ring domain, which is critical for its ability to catalyze ubiquitination. IUK63-/- MEF expressing the ring domain deleted protein (IUK63-delR) was still sensitive to viral infection. These results collectively indicated that IUK63 is an E3 ubiquitin ligase required for IRF3 K63 ubiquitination and activation.

Further studies will focus on the molecular basis of this new finding, including 1) How does TBK1/IKKi activate IUK63? 2) Is this protein also required for NF-kB, or MAPK activation? 3) How and why does K63 ubiquitination of IRF3 lead to its activation?

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Cytoskeletal Abnormalities, Neutrophil Dysfunction, Recurrent Infections and Impaired Healing Due to Mutations in WDR1, Encoding Actin-Interacting Protein 1

Douglas B. Kuhns[1], Harry L. Malech[2], John I. Gallin[2], Steven M. Holland[3]
[1]*Clinical Services Program, SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702;* [2]*Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD 20892;* [3]*Laboratory of Clinical Infectious Diseases, NIAID, NIH, Bethesda, MD 20892*

Cell motility, division, and structural integrity depend on the remodeling of the actin cytoskeleton by a highly regulated dynamic process polymerization/depolymerization. Disruption of these processes in neutrophils causes immunodeficiency. We identified four children in three families with recurrent infections and clinical manifestations ranging from mild neutropenia, severe stomatitis with oral stenosis, to death. All patients had distinctive neutrophil dysmorphology: herniation of the nuclear lobes with agranular regions within the cytosol (Fig. 1). *In vitro*, neutrophils exhibited defects in random and directed migration, spreading on glass, and cell polarization. Filamentous actin in patient neutrophils was increased 4-fold compared to that observed in neutrophils from normal subjects. Using 2-D differential in-gel electrophoresis and mass spectrometry, numerous proteins were identified that were differentially expressed in neutrophil lysates of two affected siblings *vs.* neutrophil lysates of two control subjects. Actin interacting protein 1 (Aip1 encoded by *WDR1*), a double β-propeller cytosolic protein (Fig. 2), was differentially expressed.Aip1 has been shown to complex with cofilin and bind to filamentous actin and is thought to regulate cofilin-mediated severing of filamentous actin. Sequencing of *WDR1* identified biallelic mutations in all patients, and heterozygous mutations in some clinically normal relatives, confirming autosomal recessive inheritance of Aip1 defects. We obtained five discrete mutations within distinct anti-parallel β-strands of blades withing both the N- and C-terminal propellers of Aip1. This novel autosomal recessive immunodeficiency identifies a previously unappreciated role of Aip1 in cell biology, neutrophil function and host defense.

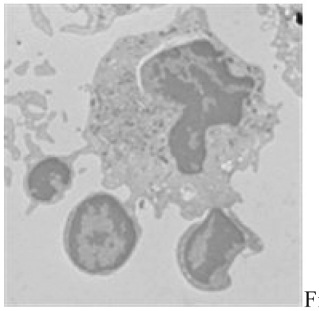

Fig. 1

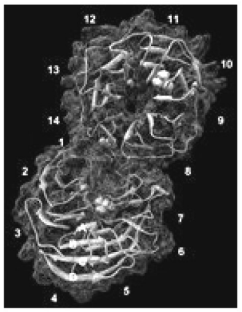

Fig. 2

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Differential Induction of Chemokines MIP-2 and KC Regulates Neutrophil Trafficking and Protects Mice from Bacterial Sepsis

Shalaka Metkar, Jack Silver, Sanna M. Goyert
CUNY Medical School, Sophie Davis School of Biomed. Education, City College of New York

Previous studies have shown that the CD14-dependent induction of systemic chemokines in response to a local bacterial infection with *E. coli* results in a delay in neutrophil recruitment to the site of infection (peritoneal cavity) and leads to bacterial proliferation and increased mortality. In contrast, in CD14-deficient mice, injection (i.p.) of *E. coli* leads to a local induction of chemokines via a CD14-independent pathway that enables rapid neutrophil recruitment, enhanced bacterial clearance and improved survival. This study was initiated to determine whether TLR4 plays a role in the CD14-independent chemokine production. Mice deficient in TLR4 or TLR4 signaling molecules (MyD88-/-TRIF-/-) injected with a lethal dose of E. coli exhibited enhanced survival, bacterial clearance, early neutrophil recruitment and chemokine induction that was low in blood and higher in the site of infection. In contrast, LPS alone did not induce neutrophil recruitment. These studies describe a third pathway for chemokine induction in the peritoneal

cavity that is both CD14- and TLR4-independent that represents a response to a non-LPS component of *E. coli*. These studies illustrate the existence of multiple pathways for inducing neutrophil-attracting chemokines and suggest that differential induction of these pathways can regulate neutrophil trafficking. GRANT SUPPORT: NIH-NIAID#AI23859, NIH-RCMI #G12RR03060.

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Interleukin-17 Is Protective against *saeR/S*-Mediated *Staphylococcus aureus* Pathogenesis and Is Dependent on Interferon-Gamma Gene Activity

Robert Watkins, Kyler Pallister, Oliwia Zurek, Jovanka Voyich
Department of Immunology and Infectious Diseases, Montana State University

Staphylococcus aureus (*S. aureus*) is the predominant bacterial cause of skin and soft tissue infections and this observation is further complicated by increasing antimicrobial resistance. To combat the rapidly emerging antibiotic resistance phenomena, immune-targeting therapies are proposed as viable alternative approaches. However, little is known regarding the mechanisms and impacts of these proposed treatments. Previously, we demonstrated the *S. aureus* two-component system, SaeR/S, was essential for full virulence and is a potent inducer of interferon-gamma genes (*ifng*) and protein (IFNg). Herein, we demonstrate that *ifng*-knockout IFNg-deficient mice (GKO) are protected against *saeR/S*-mediated skin pathogenesis. GKO mice infected with wild-type *S. aureus* (LAC) develop significantly reduced dermonecrotic lesions compared to normal mice (BALB/c). Surprisingly, neutralization of IFNg provided only negligible protection against lesion development in LAC-infected BALB/c mice. However, significantly elevated concentrations of interleukin (IL)-17 were observed early (8 hours) in the affected tissue of LAC-infected GKO mice. Neutralization of IL-17 abrogated the protection observed in LAC-infected GKO mice, as anti-IL-17 mAb-treatment produced similar dermonecrotic lesion sizes to LAC-infected BALB/c mice. Additionally, exogenous IL-17 treatment of LAC-infected BALB/c mice provided protection comparable to the lesions observed in LAC-infected GKO mice. Both GKO and BALB/c mice infected with an isogenic *saeR/S* deletion mutant (LAC*saeR/S*) developed non-dermonecrotic abscesses of similar size and duration. This is in contrast to LAC-infected mice (GKO and BALB/c), which develop open dermonecrotic lesions. From these findings, we conclude that IL-17 protects against *saeR/S*-mediated pathogenesis in an *ifng*-dependent manner and that in the absence of *saeR/S*, both *ifng* and IL-17 are inconsequential for mediating (or inhibiting) protection.

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Negative Regulation of TLR Signaling by PLZF Modulation of NFkB

Bryan R.G. Williams
Monash Institute of Medical Research

Toll-like receptors (TLRs), the primary sensors of specific microbial motifs by the innate immune system, trigger the production of inflammatory cytokines, thereby shaping innate and adaptive immunity to pathogens. TLR signalling must be tightly regulated to maintain immune balance. We show the Promyelocytic Leukemic Zinc Finger (PLZF) protein is a critical regulator of TLR-mediated signalling. PLZF repressed TLR induced, NF-κB dependent transcription by stabilizing a repressor complex that encompasses the NF-κB p50 subunit and the histone deacetylase-3(HDAC3). As a consequence, TLR activated PLZF-null macrophages express higher levels of certain NF-κB dependent transcripts, including those encoding the potent inflammatory cytokines TNFα, IL-12 and IL-6. Accordingly, PLZF-deficient animals are hypersensitive to septic shock and mount an exaggerated inflammatory response to Salmonella infection. These results provide new insights into the mechanisms regulating selectivity in the NF-κB transcriptional programme and suggest a novel strategy for the therapeutic targeting of inflammatory diseases.

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Unique Role of IRF-5 in the Innate Signaling Pathways and Regulation of the B Cell Response to Pathogens

Paula M. Pitha[1, 2], Sujayita Roy[1], Eva Szomolanyi-Tsuda[2]
[1]Johns Hopkins University; [2]University of Massachusetts Medical School

IRF-5 is a transcription factors activated by TLR7 and TLR9 during innate immune response to infection. IRF-5 activates not only Type I IFN, but also inflammatory cytokines. Most importantly a genetic variation in the IRF-5 gene shows a strong association with many autoimmune diseases including Lupus. We have shown that Irf5-/- mice have not only attenuated innate immune response to pathogens, but also a decreased level of plasma cells and plasma cell differentiation factor Blimp-1. Here we report that Irf5-/- mice have a decreased IgG2a/c response to T cell-dependent and independent antigens and to polyoma virus infection. This defect is due to the intrinsic deletion of IRF-5 in B cells, as infected SCID mice reconstituted with Irf5-/- B cells and WT T cells show a decrease in IgG2a/c expression when compared to mice that received WT B cells. In vitro, Irf5-/- B cells have diminished TLR and cytokine-induced class switching to IgG2a/c. Addressing the molecular mechanism of this defect, we show that IRF-5 regulates IgG2a/c expression by attenuating Ikaros expression; reconstitution of IRF-5 in Irf5-/- B cells down-regulates Ikaros levels and increases switching to IgG2a/c. The IRF site in ikzf1 promoter binds IRF-5, IRF-4 and IRF-8 both in vitro and in B cells in vivo. In the transient expression assay IRF-5 inhibits the transcriptional activity of IRF-8. Thus our data are consistent with B cell-intrinsic role of IRF-5 in B cell differentiation and promotion of IgG2a expression, antibody isotope that is important in the antiviral response and also in the autoimmune pathology

ABSTRACTS

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The Genetics of Anthrax Immunization

Kenneth M. Kaufman[1, 2, 3], Beth L. Cobb[1], Lori Garman[4], Nora Rachels[7], Christina Spooner[6], Renata J. Engler[6], Judith A. James[4, 5], John B. Harley[1, 2, 3]

[1]Cincinnati Children's Hospital Medical Center; [2]US Department of Veterans Affairs Medical Center; [3]University of Cincinnati; [4]Oklahoma Medical Research Foundation; [5]University of Oklahoma; [6]Walter Reed National Medical Center; [7]Womack Army Medical Center

Among all the disease causing pathogens, *Bacillus anthraces* is one of the most deadly. Vaccination is recommended for anyone with risk of exposure and is required for United States military personnel, for whom Anthrax Vaccine is prepared from a nonencapsulated strain of the bacteria that is toxigenic and whose cell-free extract contains Protective Antigen (PA), Lethal Factor (LF) and Edema Factor (EF). Vaccinations are given at 0 and 4 weeks, with three vaccinations at 6, 12, and 18 months, followed by annual boosters. The immune response generated towards anthrax varies greatly and little is known of the genetic determinates for the immune response. In order to identify genes that play a role in the anthrax vaccination immune response we performed a genome wide association study using DNA collected from US military volunteers that have been immunized against anthrax within 2 years. The antibody response towards PA, LF and EF were measured using sera collected from each sample using serological assays. PA antibody levels were used to subset samples into five categories (384 total samples). Each category was used to generate a pool of DNA samples that were genotyped on Affymetrix SNP (single nucleotide polymorphism) arrays. A quantitative trait locus analysis was then used to identify candidate loci showing an association with the anti-PA antibody response. Individual genotyping was then performed on the initial cohort of samples used in the DNA pooling experiments, as well as a second cohort of samples to confirm the original associations (total samples 938). 1536 SNPs were genotyped using the Illumina GoldenGate assay. Approximately 400 ancestry markers were used to perform population stratification analysis. The remaining markers were selected based on the previous DNA pooling experiments. The strongest association using the anti-PA antibody concentration in the QTL analysis was with the variant rs4772492 which is located in the promoter region of the TPP2 gene on chr 13 with a p-value of 0.0002. The second strongest association was with rs660811 (p-value of 0.001), located in the first intron of the TPP2 gene. This further supports the association of TPP2 with anti-PA antibody response. These were the only two markers genotyped near the TPP2 gene in our custom SNP array.

These results suggest an intriguing model of the anti-PA antibody response that is dependent on the level TPP2 expression and function. The TPP2 gene encodes tripeptidyl peptidase II protein that is essential for some MHC class I antigen presentation. An examination of the NCBI GTEx (Genotype-Tissue Expression) eQTL Browser shows that the genotype of rs4772492 is strongly associated with the expression level of the TPP2 (p-value 3.5x10-24). Decreased expression is associated with the AA genotype which corresponds with the low anti-PA antibody concentration. The variants at TPP2 may help explain the large differences in responses to anthrax vaccination.

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IL-17A and IL-25 Confer on Normal Germinal Centre B Cells de Novo Migratory Competence to CXCL12 and CXCL13

Vito Pistoia[1], Emma Di Carlo[2], Emanuela Ognio[3], Carla Guarnotta[4], Francesco Bertoni[5], Anna Corcione[1], Ignazia Prigione[1], Giulio Fraternali Orcioni[6], Claudio Tripodo[4], Elisa Ferretti[1]

[1]Laboratory of Oncology, G. Gaslini Institute, Genova, Italy; [2]Department of Oncology and Neurosciences, "G. d'Annunzio" University and Ce.S.I. Aging Research Center, "G. d'Annunzio" University Foundation, Chieti, Italy; [3]Animal Model Facility, National Institute for Cancer Research, Genova, Italy ; [4]Tumor Immunology Unit, Department of Health Science, Human Pathology Section, University of Palermo, Italy; [5]Lymphoma & Genomics Research Program, Institute of Oncology Research (IOR), and Lymphoma Unit, Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland; [6]Department of Pathology, Anatomic Pathology Division, San Martino Hospital, Genoa, Italy

Interleukin(IL)-17A and IL-17E (IL-25), two cytokines belonging to the IL-17 superfamily, are up-regulated in inflammatory and allergic reactions. Previously it was shown that IL-17A contributes to promote germinal centre (GC) formation in different mouse models of human autoimmune or infectious diseases.

In this study we have addressed the expression of receptors for IL-17A and IL-25 and the functional activity of both cytokines on human GC B cells freshly isolated from tonsil. First, we demonstrated by flow cytometry that GC B cells expressed the complete heterodimeric receptors for IL-17A (IL-17RA and IL-17RC chains) and IL-25 (IL-17RA and IL-17RB chains). Such expression was confirmed by immunohistochemical analyses of tonsil tissue sections. When GC B cells were incubated in vitro with either IL-17A or IL-25, the phosphorylated form of NF-kB p65 was found to be upregulated by both cytokines, indicating the occurrence of signalling. No differences in the proportion of apoptotic or proliferating cells in IL-17A or IL-25 treated vs untreated cells were observed. Moreover, we demonstrated that treatment of GC B cells with IL-17A or IL-25 conferred upon these cells migratory competence to CXCL12 and CXCL13, two chemokines involved in the positioning of lymphocytes in secondary lymphoid organs. These effects were driven by 17A- or IL-25- induced modulation of RGS16 expression in GC B cells through a NF-kB dependent mechanism, in the absence of changes in the expression of CXCR4 and CXCR5 in cytokine-treated cells. These data support the hypothesis of a role of IL-17A and IL-25 in the control of the trafficking of GC B cells in the GC microenvironment.

ABSTRACTS

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Human Herpesvirus 8 Infection Induces a Potent Cytokine and Chemokine Polyfunctional Response in Primary Memory B Lymphocytes

Emilee Knowlton[1], Giovanna Rappocciolo[1], Sagar Nadgir[1], Paolo Piazza[1], Mariel Jais[1], Frank Jenkins[1, 2], Charles Rinaldo[1, 2]
[1]Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania; [2]Deparment of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Objectives/Research Questions: DC-SIGN expressing B cells are a major target of human herpesvirus 8 (HHV-8), the etiologic agent of Kaposi's sarcoma (KS) and some B cell lymphomas. Cytokines/chemokines produced by effector B cells could play an important role in KS development. We therefore determined the subset of B cells that supports HHV-8 infection and replication and their cytokine/chemokine profile.
Methods: B cells were infected with purified HHV-8, UV-light inactivated HHV-8 (UV-HHV-8) or exposed to soluble HHV-8 glycoprotein B (gB). B cell phenotypes and intracellular cytokine/chemokine production were determined by flow cytometry. Secreted immune mediators were detected by Cytometric Bead Array. Viral replication was measured by PCR, flow cytometry and TCID50 assay for HHV-8 DNA, proteins and infectious virions, respectively.
Results: HHV-8 DNA levels, protein expression and infectious virion production increased to peak levels by 2 days. HHV-8 infected B cells were exclusively DC-SIGN+/IgM+, with variable expression of CD20, CD27, CD38 and IgD. Infected B cells produced elevated levels of TNF-α, IL-6, IL-8, MIP-1α and MIP-1β, with a predominance of ≥3 cytokines/chemokines per cell. UV-HHV-8 and gB induced lower levels of cytokines/chemokines in DC-SIGN positive and negative B cells.
Conclusions: Productive HHV-8 replication elicits the broadest and greatest magnitude of immune mediators and polyfunctional activity in memory B cells. This could be important in theinduction of KS and HHV-8 associated B cell lymphomas.

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Mucosal Tolerance Therapeutic Stimulates Potent Regulatory B Cells for Protection in a BTLA-Dependent Fashion against Myelin Oligodendrocyte Glycoprotein (MOG)-Induced Experimental Autoimmune Encephalomyelitis (EAE)

David W. Pascual[1, 2], Eduardo Huarte[1], SangMu Jun[1, 2]
[1]Dept. Immunol. & Infect. Dis., Montana State University, Bozeman, MT; [2]Dept. Infect. Dis. & Path., University of Florida, Gainesville, FL

Recently, the tolerizing agent, MOG, fused to reovirus protein σ1 (MOG-pσ1), was shown to resolve EAE at the peak of disease within 24 hrs of mucosal treatment via rapid recruitment of regulatory T cells into the central nervous system (CNS) in an interferon-producing killer dendritic cell (IKDC)-dependent fashion. This tolerogen could resolve EAE following a single nasal or oral dose and lessen the anti-MOG Ab titers. Recent evidence also found that (μMT) mice deficient of B cells were less responsive to MOG-pσ1 intervention, suggesting a B cell component necessary for successful immunotherapy. Characterization of the responsive B cells revealed these to be IL-10-producing $CD1d^{-}$ $CD5^{+}$ $B220^{+}$ B cells. Adoptive transfer of $CD5^{+}B220^{+}$, not $CD5^{-}B220^{+}$, B cells from MOG-pσ1-treated mice into EAE recipients resolved clinical disease and dramatically reduced IL-17 and IFN-γ with concomitant enhanced IL-10. Adoptive transfer of $CD5^{+}B220^{+}$ regulatory B (B_{reg}) cells from MOG-pσ1-treated $IL\text{-}10^{-/-}$ mice was unable to resolve EAE recipients, further emphasizing the relevance of IL-10 for protection. To begin to understand the mode of action by these B_{reg} cells, surface activation molecules were examined, and elevated expression of Herpes virus entry mediator (HVEM) and the co-inhibitory molecule, B and T lymphocyte attenuator (BTLA) were found. $BTLA^{-/-}$ mice showed reduced numbers of B_{reg} cells and exacerbated EAE. Adoptive transfer of as few as 30, 000 MOG-pσ1-stimulated B6 B_{reg} cells into $BTLA^{-/-}$ EAE recipients protected mice against disease. Protection was mediated by reduction of IL-17 and IFN-γ and an elevation in TGF-ß. The capacity of B_{reg} cells to migrate into the CNS to dampen the action of encephalitogenic $CD4^{+}$ T cells provides for one mechanism of MOG-pσ1's therapeutic effect. These data further confirm the feasibility of using pσ1 as a mucosal delivery platform for tolerance induction applied specifically to treat autoimmunity. This work is supported by NIH AI-078938.

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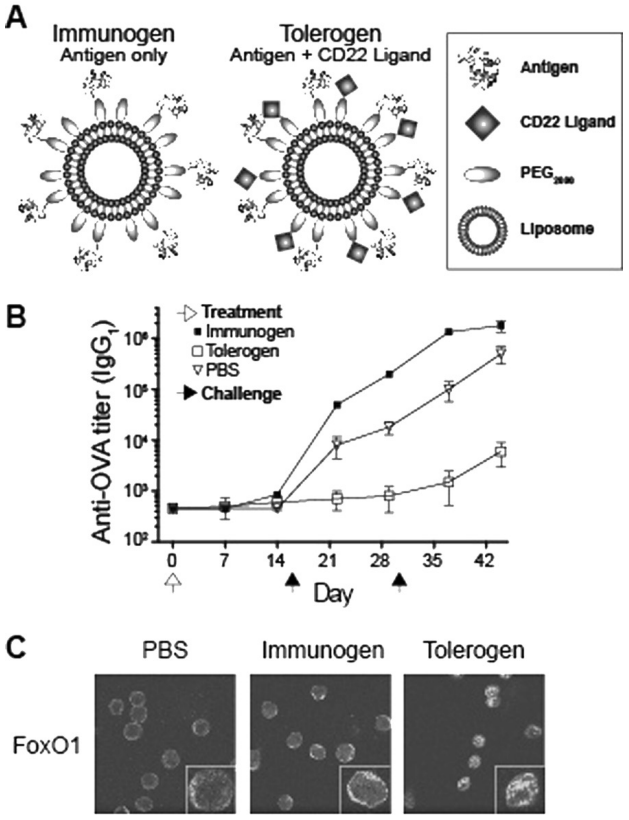
Siglecs in B Cell Tolerance

Matthew S. Macauley, Fabian Pfrengle, Christoph Rademacher, Corwin Nycholat, James C . Paulson
The Scripps Research Institute

Mechanisms that enforce B cell tolerance prevent an autoimmune response by self-reactive B lymphocytes that reach the periphery due to incomplete central tolerance or through hypermutation of the BCR in germinal centers. Maintenance of peripheral B cell tolerance to cell surface self-antigens is still poorly understood, and of high interest with respect to the roles of B cells in autoimmune diseases. The B cell receptor is highly regulated by co-receptors that are thought to aid in distinguishing 'self' from 'non-self' and minimize inappropriate activation to self-antigens. The B cell siglecs, CD22 and Siglec-G/10, are unique among inhibitory co-receptors, which recognize sialic acid-containing glycans as self-ligands that are expressed on all mammalian cells. Previously, we showed that multivalent presentation of high affinity CD22 ligands in *cis* with a T-independent antigen can induce tolerance to B cells (Duong *et al*., J. Exp. Med., 2010). To investigate the relevance to T-dependent antigens we developed a novel liposomal nanoparticle platform for presentation of both antigen and synthetic high affinity siglec ligands specific for CD22 or Siglec-G (**panel A**). Using this platform, robust B cell tolerance is achieved towards both T-independent and T-dependent antigens in mice (**panel B**). Tolerance is also achieved by presentation of antigens on cells expressing natural ligands of siglecs. Mechanistic studies demonstrate that tolerance induction is antigen-specific, siglec-dependent, and is the result of induction of apoptosis in the antigen-specific B cells. The mechanism of apoptosis was investigated in detail and found to involve strong siglec-mediated inhibition of

basal BCR signaling, which resulted in inhibition of the PI3K/Akt survival pathway. This result was more readily apparent by the observed nuclear import of FoxO1 (**panel C**). These studies support the hypothesis that CD22 and Siglec-G/10 participate in maintaining peripheral B cell tolerance by recognition of sialic acid-containing glycans as 'self'. This method has potential for antigen specific tolerization of B cells in a therapeutic setting. (NIH grants AI050143, AI099141, CA013889 and HFSP Fellowship LT001099/2010-L)



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'Slings' Enable Neutrophil Rolling during Inflammation

Prithu Sundd[1], Edgar Gutierrez[2], Ekaterina K. Koltsova[1], Yoshihiro Kuwano[1], Satoru Fukuda[3], Maria K. Pospieszalska[1], Alex Groisman[2], Klaus Ley[1]

[1]Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, 92037.; [2]Department of Physics, University of California San Diego, La Jolla, CA, 92093.; [3]Laboratory of Electron Microscopy, University of Tokyo, Tokyo, Japan.

Leukocyte recruitment to the sites of inflammation involves leukocyte rolling along the inflamed venules. Rolling is mediated by P-selectin on the endothelium binding to P-selectin glycoprotein ligand-1 (PSGL-1) constitutively expressed on leukocytes. Most leukocytes can roll along the walls of venules at low shear stress (1 dyn/cm^2), but neutrophils have the ability to roll at 10-fold higher shear stress in microvessels *in vivo*. Although the shear-resistant neutrophil rolling is known to be facilitated by cell flattening and pulling of long membrane tethers, the mechanisms that enable neutrophil rolling during inflammation are poorly understood. Isolated mouse bone marrow neutrophils stained with an intercalating membrane dye (DiI or DiO) were allowed to roll on a P-selectin (20 molecules/μm^2) coated cover slip in a microfluidic device at a shear stress of 6 to 10 dyn/cm^2 and footprints recorded using dual-color quantitative dynamic footprinting (DqDF). Epifluorescence intravital microscopy was used to observe rolling of neutrophils in the mice cremaster venules. As anticipated, rolling neutrophils formed long membrane tethers (> 10 μm) at the rear. However, following detachment these long tethers did not retract as postulated, but instead persisted and appeared as 'slings' at the front of rolling cells. Rolling neutrophils formed slings in a model of acute inflammation *in vivo* and on P-selectin *in vitro*, where PSGL-1 was presented as discrete sticky patches (1.6 μm apart) while LFA-1 was expressed over the entire length on slings. As neutrophils rolled forward, slings wrapped around the rolling cells and underwent a step-wise peeling from the P-selectin substrate. As each PSGL-1 patch failed, the rolling neutrophil was unable to accelerate because a new downstream patch on the same sling was already lined up and became load-bearing. LFA-1 on the sling interacted with inter-cellular-adhesion-molecule (ICAM)-2 on the neutrophil surface in *trans* to facilitate wrapping of slings and blocking this interaction resulted in less tight wrapping and thus, reduced rolling velocity on P-selectin substrate. The discovery of slings provides a mechanism by which neutrophils rolling at high shear stress pave their own way for enhanced rolling. Besides stabilizing rolling, slings are unique structures that also enable rolling neutrophils to present LFA-1 to its ligand ICAM-2 in *trans*. This study was supported by the NCRP-Scientist Development Grant 11SDG7340005 from the American Heart Association (P.S.) and NIH EB02185 (K.L.).

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Origin of Tumor Elicited Inflammation: Pro-inflammatory Cytokines Promote Colorectal Tumorigenesis

Sergei Grivennikov[1, 2], Kepeng Wang[2], Michael Karin[2], Daniel Mucida[3], Hilde Cheroutre[3], Giorgio Trinchieri[4], C. Andrew Stewart[4], Eric Fearon[5], Dominik Jauch[2]

[1]Fox Chase Cancer Center; [2]University of California San Diego; [3]La Jolla Institute for Allergy and Immunology; [4]NCI-Frederick/NIH; [5]University of Michigan Ann Arbor

Inflammation is an important tumor promoter, as initially shown in cancers, whose development is preceded by chronic inflammatory condition. However, non-steroid antiinflammatory drugs curtail tumor progression and decrease cancer-related deaths in many seemingly 'non-inflammatory cancers. Indeed, most of solid tumors exhibit immune cell infiltrates and enhanced expression of inflammatory mediators and must have a mechanism to trigger such 'tumor- elicited inflammation'. What are the molecular and cellular mechanisms of tumor elicited inflammation is poorly understood. Interleukin 23 (IL-23) is upregulated in "inflammatory" colitis-associated cancer (CAC) and in spontaneous colorectal cancer (CRC). We demonstrate that IL-23 is an important tumor promoter during CAC and CRC tumorigenesis. IL-23 is expressed by tumor-associated myeloid cells, such as tumor-associated macrophages and dendritic cells, and acts on various hematopoietic cells to trigger

the production of pro-tumorigenic cytokines, such as IL-6, IL-17 or IL-22. These cytokines particularly act of intestinal epithelial and pre-malignant cells promoting their survival and proliferation. These cytokines also act on the cells of innate and adaptive arms of the immunity in the tumor microenvironment. Importantly, IL-23 and other cytokines are induced by commensal microbiota, because tumor areas have defective barrier function, allowing translocation of microbial products. Defects in protective barriers are induced as early as key oncogenic mutations in colorectal cancer appear. Therefore oncogenic genetic alteration, disturbance in tissue homeostasis and tumor elicited inflammation are tightly linked and regulated by pro-inflammatory cytokines.

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SAA, ApoE and LBP Modify Macrophage's Innate Immune Response Induced by the Malarial Hemozoin

Fikregabrail A. Kassa[1, 2], Marina T. Shio[1, 2], Martin Olivier[1, 2]

[1]Department of Microbiology and Immunology, McGill University, Montréal, Québec, Canada; [2]Center for the Study of Host Resistance and the Research Institute of McGill University Health Center, McGill University, Montréal, Québec, Canada

Malaria is one of the most important infectious diseases worldwide and still remains to be the major cause of death in tropical countries. The intraerythrocytic stage of the malaria parasite results in the production and release of hemozoin (HZ), which is responsible for some of the clinical manifestation of the disease. Recently, using novel proteomic and biochemical approaches, we have identified the host serum proteins that interact with the malarial HZ. We have shown that hemozoin specifically interacts with serum amyloid A (SAA), apolipoprotein E (ApoE), and LPS binding protein (LBP) to mention a few. In our current work, we were interested to determine the impact of these serum proteins on the macrophage's innate immune response triggered by HZ. Previous work from our lab showed that HZ induces IL-1β production via the NLRP3 inflammasome. Using PMA-differentiated human monocytic cells (THP-1 cells), we looked at the production of IL-1β. This latter, along with TNF-a, is considered to be the major contributor for malaria pathology. Our results show that SAA bound to HZ induces the production of IL-1β in a dose dependent manner, whereas increasing concentration of ApoE abrogates this concentration-dependent production of IL-1β. Furthermore, HZ-SAA and hemozoin-ApoE complexes phosphorylate the MAPKs specifically ERK1/2 and JNK. This is in agreement with the observation that SAA interacting with CD36 activates downstream signaling pathways via LYN resulting in the activation of JNK and ERK1/2. Apart from IL-1β production, HZ bound serum proteins also modulate ROS production and phagocytosis in PMA-differentiated THP-1 cells. Interestingly, LBP enhances the recognition and phagocytosis of HZ by THP-1 cells. Overall, our results show that serum inflammatory proteins adhering on HZ modify its capacity to stimulate phagocytosis, IL-1β and ROS productions by THP-1 cells upon stimulation and therefore strongly suggest that such cooperation could greatly influence the development of malaria-related pathologies.

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Topical Administration of G-CSF Promotes Resolution of *Staphylococcus aureus* Wound Infection in Aged Mice

Aleah L. Brubaker, Elizabeth J. Kovacs

Department of Surgery, Burn & Shock Trauma Institute; Cell Biology, Neurobiology & Anatomy Program; Stritch School of Medicine, Loyola University Chicago Health Sciences Division, Maywood, IL, USA

This study tested the hypothesis that heightened bacterial colonization and delayed wound closure in aged mice could be attenuated by granulocyte-colony stimulating factor (G-CSF) treatment. To test this, we developed a murine model of cutaneous *Staphylococcus aureus* (*S. aureus*) wound infection in young and aged BALB/c mice. Young (3-4 month) and aged (18-20 month) BALB/c mice received six, 3 mm full-thickness, dorsal excisional wounds followed by topical application of 10^3 CFU/wound of *S. aureus*. At days 1, 3, 7 and 10, mice were sacrificed and wounds examined for bacterial colonization, wound size, leukocyte recruitment and chemokine levels. Over the course of ten days, aged mice had elevated wound bacterial levels, protracted wound closure and reduced wound neutrophil accumulation following *S. aureus* wound infection relative to young mice ($p<0.05$). Interestingly, diminished neutrophil recruitment in aged mice was associated with increased levels of the neutrophil chemokines CXCL1 and CXCL2 in aged mice as compared to young ($p<0.05$). Relative to young, neutrophils from aged mice demonstrated reduced chemotaxis to CXCL1 in both *in vitro* and *in vivo* assays ($p<0.05$), suggesting a neutrophil migratory defect with advanced age. While G-CSF has long been reported to enhance neutrophil survival and chemotaxis, others have shown that G-CSF treatment improves wound closure in aseptic wound models. Given these observations, our next objective was to determine if local G-CSF administration after injury could reverse age-associated differences in wound bacterial burden and closure by increasing wound neutrophil recruitment. Young and aged mice received three dorsal, subcutaneous injections of G-CSF (250 ng/50 ul/injection) or saline control (50 ul/injection) 30 minutes after *S. aureus* wound infection. Mice were sacrificed at days 3 and 7 post wound infection and bacterial colonization, wound size and wound leukocyte accumulation. At days 3 and 7 after wound infection, bacterial colonization was reduced 75% and 97%, respectively, in G-CSF-treated aged as compared to saline-treated aged mice and were comparable to levels observed in saline-treated young mice ($p<0.05$). Bacterial colonization was similar between young saline and G-CSF treated animals. Wound size was reduced in G-CSF-treated aged mice ($p<0.05$) to levels in young mice. Local G-CSF did not alter wound size in young mice. Topical G-CSF enhanced neutrophil wound accumulation 2-fold in aged mice ($p<0.05$), whereas there was no G-CSF-induced change in young mice. These data demonstrate that local G-CSF enhances bacterial clearance and wound closure in an age-dependent manner. Moreover, G-CSF may be of therapeutic potential in the setting of post-operative wound infection or chronic, non-healing wounds in the elderly.

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ABSTRACTS

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Cell-Intrinsic Control of Macrophage Activation by CD39 and Implications for Treating Inflammatory Disease

Heather Cohen[1, 2], David Mosser[1, 2]
[1]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park; [2]The Maryland Pathogen Research Institute, University of Maryland, College Park

Macrophages play vital roles in both the progression and resolution of inflammatory disease. However the factors released in tissue that orchestrate these disparate macrophage functions remain elusive. Extracellular ATP has recently been implicated as an important 'danger signal' released at the site of injury or infection to promote the recruitment of anti-microbial immune cells and the production inflammatory cytokines. Excessive levels of ATP may lead to chronic inflammation and severe tissue damage. ATP present in the extracellular milieu is largely regulated by CD39, an ecto-enzyme that hydrolyzes ATP and ADP to generate AMP, which can then be further degraded to adenosine. Here, we demonstrate that classically activated macrophages exposed to physiologically relevant concentrations of ATP readily upregulate anti-inflammatory genes including IL-10, arg1, sphk1 and hb-egf and downregulate inflammatory TNFα and IL-12 production. Thus, extracellular ATP actually generates immunosuppressive macrophages. This modulation in cytokine production is dependent on ATP hydrolysis, as macrophages exposed to ATPγs, a non-hydrolyzable ATP analog, did not attenuate inflammatory cytokine production. We provide biochemical and functional evidence that macrophages rapidly convert adenine nucleotides into immunosuppressive adenosine, and that the rapid hydrolysis of ATP by macrophages is dependent on CD39. Moreover, we show that CD39-null macrophages are hyper-inflammatory in response to LPS, and that the addition of a small number of CD39-deficient macrophages to wild-type mice results in lethal endotoxic shock when these mice are administered low levels of LPS. Our results provide evidence that macrophages actively convert extracellular ATP to adenosine via CD39. This conversion represents a "molecular switch" that controls the activation state of macrophages. These data reveal a novel mechanism that macrophages utilize to control their own activation state to limit the progression of inflammation, and suggest that the development of therapeutics targeting CD39 on macrophages may represent promising new ways to treat inflammatory diseases.

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SHIP Represses Auto-inflammation and Is Required for Intestinal Immune Homeostasis

Eyler N. Ngoh, Shelley B. Weisser, Hayley K. Brugger, Roger Jen, Aaron Hirschfeld, Stuart E. Turvey, Laura M. Sly
Department of Pediatrics, Child & Family Research Institute, BC Children's Hospital, and the University of British Columbia

Crohn's Disease (CD) is a type of inflammatory bowel disease characterized by inflammation in the gastrointestinal tract. Current thinking is that it occurs in genetically susceptible individuals due to complex interactions between the intestinal microbiota and the immune system. The innate immune response is pivotal in mucosal immune responses and it has been suggested that CD may result from a primary immune dysfunction in macrophages. Macrophages contain multi-protein complexes called inflammasomes that mediate the maturation and secretion of IL-1β, an early innate immune effector and key driver of auto-inflammation.

The SH2 domain-containing inositol 5´-phosphatase, SHIP, is a hematopoietic-restricted negative regulator of class I PI3-kinases (PI3K). PI3K is essential for fundamental biological processes including cell proliferation, differentiation, and immune responses. As such, SHIP-/- cells are hyper-responsive to growth factor and immune stimuli. We have reported that SHIP-/- mice develop spontaneous intestinal inflammation that shares key pathological features with CD. These mice develop spontaneous, discontinuous, inflammation in the distal ileum, the most common site of presentation of inflammation in people with CD. In addition, inflammation is accompanied by muscle thickening, accumulation of mesenchymal cells, and excessive collagen deposition. These features are characteristics of intestinal fibrosis, a serious complication that occurs in one in three people with CD.

Herein, we demonstrate that ileal homogenates from SHIP knockout mice contain high levels of IL-1β. *Ex vivo* SHIP-/- macrophages produced significantly more IL-1β than their wild type counterparts upon inflammasome activation and increased IL-1β production was dependent on toll-like receptor activation of the p110α subunit of class I PI3K. Intestinal pathology was dramatically reduced in SHIP-/- mice by depleting macrophages with clodronate-containing liposomes or by treating mice with the IL-1 receptor antagonist, anakinra.

Furthermore, we have found that SHIP protein levels are low in PBMCs and ileal biopsies in a subset of subjects with ileal CD. A single nucleotide polymorphism in the autophagy-related gene, ATG16L1, is a susceptibility locus for CD and we have found that reduced SHIP expression correlated with expression of the ATG16L1 SNP in a gene dose-dependent manner. SHIP protein levels are normally increased upon induction of autophagy. However, siRNA knockdown of ATG16L1 blocked autophagy-induced SHIP up-regulation and was reduced in PBMCs from subjects with the ATG16L1 susceptibility SNP. Importantly, PBMCs from subjects with low SHIP levels were hyper-responsive to inflammasome activation and produced more IL-1β than subjects with high SHIP protein levels.

Taken together, our data suggest that CD may be caused by auto-inflammation in some people and that these people may be amenable to treatment with anakinra or other IL-1/IL-R antagonists.

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Transcriptomic Analysis of Manipulation of the NOD2 Signaling Pathway

Justine Tigno-Aranjuez, Derek Abbott
Dept. of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Alterations in the intracellular bacterial-sensing protein, NOD2, are tightly linked to inflammatory disease. Loss-of-function NOD2 polymorphisms are responsible for approximately 20% of genetic Crohn's disease while gain-of-function mutations cause Early Onset Sarcoidosis. Hyperactive NOD2 signaling has also been linked to such inflammatory and autoimmune diseases as

asthma, sarcoidosis, multiple sclerosis and inflammatory arthritis. Given this clinical relevance, we have been studying the basic signal transduction pathways underlying NOD2 signaling in hopes of identifying pharmacologic agents and targets that can alleviate NOD2-driven inflammatory disease. To this end, we have recently started using Next-generation sequencing (RNA-seq)technologies to illuminate the cell biologic pathways driven by NOD2 signaling and the effect of pharmacologic manipulation of NOD2 signaling on these pathways. Because RNA-seq has greatly increased linear range and greater sensitivity than classic microarray analysis, it gives a greater breadth of information. This breadth is particularly helpful in deciphering signaling pathways with a low signal-to-noise ration like that found in the NOD2 signaling pathway.Our analysis identifies a number of unexpected transcription factor pathways affected by NOD2 signaling, a number of unexpected cellular processes affected by NOD2 signaling and a surprising specificity in signal transduction revealed by selective pharmacologic manipulation of the NOD2 signaling pathway. This work further identifies potential biomarkers that can help predict NOD2-inhibitor efficacy in inflammatory disease. In all, Next-gen sequencing technology has illustrated a number of potentially important NOD2-driven signaling pathways that could be manipulated for therapeutic gain.

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A New Branch of TLR3 Signaling

Ganes C. Sen, Michifumi Yamashita, Saurabh Chattopadhyay
Cleveland Clinic Foundation

Toll like receptors (TLRs) recognize specific microbial products and elicit innate immune signals to activate specific transcription factors that induce protective proteins, such as interferon. TLR3 recognizes double-stranded (ds) RNA, generated by virus infected or apoptotic cells. Genetically TLR3 has been linked to several human diseases, including some with no known viral etiology. Unlike other TLRs, TLR3 requires phosphorylation of two specific tyrosine residues, in its cytoplasmic domain, to initiate signaling. We now report that two protein tyrosine kinases, epidermal growth factor receptor (EGFR) and Src, carry out this process. DsRNA binding to TLR3 exposes its cytoplasmic site for EGFR binding, a process that does not require TLR3 Tyr residues, TRIF or EGFR kinase activity. The TLR3-EGFR complex recruits Src, which is activated by auto-phosphorylation. Active Src and EGFR phosphorylate the two tyrosine residues of TLR3, a step essential for the recruitment of the obligatory adaptor protein, TRIF, leading to gene induction and antiviral effects. Thus, a connection between antiviral innate immunity and cell growth regulators has been revealed. This study also revealed a new branch of TLR3 signaling, mediated by Src activated by TLR3-binding; this branch does not require gene induction, but it affects many cellular properties, such as cell migration, adhesion and proliferation. For these effects of TLR3 signaling, Src, but not the adaptor proteins TRIF and MyD88, were needed. The response was biphasic: upon dsRNA-treatment of TLR3 expressing cells, we observed an immediate increase in cell motility followed by its strong inhibition. Our results indicate that the first phase was mediated by dsRNA- induced phosphorylation and activation of Src whereas the second phase resulted from the sequestration of activated Src in lipid rafts, thus decreasing its active cytoplasmic pool. In mice, injection of dsRNA inhibited the recruitment of macrophages to the peritoneal cavity in a TLR3-dependent fashion. These results demonstrate that activated TLR3 can engage Src to trigger multiple cellular effects. This study also provides a rare example of TLR-mediated cellular effects, that do not require gene induction, and the first example of an adaptor-independent effect of any TLR.

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Cytopenia and Immunosuppression Caused by NLRP1 Activation

Ben A. Croker[1], Seth L. Masters[1], Motti Gerlic[1], Donald Metcalf[1], Simon Preston[1], Marc Pellegrini[1], Joanne A. O'Donnell[1], Kate McArthur[1], Daniel L. Kastner[2], Lionel Feigenbaum[3], Douglas J. Hilton[1], Warren S. Alexander[1], Benjamin T. Kile[1]
[1]The Walter and Eliza Hall Institute of Medical Research and The Department of Medical Biology, The University of Melbourne, Victoria, Australia; [2]Inflammatory Disease Section, National Human Genome Research Institute, National Institutes of Health, Bethesda MD, USA; [3]Science Applications International Corporation, National Cancer Institute, Frederick MD, USA

Peripheral blood cytopenias in patients with systemic infection are well-established predictors of fatal outcome despite antimicrobial therapy. Here we define a role for NLRP1a-induced Caspase-1-dependent death, known as pyroptosis, in infection-induced cytopenias. Active NLRP1a induced a lethal systemic inflammatory disease that was driven by Caspase-1 and IL-1β but was independent of ASC and ameliorated by IL-18. Surprisingly, in the absence of IL-1β-driven inflammation, active NLRP1a triggered pyroptosis of hematopoietic cells resulting in leukopenia at steady state. During periods of hematopoietic stress induced by viral infection, active NLRP1a caused prolonged cytopenia and immunosuppression. Conversely, NLRP1-deficient mice showed enhanced recovery from viral infection, demonstrating that NLRP1a acts as a cellular sentinel in hematopoietic cells to alert Caspase-1 to infectious stress.

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Peptidoglycan Degradation Products Activate the Inflammasome through a Novel Mechanism Using Glycolytic Enzymes

Andrea J. Wolf, Christopher Reyes, Courtney Becker, David M. Underhill
IBD Department, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Evolutionarily, phagocytosis is the means by which organisms internalize and break down nutrients. Innate immune phagocytes have adapted this process for the degradation, sensing, and presentation of antigens in order to initiate and coordinate the immune response to pathogens. We have previously shown that the lysosomal degradation of peptidoglycan, the dominant component of the cell wall of gram positive bacteria, leads to enhanced inflammatory responses to TLR ligands as well as stimulation of the caspase-1 activating NLRP3 inflammasome complex. We have identified a previously undefined fragment of

peptidoglycan backbone that possesses NLRP3 inflammasome activating properties in both macrophages and dendritic cell. Experiments utilizing chemical inhibitors, shRNA knockdown, gene overexpression and imaging in primary phagocytes have identified a glycolytic enzyme as the intracellular receptor for peptidoglycan degradation products leading to inflammasome activation. The products of peptidoglycan degradation inhibit glycolytic enzyme function which serves as the initial step in the signaling cascade resulting in mitochondrial dysfunction that triggers inflammasome activation. Our data suggests that innate immune phagocytes have co-opted a glycolytic enzyme as an innate immune receptor in order to sense the release bacterial degradation products. This adaptation of the evolutionarily conserved metabolic pathway by innate immune cells implies more significant crosstalk between cellular metabolism and innate inflammatory processes then has previously been appreciated. NIH-T32AI089553-01 and NIH-RO1GM085796

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The Contribution of the NLRP3 Inflammasome to Air Pollution Facilitated Allergic Sensitization and Airway Inflammation

Jeremy A. Hirota[1], Matthew Gold[2], Paul Hiebert[1], Kelly McNagny[2], Darryl A. Knight[1]

[1]James Hogg Research Centre - University of British Columbia; [2]Biomedical Research Centre - University of British Columbia

Objective: Our overarching objective is to characterize the inflammatory responses mediated by the airway epithelium to various environmental stimuli. We have used PM, a clinically relevant exposure, as a stimulus for NLRP3 inflammasome mediated IL-1β production in the airway epithelium. We hypothesized that the airway epithelium expressed a NLRP3 inflammasome that mediates immune responses to PM facilitating sensitization to allergens.
Methods: Mechanistic in vitro studies were performed by exposing primary human airway epithelium cultures to PM in the presence of silencing RNA for NLRP3, and assessing IL-1β, GM-CSF, CCL-20, and TSLP protein production. In vivo exposure of ASC -/-, NLPR3 -/-, and wild-type control mice to ovalbumin, PM, or ovalbumin/PM combination was performed with outcome measurements of lung cell inflammation, goblet cell metaplasia, serum immunoglobulin profiling, lung gene expression profiling, lung and mediastinal lymph node dendritic cell phenotype.
Findings: In vitro PM exposure to primary human airway epithelium resulted in NLRP3 inflammasome mediated production of IL-1β. PM-induced epithelium production of CCL-20 and GM-CSF was sensitive to NLRP3 inhibition, suggesting that these activators of dendritic cells may be produced downstream of NLRP3 activation. In vivo ovalbumin/PM exposure resulted in development of a Th2 skewed immune response not observed for ovalbumin or PM exposure alone. ASC -/- and NLRP3 -/- animals developed similar immune profiles as wild-type littermate controls.
Conclusions: PM exposure activates the NLRP3 inflammasome in airway epithelium to induce production of inflammatory mediators, IL-1β, GM-CSF, and CCL-20. In vivo, ASC and NLRP3 proteins are not required for PM facilitated sensitization and Th2 skewed immunity to ovalbumin. These results suggest that the NLRP3 inflammasome may mediate acute inflammatory events that are mutually exclusive from allergic sensitization.
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Molecular Circuitry Responsible for the Opposing Paradigm of Endotoxin Priming and Tolerance

Hui Deng, Urmila Maitra, Liwu Li

Department of Biology, Virginia Tech, Blacksburg, VA 24061-0910

Host innate macrophages can be pre-programmed into opposing primed or tolerant states by super low dose or high dose bacterial endotoxin lipopolysaccharide (LPS). The paradigm of endotoxin priming and tolerance has significant implications in the pathogenesis and resolution of both acute and chronic inflammatory diseases. Endotoxin priming and tolerance may confer significant survival advantages to higher eukaryotes. Priming of innate immune cells may enable robust and expedient defense against invading pathogens, a mechanism crudely analogous to vaccination of the adaptive immune system. On the other hand, tolerance may promote proper homeostasis following robust innate immune responses. However, despite these survival advantages, endotoxin priming and tolerance are also closely associated with the pathogenesis of both chronic and acute human diseases. For example, despite the potential ability to limit pro-inflammatory cytokine production, endotoxin tolerance is responsible for the induction of immunosuppression in patients with sepsis shock, and this suppression leads to increased incidence to secondary infections and mortality. Endotoxin priming, on the other hand, reprograms macrophages to super-induction of proinflammatory cytokines. Increasing evidence relates this phenomenon to low-grade metabolic endotoxemia, where an elevated but physiological level of LPS in the host's bloodstream results in a higher incidence of insulin resistance, diabetes and atherosclerosis. However, the responsible mechanisms are not well understood. By utilizing wild type (WT), IRAK-1 and Toll-interacting protein (Tollip) deficient primary murine bone marrow derived macrophages (BMDM), we examined the dynamic activation status of downstream pathways including NFkB, MAPK, and PI3K triggered by low vs high dose LPS. Our studies reveal that there is an opposing intracellular circuit within macrophages that are responsible for this paradigm. This circuit involves the interleukin receptor-associated kinase 1 (IRAK-1) and Toll-interacting protein (Tollip). Super low dose and high dose LPS cause opposing modulation of IRAK-1, Tollip and PI3K pathways, and lead to opposing effects of priming and tolerance. Taken together, the pathway switching and flipping induced by super low vs high dose LPS underscores the importance of competing intracellular circuitry during the establishment of macrophage priming and tolerance.

ABSTRACTS

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The Discovery of a New Guanine Nucleotide Exchange Factor (GEF) in Leukcoytes, That Happens to be a Phospholipase

Julian Gomez Cambronero
Dept. of Biochemistry and Molecular Biology, Wright State University School of Medicine

Phospholipase D2 (PLD2) is an integral membrane enzyme that converts phosphatydyl choline into choline and phosphatidic acid, a lipid second messenger involved in cell growth, cell motility and other key functions. Apart from this well-known lipase activity, we have discovered that the enzyme PLD2 binds directly to the small GTPase Rac2, resulting in PLD2 functioning as a guanine nucleotide exchange factor (GEF), because it switches Rac2 from the GDP-bound to the GTP-bound states. This effect is large enough to be meaningful (~72% decrease for GDP dissociation and 300% increase for GTP association, both with PLD2), it has a halftime of ~7 min, is enhanced with increasing PLD2 concentrations, and compares favorably with other known GEFs in leukocytes, such as Vav-1. PLD2's novel GEF function is demonstrated in living cells, because silencing PLD2 results in reduced Rac2 activity, whereas PLD2-initiated Rac2 activation enhances cell adhesion, chemotaxis, and phagocytosis of macrophages. There are several known GEFs, but we report here for the first time that a phospholipase can be a GEF. The benefit for the cell is that PLD2 brings spatially separated molecules together in a membrane environment, ready for fast intracellular signaling and cell function. Recent data from our lab have uncovered the domains (PX and PH) and amino acid residues responsible for the GEF activity of PLD2 and their physiological relevance in vivo for leukocyte chemotaxis and phagocytosis. Interestingly, the catalytic site makeup provides a new biochemical mechanism of action of this dual GEF-lipase. Further, knowing the exact amino acids of the catalytic site will be crucial for the rational design of drugs that can suppress this activity in human pathologies.

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An IFN Gamma-IL15-IL32 Pathway in Human Macrophages Induces Vitamin D-Dependent Antimicrobial Program against Mycobacteria

Dennis Montoya[1], Poorva Vaidya[2], Grace Yim[1], Marcos Munoz[1], Rosane M. Teles[1], Robert L. Modlin[1]
[1]Division of Dermatology, Department of Medicine, David Geffen School of Medicine at University of California Los Angeles; [2]Department of Molecular, Cell, & Developmental Biology, University of California Los Angeles

Adequate circulating 25-hydroxyvitamin D (25D) serum levels have been shown to be important for human host defense against mycobacterial infection. We previously demonstrated that in macrophages, innate (Toll-like receptor 2/1, TLR2/1) and adaptive (IFN-γ, IL-15) mechanisms converge on a common vitamin D receptor (VDR) mediated antimicrobial response through production of antimicrobial peptides essential for killing of mycobacteria. Vitamin D receptor pathway genes have been found to be more prominent in skin lesions of the self-limited form of leprosy than the clinically progressive, multibacillary form of the disease. A bioinformatics analysis of vitamin D-induced genes co-regulated in microarrays from self-limited form of leprosy lesions and IL-15 stimulated monocytes yielded IL-32γ, a cytokine known to play a protective role against tuberculosis.
Both IFN-γ and IL-15 were confirmed to upregulate transcription and protein levels of IL-32γin primary monocytes. IL-32γ was sufficient to induce CYP27b1, a key enzyme for vitamin D metabolism to similar levels as IFN-γ, IL-15, and TLR 2/1 ligand. The induced CYP27b1 was metabolically active as IL-32 treated monocytes were able to convert inactive 25D into the active 1, 25D. IL-32 stimulation also induced the VDR downstream antimicrobial peptides, cathelicidin and human beta defensin 2 in 25D-sufficient human serum and lead to the decrease of *Mycobacterium leprae* viability in infected monocytes in a vitamin D-dependent manner. Knockdown of IL-32 expression by siIL-32 abrogated IFN-γ and IL-15 induction of vitamin D antimicrobial activity compared to a control siRNA. Our data propose the following sequential model: IFN-γ→IL-15→IL-32→CYP27b1 and downstream activation of the VDR with induction of antimicrobial peptides effective at combating mycobacteria. We provide preliminary evidence that IL-32, with vitamin D supplementation, may offer an adjunct therapy against mycobacteria, especially multidrug resistant strains.
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Nlrp10 Regulates Dendritic Cell Function

Stephanie C. Eisenbarth[1], Adam Williams[2], Richard A. Flavell[2], Fayyaz S. Sutterwala[3]
[1]Department of Laboratory Medicine, Yale School of Medicine; [2]Department of Immunobiology, Yale School of Medicine; [3]Inflammation Program, Department of Internal Medicine, University of Iowa

Nucleotide-binding domain leucine-rich repeat containing receptors (also known as NOD-like receptors or NLRs) are a class of pattern recognition receptors (PRRs) that respond to host perturbation from either infectious agents or cellular stress. The function of most NLR family members has not been characterized. Nlrp10, also known as Pynod and Nalp10, is the only NLR lacking the putative ligand binding leucine rich repeat domain, and has been postulated to be a negative regulator of other NLR members including Nlrp3. To evaluate the immunological role of Nrlp10, we generated genetically modified mice that lack Nlrp10 and stimulated macrophages and dendritic cells in vitro with inflammasome and Toll-like receptors agonists. In contrast to previous findings using overexpression of Nlrp10, in vitro monocyte production of IL-1beta and pro-inflammatory cytokines was unaltered in Nlrp10-deficient cells. However, Nlrp10-deficient mice had a profound defect in adaptive immune responses to all tested adjutants including aluminum hydroxide, complete Freund's adjuvant and lipopolysaccharide. Using T cell and dendritic cell (DC) adoptive transfer experiments we discovered a DC-intrinsic defect in naïve T cell priming in the draining lymph node. This defect appears to be due to loss of migration of a specific DC subset expressing CD11b from inflamed tissues during immunization. Therefore we find no evidence that Nlrp10 functions as a negative regulator of the Nlrp3 inflammasome but instead appears to be critical for the initiation of adaptive immunity. Future work to elucidate the

molecular mechanism of Nlrp10 activation and function in dendritic cells will be critical to shed light on this new member of the NLR family of innate immune molecules.

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Geriatric Immunosenescence Differentially Affects T Lymphocyte and Antigen Presenting Cell Function

Carol Miller-Graziano, Gautam Bandyopadhyay, Sanjukta Bandyopadhyay
Department of Surgery, University of Rochester Medical Center

Elderly individuals (Age 65+) have increased infection susceptibility and constitute the majority of post-surgical septic patients. This elevated susceptibility to infectious complications is linked to depressed T cell adaptive immunity but is accompanied by increased inflammatory responses. Depressed T cell function results from increased inhibitory receptor & decreased costimulatory receptor expression, altered memory/ effector T cell ratio or loss of naive T cell repertoire. We found increased inhibitory receptor expression on seniors' T cells and significantly increased SHP-1 inhibitory phosphatase activity [Median MFI 612 in elderly T cell vs. 328 in younger] associated with depressed senior T cell capacity to proliferate [median T cell proliferation toantiCD3- 41744 in elderly vs 78669 in younger subjects] and altered transcription factor expression [significantly depressed NFκB & RORγt in elderly T cell]. Myeloid professional antigen presenting cells are crucial to initiate adaptive response. In view of the elderly's aberrant inflammatory macrophage functions, we investigated possible aging associated alterations in monocyte (MO) derived dendritic cells (DC). To test possible alterations in MO to DC differentiation and resultant deviant DC function, we stimulated freshly isolated MO from elderly (age 65+) and younger (Age < 35) subjects with IL-4 & GM-CSF to *in vitro* differentiate them into DC. We compared elderly with younger MO to DC differentiation capability, phenotype and function. Although total differentiated DC numbers were reduced, MO from elderly subjects differentiated to $CD1a^+$ DC as efficiently as younger MO (Median $CD1a^+$ DC differentiation in elderly 62% vs. 68% in younger). Differentiated elderly DC showed no decreased antigen processing capacity (as assessed by measuring processed DQ-Ovalbumen, median MFI 943.3 in elderly vs. 994.8 in younger subjects), membrane costimulatory receptor expression profile or T cell stimulatory capability compared to DC from younger subjects [allogenic T cell response to elderly DC 10221 CPM vs. proliferation to youngers' DC 10207 DPM (median)]. However, differentiated DC from elderly subjects showed reduced expressions of CD16 [median CD16 MFI 4.4 in elderly vs. 12.8 in younger]. Since CD16 expressing DC have inflammatory suppressive capacity, our results could indicate that this decreased DC suppression combined with highly effective DC generation contributes to elders' hyper-inflammatory responses.

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Dynamic T Cell-APC Interactions Sustain Chronic Inflammation in Atherosclerosis

Ekaterina Koltsova[1], Zacarias Garcia[1], Grzegorz Chodaczek[1], Sibylle von Vietinghoff[1], Elena Galkina[2], Yury Miller[3], Klaus Ley[1]
[1]La Jolla Institute for Allergy and Immunology; [2]Eastern Virginia Medical School; [3]University of California, San Diego

Atherosclerosis is an immune-mediated disease associated with lipid accumulation and formation of atherosclerotic plaque in the major arteries of the body. Vascular dendritic cells (DC) are present throughout the adventitia of the normal mouse aorta and expand under atherosclerotic conditions, but their function remains incompletely understood. Here, for the first time we have developed live cell (two photon) imaging to study the behavior and role of antigen-presenting cells in normal and atherosclerotic mouse aortas. CD11c-YFP tagged DC were motile in mouse aortas and interacted with T cells in an antigen dependent manner. In atherosclerotic *Apoe*$^{-/-}$ $CD11c^{YFP}$ mice, DC interacted with transferred T cells from *Apoe*$^{-/-}$, but not C57BL/6 mice. Interaction with DC decreased T cell migration velocity and induced secretion of inflammatory cytokines IFN-γ and TNF-α, which in turn enhanced oxLDL uptake by primary macrophages and their differentiation into 'foam-like' cells. Taken together, our data show that antigen presentation by APC to CD4 T cells in the arterial wall results in local T cell activation and production of pro-inflammatory cytokines, which maintain chronic inflammation and promote atherosclerosis by inducing foam cell formation.

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The Fifth Cardinal Sign: N(K) Cells Induce Autophagy, HMGB1 Release, Functio Laesa, and Promote Tumor Immunity

Michael T. Lotze, William J. Buchser, Xiaoyan Liang
University of Pittsburgh Cancer Institute

Natural killer (NK, N) cells are innate lymphoid cells (ILCs) derived from hematopoietic progenitors. These cells play important roles in tissue remodeling following unscheduled cell death and damage. As such, they regulate epithelial survival, senescent cell elimination, and stromal cell recruitment and remodeling. Several novel NK cell populations have been identified in addition to the paradigmatic cytolytic NK cells including: 1) lymphoid tissue-inducer (LTi) cells, playing an important role in secondary lymphoid tissue formation; 2) NK22 cells which produce interleukin 17 (IL17) and IL22, promoting epithelial survival and recruitment of neutrophils; and 3) natural helper (NH) cells, nuocytes, expressing IL5 and IL13, promoting allergic, antihelminthic, and atopic responses.

Autophagy (programmed cell survival) is a metabolic process promoting late cancer growth induced by NK cells and mediating effective cross-presentation of antigen by DCs. Administration of high-dose recombinant interleukin 2 (IL2), enhances cytolytic immune cell proliferation and delivery, promoting complete antitumor responses in 10% of treated individuals. IL-2 toxicity, attributed to a cytokine storm and an associated "systemic autophagic syndrome" could be promoted by both T-cell and NK cell induction of cell-mediated autophagy (*Buchser WJ, et al. Cell-*

mediated Autophagy Promotes Cancer Cell Survival. Cancer Res. 2012 Apr 17. PubMed PMID: 22505650). HMGB1 is detected at high levels in the serum of IL2-treated mice with translocation to the cytoplasm from the nucleus in the liver, consistent with HMGB1's release in response to stress, and ability to sustain autophagy. Limiting autophagy in mice with coadministration of chloroquine (CQ) diminishes serum levels of HMGB1, cytokines (IFNG and IL6 but not IL18), and autophagic flux, attenuating weight gain, markedly enhancing DC, T-cell, and NK cell numbers, and promoting long-term tumor control in a murine C57Bl/6 hepatic colorectal metastases model (*Liang X, et al. Inhibiting Systemic Autophagy during Interleukin 2 (IL-2) Immunotherapy Promotes Long-term Tumor Regression. Cancer Res. 2012 May 15. PubMed PMID: 22472122*).

In three experiments, CD11c number in the liver (and spleen) increased from $1.5x10^5$ to $2.5x10^5$ /g ($p<.001$) suggesting that CQ enhances IL-2 driven DC numbers as well as NK and T cell effectors. We have also demonstrated increased DC numbers in the spleen with IL-2 administration in a pulmonary melanoma metastases model and enhancement of antitumor effects in Balb/c mice bearing the renal cancer. IL2 administration to RAG knockout mice was associated with diminished tumor growth and profound susceptibility to IL2 toxicity, limited by either depletion of NK cells with antibody to asialo-GM1 or autophagy inhibition with CQ administration.

We have initiated a multicenter clinical trial of IL-2 and hydroxychloroquine with the Cytokine Working Group for patients with renal carcinoma based on these observations.

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Cellular Source And Molecular Form of TNF Defines Its Pathogenic and Protective Functions during Autoimmune Arthritis

Andrey A. Kruglov[1, 2], Lars Morawietz[3], Sergei A. Nedospasov[1, 2]
[1]Engelhardt Institute of Molecular Biology, and Lomonosov Moscow State University, Moscow, 119991, Russia.; [2]German Rheumatism Research Center (DRFZ), a Leibnitz Institute, ; [3]Institute of Pathology, Charite Medical School, Berlin, 10117, Germany.

TNF overexpression has been linked to pathogenesis of various autoimmune diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis, Crohn's disease and others. Despite the success of anti-TNF therapy in RA, various side effects were reported, including increased susceptibility to intracellular pathogens, spontaneous development of autoimmune syndromes and others, suggesting that complete elimination of TNF may not be desirable. In order to delineate cellular sources of pathogenic and protective TNF we studied collagen-induced arthritis in the panel of mice with cell-specific TNF ablation. TNF KO mice were resistant to collagen-induced arthritis as were tmTNF KI mice (mice producing only membrane-bound TNF), implicating a pathogenic role for soluble TNF. Mice with TNF deletion in B cells, characterized by reduced germinal center formation and diminished anti-collagen antibody titres, developed arthritis with significantly reduced severity, suggesting a critical role for TNF produced by B cells in autoantibody production and arthritis development. When TNF was depleted in macrophages and neutrophils, mice exhibited reduced arthritis incidence and lower disease score, clearly implicating pathogenic role of myeloid cell derived TNF in experimental arthritis. Strikingly, ablation of TNF in T cells (T-TNF KO mice) resulted in significantly higher disease incidence and exacerbated disease scores. T-TNF KO mice exhibited increased numbers of autoreactive T cells in secondary lymphoid organs during arthritis, suggesting that T-TNF is critical for dampening autoreactive T cell responses. Furthermore, mice lacking from both T and B cells still developed exacerbated disease, similar to T-TNF KO animals, despite the lack of pathogenic anti-collagen antibodies, indicating that autoantibodies are not critical for disease manifestation in the context of increased autoreactive T cell response. Finally, depletion of TNF from both myeloid cells and T cells led to significantly decreased incidence of arthritis.

Thus, our data uncover non-redundant effects of TNF from distinct cellular sources in arthritis: direct pathogenic role of TNF derived from myeloid cells in disease induction, control of autoreactive T cell development by T-cell derived TNF, and regulation of autoantibody production (via control of FDCs development and GC formation,) by soluble TNF produced by B cells.

Our findings suggest that in an "ideal" anti-TNF therapy production of TNF by T cells, most likely in membrane-bound form, should be spared.

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Cytokine Requirments for Th17 Cell Lineage Commitment Are Dictated by Priming Microenvironments

Pasare Chandrashekhar
Dept. of Immunology, UT Southwestern Medical Center

Activation of pattern recognition receptors on dendritic cells (DCs) leads to their maturation, which is critical for activation of naïve CD4 T cells. Activated DCs also secrete several pro-inflammatory cytokines, including Interleukin (IL)-12, IL-6 and IL-1, all of which play an important role in activation and differentiation of various CD4 T cell lineages. The cytokine IL-12 plays a major role in differentiation of Th1 lineage cells whereas a combination of IL-6 and Transforming growth factor-beta (TGF-beta) has been demonstrated to control and guide Th17 lineage differentiation. Our recent studies have discovered that cytokine requirements for Th17 polarization depend entirely on the site of priming. While IL-6 plays a critical role in Th17 lineage priming in mucosal tissues such as the lamina propria of the gut and the lungs, it is not required for Th17 priming in the spleen. However, IL-1R mediated MyD88 dependent signaling in CD4 T cells plays an irreplaceable role in Th17 priming in all tissues. Importantly, we find that DC populations resident in the spleen and lamina propria guide IL-6 independent and dependent pathways of Th17 differentiation, respectively. While CD103hi DCs are absent in the spleen, they are present in mucosal tissues and the skin and play an important role in regulating Th17 differentiation. Our studies have revealed that CD103hi DCs impose the requirement of IL-6 for Th17 priming in both lamina propria of the gut as well as the skin draining lymph nodes. Additionally, we have also discovered that the function of CD103hi DCs can be perturbed by gut microflora that can lead to IL-6 independent priming of Th17 cells in the intestines. These

results reveal fundamental differences by which systemic and mucosal immune systems regulate Th17 cell lineage differentiation.

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Amphisome Formation and Autophagy Are Required for Virus-Induced Production of IFN-Alpha by Primary Human Plasmacytoid Dendritic Cells

Patricia Fitzgerald-Bocarsly, Jing Deng[1], Jihong Dai, Sukhwinder Singh

UMDNJ- New Jersey Medical School

Human pDC produce large amounts of IFN-α and IFN-λ in response to viruses and synthetic TLR7 and -9 agonists and are considered to be "professional" IFN-producing cells. Unlike other cell types that produce IFN-α, pDC do not have to be infected by the viruses they respond to; rather, they can respond to inactivated viruses (including flu, HSV and HIV-1) as well as to virus-infected cells, making them powerful sentinels for detection of viral infection. However, the mechanisms of virus trafficking and virus-stimulated IFN-α production without infection in pDC remain unclear. Iwasaki and colleagues previously reported a role for constitutive autophagy in the induction of IFN-α induced in murine pDC for viruses that actively infect the pDC. In contrast, in human primary pDC, using Amnis ImageStream imaging flow cytometry, we observed that stimulation of pDC with live or inactivated HSV or HIV-1 or with influenza A virus or CpGA induced both induction of IFN-α and –λ but also aggregation of LC3B, a hallmark of autophagy. Interestingly, enhanced LC3B aggregation occurred with HSV stimulation but did not occur in the context of uptake of FITC-dextran by pDC or upon stimulation with LPS; moreover, the aggregation of LC3B in response to HSV was prevented when TLR9 signaling was inhibited using a TLR9 inhibitory ODN. Inhibition of autophagy using the PI3K inhibitors 3-methyl adenine, wortmannin and LY29004 led to inhibition of both LC3B aggregation and IFN-α production; these inhibitors had no effect on uptake of GFP-HSV, indicating a post-internalization step was involved. Moreover, using electron microscopy, we observed double-membrane autophagosomes formed within 2hr stimulation of pDC with HSV, with HSV particles clearly visible within the structure. We also found theearly endosomal marker, Rab5, colocalized with the autophagosomal marker, LC3, in pDC after stimulation with HSV-1, indicating amphisomes were induced. These amphisomes further accumulated when autophagic flux was blocked by chloroquine treatment. Knockdown of autophagy pathway proteins Beclin 1 and ATG7 in primary pDC using siRNA resulted in a significant inhibition of IFN-a production and release in pDC. We further investigated the interactions between early endosomes, autophagosomes and TLR9 after HSV stimulation using imaging flow: HSV-GFP particles were internalized into early endosomes by endocytosis and were subsequently transported to amphisomes; concurrent with the trafficking of HSV-GFP, TLR9 rapidly redistributed to HSV-accumulating amphisomes by colocalizing with LC3 within 30 min for ligand binding and signal transduction. Collectively, our studies unveil a previously unknown mechanism for autophagy and amphisome formation in virus-induced IFN-α production involving the recognition of virus and the recruitment of TLR9 to signaling compartments in human pDC.

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Role of SRC and PI3K Signaling in Leukocyte "Entrainment" during Tumor Metastasis

J Sai[1, 2, 3], OE Hawkins[1, 2, 3], CM McClain[3], A Richmond[1, 2, 3]

[1]Department of Veterans Affairs, Nashville, TN; [2]Department of Cancer Biology; [3]Vanderbilt University School of Medicine, Nashville, TN

Chemokines recruit myeloid cells to tumors and to the pre-metastatic niche. While in many cases this may prove to be a tumor promoting process, in other cases chemokines may entrain "anti-tumor neutrophils" to inhibit metastatic seeding of tumor cells. activate PI3K and SRC-kinases, which are frequent targets for chemotherapy. To determine how loss of PI3K or SRC activity affects the leukocytes in the tumor or pre-metastatic niche, poorly metastatic B16F0 melanoma cells were implanted into WT-C57Bl/6 mice or *hck/fgr/lyn*-/- C57Bl/6 mice and allowed to grow 1 week to potentially enable "entrainment" of leukocytes. Subsequently, highly aggressive, GFP tagged B16F10 melanoma cells were injected into the tail vein of mice to determine whether loss of *hck/fgr/lyn* altered the ability of B16F0 tumors to entrain leukocytes and therefore affect implantation and growth of B16F10 cells. Tumors arising from the poorly metastatic B16F0 cells were larger in the hck/fgr/lyn null mice (1639mm3±249) compared to wild type mice (649mm3 ±87), and the lung lesions of GFP-B16F10 were much larger in *hck/fgr/lyn* null mice, while WT mice showed more numerous, but very small clusters of 4-5 tumor cells. Thus, loss of *hck/fgr/lyn* may impair 'entrainment' of leukocytes and allow unrestricted growth of circulating tumor cells in the lung. We observed ~2 fold increase in PMNs and immature myeloid cells (iMCs) that infiltrate the tumor and small increases in CD4 and CD8 T cells. The % of IL-4 expressing macrophages (M2) in the B16F0 primary tumor and in the lung of tumor bearing *hck/fgr/lyn* null mice was also increased. Since *hck/fgr/lyn* are predominately expressed in hematopoietic cells, our data suggest that loss of SRC activity in hematopoietic cells increases the population of pro-tumor myeloid cells in the lung and enhances outgrowth of metastatic tumor foci. When similar experiments were performed with PI3K?/DOCK2 null C57Bl/6 mice bearing PyMT breast cancer tumors, we observed a decrease in tumor volume (38%) but an increase in circulating tumor cells in the PI3K?/DOCK2 mice as compared to WT mice. Moreover loss of PI3K?-/-/DOCK2-/- resulted in more macrophages/PMNs or immature myeloid cells in the tumor and/or the lung. Currently SRC kinase inhibitors and PI3K inhibitors are being used in the clinic to treat breast cancer patients. Our data suggest that these inhibitors may shrink the primary tumor, but promote metastasis, due to loss of entrainment.

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ABSTRACTS

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TREM-Like Transcript 2 (TLT2) Regulates Intrinsic and Extrinsic Processes That Promote Neutrophil Recruitment and Activation Contributing to Inflammation and Innate Host Defense

Louis B. Justement, Matthew M. Halpert, Kimberly A. Thomas, R Glenn King

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

TLT2 is expressed on a range of immune cell types, including neutrophils, macrophages and B lymphocytes. Ligation of TLT2 on neutrophils enhances their response to agonists that bind to G-protein coupled receptors (GPCR). To date, TLT2 ligation has been shown to enhance ROS production, degranulation and chemotaxis in response to fMLF, C5a, CXCL1 and CXCL2. Importantly, ligation of TLT2 does not enhance neutrophil chemotaxis or degranulation in response to GM-CSF, which activates a tyrosine-based signaling cascade. Moreover, TLT2 ligation alone does not induce neutrophil activation or migration, demonstrating that signaling via this receptor acts to potentiate the neutrophil response to other agonists. Thus, TLT2 exerts an intrinsic effect on neutrophils leading to enhanced responsiveness to GPCR agonists that promote activation and migration. Recently, studies have shown that administration of anti-TLT2 mAb in vivo drives neutrophil recruitment in the absence of other stimuli. Because TLT2 ligation alone does not drive neutrophil migration, studies were performed to determine if administration of anti-TLT2 mAb leads to the production of chemotactic factors. Indeed, it was determined that TLT2 ligation in vivo drives the production of G-CSF, CXCL1 and CXCL2. Additional studies determined that resident macrophages contribute to the production of these factors, although other cells types may also be important. These data suggest that TLT2 exerts an extrinsic effect on macrophages leading to the production of chemokines that in turn recruit neutrophils leading to a positive feed forward loop. Importantly, this positive feed forward loop can significantly impact the outcome of physiological processes that involve neutrophils. Studies demonstrated that intravenous administration of anti-TLT2 mAb enhances the inflammatory response in the croton oil ear model. Conversely, intravenous administration of anti-TLT2 mAb protects mice from intratracheal challenge with S. pneumonia. These results demonstrate the importance of TLT2-dependent processes in inflammation and innate immunity.

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Crosstalk between Toll-Like Receptor and Cytokine Signaling in Promoting Gastric Tumorigenesis Independent of Inflammation

Brendan Jenkins[1], Hazel Tye[1], Catherine Kennedy[1], Meri Najdovska[1], Louise McLeod[1], William Sievert[2], Masanobu Oshima[3], Patrick Tan[4]

[1]Monash University, Australia; [2]Monash Medical Centre, Australia; [3]University of Kanazawa, Japan; [4]Genome Institute of Singapore, Singapore

Gastric cancer (GC) is the second most lethal cancer in the world and represents a growing number of cancers linked with inflammation. While it is accepted that deregulated interactions between gastric microbes (i.e. H. pylori) and the host innate immune system are likely to be involved in the pathogenesis of gastric inflammation (gastritis) and GC, the identity of oncogenic inflammatory/immune regulators in the host gastric mucosa remains obscure. On this note, uncontrolled activation of the pro-inflammatory and oncogenic transcription factor signal transducer and activator of transcription (STAT) 3 is implicated in various inflammation-associated cancers, including up to 50% of human GC cases. However, the downstream molecular consequences of aberrant STAT3 activation in promoting gastric inflammation and tumors are ill-defined.

We report here our investigation into the role of Toll-like receptors (TLRs), which are key components of the innate immune system primarily known to trigger an inflammatory response upon pathogen detection, during GC. For this purpose, we used 2 independent gastritis/GC mouse models characterized by STAT3 hyper-activation, 1) gp130F/F mice carrying a specific "knock-in" mutation in the interleukin (IL)-6 cytokine family co-receptor gp130 which abolishes a negative feedback mechanism, and 2) K19-Wnt1/C2mE transgenic mice displaying the simultaneous activation of the cyclooxygenase-2, prostaglandin E2 and Wnt pathways. Among the TLR family, gastric STAT3 hyperactivation in these mice significantly upregulated the expression of TLR2, and TLR2 was identified as a bona fide STAT3 target gene. In addition, genetic deletion of TLR2 in gp130F/F:Tlr2-/- mice severely reduced the gastric tumor mass by 50%. However, unexpectedly, the level of gastritis was comparable to gp130F/F mice. Immunohistochemical analyses revealed that gp130F/F:Tlr2-/- mice had increased TUNEL-positive apoptotic cells and reduced PCNA-positive cells in the gastric mucosal epithelium, thus implicating a role for TLR2 in gastric epithelial cell proliferation and survival. Consistent with our mouse data, we identified that activating TLR2 using synthetic lipopeptides in human gastric epithelial cells promoted cell proliferation via multiple signaling pathways. In human GC, both increased STAT3 pathway activation and TLR2 expression were negatively associated with patient survival. Collectively, our data depict an unexpected role for TLR2 in gastric tumorigenesis independent of inflammation, whereby increased STAT3 activation results in over-expression of TLR2 to promote gastric epithelial cell growth.

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Programmed Death Ligand-1 Expression Attenuates Liver Sinusoidal Endothelial Cell Injury in Sepsis

Noelle A. Hutchins[1, 2], Joshua B. Borgerding[2], Chun-Shiang Chung[2], Alfred Ayala[1, 2]

[1]Pathobiology Graduate Program, Brown University Warren Alpert School of Medicine; [2]Division of Surgical Research, Rhode Island Hospital

Endothelial cell (EC) dysfunction contributes to multiple organ injury—the primary cause of fatality seen in critically ill patients. Although EC dysfunction has been well characterized in the heart and lungs, it still remains unclear in the liver. Our laboratory studies sepsis in order to understand how changes in liver immune tolerance contribute to the pathophysiology of organ injury. It has been proposed that liver sinusoidal endothelial cells (LSECs) maintain immune tolerance by directly ligating the co-inhibitory

receptor, programmed-death receptor-1 (PD-1), on leukocytes via their cell surface expression of PD-ligand-1 (PD-L1). Our laboratory has previously discovered that PD-1-/- animals have improved survival after sepsis, which is associated with reducing inflammatory cytokine levels and enhancing macrophage function. Although it has been reported that LSEC express PD-L1, the role of PD-L1 in the septic liver remains unknown. *Thus, we hypothesized that PD-L1 initially protects LSECs from injury, while increased PD-1+ expressing leukocyte interactions result in the breakdown of liver immune tolerance*. We initially noted that the increased EC permeability from liver tissue after sepsis induced by cecal ligation and puncture (CLP) in wild-type (WT) animals decreased in CLP PD-L1-/- animals. There is also more pSTAT3 protein expression in whole liver homogenates after CLP, and less pSTAT3 in CLP PD-L1-/- animals. We phenotyped LSECs as $CD146^{+}CD45^{-}$ expressing cells, and found an increased expression of PD-L1, 24h post-CLP in WT mice. To determine whether PD-L1 directly regulates LSEC injury, we found that LSECs from CLP PD-L1-/- mice exhibit significantly less apoptosis compared to CLP WT mice. There was a two-fold decrease in LSEC cell number after CLP WT, and these numbers were restored in CLP PD-L1-/- animals. LSECs from CLP WT mice had significantly lower expression of angiogenic marker VEGF-R2 vs. Shams, and this was rescued in CLP PD-L1-/- mice. Finally, we also found that LSECs from CLP WT mice undergo less proliferation compared to CLP PDL1-/- animals. Interestingly, PD-L1's role in LSEC function during sepsis appears independent of Fas:FasL signaling, since the increased Fas receptor expression in CLP PD-L1-/- mice remains unchanged compared to CLP WT mice. Our data suggests that PD-1 (leukocyte): PD-L1 (EC) signaling maintains immune tolerance in Sham animals. However, the increased expression by PD-L1 alone or in combination with other signals ultimately leads to increased LSEC injury and STAT3 activation, while suppressing proliferation and angiogenesis—essential to sustain vascular integrity.

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AMPK Coordinates PI3K/Akt/mTOR and STAT3/SOCS3 Signaling in Macrophages in Response to IL-10

Yanfang Zhu, Jill Suttles
University of Louisville

We reported previously that AMP-activated protein kinase (AMPK) is a negative regulator of macrophage inflammatory function and is activated by stimulation with IL-10. With use of macrophages generated from AMPK-deficient mice, we demonstrate that AMPK is required for IL-10 activation of phosphatidylinositol 3-kinase (PI3K) and its downstream targets PDK1 and Akt. Consistent with these results, macrophages expressing constitutively active AMPK (CA-AMPK) displayed repressed activation of the negative regulator of PI3K, PTEN. These data indicate that IL-10-induced AMPK activity acts to positively regulate PI3K/Akt signaling. In support of this, IL-10 activation of cAMP response element-binding (CREB), a downstream target of the Akt/GSK3beta pathway was enhanced in macrophages expressing CA-AMPK, and AMPK-deficiency resulted in decreased phosphorylation of p70 S6 Kinase (S6K), a substrate of mammalian target of rapamycin complex 1 (mTORC1), indicating impaired mTORC1 activity. Akt has been demonstrated as a positive regulator of mTORC1. Thus, our data suggest that, in macrophages, AMPK promotes mTORC1 activity via Akt activation. Others reported that phosphorylation by mTOR is necessary for full activation of signal transducer and activator of transcription 3 (STAT3). We found elevated STAT3 activation in macrophages expressing CA-AMPK macrophages, as well as enhanced expression of suppressor of cytokine signaling 3 (SOCS3) in response to IL-10 stimulation. Both STAT3 activation and SOCS3 expression in response to IL-10 were reduced in the absence of AMPK expression. Taken together our results indicate that AMPK plays a key role in IL-10 signal transduction and the induction of the anti-inflammatory profile of IL-10-stimulated macrophages.

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Pathological Dysregulation of the GAIT Translational Control System

Paul L. Fox, Jie Jia
Department of Cell Biology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue/NC10, Cleveland, OH 44195, USA

Multiple eukaryotic ribosomal proteins (RP) are co-opted for extraribosomal "moonlighting" activities, but paradoxically, RPs exhibit rapid turnover when not ribosome-bound. In one illustrative case of a functional extraribosomal RP, interferon (IFN)-gamma induces RP L13a release from the ribosome and assembly into the IFN-Gamma-Activated Inhibitor of Translation (GAIT) complex for translational control of a subset of inflammation-related proteins. Here we show that the GAIT complex constituent glyceraldehyde-3-phosphate (GAPDH) functions as a chaperone, shielding newly released L13a from proteasomal degradation. However, the protective activity of GAPDH is lost following myeloid cell treatment with oxidatively-modified low density lipoprotein (LDLox) and IFN-gamma. Together, these agonists stimulate S-nitrosylation of GAPDH at Cys247, which prevents its interaction with L13a, resulting in ubiquitination and proteasomal degradation of essentially the entire cell complement of L13a. Consequent failure of GAIT complex assembly and defective translational control results in prolonged macrophage expression of GAIT target proteins, including vascular endothelial growth factor-A and ceruloplasmin. Remarkably, GAPDH is S-nitrosylated and L13a is specifically depleted in macrophages of atherosclerotic lesions from cholesterol-fed rabbits and patients with cardiovascular disease. Our studies establish a new molecular mechanism by which LDLox can exacerbate inflammatory gene expression in atherosclerosis and possibly other inflammatory disorders. Moreover, the results suggest that evolution of extraribosomal ribosomal protein activities might require co-evolution of protective chaperones, and pathological disruption of either protein, or their interaction, presents an alternative mechanism of diseases due to RP defects, and new targets for therapeutic intervention.

ABSTRACTS

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Inhibition of iNOS by miR-146A Allows Tumor Cells to Escape Macrophage-Induced Death

Michal A. Rahat, Miriam Walter, Nitza Lahat
Immunology Research Unit, Carmel Medical Center and The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Haifa, 34362, Israel.

Infiltrating macrophages can potentially kill tumor cells using their high output inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production, but in fact secrete low, pro-angiogenic amounts of NO due to the microenvirnment. Tumor cell iNOS expression is often reduced or lost in high grade carcinomas and metastatic cells. Upon induction with IFNγ (100U/ml) and LPS (1μg/ml), iNOS mRNA is accumulated in the mouse renal cell (RENCA), colon (CT26) and prostate (TRAMP-C2) carcinoma cell lines, but only TRAMP-C2 cells express iNOS protein and produce NO. Expression of miR-146a is negatively correlated to iNOS expression, and was higher in RENCA and CT26 cells. Only TRAMP-C2 cells that produce NO (31±5μM) exhibited increased cell death when co-cultured with stimulated RAW 264.7 macrophages, whereas RENCA and CT26 cells that did not produce NO were resistant to death. Transfection of anti-miR-146a into RENCA and CT26 restored NO production (7.1±2 and 12.7±4μM) in comparison to the negative control (anti-miR-NC, 1.7±0.4 and 1.2±0.3μM respectively, $p<0.05$), and increased their death (by 50±4% and 24±1%, respectively, $p<0.01$)when co-cultured with stimulated RAW 264.7 macrophages. When injected into BALB/c mice, anti-miR-NC transfected RENCA cells developed s.c. tumors, whereas RENCA cells transfected with anti-miR-146a did not, suggesting early tumor cell death mediated by the immune system *in vivo*. As miR-146a may enhance or inhibit the expression of additional genes related to death, we next performed a gene array analysis, and observed changes in the expression of 7 candidate genes, which are currently being validated. Thus, miR-146a regulates tumor susceptibility to macrophage-induced death through its ability to regulate iNOS expression and tumor endogenous NO production, as well as other potential genes that may synergize with NO.

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Temporal and Spatial Expression of Mouse microRNAs 125-5p, 135a, 16, 214, 182, and 30c Following Genital *Chlamydia trachomatis* Infection

Bernard Arulanandam, Rishein Gupta, Shradha Wali, Bharat Chaganty, Neal Guentzel, James Chambers
South Texas Center for Emerging Infectious Disease and Center of Excellence in Infection Genomics, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249.

Chlamydia trachomatis (CT) is the leading cause of sexually transmitted infections and associated reproductive damage in humans. The contribution of host immunity to the development of genital pathology is an area of active investigation. Given the influence of microRNAs (miRNAs) as important immune regulators, we examined the modulation of miRNAs following chlamydial genital challenge. Chlamydial elementary bodies (5X104EBs) were inoculated intravaginally in 4-6 week old C57BL/6 mice followed by euthanasia at days 6 and 12 post challenge. The removed genital tract (GT) was divided into lower (LGT; vagina and cervix), and upper (UGT; uterine horns, oviducts, and ovaries) regions for RNA extraction. Reverse-transcriptase real time PCR was performed using a pathway focused Immunopathology RT2miRNA array (MAM104, SABioSciences, CA). The miRNAs of LGT from infected mice 6 days post challenge were significantly down-regulated: mmu-miR-125b-5p (fold change: -17.34; p value = 0.014), mmu-miR-16 (-8.87; p value = 0.014), mmu-mir-214(-4.30; p value = 0.043), mmu-mir-182 (-19.29; p value = 0.0004), and mmu-miR-30c (-19.15, p value = 0.035) or upregulated: mmu-miR-135a (13.89; p value = 0.034), compared to mock challenged mice. In contrast, at the same time points in the UGT, these miRNAs were found to be down-regulated but not significantly when compared to infected LGT. By day 12 post challenge, minimal differences were observed in expression of these specific miRNAs in both LGT and UGT when compared to mock animals. These initial results suggest that miRNAs involved in chlamydial infection may be spatially and temporally regulated, and are finely tuned in the region of infection, i.e., lower vs upper, and the stage of infection (early, i.e., 6 days vs established, i.e., 12 days post infection). To this end, it is reported that mir-125b controls naïve T-cell differentiation, mir135a plays a role in embryonic pre-implantation, miR-16 influences neutrophil senescence and mir-214 affects monocyte apoptosis. Given the differential induction of these miRNAs within the genital compartment, we are currently assessing their involvement in bacterial ascension using non-invasive in vivo whole animal body imaging. Moreover, in vivo findings are being validated using in vitro miRNA knockdown assays for assessment of the functional roles of these miRNAs in initiation of host immune responses. Overall, this line of investigation provides insight into immunomodulatory changes that may contribute to bacterial ascension and subsequent reproductive injury following genital CT infection.

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Selective Regulation of Macrophage TLR Responses by Notch Signaling Components

Xiaoyu Hu[1, 2], Sinead Smith[1], Yingli Shang[1], Haixia Xu[1], Jimmy Zhu[1], Jan Kitajewski[3], Paul Saftig[4], Chao Shi[5,6], Lionel B. Ivashkiv[1, 2]
[1]Hospital for Special Surgery; [2]Weill Cornell Medical College; [3]Columbia University Medical Center; [4]University of Würzburg; [5]Memorial Sloan Kettering Cancer Center; [6]Rockefeller University

Toll-like receptor responses are tightly controlled by multiple positive and negative regulatory mechanisms to ensure adequate immune responses yet avoiding excessive inflammation. Here, we show that TLR4 responses in macrophages are regulated by the Notch pathway components that are best known for their role in developmental processes. In macrophages, signaling by Notch1 receptor through ADAM10 selectively promotes TLR4-induced expression of prototypical M1 genes such as Il12a, Il12b, and Nos2. Consistent with the effects of upstream Notch receptor

signaling, the master transcription regulator RBP-J is required for macrophage M1 polarization and host defense against Listeria monocytogenes. One mechanism by which Notch pathway promotes M1 gene expression is RBP-J-dependent accumulation of interferon regulatory factor 8 (IRF8), a transcription factor essential for expression of M1 genes. The above observations suggest that Notch signaling positively cooperates with TLR signaling to promote TLR-induced inflammatory macrophage polarization. However, certain Notch pathway components can also negatively regulate TLR responses. A canonical Notch target gene hairy and enhancer of split 1 (Hes1) encodes a transcription repressor that suppresses TLR4-induced expression of CXCL1, a chemokine important for migration of neutrophils to inflammatory sites. Hes1 deficiency in mice leads to increased recruitment of neutrophils in a model of LPS-induced peritonitis, suggesting that Hes1 restrains TLR4-mediated neutrophil response. Taken together, these results identify Notch pathway components as selective regulators of TLR responses and suggest that therapeutics targeting the Notch pathway could be developed to modulate immunity and inflammation in human diseases.

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The Functional Mechanisms of IL-17 Family Cytokines in Host Defense and Autoimmune Inflammation

Youcun Qian1, Shu Zhu1, Xinyang Song

Institute of Health Sciences, Shanghai Institutes for Biological Sciences & Shanghai Jiaotong University School of Medicine, 225 South Chongqing Road Shanghai 200025, P.R.China

Interleukin-17A (IL-17A or IL-17), produced by Th17 cells as well as some innate immune cells, not only plays a critical role in host defense against infections but also contributes to the inflammatory pathogenesis of autoimmune diseases. Its functional mechanisms remain incompletely understood. Here we identify a novel mechanism for IL-17 through regulation of miR-23b expression during autoimmune pathogenesis. miR-23b was commonly downregulated in inflammatory lesions of humans with lupus or rheumatoid arthritis, as well as in the mouse models of lupus, rheumatoid arthritis or multiple sclerosis, where IL-17 was upregulated. IL-17 downregulated miR-23b expression in primary stromal cells and was essential for the downregulation of miR-23b during autoimmune pathogenesis. In turn, miR-23b suppressed IL-17-, TNF-a or IL-1ß-induced NF-κB activation and inflammatory cytokine expression by targeting TAB2, TAB3 and IKK-a and, consequently, repressed autoimmune inflammation. Thus, IL-17 contributes to autoimmune pathogenesis by suppressing miR-23b expression in radio-resistant resident cells and promoting proinflammatory cytokine expression.

IL-17 family contains six cytokines (IL-17A to IL-17F). IL-17A and IL-17F are critical for host defense against bacterial infections while IL-17E (also named IL-25) is essential for Th2 mediated biology. The function of the other cytokines is not well understood. Here we found that IL-17C is the cytokine for the orphan receptor, Interleukin 17 receptor E (IL-17RE), and plays an essential role in host mucosal defense against bacterial infection. IL-17C activated downstream signaling through IL-17RE-IL-17RA complex for the induction of genes encoding antibacterial peptides as well as proinflammatory molecules. IL-17C was upregulated in colon epithelial cells during infection with Citrobacter rodentium and acted in synergy with IL-22 to induce the expression of antibacterial peptides in colon epithelial cells. Loss of IL-17C-mediated signaling in IL-17RE-deficient mice led to lower expression of genes encoding antibacterial molecules, greater bacterial burden and early mortality during infection. Together our data identify IL-17RE as a receptor of IL-17C that regulates early innate immunity to intestinal pathogens.

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Reciprocal Interaction between NK Cells and DCs Regulates Th17 Immune Response by Controlling the Innate IFN-γ/IL-27 Axis

Rachel R. Caspi, Jun Chen, Phyllis B. Silver, Reiko Horai, Mary J. Mattapallil[1], Ru Zhou, WaiPo Chong

Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892-1857, USA.

Experimental autoimmune uveitis (EAU) is a model for human uveitis, a potentially blinding autoimmune disease that targets the neuroretina. Mice deficient in IFN-γ develop severe EAU with elevated Th17 responses. This protective effect of IFN-γ appears at odds with the ability of IFN-γ-producing Th1 effector T cells to induce EAU (1, 2). We previously showed that it is innate IFN-γ that is protective, whereas adaptive IFN-γ is pathogenic (1 3). We now demonstrate that NK cells, which are a major cellular source of innate IFN-γ, interact with DCs in draining lymph nodes of EAU-challenged mice to ameliorate disease by controlling the innate IFN-γ/IL-27 axis. We found that after immunization, both NK cells and DCs were recruited to the draining lymph nodes, with recruitment of NK cells being at least partially dependent on CXCR3. IFN-γ-/- recipients of IFN-γ+/+ wild type (WT) NK cells developed reduced disease scores compared to recipients of control IFN-γ-/- NK cells. DCs from recipients of WT NK cells, but not of IFN-γ-/- NK cells, produced high levels of IL-27, a strong suppressor of the Th17 response (4). Finally, neutralization of IL-27 in IFN-γ-/- recipients of WT NK cells abolished their protective effect on EAU. In vitro experiments revealed that LPS-matured DCs induced WT NK cells to produce IFN-γ, and that IFN-γ caused DCs to produce IL-27. Interestingly, IL-27 also stimulated NK cells to produce more IFN-γ, indicating the existence of a positive feedback loop between NK cells and DCs for IFN-γ and IL-27 production. Our results suggest that the autopathogenic Th17 response is regulated by the interaction between NK cells and DCs through the IFN- γ/IL-27 axis.

References:

1. Tarrant, T. K., P. B. Silver, J. L. Wahl ten, L. V. Rizzo, C. C. Chan, B. Wiggert, and R. R. Caspi. J Exp Med 189:219-230, 1999.
2. Luger D, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, Bowman EP, Sgambellone NM, Chan CC, Caspi RR. J Exp Med. 2008;205:799-810.
3. Grajewski RS, Hansen AM, Agarwal RK, Kronenberg M, Sidobre S, Su SB, Silver PB, Tsuji M, Franck RW, Lawton AP, Chan CC and Caspi RR. J, Immunol. 2008;181:4791-4797.
4. Stumhofer JS, Hunter CA. Immunol Lett. 2008;117:123-30.

ABSTRACTS

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Evidence for Ethanol-Induced Conformational Change of Toll-Like Receptor (TLR)-3 and an Ethanol Binding Site

Stephen B. Pruett[1], Le Vu[2], Edwin Lewis[2]
[1]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University; [2]Department of Chemistry, Mississippi State University

The molecular mechanism by which ethanol acts to suppress a wide variety of immunological responses is not known. Ethanol inhibits initiation of signaling through toll-like receptors (TLR), and we and others have reported that it acts on TLR4 by preventing movement of components of the receptor complex into lipid rafts. However, it is not known if the mechanism involves altered protein conformation or altered raft properties. Ethanol also inhibits signaling through TLR3, and the mechanism is unknown. We report here the use of molecular modeling software to identify a probable ethanol binding site on TLR3, and the estimated binding affinity is within the range of ethanol concentrations reported in alcohol abusers. To determine if ethanol binding or other effects of ethanol cause changes in TLR3 conformation, circular dichroism was used to detect conformational changes in TLR3-ligand complexes with a series of ethanol concentrations. The results demonstrate that ethanol at 20-100 mM causes conformational changes in both mouse and human TLR3-ligand complexes. The pattern of change is dose-dependent, and there is a change in slope from positive to negative near the estimated binding constant of the ethanol binding site identified in this study. This is the first report providing direct physical evidence that ethanol acts on an important immunological receptor by changing its conformation. This work was funded by grant R01AA009505 from the National Institute on Alcohol Abuse and Alcoholism.

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Peroxisome Proliferator-Activated Receptor-γ Agonists Protect Neurons and Microglia from Ethanol in the Developing Cerebellum

Paul D. Drew, Kevin D. Phelan, James C. Douglas, Cynthia J.M. Kane
University of Arkansas for Medical Sciences

Fetal alcohol spectrum disorders (FASD) result from exposure of the developing fetus to ethanol. FASD are the leading cause of mental retardation in the United States and result in significant lifetime disability. In the central nervous system, (CNS), fetal ethanol exposure can result in neurodevelopmental anomalies, and these anomalies are best characterized in the cerebellum. The current studies evaluated the effects of ethanol in a newly developed mouse model of FASD as well as on primary cultures of microglia and cerebellar neurons. We demonstrate that ethanol decreases the viability of cultured cerebellar granule cells and microglia in vitro. Surviving microglia exhibited an altered morphology in response to ethanol and produced inflammatory molecules characteristic of activated microglia. Furthermore, we demonstrate that ethanol is also toxic to neurons and microglia in vivo in the mouse model of FASD. Importantly, we demonstrate that the peroxisome proliferator-activated receptor-γ agonists pioglitazone and 15-deoxy-Δ12, 15 prostaglandin J2 protect cerebellar neurons and microglia in vitro and in vivo from the toxic effects of ethanol. We and others have previously demonstrated that PPAR-γ agonists are potent suppressors of glial activation, suggesting that PPAR-γ agonists protect neural cells at least in part by suppressing immune activation in the CNS. Collectively, these studies suggest that modulation of PPAR-γ signaling may represent a novel treatment paradigm for protecting the developing CNS from the toxic effects of ethanol.

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Epidermal Nicotinic Receptor Activation Prior to Skin Wound Infection Impairs Antimicrobial Peptide Responses and Leukocyte Recruitment to Increase Bacterial Survival

Jennifer K. Plichta, Tina Griffin, Katherine A. Radek
Loyola University Chicago, Health Sciences Campus, Department of Surgery

Antimicrobial peptide (AMP) activity is a vital component of the innate immune system that can directly facilitate tissue repair, including inflammatory cell recruitment and microbial defense mechanisms. Both nicotine usage and stress delay skin wound healing and increase the frequency of wound infection, which likely transpires partially via the activation of nicotinic receptors (nAChR). Keratinocytes possess a non-neuronal cholinergic system comprised of acetylcholine (ACh) and acetylcholine nicotinic and muscarinic receptors. We previously found that psychological stress and topical nicotine diminished epidermal AMP production and activity in mouse skin, which was reversed by topical application of the α7-nAChR antagonist, α-Bungarotoxin (α-Bung). Here, we evaluated the direct effects of nAChR activation via nicotine on cutaneous AMP responses in keratinocyte raft cultures and mouse wounds, and also the inflammatory cell recruitment during cutaneous wound infection. First, we stimulated keratinocyte organotypic raft cultures with 1, 25-dihydroxy Vitamin D3 (VD3) and the Toll-like receptor (TLR) 2/6 ligand Macrophage-activating lipoprotein-2 (MALP2). VD3 enhances the detection of microbial components, such as MALP-2, in keratinocytes through TLR2 to increase AMPs at the site of injury and/or infection. Rafts co-stimulated with 0.01nM ACh exhibited a robust decrease in cathelicidin AMP and TLR2 gene and protein expression compared to VD3/MALP2, assessed by qPCR and immunohistochemistry (IHC), which was reversed by α-Bung. Next, male C57Bl/6 mice were subjected to three days of topical nicotine treatment (i.e. local activation of epidermal nAChRs) prior to topical wound infection with *Staphylococcus aureus* (10^6 CFU). Wounds were harvested after 24 hours to assess Macrophage Inflammatory Protein-2 (MIP-2), and cathelicidin (CRAMP) and β-defensin-3 (mBD3) and mBD-14 AMP responses. Mice subjected to nicotine exhibited a ~60% significant reduction in MIP-2 (1.00±0.15 vs. 0.25±0.24) and ~50% reduction in cathelicidin (1.01±0.24 vs. 0.49±0.06) gene expression compared to vehicle ($p<0.05$), but no change was seen with mBD-3 or mBD-14. We then confirmed a reduction in the protein abundance and difference in localization of epidermal cathelicidin in nicotine treated mice by IHC compared to vehicle. Furthermore, we observed a potent reduction in neutrophil (anti-GR-1) and macrophage (anti-MOMA-2) abundance with

nicotine treatment by IHC in infected wounds, which paralleleda significant increase in the number of surviving bacteria (~10-fold) in wounds. Collectively, we propose that epithelial nAChR activation prior to wound infection diminishes AMP production and AMP-dependent responses, such as proinflammatory molecule production and leukocyte recruitment. These data further delineate the non-neuronal cutaneous interaction and suggest that excess or prolonged non-neuronal epidermal nAChR activation may increase the risk for skin infection.

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Chronic Alcohol-Induced IL-1ß Is Mediated by the NALP3/ASC Inflammasome Activation in a TLR4-Independent Manner in the Brain and Prevented by IL-1 Receptor Antagonist Treatment

Dora Lippai, Shashi Bala, Jan Petrasek, Timea Csak, Ivan Levin, Evelyn A. Kurt-Jones, Gyongyi Szabo
University of Massachusetts Medical School

Introduction: Alcohol-induced neuroinflammation is mediated by pro-inflammatory cytokines including interleukin-1-beta (IL-1β). IL-1β production requires caspase-1 activation by inflammasomes, the multiprotein complexes assembled in response to danger signals.

Aim: To test the hypothesis that alcohol-induced inflammasome activation contributes to increased IL-1β production and neuroinflammation in the brain.

Methods: WT, TLR4-, NALP3- and ASC-deficient (KO) mice received ethanol containing or isocaloric control diet for 5 weeks, some received IL-1 receptor antagonist (IL-1ra) or saline treatment. Inflammasome activation, pro-inflammatory cytokines, endotoxin and HMGB1 were measured in the cerebellum.

Results: Expression of inflammasome components (NLRP1, NLRP3, ASC) and pro-inflammatory cytokines (TNFa, MCP-1) was increased in the brain of alcohol-fed compared to control mice. Increased caspase-1 activity and IL-1β protein expression in ethanol-fed mice indicated inflammasome activation. TLR4 deficiency protected from TNFa and MCP-1 but not IL-1β increase and lipopolysaccharide was not detected in the brain. However, we found upregulation of the endogenous danger molecule, HMGB1, in its phosphorylated and acetylated forms and increased expression of HMGB1 receptors (TLR2, TLR4, TLR9, RAGE) in alcohol-fed mice. Deficiency in the inflammasome components, NALP3 or ASC, protected mice from alcohol-induced brain IL-1β or caspase-1 activation. Importantly, treatment with IL-1 receptor antagonist, anakinra, prevented inflammasome activation, IL-1β and TNFα increase in the brain of alcohol-fed mice.

Conclusion: Activation of NALP3 inflammasome/caspase-1 induces IL-1β and amplifies alcohol-induced neuroinflammation independent of TLR4. HMGB1 is an alcohol-induced endogenous danger signal in the brain and disruption of IL-1/IL-1R signaling with IL-1ra treatment prevents inflammasome activation and alcohol-induced neuroinflammation.

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Lymphocyte Metabolism in CD4 T Cell Subsets

Jeffrey Rathmell
Dept. of Pharmacology and Cancer Biology, Duke University

Protective adaptive immune responses require that T cells transition from a quiescent state to a highly proliferative state with effector functions. This switch leads to a significant change in the metabolic demands, as activated T cells require greatly increased biosynthetic capacity to support cell growth and proliferation. To support this, T cells undergo dramatic metabolic reprogramming to increase glucose uptake and glycolysis. Importantly, this activated metabolic program is not uniform and CD4 T cell metabolic programs shift upon differentiation into effector (Th1, Th2, Th17) or regulatory (Treg) subsets. We have examined the metabolic profiles of CD4 T cell activation and subsets and found that effector T cells are glycolytic while Treg utilize primarily lipids and oxidative pathways. To directly test this model in vivo, we have examined animal models for over expression or deficiency in the glucose transporter, Glut1. Glut1 transgenic (Glut1 tg) animals accumulate Teff while Treg are not changed. In contrast, T cell specific conditional knockout of Glut1 leads to reduced T cell numbers and effector function, while Treg are unaffected. Thus changes in glucose metabolism in vivo selectively impact effector T cells over Treg. Manipulating metabolic pathways, therefore, may provide a new approach to selectively target Teff in inflammatory diseases.

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Mechanisms of Macrophage Innate Immunity Inhibition by *Mycobacterium tuberculosis*

Jim Sun, Alice Lau, Vijender Singh, Zakaria Hmama
Department of Medicine, University of British Columbia

The ability of Mycobacterium tuberculosis (Mtb) to adapt and thrive intracellularly relies on a variety of strategies to alter mechanisms of the host innate immunity. In particular, interference with phagosome biogenesis was highlighted as a significant aspect of Mtb persistence and replication within the macrophage. How Mtb circumvents phagosomal acidity, bactericidal enzymes, and reactive oxygen species (ROS) is a central question for many cellular microbiologists.

ROS are produced by phagocyte NADPH oxidase (NOX2) and were classified 30 years ago as powerful microbicidal agents against many intracellular pathogens. Recent years have seen a growing body of evidence to suggest a crucial role for ROS in the control of mycobacterial infection. In particular, one group has recently identified Mtb nuoG as a potential virulence factor operating at the level of NOX2 by mechanisms yet to be defined. While the role of NOX2 in innate immunity is well established, recent reports suggest that it might act beyond the control of intracellular infections to trigger macrophage apoptosis a central event that paves the road to adaptive immunity.

Our laboratory identified Mtb nucleoside diphosphate kinase (Ndk) as a GTPase Activating Protein (GAP) acting on Rab5 and Rab7 GTPases, leading ultimately to defective phagosomal recruitment of EEA1 and RILP respectively. In recent studies, we examined whether Ndk GAP activity extends to other GTPases, with a

particular focus on Rho GTPases. We found that Mtb Ndk binds to and specifically inactivates Rac1 leading to inhibition of NOX2 assembly and activation in the macrophage. We also established a link between Ndk-dependent NOX2 attenuation and inhibition of apoptosis response to Mtb. We have demonstrated that knock down of nucleoside diphosphate kinase (Ndk) attenuated Mtb survival within the macrophage and reduced significantly its virulence in the mouse. At the molecular level, Ndk was shown to bind to and inactivate the small GTPase Rac1, leading to the exclusion of its binding partner p67phox from Mtb phagosomes. This resulted ultimately in a defect of NADPH oxidase assembly and activation along with attenuation of apoptosis. Furthermore, knock down of Ndk converted Mtb into a strain that induced macrophage oxidative burst and programmed cell death. Restoration of these phagosomal events correlated with increased susceptibility of Mtb to intracellular killing consistent with the attenuation of in vivo virulence reported above. Altogether, our findings demonstrated that Ndk is a bona fide mycobacterial virulence factor that attenuates host innate immunity.

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Regulatory States of N-Formyl Peptide Receptor in Human Neutrophils: Modulation and Partial Purification of Phosphoforms of FPR1

A J. Jesaitis[1], C I. Lord[1], J M. Gripentrog[1], M Riesselman[1], W Maaty[2], B Bothner[2], G Keren-Aviram[2], E A. Dratz[2]

[1]Department of Microbiology, Montana State University; [2]Department of Chemistry and Biochemistry, Montana State University

Phosphorylation of FPR is important in control of neutrophil responses to formyl peptides. By adjusting the phosphorylation state of the FPR1 C-terminus, FPR1 interactions with regulatory proteins can be controlled. Two antibodies, NFPRa and NFPRb (formerly NFPR1, NFPR2; J.Immunol 179:2520, 2007) were used to monitor and purify total and nonphosphorylated, respectively, FPR1 from human peripheral blood neutrophils. Neutrophils exposed to [fMLF] ranging from 0 to 10 μM were solubilized, immunoblotted with NFPRa or NFPRb primary mAbs, and quantitated by infrared fluorescence detection. NFPRb binding showed a specific and saturable dependence on prior exposure of neutrophils to fMLF with and EC_{50}=8.1 X 10^{-8} M with little change in the binding of NFPRa. Pretreatment of neutrophils with 5 μg/ml cytochalasin B (CB) had no significant effect on the EC_{50} for NFPRb but caused a 10-20% increase in the binding of NFPRa. Pertussis toxin (PTX) exposure had minimal effect on NFPRb binding to FPR1, while okadaic acid (OKA) exposure resulted in a 3 fold shift of EC_{50} to higher concentrations. These results suggest that the phosphorylation of FPR1 was not under the control of PTX substrate Giα and that the effects of the S/T phosphatase inhibitor, OKA, on FPR1 phosphorylation are complex. Higher numbers (1.5 - 2.0 X109) of neutrophils, exposed in the same way to 0, 1uM fMLF, and 1 uM fMLF+5 ug/ml CB, followed by dilution in ice cold buffer were also prepared in bulk to produce two stimulated and one unstimulated populations of neutrophils. These cells were then lysed and solubilized by 1% dodecylmaltoside in the presence of protease and phosphatase inhibitors. The extracts were cleared by low (500 X g) and high speed (100, 000 x g) centrifugation and passage over a cyanogen bromide sepaharose matrix coupled to isotype-matched irrelevant antibody. The cleared extracts were then sequentially passed over a NFPRb affinity matrix followed by passage of the post-bind over a NFPRa matrix. After washing, FPR1 was eluted using epitope-mimicking peptides as well as low pH and 1% SDS. Based on quantitative immunoblot analysis using NFPRa, 40 to 60% of FPR1a-binding activity in the cleared extracts (~ 80% of the total) was recovered from the immunoaffinity matrices. FPR content was confirmed by in-gel trypsinolysis of glycosylated or deglycosylated FPR1 bands followed by tryptic peptide identification by MALDI-TOF mass spectrometry. Preliminary MS analysis of FPR copurifying bands from (fMLF+CB)-stimulated samples suggests that CD11b, β-actin, Giα, Goα, and rap1A may be associated with FPR either directly or indirectly.

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Cathelicidins Enhance Host Defence against Bacterial and Viral Lung Infections via Antimicrobial Activity and Induction of Protective Inflammation

Donald J . Davidson[1], Silke M. Logermann[1], Emily Gwyer Findlay[1], Brian McHugh[1], Paula E. Beaumont[1], Peter G. Barlow[2, 1], Annie Mackellar[1], Richard L. Gallo[3], John R W. Govan[4], Paul M. Fitch[1], Jürgen Schwarze[1]

[1]MRC Centre for Inflammation Research, University of Edinburgh, Queens Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, UK; [2]Centre for Nano Safety, Edinburgh Napier University, Edinburgh EH11 4BN, Scotland, UK; [3]Division of Dermatology, Department of Medicine, University of California at San Diego and VA San Diego Health Care System, San Diego, California, USA; [4]University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, Scotland, UK

Cathelicidins are cationic host-defence peptides (CHDP) with key roles in innate immunity. Initially described primarily as bactericidal agents, we have characterised cathelicidins as multifunctional antimicrobial immunomodulators, modifying host responses to pathogens.

The sole human cathelicidin hCAP-18/LL-37 (encoded by the *CAMP* gene) is pre-formed in neutrophils and inducible in epithelial cells and macrophages during infection and inflammation. Mice deficient in the murine orthologue of *CAMP* (*Camp*) have increased susceptibility to bacterial infection in multiple systems, demonstrating the critical and non-redundant nature of cathelicidin-mediated protection in host defence against infection. However, the mechanisms underpinning this protection remain unclear, and the role of cathelicidins in host defence against viral infections has not been well characterised.

Using *in vitro* and *in vivo* models of pulmonary infection with human respiratory pathogens *Pseudomonas aeruginosa*, respiratory syncytial virus (RSV) and influenza A virus (IAV), we demonstrate key antimicrobial and host immunomodulatory properties of cathelicidins.

We report on different mechanisms by which cathelicidins promote pathogen clearance and modulate host-pathogen interactions to protect against pulmonary infection, including: 1) anti-viral

activity against both RSV and IAV, and induction of an antiviral state in airway epithelial cells, and 2) induction of protective cellular inflammatory responses and enhanced pathogen clearance after infection, which was defective in cathelicidin knockout animals (*Camp-/-*), but correctable by therapeutic cathelicidin administration.

These data demonstrate key roles for cathelicidins in host defence against pathogenic bacterial and viral lung infections and the potential to inform the development of synthetic analogues to modulate specific host-pathogen interactions as novel antimicrobial therapeutics.

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Impact of *Leishmania* Infection on Macrophage NLRP3 Inflammasome Network

Marina T. Shio, Martin Olivier

Departments of Medicine and of Microbiology and Immunology, The Research Institute of the McGill University Health Centre, Centre for the Study of Host Resistance, Faculty of Medicine, McGill University, Montréal, Québec, Canada.

Innate inflammatory responses under Nod-Like Receptors (NLR) regulation play a critical role in controlling pathogens. However, protozoan parasites such as *Leishmania* evolved strategies to avoid phagocyte activation by seizing control of key signaling pathways, therefore favoring their invasion and survival within the host cell. In fact, *Leishmania* is known to alter NF-κB-dependent signaling, as well as to avoid induction and to tame-down IL-1β secretion by macrophages. As NLRP3 inflammasome complex is known to be critically involved in IL-1β secretion by macrophages, it is possible that *Leishmania* can also alter NLR-related pathways to avoid its detection as a "danger signal" and further secure its survival by silently enter macrophages potentially harsh environment. Since NLRP3 was found to be critical for the recognition of various inorganic crystals such as malarial hemozoin (HZ), silica, asbestos and uric acid (MSU) all concurring to IL-1β secretion by macrophages, we used those crystals, and HZ in particular, to ask whether the capacity of *Leishmania* parasite to tame-down IL-1β secretion could involve the alteration of NLRP3 inflammasome pathway. Using PMA-differentiated THP-1 cells pre-infected with *Leishmania* (*L.*) *mexicana* we observed a substantial inhibition of IL-1β production induced by HZ, silica and MSU. In addition, different species of *Leishmania* were capable to inhibit HZ-induced IL-1β production and using *L. major* being deficient for the metalloprotease GP63 we found that this virulence factor produced by *Leishmania* parasites was involved in the inhibition of IL-1β production. Similar results were obtained using culture supernatant of *L. major* WT or GP63 deficient, suggesting that secreted form of GP63 also can inhibit HZ-induced IL-1β production. As GP63 exist as secreted form or at the surface of secreted exosome vesicles, we purified those latters from *L. mexicana* cultures and evaluate its effect on IL-1β production. Purified GP63 and GP63-containing exosomes inhibited IL-1β production and caspase-1 cleavage, suggesting their effect upstream of caspase-1. Other mechanisms known to regulate NLRP3 inflammasome activation involving ROS production and signaling pathway were find to be affected by *L. mexicana* infection or purified GP63. Collectively, our study permits to report the first demonstration that a pathogen, for instance the protozoan Leishmania, can downregulate NLRP3 inflammasome complex through the action of its surface metalloprotease GP63. A better understaning on how pathogens can dodge innate inflammatory can lead to the development of new way to treat infections and other pathologies.

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Δ 9-Tetrahydrocannabinol [THC] Reduces Inflammation and HIV-1 Infection of Target Cells

Julie C. Williams[1], Sofia Appelberg[1], Amanda C . Lowe[1], Yun Mei[1], Caroline Reist[1], Melanie Cash[1], Mark A. Wallet[1], Li Yin[1], Bruce Goldberger[1], Tom Klein[2], Sharon Nichols[3], John W. Sleasman[2], Maureen M. Goodenow[1]

[1]University of Florida; [2]University of South Florida; [3]University of California, San Deigo

Use of cannabis is reported to be as high as 81% among HIV-1-infected adolescents.The major psychoactive constituent of cannabis, Δ9-tetrahydrocannabinol [THC], mediates anti-inflammatory effects through interaction with cannabinoid 2 [CB2] receptors on immune cells. As most studies of THC and immunity have been conducted in rodents, the role of THC in modulating human immunity and HIV-1 immune pathogenesis remains unclear. The impact of cannabis use on immune activation was investigated by a study combining in vivo and ex vivo approaches.

Expression of THC receptors CB2, CB1, and GPR55 by human monocytes, monocyte derived macrophages, and undifferentiated or PMA differentiated THP-1, HL-60 and U937 monocytic cell lines was evaluated by nested reverse transcriptase PCR. CB2 mRNA levels decreased 40% with monocyte differentiation, while mRNA levels for CB1 increased 40-fold or remain unchanged for GPR55. Modulation of inflammation was evaluated by pretreatment of cells with a range of THC doses followed by immune activation via different pathways. THC treatment reduced: 1)TNF-induced ICAM-1 protein levels in undifferentiated THP-1 cells and monocytes [$p<0.05$]; 2) IL-6 levels produced by LPS-activated peripheral blood mononuclear cell [PBMC] [$p<0.001$] and monocyte cultures [$p<0.001$]; and 3) IFNβ-stimulated CXCL10 production by PBMCs [$p<0.001$] and monocyte-derived macrophages [$p<0.001$]. Intracellular flow cytometry revealed that the predominant cellular source of CXCL10 and IL-6 among PBMCs was the monocytes.

Acute THC treatment of monocyte-derived macrophages or PBMCs had no effect on HIV-1 replication. However, chronic THC treatment of monocytes during differentiation into monocyte-derived macrophages or PBMCs during activation significantly inhibited HIV-1 replication as measured by p24 levels in cell supernatants. Levels of HIV-1 receptors, CD4, CXCR4, and CCR5 were not affected by THC or ETOH vehicle control during chronic treatments.

Finally, a panel of biomarkers of immune activation associated with neurocognitive impairment or overall morbidity/mortality was measured in plasma samples from a cohort of 78 HIV-1-infected and 35 uninfected subjects. In the HIV-1-infected group 35.8% tested positive for cannabinoids compared to 37.1% in the control group. For the vast majority of biomarkers, cannabis use failed to correlate with immune activation in either group. Significantly decreased

levels of sCD27 were observed in cannabinoid-positive subjects compared to cannabinoid-negative subjects in the uninfected group [p=0.014].
THC reduces inflammation and HIV-1 infection of target cells. However, futher investigation into the mechanism of the inhibitory affects of THC is required.
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A Fluidics Approach Reveals *Toxoplasma gondii* Modulation of Human Monocyte Interactions with Vascular Endothelium

Melissa B. Lodoen[1], Katherine S. Harker[1], Norikiyo Ueno[1], Tingting Wang[2], Wendy Liu[2]
[1]Department of Molecular Biology and Biochemistry and the Institute for Immunology, University of California, Irvine ; [2]Departments of Biomedical Engineering, Chemical Engineering and Materials Science, University of California, Irvine

Toxoplasma gondii is an obligate intracellular parasite that infects approximately one third of the human population worldwide and can cause life-threatening disease in immune compromised individuals and the developing fetus. During T. gondii infection, the parasite disseminates in the circulation and establishes a chronic infection in tissues, including the brain and the eye. Previous research suggests that the infection of circulating immune cells, such as monocytes, facilitates parasite dissemination. However, it is not known how T. gondii-infected cells interact with the vascular endothelium to exit the circulation and enter tissues. We have developed an in vitro fluidics system coupled with time-lapse fluorescence microscopy to examine the effects of T. gondii infection on human monocyte-endothelial cell interactions. Fluorescently-labeled infected and uninfected human monocytes were flowed over human umbilical vein endothelial cells (HUVEC) under conditions of physiologic shear stress (0.5 dyn/cm2), and time-lapse imaging was performed. Monocyte interactions with the endothelium were analyzed using cell tracking software to quantify the average velocity, pathlength, and displacement of monocytes during rolling and searching behaviors. Infected monocytes exhibited enhanced interactions with the endothelium and rolled at a significantly higher velocity and greater pathlength than uninfected monocytes before firmly adhering. The searching behaviors of infected and uninfected monocytes appear to be similar. To investigate the molecular basis for these phenotypes, the cell surface expression of adhesion molecules on monocytes and endothelial cells was examined by flow cytometry. High expression of the integrins LFA-1 (αLβ2) and VLA-4 (α4β1) on the monocytes and of their respective ligands, ICAM-1 and VCAM-1, on the activated endothelium was observed. The total expression levels of these integrins were not dramatically changed by infection, but confocal microscopy revealed that T. gondii alters integrin clustering and the ability of infected cells to form firm adhesions. Parasite inhibition of integrin redistribution may underlie the enhanced rolling of infected human monocytes on vascular endothelium.

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A Comprehensive Map of the Human Complement System from Signaling Gateway Molecule Pages

Ashok Reddy Dinasarapu[1], Anjana Chandrasekhar[1], Shankar Subramaniam[1, 2, 3]
[1]Department of Bioengineering, University of California San Diego (UCSD), 9500 Gilman Drive, La Jolla, CA 92093.; [2]Department of Cellular and Molecular Medicine, University of California San Diego (UCSD), 9500 Gilman Drive, La Jolla, CA 92093.; [3]San Diego Super Computer Center, University of California San Diego (UCSD), 9500 Gilman Drive, La Jolla, CA 92093.

The human complement activation takes place through one or more of the well-established pathways (alternative, classical, and lectin) consisting of plasma and membrane-bound proteins. All three pathways converge at the level of C3 and are controlled by regulators. The entire network is considered as a simple recognition and elimination system of host immune-complexes and apoptotic cells and pathogens, and therefore promotes host immune homeostasis. The complement system is also involved in cross-talk with other processes related to coagulation, lipid metabolism and cancer. However, many pathogens counteract complement attack through a range of different mechanisms, such as acquisition of host complement regulators to the surface of pathogen, or secretion of complement inactivation factors. In order to have a holistic view of the entire complement network, we have generated expert-authored and peer-reviewed data on each of the 50 known complement and its associated proteins. We created this network of the human complement system under the umbrella of the Signaling Gateway (www.signaling-gateway.org/molecule) project. The Signaling Gateway Molecule Pages database publishes curated data on each protein involved in cellular signaling. Each molecule page (on a given protein) provides a textual description and a structured data which exists in different forms, called functional states, participating in cellular signaling. These states range from the native state of the protein to subsequent states such as, those with post-translational modifications, interacting with one or more proteins or small molecules, and or which change cellular location. Transitions between these states are associated with a combination of one or more modifications, interactions and translocations, which might be associated with one or more biological processes. In a characterized biological state, a molecule can function as one of several entities or their combinations, which includes channel, receptor, enzyme, transcription factor and transporter. We thus provide a unique curated database of the human complement system in its entirety, which along with a comprehensive network of transition maps, with qualitative and quantitative information. Furthermore, we export the data to BioPAX, SBML and XML formats. Such bioinformatics tools incorporated in the Molecule Pages can be used by the biologists to extract the relevant published information and design experiments to both understand and fine tune the regulation of the complement proteins in a context specific manner. As a future exercise, we aim to develop the complement system ontology for uniform representation and integration over different species.

ABSTRACTS

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Constitutive Gene Expression in Monocytes from Chronic HIV-1 Infection Overlaps with Acute Toll-Like Receptor Induced Monocyte Activation Profiles

Luis J. Montaner[1], Bethsebah Gekonge[1], Malavika S. Giri[1], Andrew V. Kossenkov[1], Michael Nebozyhn[1], Malik Yousef[1], Karam Mounzer[2], Louise Showe[1]

[1]The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104; [2]Philadelphia Field Initiating Group for HIV-1 Trials, Philadelphia, PA 19104

Toll-like receptor (TLR)-mediated signalling has emerged as a major player in promoting chronic immune activation in the host. Elevated TLR expression/signalling in monocyte/macrophages (M/M) has been shown to result from *in vivo* microbial translocation, upregulation of TLR expression in cells, HIV-encoded TLR ligand interactions and HIV-RNA mediated upregulation of TLR expression. We sought to determine whether *in vivo* circulating monocytes in HIV-1 infection exhibit gene expression patterns similar to those elicited by acute TLR-2 activation. Monocytes from 12 uninfected and 13 HIV-1 infected subjects were exposed to 0.2μg/ml *Staphylococcus Aureus Cowan* (SAC), a TLR-2 agonist. Total cellular RNA was extracted using TRI reagent. Samples were isolated and analyzed individually consisting of 12 monocyte isolations from uninfected subjects as controls (C) and 12 paired 5h SAC Stimulated (CS); 13 monocyte isolations from HIV infected subjects (P) and 13 paired 5h SAC stimulated (PS). Using microarray gene expression data analysis and pathway analysis methods, we compared genes modulated in circulating monocytes in chronically HIV-1 infected individuals against acutely induced genes following TLR-2 engagement. Here we show, via differential gene expression comparisons, the presence of a constitutive *in vivo* TLR-like gene activation signature in steady-state circulating monocytes from chronically HIV-1 infected subjects. The TLR2-like gene signature was defined as an 82 gene subset of 376 genes previously described by us as constitutively modulated in *in vivo* HIV-1 monocytes, based on their overlap with *de novo* TLR2-induced genes in uninfected subjects' monocytes. Additional comparison of *in vivo* gene networks with available datasets from acute TLR activations in M/M expanded the overlap to 151-gene concordance among the 376 differential genes with emphasis on ERK/MAPK, TNF/IL6 (NFκB) and p53 gene networks. TLR2 stimulation of monocytes from HIV-1 infected subjects resulted in further upregulation of inflammatory genes indicative of a sustained transcriptional potential upon stimulation.

Our study supports the interpretation that circulating monocytes in HIV-1 infection are not "undifferentiated" or "resting" cells but represent a constitutively activated or "mature" MDM-like monocyte with differential gene expression likely to impact inflammatory monocyte function upon recruitment towards tissue macrophages and/or precursors to dendritic cells.

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Of Mice and Men… and Pig: The Pig Mononuclear Phagocyte System Is a Relevant Inflammatory Model for Humans

Ronan Kapetanovic[1], Lynsey Fairbairn[1], David P. Sester[2], Alan L. Archibald[1], David A. Hume[1]

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK; [2]Innate Immunity Laboratory, School of Chemistry and Molecular Biosciences, University of Queensland, Australia

The mouse has been the major model employed for innate immune studies, but there are undeniable differences between species in this system. The inflammation response has also diverged between these two species; for example, mice are 10^5 more resistant to endotoxin shock than humans. For this reason, we investigated the porcine mononuclear phagocyte system. Pigs offer several advantages as closer evolutionary distance to humans and the larger size of the animal. In particular, it is easier to harvest large numbers of lung macrophages.

We have isolated a large number of mononuclear cells from 3 compartments (lung, bone-marrow and blood) of 25 pigs from 5 breeds (Landrace, Large White, Pietran, Duroc and Hampshire). TNF production in response to lipopolysaccharide (LPS) was assayed and the expressions of common macrophages markers were investigated by flow cytometry, as well as the capacity to phagocyte bacteria. We analysed the gene expression in response to LPS using a newly-generated and annotated pig expression array and compared the gene expression profiles of macrophages from the different breeds and compartments; alveolar (AM), bone-marrow-derived (BMDM), monocyte subsets and monocyte-derived macrophages (MDM).

We found that isolated macrophage populations from pigs resemble those of humans. All type of macrophages expresses CD172a, CD16 and the LPS co-receptor CD14. Interestingly, CD163 expression (a C type lectin) was lower in presence of rh-CSF-1. It was retained on AM and monocyte, where its expression defined subsets and was expressed inversely with CD14. Like peripheral blood monocytes, AM comprised also 2 subpopulations that differed in adherence, LPS response, phagocytosis and expression of CD163. Thus, human and pig macrophages seem to share the same subpopulation proprieties. $CD14^{++}$ porcine monocytes resembled $CD14^{++}$ human monocytes in the expression profile and, as in human, we found that alveolar macrophages is a heterogeneous population. Macrophages from these 2 species have also in common the expression of LPS-inducible genes (STAT4, IDO, CCL20, Cyp27B1) that are not induced in mouse macrophages, and failed to induce iNOS. Finally, pig breeds showed no great differences in response to LPS. The few genes differentially expressed included cytochrome CYP3A29, the metalloproteinase MMP1, STEAP4 and the N-Myc interactor NMI. In conclusion, we have isolated and characterised the gene expression profiles of pig macrophages in multiple differentiation and activation states. The data support the use of the pig as a model of innate immunity that more closely resembles humans, and is economically important in its own right.

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Role of the Inflammasome in Melioidosis

Fabio Re[2], Manoranjan Sahoo[2], Mark Miller[1], Laura Barrio[2]

[1]University of Tennessee Health Science Center; [2]Rosalind Franklin University of Medicine and Science

Burkholderia pseudomallei is a Gram-negative bacterium that infects macrophages and other cell types and causes melioidosis. We have recently shown that the Nod-like receptors (NLR) NLRP3 and NLRC4 differentially regulate pyroptosis and production of IL-1β and IL-18 in response to *B. pseudomallei* infection. Pyroptosis and IL-18 production were equally important for resistance to *B. pseudomallei*. Surprisingly, IL-1β was found to be deleterious in melioidosis. The detrimental role of IL-1β during melioidosis was due, in part, to excessive recruitment of neutrophils to the lung. We showed that neutrophils do not express NLRC4, fail to undergo pyroptosis, and, therefore, may be permissive to *B. pseudomallei* intracellular replication leading to increased bacterial burden and morbidity/mortality. Other pathways downstream of IL-1R may contribute to the deleterious effect and are being investigated. Preliminary results also indicate that while deleterious during acute infection, IL-1 may prevent establishment of latent, persistent infection. Results will be presented that characterize the role of other cytokines including IFNγ and IL-22 during acute and chronic infection with *B. pseudomallei* and *B. thailandensis*.

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Molecular Phenotype of HIV-1-Induced Macrophage Priming Defined by Systems Biology: A Role for Guanylate Binding Proteins

Mark A. Wallet[1], Jian-Liang Li[2], Julie C. Williams[1], Maureen M. Goodenow[1]

[1]University of Florida, Gainesville, FL; [2]Sanford-Burnham Medical Research Institute, Orlando, FL

HIV-1 infection causes a vast array of immune alterations including systemic monocyte/macrophage activation due to viral replication and elevated circulating toll-like receptor [TLR] ligands emanating from a compromised gut barrier or opportunistic infections. Infection of macrophages by HIV-1 induces a primed phenotype resembling interferon [IFN]-induced priming whereby the cells are hypersensitive to activation by TLR ligands. We hypothesized that HIV-1 infection and/or replication usurps IFN-induced priming mechanisms to alter the macrophage activation state.

A systems biology strategy was developed to interrogate molecular pathways leading to the primed phenotype under IFN-induced versus HIV-1 induced conditions in primary human monocyte-derived macrophages [MDM]. Affymetrix U133 plus 2.0 cDNA microarray chips were used to identify transcriptome profiles of resting, partially activated (lipopolysaccharide [LPS] only), IFN-γ-primed, or classically activated (IFN-γ-primed + LPS) MDM. Transcriptome profiles were analyzed using Linear Models for Microarray Analysis. A biomarker gene set capable of distinguishing different activation states was extracted and applied to compare HIV-1 primed or HIV-1 + LPS-induced macrophage activation. Intracellular staining for HIV-1 p24 and tumor necrosis factor [TNF] was used for flow cytometry to determine the relationship between HIV-1 infection and priming on an individual cell basis. Only HIV-1 infected macrophages were primed in culture. While the majority of IFN-induced genes were unaffected by HIV-1 infection, genes encoding guanylate-binding proteins [GBPs] were selectively increased by virus. Expression of TNF transcripts and secretion of TNF were enhanced by HIV-1 priming, similar to IFN-γ-primed macrophages, when cells were activated with LPS. In contrast, a signature effect of IFNs, suppression of IL-10, was not detected in HIV-1 primed cells. Overall, the functional phenotype and molecular profile of HIV-1 induced macrophage priming are dissimilar to IFN-induced priming. Studies identified a novel class of genes, GBPs, which are induced by HIV-1 infection and may precipitate the HIV-1 priming phenotype.

While the role of GBPs in immunity is largely unknown, GBP5 is involved in inflammasome assembly and GBP4 modulates interferon regulatory factors [IRFs]. GBPs may enhance HIV-1 replication/persistence or contribute to shielding HIV-1 from detection by the innate immune system.

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IL-17 Confers Protection against Attenuated, but Not Virulent *Francisella tularensis* Infections

Jerod Skyberg[1], MaryClare Rollins[1], Marjorie Sutherland[2], Belisle John[2], David Pascual[1]

[1]Immunology and Infectious Diseases, Montana State University, Bozeman, MT 59718, United States of America; [2]Rocky Mountain Regional Center for Excellence in Bioterrorism and Emerging Infectious Diseases and the Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado 80523, United States of America

Francisella tularensis is a highly infectious intracellular bacterium that causes the zoonotic infection, tularemia. While much literature exists on the host response to *F. tularensis* infection, the vast majority of work has been conducted using attenuated strains of *Francisella*, which do not cause disease in humans. However, emerging data indicate the protective immune response against attenuated versus type A *F. tularensis* differs. Several groups have recently reported IL-17 confers protection against the Live Vaccine Strain (LVS) of *Francisella*. While we, too, have found that IL-17-impaired mice are more susceptible to *F. tularensis* LVS infection, our studies, using a fully virulent type A strain of *F. tularensis*, indicate mice with impaired IL-17 signaling display equivalent weight loss and organ burdens following primary pulmonary challenge as do wild-type mice. In addition, oral LVS vaccination conferred equivalent protection against pulmonary challenge with type A *F. tularensis* in both IL-17-impaired and wild-type mice . To assess the role of IL-17 in a convalescent model of type A *F. tularensis* infection, mice were treated with a suboptimal dose of antibiotics in order to extend the time to mortality. While IFN-γ was found to be critically important for protection in this model, IL-17 neutralization from either wild-type or IFN-γ-/- mice had no effect on susceptibility to infection. Collectively, these results demonstrate IL-17 is dispensable for host immunity to type A *F. tularensis* infection and protective immunity differs between attenuated and virulent strains of *F. tularensis*.

ABSTRACTS

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The Role of Host Components in Regulation of Virulence in *Staphylococcus aureus*

Oliwia W. Zurek[1], Tyler K. Nygaard[1], Robert L. Watkins[1], Kyler B. Pallister[1], Victor J. Torres[2], Alexander R. Horswill[3], Jovanka M. Voyich[1]

[1]Montana State University; [2]New York University School of Medicine; [3]University of Iowa

Staphylococcus aureus (*S. aureus*) is a highly adaptable pathogen that can cause skin abscesses, tissue necrosis, and sepsis. *S. aureus* success in hospitals and community can be partially attributed to its ability to colonize and subsequently infect a wide variety of host tissues. This capacity is dependent on elaborate two-component gene-regulatory systems that tightly control expression of virulence and immunomodulatory factors in response to different stimuli. The *S. aureus* exoprotein expression (SaeR/S) system is one of the sixteen two-component systems that is recognized as a major regulator of *S. aureus* virulence. Despite its important role during infection, it is unclear how this system becomes activated and which of the *saeR/S*-regulated factors are responsible for *saeR/S*-mediated virulence. Using QuantiGene 2.0 assays, we addressed this absence of knowledge by measuring gene expression changes in wild type, Δ*saeR/S*, Δ*saeR/S*+comp and Δ*agr S. aureus* strains in response to human and mouse neutrophils as well as antimicrobial peptides produced by the innate immune system. We found that only some of the *saeR/S* effectors, as opposed to the entire *saeR/S*-regulated virulon, were activated within ten minutes of interacting with human neutrophils and/or cationic peptides. Furthermore, human α-defensin had the most robust effect on *saeR/S* targets, which matched the expression profile of USA300 treated with human neutrophils while β-defensin, dermcidin, LL-37, H2O2 and mouse neutrophils lacking α-defensin had no effect on the levels of gene expression. Together with our published data, these findings show that regulation of the SaeR/S system is highly dependent on the host environment and is essential for *S. aureus* pathogenesis.

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Nox2-Derived Reactive Oxygen Species Are Required for Neutrophil Chemotaxis Signaling and Are Regulated by CLC-3

A. P. Volk, K. Goss, K. Merrill, S. Stolz[1], J. G. Moreland[1] *The University of Iowa, Iowa City, IA, Departments of Pediatrics*

Polymorphonuclear leukocyte (PMN) chemotaxis to invading microbes is a critical host defense mechanism. We have previously demonstrated a requirement for the chloride/proton antiporter, CLC-3, both for shape change during PMN chemotaxis (1) and for the regulation of Nox2 (2). Recent studies indicate that Nox2-derived ROS signals are required to maintain the PMN leading edge during chemotaxis (3) (4). We postulated that the impaired chemotaxis seen in the absence of functional CLC-3 may be secondary to a lack of intracellular ROS signaling. We hypothesized that Nox2-derived ROS are required for PMN chemotaxis signaling and ROS generation is regulated by CLC-3.

To evaluate the relationship between CLC-3 and intracellular ROS signaling during chemotaxis, CLC-3 WT and null PMNs were evaluated using EZ-TAXIScan assays. PMN motility parameters (including chemotactic index (CI) and instantaneous velocity (IV)) were compared, as described (1). CLC-3 null PMNs had impaired chemotaxis to fMLF compared to WT PMNs. Exogenous H2O2 (100μM) treatment rescued CLC-3 PMN directionality and velocity to greater than WT levels (N=5). In view of the rescue of ClC-3 deficient PMNs with H2O2, we next evaluated the role of Nox2-derived ROS in PMN chemotaxis using four model systems: 1&2) human control vs. Nox2 deficient PMNs (genetic (CGD) or diphenylene iodonium (DPI)); 3) murine WT vs. Nox2-deficient PMNs; and 4) differentiated control vs. Nox2-deficient PLB cells. PMN chemotaxis to fMLF was studied using the EZ-TAZIScan assay (5) and the 3-dimensional Dynamic Imaging Analysis System (3D-DIAS) (6). DPI-treated PMNs showed significantly impaired chemotaxis, in response to high (1μM), but not to lower (100nM), fMLF concentrations, (N=6). Chemotaxis to fMLF was significantly diminished in X-CGD (gp91phox null) PLB cells compared to controls (N=4), but was restored to control levels in X-CGD PLB cells transfected to overexpress gp91phox (N=3). Interestingly, CGD patient PMNs showed no difference in directionality but impaired velocity during chemotaxis to fMLF compared to controls (N=4). However, comparing 3-D shape change parameters, CGD PMNs showed significantly increased height, surface area and volume and significantly decreased 3D roundness compared to controls (N= 4 control, 1 p47phox deficient, and 3 gp91phox deficient patients). DPI-treated PMNs had abnormal shape change during chemotaxis, similar to that seen in CGD PMNs (N= 9 control and 7 DPI exp).

Using complimentary assays and cell systems to evaluate the role of Nox2 in PMN chemotaxis, we showed that cells lacking functional Nox2 had impaired chemotaxis in response to fMLF. Furthermore, evaluation of 3D shape change during migration showed that Nox2-deficient cells fail to change shape normally. We have also identified CLC-3 as a chloride/ proton antiporter involved in ROS signaling during PMN chemotaxis, likely through maintenance of Nox2 activation in response to chemoattractants.

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Vitamin D Enhances IL-1ß Secretion and Restricts Growth of *Mycobacterium tuberculosis* in Macrophages from TB Patients

Daniel Eklund[1], Hans Lennart Persson[2], Marie Larsson[3], Amanda Welin[4, 1], Jonna Idh[1], Jakob Paues[5], Sven-Göran Fransson[6], Olle Stendahl[1], Thomas Schön[7, 1], Maria Lerm[1, 8]

[1]Division of Microbiology and Molecular Medicine, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Sweden; [2]Division of Pulmonary Medicine, Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Department of Respiratory Medicine UHL, Centre of Surgery and Oncology, Linköping University Hospital, Sweden; [3]Division of Clinical Microbiology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Sweden; [4]Phagocyte Research Laboratory, Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden; [5]Division of Infectious Diseases, Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Sweden; [6]Division of Radiology, Department of Medical

and Health Sciences, Faculty of Health Sciences, Linköping University, Sweden; [7]Department of Clinical Microbiology and Infectious diseases, Kalmar County Hospital, Sweden; [8]Center for Infectious Medicine, Karolinska Institute, Sweden

The emergence of multidrug resistant strains of *Mycobacterium tuberculosis*, the bacterium responsible for tuberculosis (TB), has rekindled the interest in the role of nutritional supplementation of micronutrients such as vitamin D as adjuvant treatment. Here, we studied the growth of virulent M. tuberculosis in macrophages obtained from the peripheral blood of patients with and without TB. The H37Rv strain genetically modified to express Vibrio harveyi luciferase was used to determine the growth of *M. tuberculosis* by luminometry in the hMDMs from the patients. Determination of cytokine levels was performed using the bead array technique and analysis was performed using flow cytometry. No differences in intracellular growth of Mtb were observed between the different study groups. However, stimulation with vitamin D significantly enhanced the capacity of hMDMs isolated from TB patients to control the infection. This effect was not observed in hMDMs from the other groups. Interleukin (IL)-1β and IL-10 release by hMDMs was increased upon stimulation with vitamin D. We conclude that vitamin D triggers an inflammatory response in human macrophages with enhanced secretion of IL-1β and IL-10, as well as enhancing the capacity of hMDMs from patients with active TB to restrict mycobacterial growth.

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Type 1 Diabetes Associated Disruption of Mucosal Epithelial Cell Innate Immune Responses

Shannon M. Wallet[1, 2], Nadia L. Calderon[1], Tess R. Alonso[1], Dana L. Catalfamo[2], Kathleen G. Neiva[3]

[1]Department of Periodontology, College of Dentistry University of Florida; [2]Department of Oral Biology, University of Florida College of Dentistry; [3]Department of Endodontics, College of Dentistry, University of Florida

Dysfunction of mucosal innate systems impairs mucosal barrier function leading to localized and systemic inflammation and autoimmune processes. Mucosal epithelia play a role in maintaining tissue homeostasis. Toll-like receptors (TLRs) on epithelium have two functions: protection from infection and maintenance of tissue homeostasis, where balance between pro- and anti-inflammatory mediators is necessary. In type 1 diabetes (T1D) alterations in mucosal immunity are implicated in disease pathogenesis. Here we report that TLR2 ligation which induces homeostatic responses is disrupted in mucosal epithelial cells in human T1D. Mucosal epithelial cell monolayers were challenged with TLR2 or TLR4 agonists after which miRNA, protein and cyto/chemokine expression were evaluated. Expression of TGFβ, IL10, TNFα, IL6 and IL8 were evaluated to determine induction of homeostasis vs inflammation using a multiplex assay. Total RNA was probed for miR146a, mir133, miR9 and miR155 using real time PCR (qPCR). The expression and phosphorylation status of TLR signaling intermediates MyD88, TRAF6, IRAK1, and IRAK4 along with the expression of the tyrosine phosphatases PTPN5, PTPN7 and PTPRR were quantified using western blot analysis. In mucosal epithelial cells from diabetes-free participants, TLR2 induced expression of TGFβ and IL10 in the absence of TNFα, IL6 and IL8 concomitant with increased expression of miR146a and miR155 as well as iPTPN5 and PTPRR along with decreased expression and phosphorylation of TRAF6 and IRAK4 when compared to TLR4 induced signaling events. On the contrary, upon TLR2 ligation of mucosal epithelial cells from T1D participants, miR146a, miR155, PPTN5 and PTPRR expression was unaltered when compared to TLR4 ligation. miRNA regulation of TLR2 induced homeostatic responses as well as phosphatase regulation of TLR signaling cascades are altered in human T1D mucosal epithelial cell resulting in over-expression and phosphorylation of multiple TLR signaling intermediates allowing for exacerbated epithelial mediated inflammatory responses. *Research support: ADA 1-08-JF-37 and 7-11-CD-17*

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Plasma Gelsolin Enhances Lung Macrophage Host Defense against Bacterial Pneumonia in Mice

Lester Kobzik[1, 2], Zhiping Yang[1], Terry Chiou[2], Po-Shun Lee[2], Tom Stossel[2]

[1]Harvard School of Public Health, Boston, MA; [2]Brigham and Women's Hospital, Boston, MA

Plasma gelsolin (pGSN) functions as part of the 'extracellular actin scavenging system' but its potential to improve host defense against infection has not been studied. Mice (C57BL/6, male) were pre-treated with recombinant human plasma gelsolin (rhu-pGSN, 400 mg/kg, s.c.) or vehicle 2 h before infection with Strep. pneumoniae (serotype 3, 100K CFU, i.n.) and evaluated 24 h later. rhu-pGSN caused enhanced bacterial clearance (% bacterial left alive in lungs, mean±SD: control, 88 ± 38 vs. rhu-pGSN-treated, 10±5; n > 13 mice/grp) and reduced acute inflammation (% BAL PMNs: control, 30±11 vs. rhu-pGSN-treated, 8±3). Similar results were seen with aerosolized rhu-pGSN. In vitro, rhu-pGSN rapidly improved lung macrophage uptake and killing of bacteria. rhu-pGSN caused an activating phosphorylation (ser1177) of macrophage nitric oxide synthase type III (NOS3), which has important bactericidal functions in the AM. rhu-pGSN failed to enhance bacterial killing in NOS3 -/- macrophages. pGSN binds sphingosine-1-phosphate (S1P), and may enhance its delivery to the S1P1 receptor. Both S1P and the S1P mimic, FTY720P, enhanced macrophage uptake and killing of bacteria, and activated macrophage NOS3 as well. Patients with acute lung injury (e.g. after influenza) are at risk for secondary bacterial pneumonia. We found free actin in lavage fluid from ARDS patients and from mice after influenza or other acute lung injury, suggesting a relative deficiency of pGSN which functions to remove harmful free extracellular actin. Treatment of mice with pGSN on day 7 after influenza (peak of enhanced susceptibility to secondary infection) caused ~15-fold improvement in bacterial clearance after pneumococcal challenge. Plasma gelsolin enhances innate resistance to pneumococcal pneumonia via lung macrophage NOS3, and is a potential immunomodulator for improving lung host defense against primary and secondary bacterial pneumonia. Support: NIH ES00002

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The Role of Simian Varicella Virus ORF 61 during Infection of Rhesus Macaques

Christine Meyer[4], Amelia Kerns[4], Kristen Haberthur[1], Jesse Dewane[4], Joshua Walker[1], Wayne Gray[3], Ilhem Messaoudi[1, 2, 4]
[1]Molecular Microbiology and Immunology Department, Oregon Health & Science University, Portland, OR 97239; [2]Division of Pathobiology and Immunology, Oregon National Primate Research Center, Beaverton, OR 97006; [3]Department of Microbiology and Immunology, University of Arkansas, Little Rock, AK 72205; [4]Vaccine and Gene Therapy Institute, Oregon Health & Science University, Beaverton OR 97006

Varicella zoster virus (VZV) is a neurotropic alphaherpesvirus that causes chickenpox during primary infection and establishes latency in sensory ganglia. Host and viral factors that control the establishment and maintenance of VZV latency are poorly understood. Infection of rhesus macaques (RMs) with the homologous simian varicella virus (SVV) recapitulates hallmarks of VZV infection. SVV open reading frame (ORF) 61 is a viral transactivator and encodes an antisense transcript that is the most frequently detected transcript during SVV latency in RMs. We therefore investigated the role of ORF 61 in the balance between lytic and latent SVV infection, by comparing disease progression, viral replication, immune response and the establishment of latency following infection with a recombinant SVV lacking ORF 61 (SVVΔORF61) to infection with wild type SVV. RMs infected with SVVΔORF61 showed comparable varicella, viral loads and viral replication kinetics observed in WT SVV infected RMs. We also did not detect any differences in the ability of SVVΔORF61 to establish latency in the sensory ganglia; viral loads were similar to wild type. Interestingly we observed a dampened adaptive immune response in RMs infected with SVVΔORF61. We measured decreased B cell and T cell proliferative bursts, reduced SVV-specific IgM and IgG antibody endpoint titers and a lower frequency of SVV-specific T cells. Our data suggests that the reduced adaptive immune response is due to alterations in the recruitment of DC populations, differences in the inflammatory cytokine levels in the plasma as well as in the abundance of SVV transcripts during acute SVVΔORF61 infection.

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HIV-1 Replicates in Multiple Primary Human Macrophage Subpopulations with Distinct Surface Marker Phenotypes

Amanda Brown, Roshni Babu
Johns Hopkins University School of Medicine

Human immunodeficiency virus type I (HIV-1), which infects T-lymphocytes and cells of the monocyte lineage, remains a formidable human pathogen despite the availability of effective antiretroviral drugs that can greatly suppress its replication. However cellular and tissue reservoirs, particularly those rich in monocyte-derived cells, remain a persistent source of HIV proteins and infectious virions. In order to better understand the role of macrophages in HIV persistent and latent infection we developed an in vitro model based on human monocyte-derived macrophages (macs) infected with a GFP-tagged HIV to begin to identify and study in a longitudinal fashion the specific macrophage subpopulations in which the virus replicates.

Monocytes isolated by percoll and ficoll density gradients were cultured in RPMI1640 with 20% FBS and 5% human serum for two days then grown in the same medium without human serum for the remaining culture period. No exogenous cytokines were added. Macrophage cell surface markers were quantified by four-color flow cytometry on three populations: on uninfected macs not exposed to virus, GFP+ macs infected with HIV and bystander GFP- macs in the HIV-exposed cultures. There was an overall trend for CD11b, CD14, CD16 and CD163 being detectable at early time points post-differentiation/infection, while activation markers CD69 and CD86 appeared much later during the 42-50 day culture period. At day 7 post-infection (pi), there was preferential replication of HIV in CD14+ macs. In contrast to uninfected macs, CD14 levels on HIV-infected cells decreased with time suggesting that viral infection downregulates this receptor. Moreover CD14+CD16+ macs represented 45% of HIV-infected cells, but only 29% and 17% of uninfected and bystander macs respectively. At day 7-14 pi, HIV replication was enriched in the CD14+CD11b+ fraction (23-26%) compared to the significantly lower level of this population in uninfected (2-5%) or bystander macs (1-8%). HIV did not alter the pattern of CD11b, CD16, CD69 or CD86 expression on macs. At day 14 pi, CD163+ HIV-infected macs represented nearly 25% of the cells while CD163+ macs remained at 12% in uninfected and 4% in bystander cells. CD163+ thereafter steadily declined after day 21 on all cell populations. The HIV receptor CD4 and co-receptor CCR5 were expressed at very low levels throughout the culture period. Expression of CD18, CD23, CD33, CD36, CD64, CD68, CD105, CD206, CD209, and TLRs were also examined.

In this macrophage culture model, cell populations resembling recently described M2 phenotypes exist in early cultures and persist throughout. In contrast M1-like phenotypic macs appeared much later with time in culture and their development did not appear to be associated with a response to HIV infection. As a significant percentage of macs infected with HIV did not display the described phenotypes, additional distinct subpopulations are also likely important for HIV replication in macrophages.

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TLR Activation of Macrophage Populations during Hypersensitivity Pneumonitis

Kelly Andrews, Ae-Kyung Yi, Elizabeth A. Fitzpatrick
University of Tennessee Health Science Center, Memphis TN 38163

Hypersensitivity Pneumonitis (HP) is an immune-mediated interstitial lung disease that develops following repeated exposure to inhaled environmental antigens. The disease is characterized by alveolitis, granuloma formation and in some patients may progress to a chronic form, which is associated with fibrotic and emphysematous changes in the lung. We used the *Saccharopolyspora rectivirgula* (SR) mouse model of HP to determine whether Toll like receptors (TLR) 2 and 9 cooperate in innate immune cell activation following SR exposure. Stimulation of bone marrow derived macrophages (BMDMs) generated from C57BL/6, MyD88$^{-/-}$ and TLR2/9$^{-/-}$ mice with SR demonstrate that chemokines recruiting neutrophils and macrophages, CXCL1,

CCL2, CCL3 and CCL4, were MyD88-dependent and almost completely dependent on TLRs 2 and 9. Following repeated in vivo exposure to SR, CXCL2 production and neutrophil recruitment were reduced in TLR2$^{-/-}$ and TLR2/9$^{-/-}$ mice suggesting that the neutrophil response was largely dependent on TLR2. RT-PCR revealed a decrease in CCL2 and CCL4 production following SR exposure of TLR2/9$^{-/-}$ mice compared to WT or single knockout (KO) mice. This correlated with a decrease in the percentage of CD11c^{+}/F4/80^{+}/CD205^{+}/SSC^{high} cells that express CD11b compared to WT and single KO mice. TLRs 2 and 9 were required for maximal expression of the co-stimulatory molecule CD86 in these cells conversely; CD40 and MHCII expression appeared to be inhibited by TLR2. Cells that had a phenotype consistent with interstitial macrophages (CD11c^{-}/CD11b^{+}/F4/80^{+}/Gr1^{-}) were dependent on TLRs 2 and 9 for maximal expression of CD80, CD86, MHCII and CD40 following SR exposure. These results suggest that both TLRs 2 and 9 contribute to activation of macrophage populations during HP; however, the response of these two macrophage population differs, which likely reflects their individual roles in disease pathogenesis. This work was supported by the National Institutes of Health (HL084172).

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Mast Cell Activation by Influenza A Virus Depends on Active Infection and Multiple Pattern-Recognition Receptors

Joshua J. Obar, Amy C. Graham, Julianne Zickovich, Kimberly Hilmer

Montana State University, Department of Immunology & Infectious Diseases, Bozeman MT 59718

Influenza A virus (IAV) is a major respiratory pathogen of both humans and animals. The lung is protected from pathogens by alveolar epithelial cells, tissue resident alveolar macrophages, dendritic cells, and mast cells. However, the role of mast cells during respiratory viral infections has been under explored. Mast cell knock-in mice were used to demonstrate their *in vivo* relevance during IAV respiratory infection. Additionally, an *in vitro* co-culture assay was used to dissect the mechanism(s) behind IAV-induced mast cell activation. Both A/WSN/33 and A/PR/8/34 cause significant immunopathology in C57BL/6 mice, but only the pathology induced by A/WSN/33 was mast cell-dependent. A/WSN/33, but not A/PR/8/34, activated mast cells during *in vitro* co-culture. Importantly, a recombinant A/PR/8/34 that expresses the hemagglutinin (HA) glycoprotein from A/WSN/33 could activate mast cells. Moreover, mast cell infection was necessary for their activation. Mast cell production of cytokines, chemokines, and leukotrienes occurs in a RIG-I/MAVS-dependent mechanism, while degranulation occurs through another pattern-recognition receptor. Importantly, human IAV isolates could activate the human mast cell line, HMC-1, demonstrating this novel inflammatory pathway could be activated during human IAV infections. Our data highlight a functional dichotomy of mast cell activation during viral infections. Furthermore, we have identified unique inflammatory cascade activated during IAV infection. Thus, mast cell or their mediator could potentially be targeted to limit morbidity following IAV infection. *This work was funded by NIH grants 5P20GM103500-09 and 5K22AI091647-02.*

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Reduced IRF1 Expression Impairs HIV-1 Transcription without Affecting the Induction of IRF1-Regulated Genes in ex Vivo PBMC

Ruey-Chyi Su[1], Andrew Plesnarski[1], Zhujun Ao[1], Frank A. . Plummer[1, 2], Xiaojian Yao[1], T. Blake Ball[1, 3]

[1]University of Manitoba, Medical Microbiology & Infectious Diseases, Winnipeg, Manitoba Canada; [2]Public Health Agency of Canada, National Microbiology Laboratories, Winnipeg, Canada; [3]Public Health Agency of Canada, National HIV & Retrovirology Laboratories, Winnipeg, Canada

Background: Not all individuals exposed to HIV become infected. In defining the early interactions between host and HIV, our studies and others acquired convincing evidence that altered regulation of a key immune-regulatory factor, interferon regulatory factor-1 (IRF1) is tightly associated with decreased HIV-1 replication. In our well-described HESN (highly HIV-exposed seronegative) cohort in Nairobi, Kenya, we also identified IRF1 polymorphisms that are tightly associated with reduced IRF1 expression and with epidemiological resistance to HIV infection. This study examined unstimulated *ex vivo* PBMC from HIV- susceptible, seronegative individuals to determine whether reducing endogenous IRF1 expression would affect HIV-1 replication, the establishment of infection, and/or IRF1 regulated immune functions. **Methods:** Endogenous IRF1 expression in ex vivo PBMC, CD4^{+} T cells and monocytes were knocked-down with IRF1 specific siRNA. IRF1 expression was assessed using flow-cytometry, prior to infection with HIV-1 virus (Bal, IIIB, or VSVg-HIV, which has a LTR-driven luciferase reporter gene). Transactivation of HIV-1 LTR was assessed by luciferase activity and p24 secretion, using ELISA. The expression of IRF1 target genes was measured using quantitative RT-PCR. **Results:** Surprisingly, reducing IRF1 expression by an average of 46±10 % at the time of infection was sufficient to diminish >90% of luciferase activity and p24 expression in PBMC, CD4^{+} T cells and enriched monocyte population, demonstrating significant impairment in HIV-1 viral gene expression. However, the IRF1's function in regulating host gene expression was not affected. Expression of IL-4, STAT1α, IFN-γ, TNF-α and IL-12Rβ1 following infection was not different between the test and control groups. Transfection procedures had no noticeable impacts on the transcription of IRF1 or LTR-driven viral genes or HIV-1 infection. **Conclusions:** Reduced IRF1 expression during the early acute phase of HIV-1 infection failed to activate the LTR-driven transcription of HIV genome, but had no detectable impacts on host anti-viral gene regulation. These findings suggest that reducing endogenous IRF1 expression may be one of the key contributors to the altered susceptibility to HIV-infection and a potential candidate for devising preventative measures, and that a modest reduction of IRF1 expression will not affect endogenous host immune responses.

ABSTRACTS

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Inflammatory Response of Macrophages to *Saccharomyces cerevisiae* Mutant Bearing Human Compatible Glycoproteins

Yoshiyuki Adachi
Immunology, Tokyo Univ. Pharm. & Life Sci.

The cell wall components of *S. cerevisiae* have been known as immunostimulants for innate immune systems. The yeast cell wall has various carbohydrate components such as beta-glucan and alpha-mannan. Macrophages recognize the beta-1, 3-glucan using dectin-1 to induce the activation signals. Furthermore, there are several candidates of the functional receptors for alpha-mannans such as MMR, SIGNR-1 and dectin-2. Macrophage activation by the gene engineered yeast strain that produces human compatible glycoproteins was examined.

Inflammatory responses of peritoneal exudate macrophages from the wild type, and dectin-1 KO mice were assessed by measuring cytokine and ROS production using ELISA and chemiluminescence, respectively. Wild type *S. cerevisiae* W303-1B and the mutant TIY-20 were used as stimuli.

Macrophages stimulated with TIY-20 produced higher level of cytokine and ROS than W303-1B. Cytokine production by dectin-1 KO mice macrophages were slightly lower than wild type mice as well as ROS production, suggesting beta-1, 3-glucan moiety of the yeast mutant has little effect on the macrophage activation. ROS production by dectin-1 KO mice macrophages stimulated with W303-1B and TIY-20 was diminished by pretreatment with dectin-2 mAb.
These results indicate that the interaction between dectin-1 and β-glucan of *S. cerevisiae* is not strongly associated with the macrophage activation. Modification of N-linked oligosaccharides may change the recognition patterns to accelerate the dectin-2-mediated signaling.

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Decreased Inflammation in Response to sarA-Mediated Biofilm Formation in a Model of CNS Catheter Infection

Jessica Snowden
University of Nebraska Medical Center

CNS catheter infections are a frequent and serious complication in the treatment of hydrocephalus. These infections are commonly caused by Staphylococcus epidermidis and Staphylococcus aureus, both known to form biofilms on the catheter surface. These studies evaluate the hypothesis that the sarA regulatory locus engenders S. aureus more resistant to the CNS immune response based on its ability to regulate robust biofilm formation. These studies utilize ACH1719, a strain of MSSA obtained from a patient with a CNS catheter infection. In vitro studies of a sarA MSSA mutant on this background confirmed that it does not form a biofilm as well as wild type MSSA. Infection was generated using our previously described model of CNS catheter associated infection, similar to the CSF shunt infections seen in humans, with comparison of the bacterial kinetics, cytokine production and inflammatory cell influx between mice infected with wild type versus sarA deficient S. aureus. Silicone catheters were pre-coated with mouse serum and either wild type or sarA deficient MSSA before being stereotactically implanted in the lateral ventricle of C57BL/6 mice. Cultures of the brain tissues and catheters confirmed that a greater number of bacteria adhered to the catheter in the wild type infected animals than sarA mutant infected animals, as expected based on the in vitro inability of the sarA mutant strain to form biofilm. The mice infected with sarA deficient MSSA also had a higher rate of spontaneous catheter clearance by day 14 after infection. The mice infected with the sarA deficient MSSA had greater weight loss, higher mortality rates and increased clinical scores of illness. Pro-inflammatory chemokines and cytokines, such as IL-17, CXCL1, MCP-1, IL-1β, MIP-2 and IL-6, were significantly increased in the sarA mutant versus wild type MSSA, when corrected for bacterial burdens. This increase was most apparent at days 7 and 14 after infection, when biofilm formation is projected to occur in the wild type MSSA infected mice, based on current culture data and previous EM images. Neutrophil and macrophage influx into the infected hemisphere, as determined by flow cytometry, were also increased in the animals infected with the sarA mutant MSSA. Overall, these results support our hypothesis that sarA regulated biofilm formation leads to a decreased inflammatory response to staphylococcal infection in the CNS. Understanding the interactions between the immune system and the biofilms that form on infected CNS catheters will allow us to explore novel management strategies for these infections.

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Pathogenic Mycobacteria Manipulate Innate Immune Responses through the Direct Binding of Lipoarabinomannan to Lactosylceramide-Enriched Microdomains in Human Neutrophils

Hitoshi Nakayama[1, 5], Hidetake Kurihara[2], Yasu S. Morita[3], Taroh Kinoshita[4], Kenji Takamori[5], Hideoki Ogawa[5], Kazuhisa Iwabuchi[5]
[1]Juntendo University School of Health care and Nursing; [2]Department of Anatomy, Juntendo University of Medicine; [3]Department of Microbiology, University of Massachusetts Amherst; [4]Department of Immunoregulation, Research Institute for Microbial Diseases, Osaka University; [5]Institute for Environmental and Gender-specific Medicine, Juntendo University School of Medicine

The recognition of pathogens by phagocytes, such as neutrophils and macrophages is crucial to the innate immunity. Phagocytes express a diverse array of pattern-recognition receptors (PRRs), which sense invading microorganisms. PRRs are able to recognize highly conserved pathogen-associated molecular patterns (PAMPs) expressed on microorganisms. To enter host cells, many pathogens target glycosphingolipids, which cluster together on cell membranes and form glycosphingolipid-enriched microdomains with signaling molecules, suggesting that glycosphingolipid-enriched microdomains are pivotal for host-pathogen interactions. Previous studies have demonstrated that pathogenic mycobacteria make use of microdomains to prevent lysosomal fusion with phagosomes. A number of studies have pointed out that lipoarabinomannan (LAM) expressed on *Mycobacteria* is involved in the prevention of lysosomal fusion with phagosomes. However, little is known about what mechanisms are related to LAM-inducible microdomain-

associated signaling that prevents lysosomal fusion. We recently found that the engulfment of non-opsonized zymosans is dependent on glycosphingolipid lactosylceramide (LacCer)-enriched microdomains in human neutrophils. Here, we investigated the role of LacCer-enriched microdomains in the phagocytosis of *Mycobacteria* in human neutrophils.

First, we examined whether LAM can bind to LacCer. Binding experiments showed that both *M. smegmatis*-derived phospho-myo-inositol-capped LAM (PILAM) and *M. tuberculosis*-derived mannose-capped LAM (ManLAM) bound specifically to LacCer-coated plate. Notably, not only PILAM-coated but also ManLAM-coated polystyrene beads were engulfed by neutrophils, indicating that monomannose chains of LAM, which is a common feature between non-pathogenic and pathogenic mycobacteria, is recognized by LacCer. Previously, Hck has been shown to be involved in lysosomal fusion. Therefore, we also tested whether Hck is associated with LacCer-enriched microdomains on LAM-coated beads-containing phagosomes. Immunoprecipitation experiments showed that Hck is associated with LacCer-enriched microdomains on PILAM-coated beads-containing phagosomes, whereas those two molecules are dissociated with each other on ManLAM-coated beads-containing phagosomes. Taken together, these results indicate that, regardless of whether *Mycobacteria* are pathogenic, their engulfment by human neutrophils is dependent on the direct binding of monomannose residues of LAM to LacCer. On the other hand, the terminal mannose residues of pathogenic mycobacteria-specific ManLAM are likely to be a causing factor to prevent Hck-associated LacCer-dependent phagosome-lysosome fusion. These findings provide the evidence that pathogenic mycobacteria manipulate glycosphingolipid-enriched microdomain-mediated innate immune signaling through LAM binding to glycosphingolipid, such as LacCer, escaping from killing by human neutrophils.

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Zfra Induction of a Non-T Cell-Dependent Immune Response in Blocking Cancer Growth

Nan-Shan Chang
National Chang Kung University

A specific self-polymerizing peptide elicits anticancer response via a novel immune cell lineage. We isolated a 31-amino-acid small protein, named Zfra (**z**inc **f**inger-like protein for **r**egulating **a**poptosis). Synthetic Zfra undergoes self-polymerization in degassed enzyme-free solutions. When nude mice were pre-injected with the full-length Zfra1-31 via tail veins (less than 40 micromoles), these mice resisted the growth of implanted neuroblastoma, glioma, melanoma, and malignant cancer cells from breast, lung, skin, and prostate cells. We have shortened Zfra to various sizes. For example, as short as 7 amino acids (Zfra4-10), Zfra is highly potent in blocking cancer growth and metastasis. Notably, alteration of Ser8 to Gly8 in Zfra resulted in failure of self-polymerization and cancer suppression, suggesting that Ser8 is central to the *in vivo* function of Zfra in preventing and blocking cancer growth. When spleen cells from Zfra-treated nude mice were transferred to naïve nude mice, these mice became resistant to the growth of implanted cancer cells (50-78% suppression), suggesting that non-T "memory" cells are involved in blocking cancer growth. NOD-SCID mice, which are defective in innate T and B cells, failed to generate anticancer response upon challenge with Zfra peptides. However, these mice acquired resistance to cancer upon receiving Zfra-treated spleen cells from nude mice. Elimination of B cells by anti-CD27 antibody suppressed the Zfra effect in cancer prevention. However, CD19 antibody enhanced the Zfra anticancer effect. Notably, antibody against cell surface hyaluronidase Hyal-2 induced cancer prevention in both nude and NOD/SCID mice. Thus, Hyal-2+ memory spleen cells, co-expressing with CD27+ and/or CD19, are responsible for preventing cancer growth.

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Identification of a Gap Junction Communication Pathway Critical in Liver Injury and Sterile Inflammation

Suraj J. Patel[1, 2, 4], Jack M. Milwid[1, 2, 4], Kevin R. King[1], Stefan Bohr[1, 4], Arvin Iracheta-Vellve[1, 4], Martin L. Yarmush[1, 3, 4]
[1]Center for Engineering in Medicine and the Department of Surgery, Massachusetts General Hospital, MA 02114, USA; [2]Harvard-MIT Division of Health Science and Technology, Harvard Medical School, Massachusetts Institute of Technology, Cambridge, MA 02139, USA; [3]Department of Biomedical Engineering, Rutgers University, Piscataway NJ 08854, USA; [4]Shriners Burns Hospital, MA 02114, USA

At the onset of sterile injury, damaged cells stimulate potent inflammatory responses that amplify the overall injury and contribute to organ dysfunction, however little is known about how this process unfolds. An important clinical example of sterile injury is drug-induced liver injury, the most common cause of acute liver failure and a significant public health crisis that limits the development and application of many therapeutic compounds and presents major challenges to clinical medicine. Here we show that drug-induced liver injury is dependent on gap junction communication to amplify sterile inflammatory signals generated in response to the initial toxic injury. We demonstrate that connexin 32 (Cx32), a key hepatic gap junction protein, is an essential mediator of sterile liver injury by showing that mice deficient in Cx32 are protected against liver damage, acute inflammation, and death caused by hepatotoxic drugs. Administration of these drugs resulted in the production of intracellular free radicals that propagated through gap junctions, damaging surrounding cells and expanding the tissue injury front. By high throughput screening, we identified small-molecule inhibitors of Cx32 that protect against liver failure and death in wild-type mice when co-administered with known hepatotoxic drugs. Administration of these selective Cx32 inhibitors significantly limited hepatocyte damage and sterile inflammation, and completely abrogated mortality, confirming the importance of hepatic gap junction communication in establishing sterile injury. These findings demonstrate inhibition of hepatic gap junctions as a viable therapeutic strategy for limiting drug-induced liver injury and potentially other forms of sterile injury.

ABSTRACTS

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In Vitro and in Vivo Interactions of Human Leukocytes and Galectin-3

Martina Sundqvist, Veronica Osla, Johan Bylund, Anna Karlsson
The Phagocyte Research Laboratory, Department of Rheumatology and Inflammation Research, University of Gothenburg, Sweden.

Galectins are β-galactoside binding, soluble lectins of which 15 have been identified in mammals. One of the best characterized members is galectin-3, which was first detected as a cell-surface molecule attached to monocytes, and subsequently shown to be involved in modulating various inflammatory processes e.g., phagocyte derived ROS-production, adhesion, apoptosis and phagocytosis. Increased serum levels of galectin-3 have been demonstrated in certain cancers and a variety of inflammatory conditions. Galectin-3 contains a N-terminal non-lectin part, responsible for oligomer formation, and a carbohydrate-recognition domain (CRD) that enables the specific binding to β-galactosides. In vitro, primed/actived neutrophils can cleave galectin-3 into truncated fragments and CRD. Here we investigate the distribution of full-length and truncated galectin-3 in circulation of healthy donors, and explore the function of these molecules in vitro.

Galectin-3 and CRD attachment to the cell surface of leukocytes was assayed by flow cytometry, using antibodies directed either to the N-terminal part (detecting only full-length galectin-3) or the CRD (detecting both full-length and truncated galectin-3). ELISA and Western blot were used to measure soluble galectin-3 and/or CRD. The activating capacity of full-length galectin-3 and CRD was determined by monitoring phagocyte derived ROS-production with chemiluminescence.

Our data demonstrate that galectin-3 is attached to leukocytes (neutrophils, monocytes and lymphocytes) in circulation and that all leukocyte populations have the ability to bind additional recombinant galectin-3 as well as CRD. Incubation of recombinant galectin-3 with isolated, primed neutrophils, lead to cleavage of the lectin into several fragments including the CRD. Both full-length and truncated galectin-3 was also detected in sera from healthy donors indicating that cleavage may occur in vivo. Full-length galectin-3 induced ROS production in primed neutrophils, whereas CRD-induced ROS-production was negligible.

Both full-length and truncated galectin-3 are present in circulation and the fact that CRD is non-activating raises the question whether CRD has an inhibitory role for galectin-3 function by blocking full-length protein binding.

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Interleukin-13 and the CD14dimCD16^{+} Monocytic Subset Are Associated with Chronic Systolic Heart Failure

Michal A. Rahat[1], Illya Spyvak[1], Idit Lavie[3], Offer Amir[2]
[1]Immunology Research Unit, Carmel Medical Center, and The Faculty of Medicine, Technion, Haifa, 34362, Israel; [2]Heart Failure Center Division of Cardiology, Carmel Medical Center, and The Faculty of Medicine, Technion, Haifa, 34362, Israel; [3]Department of Community Medicine and Epidemiology, Carmel Medical Center, and The Faculty of Medicine, Technion, Haifa, 34362, Israel

Different monocytic subsets are important in inflammation and tissue remodeling, but although heart failure (HF) is associated with local and systemic inflammation, their roles in HF are yet unknown. We searched for cytokine and monocytic biomarkers by identifying changes in specific subsets or cytokine profiles. We recruited 59 chronic systolic HF patients (aged 58±13, 44 males and 14 females), and 29 age- and gender-matched non-cardiac controls. Blood samples were analysed for monocytic subsets and serum cytokine concentrations. Compared to the controls, we found no change in the CD14^{+}CD16^{+} subset, but the CD14^{++}CD16^{-} subset was reduced (73.5±1.8% vs. 84.3±1.9% positive cells, $p<0.0001$) and the CD14^{dim}CD16^{+} subset was expanded (9.2±0.5% vs. 5.8±0.6, $p<0.0001$) in HF patients in comparison to healthy controls, and were associated with advanced NYHA class (≥3, $p<0.01$). Serum TGFβ, IFNγ, and IL-17A did not change significantly, whereas IL-10 was significantly elevated in HF patients but was not associated to any monocytic subset. In contrast, serum levels of TNFα, IL-1β, IL-13 and CRP were significantly elevated and MCP-1/CCL2 was reduced in HF patients, and all were significantly correlated to the CD14^{++}CD16^{-} subset, whereas only CRP ($p=0.22$) and IL-13 ($p=0.0017$) were significantly correlated to the CD14^{dim}CD16^{+} subset. Multivariate analysis suggested that the combination of CRP, MCP-1 and IL-3 could better predict HF than each of the cytokine alone, pointing to this profile as a new biomarker for HF. Furthermore it indicated that CD14^{dim}CD16^{+} monocytes and IL-13 are together associated with HF. The production of IL-13 by CD14^{dim}CD16^{+} was explored via intracellular flow cytometry analysis, and we could show that in HF patients, 18% of the CD14^{dim}CD16^{+} cells were positive for IL-13. Thus, we demonstrate for the first time, that CD14^{dim}CD16^{+} monocytes have a role in HF, which is mediated, at least in part, through IL-13.

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Differential Effect of Nitric Oxide on Transcriptional Regulation of Genes Associated with Alternative Activation in Fibroblasts and Macrophages

Omar A. Saldarriaga, Bruno L. Travi, Peter C. Melby
University of Texas Medical Branch, Galveston, TX

The hamster model of visceral leishmaniasis (VL) caused by Leishmania donovani closely mimics chronic progressive human VL, which is characterized by a profound splenomegaly with an inflammatory infiltrate of heavily parasitized macrophages. *L. donovani* infection induces macrophage STAT6 activation and STAT6 dependent arg1 expression. The infection is also characterized by a low iNOS induction that was related to the lack

of critical promoter sequences in the hamster iNOS promoter that affected basal and inducible activity. To establish the biological significance of the promoter sequences and iNOS expression in hamster cells we transduced baby hamster kidney fibroblasts (BHK) with lentiviral vectors expressing the mouse iNOS open reading frame (ORF) under the control of either the mouse iNOS proximal promoter (msPP/msORF) or the hamster iNOS proximal promoter (htPP/msORF). After stimulation with IFN-γ/LPS, BHK cells stably transduced with the msPP/msORF expressed significantly higher mouse iNOS mRNA, produced more NO, and showed increased ability to control the intracellular parasite *Leishmania donovani* when compared with BHK cells transduced with htPP/ms/ORF or the empty vector. Remarkably, the genetic reconstitution of iNOS in BHK cells led to downregulation of the transcription of hamster arg1 and CCL17 mRNAs, genes that are expressed as part of a macrophage alternative activation phenotype. In addition, an NO donor (GSNO) downregulated the IFN-γ/LPS induced expression of arg1 and CCL17 in BHK cells but not in macrophages. GSNO also reduced the IL-4 induced STAT-6 luciferase reporter activity in BHK cells. IFN-γ alone, which is highly upregulated in spleen during *L.donovani* infection, significantly upregulated the expression of arg1 and CCL17 in BHK cells. IFN-γ also showed a synergistic effect with IL-4 in the upregulation of CCL17 in BHK cells and arg1 and CCL17 in macrophages. We have also determined the arg1 and CCL17 transcriptional start sites and cloned 1.6 and 3.9 kb of the upstream promoter sequences, respectively. *In silico* analysis of these promoter sequences revealed the predicted binding element for STAT6, which is a transcriptional regulator of arg1 and CCL17. Collectively, these data indicate that the generation of NO contributes to the defense against an intracellular pathogen through both direct microbicidal activity and potentially by suppressing the transcription of genes associated with the alternative activation phenotype. The fact that IFN-γ upregulates the expression of these alternative activated genes in the absence of NO opens the possibility of explaining the contribution of this cytokine, which is typically considered to be protective, to the immunopathogenesis of VL.

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Enhanced Cysteinyl Type 1 Receptor Expression in T Cells from House Dust Mite-Allergic Individuals Following Allergen Stimulation

Marek Rola-Pleszczynski, Maryse Thivierge, Sylvie Turcotte, Jana Stankova
Université de Sherbrooke

Cysteinyl-leukotriene receptor type 1 ($CysLT_1$)-mediated signalling plays a major role in allergic rhinitis and asthma, and T cells are involved in the initiation and regulation of allergic inflammation. House dust mites (HDM) are a major source of allergens that contribute to the rising incidence of allergic asthma. In this study, peripheral blood mononuclear cells from HDM-allergic or non-allergic individuals were incubated with Dermatophagoides pteronyssinus allergen Der p 1 and $CysLT_1$ and $CysLT_2$ expression on T cell subsets was analyzed by flow cytometry, and responsiveness to LTD4 was assessed in an intracellular Ca^{++} flux assay. Whereas baseline $CysLT_1$ expression was similar in both groups of donors, Der p 1 significantly enhanced $CysLT_1$ expression in $CD4^+$ and $CD8^+$ T cells of HDM-allergic individuals, but not in non-HDM-allergic individuals, and induced enhanced responsiveness of $CD4^+$ T cells to LTD_4 in terms of calcium mobilisation. This effect was prevented by the $CysLT_1$ antagonists montelukast and MK 571. Der p 1 also significantly induced IL-4 and IL-10 production, and neutralizing Ab to IL-4 effectively prevented both the enhanced $CysLT_1$ expression and the enhanced responsiveness of T cells to LTD_4 induced by Der p 1. In HDM-allergic individuals, Der p 1 also induced T cell proliferation and a Th2-biased phenotype. Moreover, stimulation of non-allergic $CD4^+$ T cells with anti-CD3 Ab or with IL-4 also was able to significantly enhance their expression of $CysLT_1$ and their responsiveness to LTD_4 in terms of calcium flux. Our data suggest that, in allergen-sensitized individual, exposure to allergen can enhance T cell expression of cysteinyl-leukotriene receptors through a mechanism involving IL-4 production. This, in turn, would induce $CD4^+$ T cell responsiveness to cysteinyl-leukotrienes and Th2 cell activation.

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IME722 Attenuates Fibrotic Responses through Inhibition of CTGF and Inflammatory Cytokines

Jiyeon Ahn[1], Mi-Hyoung Kim[1,2], Hee-Jong Woo[2], Jie-Young Song[1]
[1]Korea Institute of Radiological & Medical Sciences (KIRAMS); [2]Seoul National University

Fibrogenesis occurs in various organs, including lung, liver, kidney, eye, skin and heart, due to an excess of biological events involved in aberrant wound healing responses. Although various therapeutic approaches for fibrosis have been investigated, an effective anti-fibrotic treatment has not been established yet. To identify small molecules that inhibit fibrotic responses induced by transforming growth factor-beta (TGF-β), which is one of the major fibrogenic cytokines, we screened a chemical library containing 8, 000 synthetic compounds using a cell-based luciferase assay system. Among those small chemical compounds, IME722 significantly decreased the reporter activity which was enhanced by TGF-β in a dose-dependent manner without cytotoxicity. IME722 remarkably reduced the protein expression of typical fibrotic markers, such as α-smooth muscle actin, collagen and fibronectin, on lung fibroblasts (CCD-18Lu) and hepatic stellate cells (HSC-T6) in the presence of TGF-β. To elucidate the underlying molecular mechanisms of IME722, we determined whether IME722 regulates Smad and non-Smad pathway of TGF-β signal transduction. IME722 did not regulate TGF-β-mediated receptor-regulated Smads (R-Smads; Smad-2 and -3), inhibitory Smad (I-Smad; Samd-7) or MAPK signaling, one of non-Smad pathway. However, IME722 significantly decreased TGF-β-induced connective tissue growth factor (CTGF) and plasminogen activator inhibitor (PAI)-1 expression. In addition, IME722 decreased LPS-induced NF-κB transcriptional activity in HSC-T6 cells which were stably transfected with pNF-κB-Luc reporter gene. RT-PCR analysis demonstrated that IME722 suppresses proinflammatory cytokines, such as IL-1β and IL-6 and inducible nitric oxide synthase (iNOS) in LPS-stimulated macrophage. IME722 treatment significantly reduced injury of alveolar epithelium, infiltration of inflammatory cells, and accumulation of collagens in bleomycin-induced fibrotic

lungs of C57BL6 mice. These results suggest that IME722 could be a novel promising candidate for the treatment of lung and liver fibrosis via inhibition of CTGF and inflammatory cytokines.

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Hyperglycemia-Induced Autoinflammation in Retinal Glia Cells

Derrick J. Feenstra, Prathiba Jayaguru, Susanne Mohr
Michigan State University, Department of Physiology, East Lansing, MI, USA

Purpose: Chronic inflammation is the underlying cause for many of today's most prevalent diseases such as atherosclerosis and diabetes. Recently, the concept of autoinflammation has been introduced to better understand mechanisms associated with chronic tissue inflammation. Autoinflammation is characterized as a predominantly interleukin-1β (IL-1β) driven, continuous feedback mechanism that is triggered and maintained in the absence of bacterial infection or T-cell activation. In diabetic retinopathy, a major complication of diabetes, pro-inflammatory cytokines are chronically elevated in the retina and IL-1β seems to be crucial for disease development. Blocking IL-1β signaling by knock-out of the IL-1 receptor inhibits disease progression suggesting that diabetic retinopathy could be considered an autoinflammatory disease. Glia cells are the major source for IL-1β production and the potential origin of autoinflammation in the retina under hyperglycemic conditions. Therefore, the focus of this study is to understand mechanisms leading to continuous IL-1β production by glia cells and to identify potential autoinflammatory feedback mechanisms in the retinal glia cells and tissue caused by hyperglycemia.
Methods: Activation patterns of Caspase-1, the enzyme that produces active IL-1β, were determined from retinas of normal and diabetic wild type (C57BL/6) and IL-1β receptor knock-out (IL-1R1-/-) mice using a Caspase-1 specific fluorescence substrate (YVAD-AFC). Retinal Müller cells, the principle glia cells of the retina, were treated with 5mM glucose, 25mM glucose, or 25mM glucose plus IL-1 receptor antagonist (IL-1ra, 50-100ng/ml) medium for up to 96 hours. Caspase-1 activity and oxidative stress was measured. Protein levels for IL-1β were determined by ELISA.
Results: Hyperglycemia induced a multi-phasic pattern of Caspase-1 activation and subsequent IL-1β production in retinal tissue and Müller cells compared to controls. The IL-1ra significantly decreased late phase Caspase-1 activity by 30.48±10.61% and formation of hyperglycemia-induced oxidative stress by 81.30±7.53% compared to high glucose alone in vitro. In vivo, knock-out of the IL-1 receptor prevented late phase activation of Caspase-1 in the diabetic retina. IL-1β induced caspase-1 activity by 53.72±5.00% above control. Removal of hyperglycemic stimulus did not prevent late phase caspase-1 activation.
Conclusions: Hyperglycemia induces an autoinflammatory feedback cycle within retinal glia cells and tissue. This feedback cycle is maintained by the continuous activation of the Caspase-1 and subsequent production of IL-1β acting via the IL-1 receptor. Once activated this autoinflammatory feedback cycle becomes independent of the original high glucose stimulus and is self-sustained.

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Inflammasome Complex Components, ASC and Caspase-1, Mediate Alcoholic Steatohepatitis via Interleukin-1 in Mice

Jan Petrasek, Shashi Bala, Dora Lippai, Karen Kodys, Evelyn A. Kurt-Jones, Gyongyi Szabo
University of Massachusetts Medical School

Background: Steatosis and increase in inflammatory cytokines, including interleukin (IL)-1β, are key components of alcoholic liver disease (ALD). NOD-like receptors, including NLRP1 and NLRP3, are intracellular sensors of danger signals that through the adaptor molecule, ASC, activate the multiprotein inflammasome complex. Inflammasome activation leads to caspase-1 (Casp1) activation and subsequent cleavage of pro-IL-1β. The role of inflammasome in ALD is unknown.
Aim: To investigate the role of inflammasome activation and the contribution of ASC and Casp-1 in ALD.
Methods: Wild-type (WT), IL-1 receptor (IL-1R1)-, ASC- or Casp1 - deficient mice were fed with Lieber-DeCarli ethanol or control diet for 4 weeks. Some mice received recombinant IL-1R1 antagonist (IL-1Ra), Anakinra.
Results: Alcohol feeding to WT mice resulted in liver injury (ALT), steatosis and upregulation of inflammatory cytokines and multiple inflammasome components, including the ligand sensors NLRP1, NLRP3, NOD2, NLRX1, the adaptor ASC, and the effector protein pro-Casp1. Consistent with inflammasome activation, there was increased cleavage of Casp1 in the livers of alcohol-fed mice as indicated by increased Casp1 p10, which corresponded with increased active IL-1β levels. We found that alcohol-fed, Casp1-deficient mice failed to upregulate IL-1β and showed significantly lower levels of serum ALT, inflammatory cytokines and liver triglycerides compared to WT mice. To evaluate the role of upstream components of the inflammasome, we tested ASC-deficient mice and found protection from alcohol-induced liver damage, steatosis and inflammation. These findings confirmed our hypothesis that inflammasome activation was crucial in the development of ALD. Activation of Casp1 is required for maturation of IL-1β. Alcohol feeding in IL-1R1-deficient mice revealed significantly reduced liver injury, inflammation and steatosis compared to WT mice suggesting that the effect of inflammasome is mediated via the IL-1/IL-1R1 pathway in ALD. Using IL-1R1-KO chimeric mice, we determined that the IL-1R signaling in liver parenchymal cells contributed to steatosis while IL-1R1 on myeloid cells augmented inflammatory response. The effect of IL-1 is inhibited by the naturally occurring IL-1Ra. Treatment of mice with recombinant IL-1Ra ameliorated alcohol-induced liver steatosis, inflammation and injury.
Conclusions: Our novel data show that inflammasome activation, including the ASC adaptor and Casp-1, is a key component of ALD and that IL-1 signaling is required for the development of alcoholic steatohepatitis. We show for the first time that IL-1R has distinct roles in parenchymal and myeloid cells in the liver. Our results demonstrate that pharmacological inhibition of IL-1R1 using IL-1Ra ameliorates ALD. (Supported by NIAAA1RO1AA017729)

ABSTRACTS

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Lack of TNF-Rp55 Impairs Thrombus Resolution through Reduced Expression of MMPs and uPA

Yuko Ishida[1], Mizuo Nosaka[1], Akihiko Kimura[1], Mariko Kawaguchi[1], Yumi Kuninaka[1], Naofumi Mukaida[2], Toshikazu Kondo[1]

[1]Department of Forensic Medicine, Wakayama Medical University; [2]Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University

Deep vein thrombosis (DVT) is multifactorial and often results from a combination of risk factors such as genetic conditions, obesity, drugs, pregnancy, aging, trauma, and malignancy. DVT is frequently complicated with severe morbidity, leading sometimes to mortality. We examined the pathophysiological roles of TNFalpha-TNF-Rp55 axis in the resolution of DVT by the use of TNF-Rp55 KO mice. Upon the ligation of the inferior vena cava (IVC) of WT mice, venous thrombi formed and grew progressively until 5 days, and the thrombus weight decreased less than 50% at 14 days. Concomitantly, intrathrombotic gene expression of TNFalpha and TNF-Rp55 were elevated as post-ligation intervals were extended. When TNFalpha-Rp55 KO mice were treated in the same manner, thrombus size was larger later than 5 days, compared with WT mice. Intrathrombotic collagen-positive areas were more evident later than 10 days after IVC ligation in TNF-Rp55 KO mice, compared with WT mice. Moreover, the blood flow of thrombosed IVC was more recovered in WT than in TNF-Rp55 KO mice. Furthermore, intrathrombotic uPA, MMP-9 and MMP-2 mRNA expression was significantly reduced in TNF-Rp55 KO mice than WT ones. Supportingly, the administration of anti-TNFalpha mAb delayed the thrombus resolution in WT mice. Furthermore, TNFalpha treatment enhanced gene expression of uPA, MMP-9 and MMP-2 in WT-derived macrophages but not TNF-Rp55 KO-derived ones. Collectively, TNFalpha-TNF-Rp55 axis can be a good molecular target for the DVT treatment.

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Co-activation of CXC Chemokine Receptor 4 with Ubiquitin and Stromal Cell-Derived Factor 1 Alpha Results in Synergistic Effects on Selective Cell Signals in THP-1 Cells

Jeffrey D. Davis, Matthias Majetschak

Loyola University Chicago

We have shown previously that extracellular ubiquitin functions as an endogenous immune modulator with anti-inflammatory properties and that treatment with exogenous ubiquitin modulates inflammation and confers organ protection in a variety of disease models. Subsequently, we identified extracellular ubiquitin as an endogenous agonist of CXC chemokine receptor (CXCR) 4 and provided evidence that the binding site for ubiquitin on CXCR4 is distinct from the CXCR4 binding site of the cognate ligand stromal-cell derived factor (SDF)-1α. As both, SDF-1α and ubiquitin, are present in the systemic circulation and at local sites, simultaneous activation of CXCR4 with SDF-1α and ubiquitin appears to be the physiological relevant activation mechanism. However, the cellular signaling events after co-activation of CXCR4 with both natural ligands are unknown. Thus, we studied signaling events after separate and simultaneous activation of CXCR4 with ubiquitin and SDF-1α in THP-1 cells, utilizing intracellular Ca^{2+} fluxes and cellular cyclic adenosine monophosphate (cAMP) levels as read-outs. Intracellular calcium was measured using the Fluo-4 NW calcium assay. Fluorescence signals were measured continuously before and after spiking cells with the CXCR4 agonists. Cyclic AMP levels were quantified in forskolin stimulated THP-1 cells after 15 min of incubation with the CXCR4 agonists using a cAMP complete enzyme immunoassay.

When tested at ligand concentrations of 100 pM, 1 nM and 10 nM, co-stimulation of THP-1 cells with equal amounts of ubiquitin and SDF-1α resulted in synergistic effects on Ca^{2+} fluxes, as compared with stimulation with each agonist alone (area under curve: ubiquitin – 196±50, SDF-1α: 330±165; both: 991±320). Pretreatment with both ligands desensitized ligand induced Ca^{2+} fluxes in homologous stimulation experiments at the highest ligand concentration (10 nM), but did not affect Ca^{2+} fluxes in heterologous stimulation experiments. Co-stimulation of THP-1 cells with both ligands resulted in additive effects on cellular cAMP levels.

These data suggest that simultaneous activation of CXCR4 with its natural ligands results in synergistic effects on selective signaling pathways. Thus, the ratio of the biologically relevant ubiquitin/SDF-1α concentration appears to modulate CXCR4 mediated signaling events. Furthermore, sequential activation with both ligands via their specific binding sites on the receptor may enable sustained CXCR4 signaling and activation of CXCR4 mediated effects on cell function.

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Immunophenotyping and Clinical Relevance of Myeloid-Derived Suppressor Cells in Cancer Patients

Sven Brandau[1], Sokratis Trellakis[1], Kirsten Bruderek[1], Claudia A. Dumitru[1], Lisa Zimmer[2], Stephan Lang[1]

[1]Department of Otorhinolaryngology, University Hospital Essen, Essen, Germany; [2]Department of Dermatology, University Hospital Essen, Essen, Germany

Myeloid-derived suppressor cells (MDSC) are a heterogeneous subset of immunosuppressive myeloid cells originally described in tumor–bearing mice. Characterization, function and clinical relevance of human MDSC is in it´s infancy and requires further definition. We developed an immunophenotyping panel of six markers, which allowed a clear discrimination of granulocytic MDSC (G-MDSC) from monocytic MDSC (M-MDSC) and immature myeloid cells.

Many human clinical studies rely on cryopreserved blood samples. Thus, we next tested the influence of freezing/thawing procedures on immunophenotyping and enumeration of human MDSC subsets. G-MDSC and M-MDSC subsets were sensitive to cryopreservation with immature CD16-negative G-MDSC showing the highest sensitivity. Analysis of patients with head and neck squamous cell carcinoma (HNSCC) (n=34) and melanoma (n=19) revealed that M-MDSC were more frequent than G-MDSC with G-MDSC being extremely rare in melanoma. Frequency of G-MDSC in HNSCC correlated with primary tumor size but not with N-status.

To test which factors may be involved in expansion of G-MDSC, we analyzed correlations between G-MDSC frequencies, clinical

parameters and granulocyte-related factors in the peripheral blood of HNSCC patients. We found that G-MDSC frequencies associated with the level of CCL4 and CXCL8, but not with colony-stimulating factors, IL-6 and various other cytokines.

Our results indicate that the frequency of human MDSCs varies between tumor types and that their enumeration requires the immediate analysis of fresh blood samples. Increased G-MDSC frequencies correlate with advanced disease and increased concentrations of neutrophil effector chemokines, but unexpectedly not with growth factors or IL-6. These data suggest that accumulation of G-MDSC in HNSCC patients is not driven by increased blood concentrations of classical colony-stimulating factors.

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Cationic Innate Defence Regulator Peptides as Immune-Modulatory Therapeutics for Inflammatory Arthritis

Leola N.Y. Chow, Ka-Yee G. Choi, Neeloffer Mookherjee

Manitoba Centre for Proteomics and Systems Biology, Departments of Internal Medicine and Immunology, University of Manitoba, Winnipeg, Canada.

Breakdown in the regulation of inflammation leads to various chronic inflammatory diseases such as rheumatoid arthritis (RA), inflammatory bowel disease and certain cancers. A major consideration for many therapies for these diseases is compromised immune function resulting in increased risk of infections and neoplasms. This highlights the importance of developing alternate therapeutic strategies for chronic inflammatory diseases without compromising host immune function. It was previously demonstrated in both *in-vitro* and *in-vivo* studies that synthetic derivatives of cationic host defence (antimicrobial) peptides, known as innate defence regulator (IDR) peptides, can selectively suppress pathogen-induced inflammatory responses, while maintaining immune responses required for resolution of infections. The objective of this study was to investigate the potential of IDR-peptides in selectively suppressing immune-mediated 'sterile' inflammation using RA as a disease model.

We have demonstrated that IDR-peptides derived from cathelicidin host defence (antimicrobial) peptides, can significantly suppress inflammatory cytokines such as TNF-α, IL-1β and IL-6, as well as enzymes such as MMP-3 in synovial fibroblasts (a critical cell type in inflammatory arthritis), under conditions that mediates cartilage and bone destruction in arthritis. In contrast, these peptides enhanced anti-inflammatory proteins such as IL-1RA, while differentially modulating chemokine responses that are required for resolution of infections. We explored the molecular mechanism of regulation of immune-mediated inflammation by IDR-peptides in synovial fibroblasts, by employing quantitative proteomics. Computational interrogation of mammalian immunity-related proteins demonstrated that several members of the inflammatory NF-κB and MAPK pathways were altered by our lead IDR-peptide. We confirmed the identified protein targets and pathways using various immunochemical assays, and demonstrated that the IDR-peptides interfered with the activation of NF-κB/Rel family of transcription factors and signaling pathways e.g. MAPK ERK1/2 and JNK, in synovial fibroblasts isolated from patients with RA and osteoarthritis *ex-vivo*. We further examined the impact of these IDR-peptides in a murine model of collagen-induced arthritis, and have established the ability of IDR-peptides to prevent inflammatory arthritis *in*-vivo. Overall, our results suggest that cationic IDR-peptides may be valuable in controlling the destructive effects of inflammatory arthritis without abrogating immune function required for resolution of infections. Our study provides a rationale for further examining the use of IDR-peptides as potential therapeutics for the management of inflammatory arthritis and possibly other diseases characterized by chronic inflammation.

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Circulating Neutrophils from Children with Inflammatory Bowel Disease Display a Primed Phenotype

Jessica G. Moreland[1], Emily A. Gross[1], Brieanna M. Hilkin[1], Riad Rahhal[2], Melissa J. Jensen[2]

[1]Department of Pediatrics and the Inflammation Program/ University of Iowa; [2]Department of Pediatrics/University of Iowa

Inflammatory bowel disease (IBD), including Crohn's disease and Ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract which cause significant morbidity in both adults and children. The chronic inflammation of IBD can include systemic manifestations, with neutrophils (PMN) and the circulating cytokine TNF-α as potential mediators in this process. Although PMN are unequivocally required for microbial killing, they can also cause host tissue damage secondary to inappropriate activation. PMN priming is an intermediate state of activation whereby exposure to a priming stimulus enhances responsiveness to subsequent stimuli. Priming is elicited by both host and bacterial products, including TNF-α, and has recently been demonstrated to occur in chronic inflammatory diseases in vivo. We hypothesized that pediatric patients with IBD would have primed PMN in the circulation in comparison to age-matched controls without systemic inflammatory disease. Furthermore, we postulated that the extent of disease activity would correlate with the degree of priming. After IRB approval and informed consent, PMN were isolated from pediatric IBD patients and age-matched controls. Clinical disease activity indices and medication information were also collected. PMN from Crohn's disease patients displayed significantly enhanced fMLF-elicited NADPH oxidase activity in comparison to PMN from age-matched controls, and PMN from patients with both Crohn's and UC had enhanced TNF-α priming of NADPH oxidase activity as measured by chemiluminescence. Cell surface expression of the active form of CD11b was significantly enhanced in resting PMN from UC patients. As an additional measure of cell activation, elastase release in response to TNF-α alone, or TNF-α followed by PMA was measured using a fluorometric activity assay. PMN from IBD patients demonstrated 1.7-fold ($p=0.02$, N=6 control, 25 IBD patients) increased elastase release by activity assay following stimulation with low concentration of TNF-α (1ng/ml x 30 min.), and a 1.6-fold increase in PMA-induced elastase release ($p= 0.01$). In summary, circulating PMN from pediatric patients

with IBD display multiple phenotypic alterations consistent with a primed phenotype under resting conditions. These phenotypic changes include enhanced NADPH oxidase activity in response to fMLF only or TNF-α followed by fMLF, altered cell surface protein expression, and priming of elastase release from azurophilic granules in response to TNF-α or TNF-α followed by PMA. We conclude that IBD elicits ongoing changes in the circulating neutrophil phenotype, and we speculate that PMN priming during IBD may contribute to host tissue damage and patient morbidity.

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Acute Changes in T Lymphocyte Subsets in Peyer's Patches Following Combined Radiation Injury in a Murine Model

Stewart R. Carter[1, 2], Aleah L. Brubaker[1, 2], Jessica L. Palmer[1, 2], Richard L. Gamelli[1, 2], Elizabeth J. Kovacs[1, 2, 3]

[1]Burn and Shock Trauma Institute, Loyola University Chicago, Stritch School of Medicine, Maywood, IL, 60153; [2]Department of Surgery, Loyola University Chicago, Stritch School of Medicine, Maywood, IL, 60153; [3]Department of Microbiology and Immunology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL, 60153

Nuclear disaster victims often experience combined radiation injury (CRI) with trauma or burns. Mortality observed in CRI is associated with gastrointestinal involvement, with symptoms of severe diarrhea, anorexia, dehydration, electrolyte abnormalities and bacterial translocation with resultant sepsis. Gut-associated lymphoid tissue (GALT) is the largest immune organ in the body, therefore, gut immune suppression may contribute to mortality in CRI. Mice received whole body irradiation (5-5.5 Gy) alone or with 15% total body surface area (TBSA) scald burn and systemic and intestinal responses wereexamined at 24, 48 and 72 hours post injury. Complete blood count (CBC) and analysis of serum TNF-α and IL-6 by ELISA were performed. Peyer's patches were isolated and examined by flow cytometry. Whole blood lymphocyte counts were 50% lower in irradiated groups relative to sham and burn injury alone at all time points examined ($P<0.05$). Radiation affected Peyer's patches similarly. Proportions of CD4+ lymphocytes in radiation alone and CRI were approximately half that of non-radiation groups in Peyer'spatches at similar time points ($P<0.05$). Percentages of CD8+ lymphocytes in Peyer's patches were 2.5-fold higher in radiation alone and 1.8-fold in CRI relative to sham at 24hrs ($P<0.05$). Proportions of Peyer's patch CD8+ lymphocytes in CRI remained elevated at 48 and 72hrs and were 2 and 4 times greater respectively relative to sham ($P<0.05$). Myeloid-derived suppressor cells (MDSCs) isolated from Peyer's patches were 4 to 6-fold higher in injury groups at 24hrs ($P<0.05$) and remained elevated at 48hrs in radiation treated groups ($P<0.05$). Serum TNF-α was only detectable in the CRI group with a 20-fold increase relative to all groups at 48hrs ($P<0.05$). Circulating IL-6 was elevated greater than 12-fold in burn alone mice at 24hrs and in the CRI group at both 24 and 48hr time points as compared to sham animals. CRI appears to have the strongest effect on lymphocyte subset changes in injured animals. Further investigation is needed to determine T-cell viability and if increased MDSCs and pro-inflammatory cytokines result in altered lymphocyte function. Changes in Peyer's patch lymphocyte number and function may contribute to gut immune dysfunction and elevated morbidity and mortality following CRI.

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Enhanced Chemokine Expression in Mononuclear Phagocytes Undergoing Endoplasmic Reticulum Stress Response Exhibits TLR Specificity

Chenyang Zhao, Shyamasree Datta, Paul Pavicic, Jr., Michael Novotny, Thomas Hamilton

Dept. of Immunology, Cleveland Clinic

Inflammatory response to injury or infectious challenge is now recognized to be sensitive to the activation of endoplasmic reticulum (ER) stress pathways. Importantly, cell stress pathways have been observed to intersect with and selectively modify the magnitude of inflammatory cytokine production in myeloid cell populations. The mechanistic bases for such changes, however, remain poorly understood. In order to further define the molecular interface between the two processes, we have examined patterns of chemokine gene expression and intracellular signaling in mouse macrophages stimulated with various ligands for Toll-Like Receptors while undergoing ER stress. Though coincidental initiation of cellular stress and TLR signaling had little or no effect, both the magnitude and duration of TLR-stimulated CXCL1 expression were markedly enhanced in macrophages where a stress response was fully engaged prior to exposure to TLR ligand. Though chemokine mRNAs are known to be controlled by mRNA half-life, the enhanced expression observed in stressed cells was mediated through alterations in gene transcription. Furthermore, the effects of cell stress exhibited strong TLR selectivity. In macrophages treated with tunicamycin, an inhibitor of N-linked glycosylation and potent activator of ER stress, chemokine expression was strongly enhanced in bone marrow derived macrophages stimulated with ligands for TLR3 and TLR4 but not TLR2. Furthermore, macrophages from mice deficient in TRIF had little or no sensitivity to ER stress, indicating that the TLR specificity reflects the involvement of this TLR signaling adaptor. In RAW264.7 macrophages, depletion of RIP1, a kinase selectively associated with the TRIF pathway, abrogated the stress-mediated enhancement of cytokine expression but did not alter response to the TLR ligand alone suggesting that this kinase is a primary mechanistic target of cell stress. Consistent with this, stress altered the temporal pattern for TLR3/4-mediated activation of NFkB but not IRF3. The findings define a novel interaction mechanism that shows dramatic impact on the magnitude and duration of selective TLR-induced chemokine and cytokine gene expression and further establishes the importance of the cell stress pathways in coordinating the outcomes of cellular and tissue injury.

ABSTRACTS

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PHLDA1 Is a Negative Regulator for TLR2-Mediated Foam Cell Formation

Dae-Weon Park[1], Seung-Eun Yeo[1], Eui-Soon Park[2], Jaerang Rho[2], Yoe-Sik Bae[3], Suk-Hwan Baek[1]

[1]Department of Biochemistry & Molecular Biology, College of Medicine, Yeungnam University, Daegu, 705-717, Korea; [2]Department of Microbiology and Molecular Biology, Chungnam National University, Daejeon 305-764, Korea; [3]Department of Biological Sciences, Sungkyunkwan University, Suwon, 440-746, Korea

Toll like receptor 2 (TLR2)-mediated signaling cascades and gene regulation are mainly involved in the various diseases related to inflammation. To identify molecules involved in the progression of TLR2 signaling, the expression pattern of genes in bone marrow-derived macrophages (BMDMs) and Raw264.7 cells by TLR2 agonist was compared by gene chip array. We found that PHLDA1 is a novel gene up-regulated by TLR2 activation and is determined by the detail mechanisms for PHLDA1 expression in macrophages. Treatment with Pam3CSK4 increased PHLDA1 mRNA and protein expression levels depending on TLR2. PHLDA1 expression by Pam3CSK4 is inhibited by JAK2 siRNA and AG490, proving it JAK2-dependent. Pam3CSK4 stimulated STAT3 phosphorylation, and STAT3 knock-down by siRNA diminished PHLDA1 expression by Pam3CSK4. JAK inhibitor attenuated ERK1/2 phosphorylation by Pam3CSK4, while U0126 inhibited STAT3 phosphorylation and PHLDA1 expressions. These results suggested that ERK1/2 regulates PHLDA1 expression according to JAK2 activation. We also found that PHLDA1 is a novel regulator in TLR2-mediated foam cell formation. Gain- and loss-of-function experiments showed that PHLDA1 modulated foam cell formation by activating TLR2. These findings suggest that JAK2-ERK1/2-STAT3 pathway is a key regulator of PHLDA1 expression in macrophages and may play a vital role eliciting TLR2-mediated foam cell formation.

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Inhibition of Inflammasome Activation by Ligation of Fcγ Receptors

John R. Janczy[1, 2], Ceren Ciraci[2, 3], Sophie Joly[2, 3], Matthew Drescher[2], Suzanna L. Cassel[2, 3], Fayyaz S. Sutterwala[1, 2, 3]

[1]Graduate Program in Immunology, University of Iowa, Iowa City, IA; [2]Inflammation Program, University of Iowa, Iowa City, IA; [3]Department of Internal Medicine, University of Iowa, Iowa City, IA

Activation of the immunoglobulin G (IgG) Fc receptors (FcγR) by antigen-antibody immune complexes is known to elicit broad effects on both innate and adaptive immune systems. It has been shown previously that FcγR ligation by IgG immune complexes modulates cytokine production from antigen presenting cells; specifically enhancing IL-10 production while simultaneously suppressing IL-12p40 production. However, the effect of FcγR ligation upon the production of the proinflammatory cytokine IL-1β has not been investigated. Processing and secretion of IL-1β requires activation of the cysteine protease caspase-1 by a multiprotein complex called the inflammasome. Here we demonstrate that treatment of macrophages with immune complexes effectively inhibits Nlrp3 inflammasome activation and the subsequent processing and secretion of IL-1β. This suppression was not due to suppressed synthesis of inflammasome components or pro-IL-1β. Rather immune complex treatment prevented inflammasome assembly and subsequent caspase-1 activation. These data suggest that the generation of antigen specific adaptive immune responses may have a negative regulatory role in suppressing early innate inflammatory responses

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Mitochondrial Cardiolipin Regulates Nlrp3 Inflammasome Activation

Fayyaz S. Sutterwala[1, 2], Qiong He[2], Shankar S. Iyer[1], Alicia K . Olivier[3], Stephanie C. Eisenbarth[4], William M. Nauseef[1, 2], Suzanne L. Cassel[1]

[1]Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA ; [2]Graduate Program in Immunology, University of Iowa Carver College of Medicine, Iowa City, IA ; [3]Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA ; [4]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT

Nlrp3 inflammasome activation has been shown to occur in response to numerous agonists but the specific mechanism by which this takes place remains unclear. All previously evaluated activators of the Nlrp3 inflammasome have been shown to induce the generation of mitochondrial reactive oxygen species (ROS), suggesting a model in which ROS is a required upstream mediator of Nlrp3 inflammasome activation. We have identified a novel Nlrp3 agonist, the oxazolidinone antibiotic linezolid, which activates the Nlrp3 inflammasome independently of ROS. Importantly, the pathways for ROS-dependent and ROS-independent Nlrp3 activation converge upon mitochondrial dysfunction and specifically the mitochondrial lipid cardiolipin. We demonstrate that cardiolipin binds directly to Nlrp3 and that interference with cardiolipin synthesis specifically inhibits Nlrp3 inflammasome activation. Together these data suggest that mitochondria play a critical role in the activation of the Nlrp3 inflammasome through the direct binding of Nlrp3 to cardiolipin.

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PLA2 Inhibitors Abrogate Intracellular Processing of NADPH-Oxidase Derived Radicals – A Role for Arachidonic Acid in Granule-Granule Fusion in Human Neutrophils?

Halla Björnsdottir, Johan Bylund, Anna Karlsson

The Phagocyte Research Laboratory, Department of Rheumatology & Inflammation Research, University of Gothenburg, Sweden

Background: Neutrophils are capable of producing superoxide at two distinct sites in the cell, at the cell membrane (extracellular) and within intracellular organelles, presumably granules (intracellular). Superoxide is then further processed to a variety of reactive oxygen species (ROS) by interaction with e.g. myeloperoxidase (MPO). Different agonists give rise to either intra- or extracellular ROS production, or both. Arachidonic acid, released from cellular membranes by phospholipase A2 (PLA2), has been implicated as an important factor in the assembly and/or activation of the

NADPH oxidase. The aim of this study was to investigate the effect of PLA2 on intra- and extracellular ROS production in intact human neutrophils.

Methods: ROS production was measured with the luminol/isoluminol-enhanced chemiluminescence (CL) technique where intracellular measurements are dependent on MPO, as well as with the MPO-independent p-hydroxyphenylacetate (PHPA) method. The involvement of PLA2 in extra- and intracellular ROS production was investigated using PLA2 inhibitors with suggested specificities for different PLA2 isozymes.

Results: Three different PLA2 inhibitors abrogated intracellular O2- production without affecting extracellular O2- production when measured by the peroxidase-dependent CL technique. The effect was neither due to inhibition of the cellular peroxidase (MPO), nor to inhibition of the intracellular NADPH-oxidase per se as measured by the MPO-independent PHPA method. This suggests that these PLA2 inhibitors prevent the detection of ROS by abrogating the contact of superoxide with MPO that is necessary for detection by the CL technique.

Conclusions: We suggest that inhibition of PLA2 may prevent intracellular processing of ROS by inhibiting fusion of MPO-containing azurophil granules with NADPH oxidase-containing specific/gelatinase granules.

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Src Kinase Is Associated with Immune Complex–Mediated Disruption of IFN-γ Activation of Monocytes

Anna McGrath, Gunther Boekhoudt, Gerald Feldman
U.S. Food and Drug Administration, Division of Monoclonal Antibodies, Office of Biotechnology Products, Office of Pharmaceutical Science, Center for Drug Evaluation and Research

We have previously shown that immune complexes (ICs) inhibit IFN-γ induced activation of monocytes by interfering with the JAK/STAT signaling pathway. This effect appears to be due in part to the SRC kinase family. However, the SRC family consists of nine members, and the one responsible for IC inhibition of monocyte activation has yet to be identified. In this study, human monocytes were transfected with various SRC family siRNA in order to determine which SRC kinase is involved. Using phosphoflow analysis, we observed that high levels of pSTAT were induced upon treatment with IFN-γ, and that a significant reduction of pSTAT occurred in cells pre-treated with ICs. However, pSTAT levels were not reduced in cells that had been transfected with Src siRNA. Western blot analysis confirmed that the Src siRNA did knock down Src protein levels, which correlated with the phosphoflow data. RT-PCR analysis revealed that levels of IFN inducible protein 10 (IP-10) were also rescued in Src siRNA transfected cells compared to non-transfected cells. In contrast, the inhibitory effects of ICs were not reversed when cells were transfected with siRNA targeting the SRC Family members Fyn, Lyn, Lck, or Hck. Together these findings suggest that Src is the main SRC kinase involved in IC-mediated inhibition of IFN-γ induced activation of monocytes.

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CHOP Enhances TLR-Mediated Macrophage IL-6 Expression and Contributes to Inflammatory Colitis in Mice

Shyamasree Datta, Chenyang Zhao, Paul Pavicic, Jr., Michael Novotny, Thomas Hamilton
Dept. of Immunology, Cleveland Clinic

Endoplasmic reticulum (ER) stress is increasingly recognized as an important contributing process in the control of inflammatory responses, particularly in cells of myeloid lineage. cEBP homologous protein (CHOP), an ER stress-induced gene product that promotes apoptosis, has also been linked with enhanced expression of IL-6 and the p19 subunit of IL-23. In the current study we find that engagement of ER stress in mouse macrophages enhances TLR-induced IL-6 expression via multiple mechanisms, including CHOP-dependent prolongation of gene transcription. In irradiated mice reconstituted with wild type or CHOP-/- bone marrow, we also observed that CHOP deficiency in the hematopoetic compartment resulted in marked protection from acute dextran sodium sulfate-induced colitis. Mice with bone marrow from CHOP-/- donors exhibited reduced weight loss and less severe clinical disease. Moreover, levels of cytokine expression within the injured colon were significantly less in mice with CHOP-/- hematopoetic cells. While mice reconstituted with wild type bone marrow all died, a majority of mice receiving CHOP-/- bone marrow recovered. These findings demonstrate the importance of CHOP as a mediator of stress-induced amplification of myeloid inflammatory gene expression and the impact of CHOP expression in the hematopoetic compartment on the outcome of inflammatory injury.

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Bone Marrow-Derived Eosinophils: A Tool for Studying the Role of Eosinophils in Innate Immunity

Kimberly D. Dyer, Eva M. Sturm, Helene F. Rosenberg
Eosinophil Biology Section of the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Among the difficulties encountered by eosinophil biologists is the fact that eosinophil-specific events represent only a fraction of the ongoing hematopoietic activity in bone marrow at any given time even under profound Th2 stimulation. Historically, cytokine-stimulated protocols have been relatively ineffective at generating large quantities of eosinophils from mouse bone marrow progenitors. We have devised an *ex vivo* culture system which generates large numbers of phenotypically and functionally mature eosinophils at high purity from unselected mouse bone marrow progenitors. Bone marrow derived eosinophils (bmEos) are similar to those isolated from the spleen and peripheral blood of interlekin-5 transgenic mice and can be generated from gene-ablated mice on both BALB/c and C57BL/6 backgrounds.

Several research groups have used this protocol to examine the importance of various molecules in eosinophil development and function with an emphasis on the role of eosinophils in allergic inflammation. We have used this protocol to demonstrate that Pneumovirus of Mice (PVM) infects eosinophils and that this infection elicits the release of proinflammatory cytokines such

as IL-6, CCL2, CCL3 and IP-10 in a MyD88-dependent manner. We have also demonstrated that platelet activating factor (PAF) stimulates the release of EPO from mouse bmEos and eosinophils isolated from the spleen of IL5Tg mice as well as human eosinophils independent of the known PAF GPCR receptor (PAFR). Lastly, we found that while PAF and lyso-PAF stimulated the release of EPO, these phospholipid mediators did not stimulate the release of cytokines from eosinophils. IL-6 however could and did stimulate the release of stored cytokines indicating that degranulation occurs in a regulated manner. Currently we are working on a method to transplant bmEos into wild-type and eosinophil deficient ΔdblGATA mice in order to study the effects of eosinophils *in vivo*.
Our work and that of others demonstrate that these cells are useful for examining the role of eosinophils in innate immunity.

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Impaired Secretion of IL-1ß in Preterm Neonates Is Associated with a Lack of NALP3 Inflammasome/Caspase-1 Activity

Ashish A. Sharma[1, 2], Roger Jen[1, 2], Abhinav Sharma[1, 2], Mihoko Ladd[2], Kelsey Lee[1, 2], Laura M. Sly[1, 2], Helene Cote[1], Pascal M . Lavoie[1, 2, 3]
[1]University of British Columbia; [2]Child and Family Research Institute; [3]Children's and Women's Health Centre of BC

Infection due to immaturity of the innate immune system is a major cause of neonatal morbidity and mortality worldwide, especially in infants born before full term of gestation. Interleukin-1β (IL-1β) is a powerful pyrogen and major mediator of inflammation. Neonates are deficient in their ability to produce IL-1β. Cell secretion of IL-1β is regulated in a two-step process involving the activation of Toll-like receptor (TLRs) leading to the NF-κB-mediated transcription of the pro-IL-1β gene, followed by the NALP3/Caspase-1-mediated proteolytic processing of pro-IL-1β into mature (secreted) IL-1β. Here, we investigated the regulation of interleukin-1β in early life, using cord blood obtained from human neonates born prematurely or at full-term. Our data confirms that preterm neonatal cord blood (CD14+) monocytes are abundant, phenotypically immature and substantially impaired in their ability to produce IL-1β upon stimulation with lipopolysaccharide and ATP (to activate the NALP3 Inflammasome). IL-1β mRNA levels are reduced following LPS stimulation in preterm cord blood. However, intracellular levels of the pro-IL-1β protein in preterm monocytes are comparable to adults or term monocytes, indicating sufficient production of the pro-IL-1β precursor. Using a flow cytometry adaptation of the Fluorescein Caspase-1 Activity (FLICA) assay, we demonstrate a developmental attenuation in the activity of caspase-1 (within monocytes) to undetectable levels at the beginning of the first trimester of gestation. We believe that this mechanism is predominantly responsible for the lack of mature IL-1β in preterm neonates. Our results indicate a potential pharmacological target for prevention of infection in newborns.

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Pegylated Interferon-Alpha2A Monotherapy Induces Durable Suppression of HIV-1 Replication and Decreased HIV DNA Integration Following ART Interruption

Livio Azzoni[1], Andrea S. Foulkes[2], Emmanouil Papasavvas[1], Angela M. Mexas[3], Karam Mounzer[4], Pablo Tebas[3], Jeffrey M. Jacobson[5], Una O'Doherty[3], Jay Kostman[3], Luis J. Montaner[1]
[1]The Wistar Institute, Philadelphia, PA, USA; [2]University of Massachusetts, Amherst, MA, USA; [3]University of Pennsylvania School of Medicine, Philadelphia, PA, USA; [4]Philadelphia FIGHT, Philadelphia, PA, USA; [5]Drexel University, Philadelphia, PA, USA

Background: ART interruption invariably results in viral rebound, independent of achieved immune reconstitution. Prior immune-based strategies have failed to achieve durable viral suppression.
Methods: 23 HIV-1 infected, ART-suppressed individuals (VL <50 copies (c)/ml, CD4 count >450 cells/ml) were randomized to receive 180 (arm A) or 90 (arm B) μg/week of Pegylated Interferon-α2a (pIFNα). After 5 weeks of overlapping treatment, ART was discontinued and pIFNα mono-therapy was maintained up to 24 weeks; subjects with virological failure (VL≥ 400 c/ml) or Serious Adverse Events (SAEs) restarted the previous ART regimen. Primary analysis compared the observed proportion of subjects with VL < 400 copies/ml following 12 weeks of pIFNα monotherapy to an expected proportion of 0.09 (exact binomial test, SAEs treated as virologic failures).
Secondary analysis: 1) compare the proportion of subjects sustaining VL < 400 copies/ml thru week 24 with historical controls (ART interruption, no pIFNα); 2) measure integrated PBMC HIV DNA levels by Alu-PCR as correlates of viral control.
Results: Of 23 patients enrolled, 3 were excluded from the primary analysis (1 lost to follow-up, 1 incarcerated, 1 grade 2 LFTs). 9 of the 20 remaining subjects (45%) maintained a VL<400 c/ml thru week 12, which was significantly greater than the expected proportion of 0.09 (arm A, p=0.0088; arm B, p=0.0010, combined arms, p <0.0001). 4 of these 9 subjects had VL<48 copies/ml (p=0.0027, combined arms). A sensitivity analysis including the 3 excluded subjects confirmed the result (39%, arm A, p=0.0178, arm B, p= 0.0017, combined arms, p<0.0001).
The 9 subjects with VL<400 c/ml at week 12 continued pIFNα for up to 12 more weeks; at week 24, 6 out of 9 (67%) subjects had VL < 400 c/ml on pIFNα monotherapy. CD4 counts remained >300 cells/μl in all subjects. SAEs included neutropenia (n=1) and depression (n=3).
Upon ART resumption all subjects were successfully suppressed at VL<48 c/ml.
A net decrease (p=0.01) in integrated HIV DNA copies/CD4+ T cell between baseline and week 12 was observed in all subjects who remained suppressed VL<400 c/ml, but not in those with viral breakthrough > 400 c/ml.
Conclusions: We provide the first proof-of-concept that pIFNα-based immunotherapy results in durable control HIV replication following ART interruption. We also show, for the first time, that pIFNα-mediated viral control impacts HIV.

ABSTRACTS

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Lipopolysaccharide Induced Expression of the Key Genes Involved in Inflammasomes in the Spleen of HIV-1 Transgenic Rat

Sulie L. Chang[1, 2], Xin Mao[1]
[1]Seton Hall University/ Institute of NeuroImmune Pharmacology; [2]Seton Hall University/ Department of Biological Sciences

Inflammasomes are protein complexes that activate caspase-1 to up-regulate pro-inflammatory cytokines, such as IL-1β. Of the four well-characterized inflammasomes, AIM2, IPAF, NLRP1a, and NLRP3, NLRP3 can be induced by the endotoxin, lipopolysaccharide (LPS). We previously showed that LPS-induced serum levels of interleukin-1β (IL-1β) are significantly greater in the HIV-1 transgenic (HIV-1Tg) rat than in control F344 rats. In this study, we compared the expression of 84 key genes involved in inflammasome function in the spleen of HIV-1Tg rats and F344 control animals with and without LPS treatment. Adult HIV-1Tg and F344 male rats were randomly assigned to receive either LPS (250 μg/kg) or saline (i.p.). Two hours following treatment, the animals were sacrificed, and their spleens were collected. Total spleen RNA was isolated for real time PCR using the Rat Inflammasomes RT² Profiler™ PCR Array. Expression of the 84 genes examined was comparable in the spleen of the HIV-1Tg and F344 rats given saline, indicating that gene expression of the four known inflammasomes was not altered in the presence of HIV-1 viral proteins. While gene expression for the NLRP3 inflammasome, the IL-1β pro-inflammatory cytokine, the Cxcl1, Cxcl3, Ccl2, and Ccl7 chemokines, and other downstream signaling molecules, including NFkB, was up-regulated in both the HIV-1Tg and F344 rats in response to LPS, the magnitude of the up-regulation was much greater in the HIV-1Tg rats, except for NLRP3. LPS-induced up-regulation of NLRP3 was 4.5-fold greater in the spleen of F344 rats, but only 2.7-fold greater in HIV-1Tg rats. Conversely, LPS-induced up-regulation of IL-1β was 16.8-fold greater in the spleen of HIV-1Tg rats, but only 10.9-fold greater in the F344 rats. Our data suggest that there may be additional mechanisms other than NLRP3 inflammasome production underlying LPS-induced up-regulation of IL-1β in the spleen of HIV-1Tg rats (NIH DA007058 and DA016149 to SLC).

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Hyperoxaluria Leads to Increased Expression of Genes Encoding NADPH-Oxidase Complex Associated Proteins in a Rat Model

Saeed R. Khan, Sunil Joshi, Ammon B. Peck
Department of Pathology, College of Medicine, University of Florida

We have previously shown that production of reactive oxygen species (ROS) is an important contributor to renal injury and inflammation following exposure to oxalate and/or calcium-oxalate (CaOx) crystals. The present study was conducted, utilizing global transcriptome analyses, to determine if the NADPH oxidase system is activated in kidneys of rats fed a diet leading to hyperoxaluria and CaOx crystal deposition.

Age-, sex- and weight-matched rats were fed regular rat chow supplemented with 5% w/w hydroxy-L-proline (HLP). After 28 days, each rat was euthanized, their kidneys freshly explanted and dissected to obtain both cortex and medulla tissues. Total RNA was extracted from each tissue and subjected to genomic microarrays to obtain transcriptome data. KEGG was used identify gene clusters. Immunohistochemistry was used to confirm protein expressions of selected genes.

Genes encoding both membrane- and cytosolic-NADPH oxidase complex-associated proteins, together with Rac1 and Rac2, were coordinately up-regulated in both renal medulla and cortex tissues in the HLP-fed rats compared to controls. Simultaneously, genes encoding ROS scavenger proteins, catalase and superoxide dismutase, were down-regulated. Activation of NADPH oxidase appears to occur via the angiotensin-II/angiotensin-II receptor-2 pathway.

A strong up-regulation of an oxidative burst involving the NADPH oxidase system, activated via the angiotensin-II and DAG-PKC pathways, occurs in kidneys of hyperoxaluric rats.

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Combined Antagonism of the IL-4R and IKKß Inhibits Melanoma Growth

Oriana E. Hawkins[1, 2], Linda W. Horton[1, 2], Gregory D. Ayers[3], Ann Richmond[1, 2]
[1]Department of Veterans Affairs; [2]Department of Cancer Biology, Vanderbilt School of Medicine; [3]Department of Biostatistics, Vanderbilt University School of Medicine

With over 8500 deaths each year in the USA, malignant melanoma is the deadliest of all skin cancers. Constitutive activation of the nuclear factor kappa B (NF-κB) is common in melanoma, promoting tumor growth, survival and metastasis. In light of its role in tumorigenesis, targeting of the NF-κB pathway has been considered a possible therapeutic option for the treatment of melanoma. However, NF- κB activation is critical for the development, survival, migration and function of immune cells. We hypothesized that systemic inhibition of the activation of NF- κB might have a deleterious effect on the tumor immune response. Melanoma bearing C57BL/6 mice received 50 mg/kg of BMS-345541, a selective inhibitor of the inhibitor of κB kinase (IKKβ), twice daily. Tumors, spleens and bone marrow were harvested at different time points and analyzed by flow cytometry for leukocyte composition and cytokine production. Intracellular cytokine staining revealed an early induction of Interleukin-4 (IL-4) within the myeloid and B cell components of the tumor, as a result of BMS-345541 treatment. The production of IL-4 by these cells suggests an early switch to a tumor promoting T helper 2 (Th2) and M2 driven immune response, which may counteract the anti-tumor effect of IKKβ inhibition by generating a pro-tumorigenic microenvironment.

IL-4 receptor (IL-4R) expression on human melanoma cells has been previously documented; however the effects of IL-4 on these cells have generated contradictory reports. IL-4R signaling has been shown to either inhibit growth of tumor cells in vitro or to promote survival of tumor cells. In vitro IL-4 delivery did not alter either the

proliferative capacity of IL-4R expressing melanoma cell lines or their sensitivity to BMS-345541. In C57Bl/6 mice bearing a murine melanoma, BMS345541 (100 mg/kg qd) was given in combination with 100 μg of IL-4R blocking antibody on day 4 and day 12 of treatment. This combination inhibited tumor growth compared to BMS345541 treatment alone. These data suggest an important role for IL-4 in promoting melanoma growth in vivo. Dual targeting of IL-4R and NF-κB signaling pathways neutralizes the tumor promoting immune component. Currently, IL-4R antagonists are in clinical trials for the treatment of asthma, which makes the translational prospects of this study extraordinarily viable.

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Combined Radiation and Burn Injury Results in Exaggerated Early Pulmonary Inflammation

Jessica L. Palmer[1], Cory R. Deburghgraeve[1], Melanie D. Bird[1], Martin Hauer-Jensen[2], Elizabeth J. Kovacs[1]

[1]Burn and Shock Trauma Institute, Department of Surgery/ Loyola University Medical Center; [2]Division of Radiation Health, University of Arkansas for Medical Sciences, and Surgical Service/ Central Arkansas Veterans Healthcare System

In nuclear accidents or attacks, radiation exposure is often coupled with other forms of injury, such as burns, blast injury, blunt trauma, and infectious complications. Of these, burn injury is most often sustained in addition to radiation exposure. Since independently radiation exposure and burn can have deleterious effects on the lung, we examined the early effects (within 48 hours) of combined radiation injury (CRI) on parameters of lung inflammation. Eight to ten week-old C57BL/6 male mice were subjected to 5 Gray (Gy) of total body irradiation followed by a 15% total body surface area (TBSA) scald burn. In this model, there is around 30% mortality within the first few days of the injury. Lungs from the surviving animals were examined for evidence of pneumonitis. At 48 hours post-injury, pathology of the lungs from combined injury mice showed greater inflammation compared to all other treatment groups, with marked red blood cell and leukocyte congestion of the pulmonary vasculature. There was excessive leukocyte accumulation, comprised mostly of neutrophils, in the vasculature and interstitium, with occasional cells in the alveolar space. Pulmonary neutrophils were counted and found to be more numerous in both burn only and CRI lungs at 24 and 48 hours following injury ($p<0.01$). In accordance with histological evidence for pulmonary neutrophil accumulation, CRI lung myeloperoxidase (MPO) content was increased 2-fold as compared to radiation alone at 24 hours ($p<0.05$). At 24 hours, burn injury only MPO levels were also significantly elevated above sham and radiation only groups ($p<0.05$). However, at 48 hours after injury, pulmonary MPO levels in the CRI group continued to increase and were higher than all other groups ($p<0.01$). Pulmonary levels of the neutrophil chemoattractant KC (CXCL1) were 3 times above that of either injury alone ($p<0.05$). Insterestingly, macrophage inflammatory protein-2 (MIP-2, CXCL2) levels were not markedly different in lungs of treated groups as compared to sham. Monocyte chemotactic protein-1 (MCP-1, CCL2) was increased 2-fold and 3-fold in CRI lungs compared to burn injury or radiation injury, respectively ($p<0.05$). This suggests that macrophages might also be contributing to the pulmonary congestion and inflammation observed following combined injury. We are currently investigating macrophage migration, accumulation, and retention in the lungs in this model. Together, these data suggest that combined radiation and burn injury augments early pulmonary congestion and inflammation, which could lead to eventual fibrosis and organ failure. Currently, countermeasures and treatments for this unique type of injury are extremely limited. Further research is needed to elucidate the mechanisms behind the negative synergistic effects of combined injury in order to develop appropriate treatments. NIH R21/R33 AI080528 (EJK), and U19 AI67798 (MH-J).

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Selenoprotein K Interacts with Vav1 in Macrophages to Inhibit Rac1 Activity

Robert L. Norton, Zhi Huang, Fukun W. Hoffmann, Peter R. Hoffmann

Cell & Molecular Biology, University of Hawaii at Manoa, Honolulu, HI

Selenium is a mineral micronutrient that is essential in a variety of biological systems, including immune responses. Selenium is used for biosynthesis of the amino acid selenocysteine, which is used by selenoproteins to carry out biological functions. In humans, 25 selenoproteins have been identified and Selenoprotein K (SelK) was recently identified as an endoplasmic reticulum transmembrane protein important for calcium flux during the activation of immune cells. Vav1 is a guanine exchange factor that activates Rac1 and leads to actin polymerization and cytoskeletal rearrangement. Implementation of the SH3 hunter software predicted that the SH3-binding domain of SelK associates with one of the two SH3 domains of Vav1. We were able to show that SelK co-immunoprecipitates with Vav1 from mouse bone marrow derived macrophage (BMDM) whole cell lysates. By pull down assay, we saw higher levels of activated, GTP bound Rac1 in KO BMDM's compared to WT BMDM's when treated with RANTES. This suggests that SelK could be involved in Rac1 activation. Studies are underway to determine if Vav1 is being sequestered by SelK, preventing it from becoming activated. Macrophage activation might cause SelK to release Vav1, leading ultimately to actin polymerization, cytoskeletal rearrangement and cell motility. Future experiments will determine if this co-immunoprecipitation is abolished when the SH3 binding domain of SelK is deleted or when the SH3 motif of Vav1 is deleted.

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Molecular Mechanisms Responsible for the Selective and Low-Grade Induction of Pro-inflammatory Mediators by Lipopolysaccharide

Bianca Baker, Urmila Maitra, Hui Deng, Trevor Glaros, Liwu Li

Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061-0910

Low dose endotoxemia is prevalent in humans with adverse health conditions, and correlates with the pathogenesis of chronic

inflammatory diseases such as atherosclerosis, diabetes, and neurological inflammation. However, the underlying molecular mechanisms are poorly understood. Here, we demonstrated that subclinical low dose lipopolysaccharide (LPS) skews macrophages into a mild pro-inflammatory state, through cell surface toll-like-receptor 4 (TLR4), interleukin-1 receptor associated kinase-1 (IRAK-1), and toll-interacting-protein (Tollip). Unlike high dose LPS, low dose LPS does not induce robust activation of nuclear factor kappa-B (NFκB), mitogen-activated protein kinases (MAPK) or anti-inflammatory mediators. Instead, low dose LPS induces activating transcription factor 2 (ATF2) through Tollip-mediated generation of mitochondrial reactive oxygen species (ROS), allowing mild induction of pro-inflammatory mediators. Mitochondrial serves as a critical hub and assembly platform for innate immunity signaling, and mitochondria dysfunction is associated with diverse inflammatory diseases. Our study further supports the notion that mitochondria are critically involved in low grade inflammation. Taken together, our data reveal novel mechanisms responsible for skewed and persistent low grade inflammation, a cardinal feature of chronic inflammatory diseases, which utilizes the mitochondria.

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VentX is a Novel Regulator of Human Macrophage Activation

Jenny (Hong) Gao[1], Xiaoming Wu[2], Jerry (Zhenglun) Zhu[2]
[1]North Shore Hospital; [2]Brigham and Women's Hospital

Macrophages play key roles in pathogenesis of autoimmune inflammatory diseases. While aberrant activation of macrophages has been implicated in pathogenesis of diseases, such as SLE, RA and inflammatory bowel diseases (IBD), the mechanisms of transcriptional control of macrophages activation remains incompletely understood. VentX is a recently appreciated hematopoietic homeobox gene, identified as a novel tumor suppressor through reverse genetic modeling of Xenopus embryogenesis. Our recent studies showed that VentX plays a key role in regulating macrophage function under both physiological and pathological conditions. The function of VentX in macrophage activation was explored by gain-of-function and loss-of-function approaches. The results of our studies showed that VentX promotes and is required for macrophage activation. We found that VentX regulates the expression of g-INF receptor, NFkB, Stat1 and AP-1 signaling pathways, which play critical role in macrophage activation. Moreover, we found that VentX and Stat1 form a positive signaling loop for pro-inflammatory activation of macrophage. Clinically, we found that VentX expression correlates with the expression of pro-inflammatory cytokines in SLE and RA patients. Moreover, we found that VentX expression can be down-regulated by immuno-modulators commonly used in the treatment of autoimmune and inflammatory diseases. The results of our studies suggested that further investigations on the mechanisms of VentX action and its potential clinical application may help advance management of autoimmune and inflammatory diseases by providing a novel basis for mechanistic exploration and a potential target of intervention.

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Adiponectin Enhances Expression of Mer Tyrosine Kinase in Macrophages to Promote Clearance of Apoptotic Cells

Manuel D. Galvan[1, 2], Suzanne S. Bohlson[1, 2]
[1]Department of Biological Sciences, Eck Institute for Global Health, University of Notre Dame, South Bend, IN, 46556; [2]Indiana University School of Medicine-South Bend, South Bend, IN 46617

Failure to efficiently clear apoptotic cells is linked to defects in development and autoimmunity. Complement component C1q stimulates monocyte/macrophage engulfment of apoptotic cells, and C1q is required for prevention of autoimmunity. We recently described a novel molecular mechanism for C1q-dependent clearance of apoptotic cells. Full length C1q, but not the collagen-like tail, triggered expression of Mer tyrosine kinase and the Mer ligand, Ga6: a receptor-ligand pair that mediates clearance of apoptotic cells. Moreover, C1q-dependent Mer expression was required for C1q-dependent enhanced engulfment of apoptotic cells in mouse bone marrow derived macrophages (BMDM). To investigate the signaling pathway required for C1q-dependent Mer expression, BMDM were stimulated with either full-length C1q or the collagen-like tail, and microarray analysis was performed to identify macrophage transcripts specific to stimulation with full length C1q. Pathway analysis identified the adiponectin signaling pathway as significantly upregulated with full length C1q but not the C1q tail region. Adiponectin is a C1q homologue that is important in regulating metabolism. Since adiponectin also regulates engulfment of apoptotic cells and autoimmunity in mice, we hypothesized that adiponectin would upregulate Mer expression and function. As predicted, adiponectin triggered expression of Mer, and the increase in Mer expression correlated with enhanced engulfment of apoptotic cells. Soluble Mer-Fc fusion protein inhibited adiponectin-dependent engulfment of apoptotic cells indicating a requirement for Mer and Mer ligands. Our results suggest that C1q and adiponectin promote engulfment of apoptotic cells via a common signaling pathway. Moreover, these data are consistent with a role for C1q and adiponectin in prevention of autoimmunity.

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IL-12 Modification of Tumor-Associated Macrophage Responsiveness via IFNγ-Dependent and Independent Mechanisms

Courtney J. George, Kimberly Head, Lihua Zhang, Jill Suttles, Robert D. Stout
University of Louisville School of Medicine, Department of Microbiology and Immunology

Understanding the impact of IL-12 on tumor-associated macrophages (TAMs) is an important component in deciphering the mechanisms related to IL-12-related immunotherapeutic effects. We have demonstrated that IL-12 treatment in vivo transiently renders tumor-associated macrophages immunocompetent and that this phenotypic change in macrophages contributes to IL-12's anti-tumor effects. Our data suggested that IL-12 enhances the ability of TAMs to respond to inflammatory stimuli which raised the following questions: 1) does IL-12 change signaling events associated with inflammatory signal transduction, and 2) is the IL-12-induced

enhancement of TAM responsiveness to inflammatory stimuli exclusively IFNγ dependent? In the present study, we demonstrate that in vitro treatment of TAMs with IL-12 followed by exposure to LPS enhances the amount of pro-inflammatory cytokines, IL-6 and TNFα with a reciprocal decrease in the anti-inflammatory cytokine, IL-10. This modulation of the TAM's inflammatory phentoype is associated with increased phosphorylation of MAPK family members p38, JNK, and p44/42. Using TAMs from IFNγ-/- tumor-bearing mice, we observed that, although the overall magnitude of pro-inflammatory cytokine release was decreased, IL-12 significantly augmented the response of TAMs to LPS. The ability of IL-12 to decrease IL-10 secretion from TAMs in response to LPS was not affected by the absence of IFNγ. However, the loss of IFNγ abolished the increased phosphorylation of p38 and JNK, while the amplified phosphorylation of p44/42 was sustained. Our results provide novel insights into the mechanism by which IL-12 may directly impact the functional phenotype of macrophages within the tumor microenvironment. Additionally, this work provides insight into the function of IL-12 in the context of IFNγ and MAPK pathways which have broad-reaching roles in the regulation of immune responses, inflammation and cancer. This study offers a corollary to the classically identified mechanisms of IL-12 in innate immunity and cancer immunotherapy.

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Mitochondrial Sirt3 NAD+-Dependent Deacetylase Regulates Oxidative Bioenergetics during the TLR4-Induced Acute Inflammatory Response

Tie F. Liu[1], Anthony Molina[2], Charles E. McCall[3]
[1]Sections of Molecular Medicine, Department of Internal Medicine, Wake Forest University School of Medicine; [2]Sections of Geriatrics, Department of Internal Medicine, Wake Forest University School of Medicine; [3]Sections of Molecular Medicine, Department of Internal Medicine, Wake Forest University School of Medicine

The role of mitochondrial bioenergetics in the acute inflammatory and immune responses is obscure. We recently reported in THP1 human promonocytes and in human and murine sepsis that Sirt 1 and 6 NAD+-dependent redox sensors differentially reprogram the acute inflammatory response of innate immune myeloid-derived phagocytes by switching from an early Warburg-like glycolytic and anabolic response to increased fatty acid flux and fatty acid beta oxidation by mitochondria during a later catabolic and adaptation response. This NAD+ dependent coupling by Sirt1 and 6 also controls epigenetic reprogramming of genes that regulate the acute inflammatory response. Here, we show that mitochondria-located NAD+ sensor Sirt3, whose expression is supported by NAD+-activated Sirt1, regulates oxidative bioenergetics during acute TLR4-induced sequential reprogramming of THP1 cells. Using the Bioscience Seahorse XF to analyze real time mitochondrial bioenergetics, we observe TLR4-induced increases in the mitochondrial oxygen consumption rate (OCR) and extracellular proton flux (ECAR), which peak at 8 h and decline by 24 h. Increased OCR is fatty acid oxidation dependent and correlates with increased expression of long chain acyl-CoA dehydrogenase (LCAD) that is rate limiting for fatty acid oxidation, iso-citrate dehydrogenase 2 (IDH2) that is rate limiting for the tricarboxylic cycle, and mitochondrial superoxide dismutase 2 (SOD2) that limits reactive oxygen species. These 3 mitochondrial proteins are directly activated by Sirt3 de-acetylation. Sirt3 levels increase in parallel with elevations in OCR, and Sirt3 knockdown: 1) attenuates TLR4-induced increases in OCR and ECAR; 2) reduces mitochondrial spare respiratory capacity; and 3) decreases deacetylation of LCAD and IDH2. We conclude the NAD+ sensing by mitochondrial Sirt3 combines with nuclear Sirt1 and 6 to regulate epigenetic and metabolic reprogramming during the acute inflammatory response.

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Detection of TLR Agonists by Hematopoietic Stem and Progenitor Cells Programs Macrophage Function

Helen S. Goodridge[1], Alberto Yanez[1], Nargess Hassanzadeh-Kiabi[1], Madelena Y. Ng[1], Javier Megias[2], Aparna Subramanian[1], George Y. Liu[1], David M. Underhill[1], Maria Luisa Gil[2]
[1]Cedars-Sinai Medical Center, Los Angeles, USA; [2]Univeristy of Valencia, Burjassot, Spain

Upon infection, myeloid cells must be rapidly mobilized to clear invading microbes and repair damaged tissues. The production of myeloid cells from hematopoietic stem and progenitor cells (HSPCs) during an infection is instructed indirectly by cytokines and growth factors produced by mature cells in the periphery when they detect microbial components. In addition, several recent reports have shown that HSPCs can directly sense microbes using Toll-like receptors (TLRs), which instruct the differentiation of macrophages, dendritic cells and neutrophils. However, although these "TLR-derived" cells exhibit myeloid cell characteristics (phagocytosis, cytokine production, antigen presentation), it isn't currently clear whether they are functionally equivalent to their colony stimulating factor (CSF)/cytokine-derived counterparts. In this study we show that TLR2-derived macrophages produce less TNF-α and reactive oxygen than M-CSF-derived macrophages. Furthermore, we show that even transient exposure of mouse and human HSPCs to TLR2/4 agonists is sufficient to program the function of macrophages subsequently derived from them using M-CSF. We also demonstrate HSPC programming by a TLR2 agonist in vivo. Our data indicate that direct detection of microbial components could influence macrophage responses long after an infection has been cleared.

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Mononuclear Myeloid-Derived Suppressor Cells (M-MDSC) in Experimental Colitis

Dmitry V. Ostanin, Elvira Kurmaeva, Seth Berney
Dept. Medicine, Center of Excellence for Arthritis and Rheumatology/LSUHealth-Shreveport

The inflammatory bowel diseases (IBD) are accompanied by extensive granulo- and myelopoiesis and infiltration of myeloid cells into colonic lamina propria (cLP) and secondary lymphoid tissues. Surprisingly little is known regarding the contribution of myeloid cells to the regulation of adaptive immunity and IBD pathogenesis. Transfer of CD4+CD45RB^{high} naïve T cells into immunodeficient recombination activating gene-1-deficient (RAG-1$^{-/-}$) recipient mice results in the development of chronic colitis, which is reminiscent to

human Crohn's disease (CD). Previously we found that neutrophils isolated from colon lamina propria (cLP) showed increased surface expression of MHC class II and CD86 and triggered proliferation of antigen-specific CD4+ T cells. In this work, we have identified Mac-1+Ly6G-Dectin-1+ myeloid cells in cLP, spleen, blood and bone marrow (BM) of colitic mice that suppressed proliferation of T cells. Analysis of surface markers by flow cytometry and morphological evaluation revealed remarkable differences between MDSCs in different tissues. Blood, BM, and spleen MDSCs were PD-L1^{low}CD115+ and were similar to monocytes, while cLP cells were macrophage-like PD-L1^{high}CD115- cells. Suppression of T cell proliferation was both antigen-dependent and –independent. It was abrogated by inhibition of nitric oxide (NO) synthases and required close proximity between cells, but was not due to arginine or cystein depletion, production of reactive oxygen species, or surface β2 integrin engagement. M-MDSCs did not trigger T cell apoptosis, but, on the contrary improved their viability. Inability of M-MDSCs to attenuate colitis was not due to their paucity in the cLP. Remarkably, cLP M-MDSCs suppressed proliferation and attenuated production of IFN-γ, IL-17, and IL-2 by effector cLP Th1/Th17 cells isolated from mice with active disease under hypoxic (1% O_2) conditions, which is physiologically relevant to chronically inflamed regions of intestine in human CD. Taken together, colitis-induced M-MDSCs share many similarities with tumor-induced MDSCs. A better understanding of how these complex interactions contribute to disease pathogenesis may reveal novel therapeutic strategies for the treatment of patients with IBD.

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JAK2 and PTPN1: Role in Cytokine Production by Platelet-Activating Factor-Stimulated Human Dendritic Cells

Geneviève Hamel-Côté, Marek Rola-Pleszczynski, Jana Stankova
Immunology Division, Department of Pediatrics, Université de Sherbrooke, Sherbrooke, Québec, Canada

Among pro-inflammatory lipids, platelet-activating factor (PAF) is a key mediator in the induction and progression of many pathophysiologic states such as atherosclerosis, where dendritic cells (DC) can act as regulators of disease progression. As some signalling pathways activated by PAF involve JAK kinases, we investigated the modulation of functional responses of DCs to PAF by one of the regulators these kinases, protein-tyrosine phosphatase non-receptor type 1 (PTPN1). Using DCs, generated from monocytes isolated from healthy volunteers, we down-regulated PTPN1 expression using siRNAs, which decreased PTPN1 protein expression by 25%. Here, we show that even if modest, this down-regulation of PTPN1 is enough to modify the profile of cytokine mRNA expression observed after PAF stimulation, without affecting maturation of DCs. In DCs transfected with PTPN1 siRNAs, as compared to those transfected with control siRNAs, PAF induced significantly higher mRNA levels of IL-6, IL-8 and CCL2 whereas IL-10 level was not significantly affected. On the other hand, PAF-induced mRNA levels of TNF alpha and TGF beta were drastically decreased in presence of PTPN1 siRNAs . Luciferase assay studies in PAF receptor-transfected HEK 293 cells showed that over-expressed PTPN1 down-regulated promoter activity of IL-6 and IL-8 in response to PAF. Our results suggest that the effects of PTPN1 on IL-6 promoter activity, in response to PAF, are dependent on JAK2 kinase as suggested by JAK2 inhibitors or over-expression of JAK2 dominant negative mutants. In addition, confocal microscopy shows co-localization of PTPN1 and Jak2 after PAF stimulation. Together, our results suggest that PTPN1 is an important modulator of PAF-induced functional responses by influencing cytokine production.

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Swine Confinement Facility Dust Modulates Phagocytosis and Oxygen Radical Production by Macrophages

Jenora T. Waterman, Radiah C. Minor, Ruth J. Pender, Steven L. Hurley
North Carolina Agricultural and Technical State University, Department of Animal Sciences

Workers in swine confinement facilities (SCF) are exposed to organic dust that often results in airway inflammation and health conditions including chronic bronchitis and asthma-like syndrome. It is well established that oxidative stress is a major contributor to onset and exacerbation of respiratory diseases; however, the exact mechanism remains unclear. We investigated the hypothesis that SCF dust induces airway oxidative stress and increased plasma protein levels in lung exudates. Bronchoalveolar lavage (BAL) fluid from female Balb/c mice that were chronically exposed to SCF dust extract (DE) [5%, 12.5% and 25%] or phosphate buffered saline via nebulization contained higher levels of vacuolated macrophages and protein content compared to mice exposed to saline. Monocytic THP-1 cells exposed to SCF DE [0.1%, 1%, and 10%] for 24 hours in vitro showed enhanced phagocytosis and vacuolation. Companion reactive oxygen species generation studies revealed hydrogen peroxide production by dust extract-exposed THP-1 cells in a dose-dependent manner. Taken together these results indicate that airway inflammation observed in SCF workers may be caused by dust modulation of inflammatory cells, particularly macrophages, ROS production and protein mediators within the airway.

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Synergistic Interaction between C5a and Nod2 Signalling in the Regulation of Chemokine Expression in Macrophages

Hui Tang, Dora Tang, Mark A. Barnes, Laura E. . Nagy
Department of Pathobiology, Cleveland Clinic, Cleveland OH

The innate immune response to pathogen infection is a complex process in which multiple pathogen-recognition receptors (PRRs) can be activated to precisely control innate immunity. Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors are two major classes of PRRs. Complement is also activated by pathogens, resulting in the generation of the potent analphylatoxin, C5a. While previous studies have shown that complement interacts synergistically with TLR4, the interactions between NOD-like receptors and complement is not well understood. Here, we report a synergistic interaction between C5a

and Nod2 signalling in RAW 264.7 macrophages. MDP, a ligand of Nod2, enhanced C5a-mediated expression of chemokine mRNA in RAW 264.7 cells. Knocking down Nod2 prevents this enhancement. C5a exerts most of its functions through C5a receptor (C5aR), but an emerging body of evidence indicates the involvement of a second C5a receptor, C5L2, which acts as a negative modulator of C5aR activity. Importantly, we found that treatment of RAW264.7 cells with MDP reduced expression of C5L2, not C5aR. Over-expression of C5L2 inhibited the synergistic interaction between MDP and C5a mediated expression of chemokine mRNAs. C5L2 was predominantly expressed at the cell surface, while C5aR distributed to both intracellular vesicles and at the plasma membrane. MDP amplified C5a mediated phosphorylation of p38 MAPK and treatment of RAW264.7 cells with an inhibitor of p38 attenuated the synergistic effects of C5a on MDP-primed cells on the expression of MIP-2 mRNA, but not MCP-1 mRNA. In contrast, inhibition of AKT prevented C5a stimulation of MCP-1 mRNA, but not MIP-2 mRNA, in MDP-primed cells. Taken together, these data demonstrated a synergistic interaction between C5a and Nod2 in the regulation of chemokine expression in macrophages, associated with a down-regulation of C5L2, a negative regulator of C5a receptor activity.

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Immunoresponsive Gene 1 Contributes to Bactericidal Activity of Macrophage-Lineage Cells by Regulating ß-Oxidation-Dependent Mitochondrial ROS Production

Christopher J. Hall, Rachel H. Boyle, Maria Vega Flores, Stefan H. Oehlers, Jonathan W. Astin, Leslie E. Sanderson, Kathryn E. Crosier, Philip S. Crosier

Department of Molecular Medicine and Pathology, School of Medical Sciences, The University of Auckland, Auckland, New Zealand

The emerging field of immunometabolism has its basis in the observation that inflammation is a 'hallmark' of many chronic metabolic disorders. Central to this link between the immune and metabolic systems is the mitochondrion. In addition to roles during cellular metabolism and apoptosis, mitochondria are becoming recognized as central players in the orchestration of inflammatory pathways and immune cell function. Recent studies have linked the production of mitochondrial reactive oxygen species (mROS), once thought to be an insignificant by-product of oxidative phosphorylation (OXPHOS), to macrophage-mediated antibacterial activity. How mitochondrial metabolism influences immune cell function, and what signaling cascades help drive cell-intrinsic metabolic modes in immune cells is poorly understood, and an area of intense interest.

Using a live zebrafish infection system we provide the first description of an immune cell function for the infection-responsive gene Immunoresponsive gene 1 (IRG1). IRG1 is a known LPS-responsive gene within macrophage-lineage cells. The encoded protein localizes to the mitochondria and is most closely related to bacterial 2-methylcitrate dehydratase (encoded by the prpD gene), an enzyme required for the catabolism of the short-chain fatty acid propionate. We describe a novel role for the zebrafish ortholog of IRG1 (Irg1) during the utilization of fatty acids as a fuel for OXPHOS and mROS production. In a zebrafish infection model, infection-responsive expression of zebrafish irg1 is specific to macrophage-lineage cells and is regulated cooperatively by glucocorticoid and JAK/STAT signaling pathways. Irg1-depleted macrophage-lineage cells are impaired in their ability to utilize fatty acids as an energy substrate for OXPHOS and mROS production and defective in their bactericidal activity and migratory capacity. These results reveal IRG1 as a key component of the immunometabolism axis, connecting infection, metabolism and macrophage function. Given its activities during metabolism and inflammation, IRG1 represents a potential target to manipulate the interface between the metabolic and inflammatory pathways.

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Vitamin A-Triggered Antimicrobial Activity against *M. tuberculosis* is Dependent on NPC2-Mediated Cholesterol Regulation and Is Blocked by HIV-Protease Inhibitors

Philip T. Liu[1, 2], Matthew Wheelwright[2], Elliot W. Kim[1], Stephan R. Krutzik[2]

[1]UCLA and Orthopaedic Hospital Department of Orthopaedic Surgery and the Orthopaedic Hospital Research Center, Los Angeles, CA 90095.; [2]Division of Dermatology, Department of Medicine, David Geffen School of Medicine at University of California, Los Angeles, CA 90095

One key function of the innate immune response is the rapid detection and elimination of microbial invaders, which can be influenced by micronutrients. A role for vitamin A in the host defense against *Mycobacterium tuberculosis* has been suggested through epidemiological and in vitro studies; however, the antimicrobial mechanism remained unclear. Here, we demonstrate that treatment of *M. tuberculosis*-infected human monocytes with either all-*trans* retinoic acid (ATRA) or 1, 25-dihydroxyvitamin D3, the biologically active forms of vitamin A and vitamin D respectively, resulted in mechanistically distinct antimicrobial activity against the pathogen. Comparison of the ATRA and 1, 25D3 elaborated gene expression profiles revealed that ATRA triggered a lipid metabolism and efflux pathway in human monocytes. Accordingly, stimulation with ATRA but not 1, 25D3 led to decreased total cellular cholesterol content and altered intracellular lipid distribution, both of which were dependent upon expression and function of Niemann-Pick disease type C2 (NPC2). Infection of human monocytes with M. tuberculosis, resulted in downregulation of NPC2 expression, which was recovered by ATRA treatement. Knockdown of NPC2 expression resulted in the loss of ATRA- but not 1, 25D3-induced antimicrobial activity against *M. tuberculosis*. The addition of HIV-protease inhibitors known to inhibit cholesterol efflux, ritonavir and nelfinavir, blocked both ATRA-induced cholesterol decrease as well as antimicrobial activity. Taken together, these results suggest that the vitamin A-mediated host defense mechanism against *M. tuberculosis* requires regulation of cellular cholesterol which can be inhibited by HIV-protease inhibitors, providing a potential mechanism for exacerbation of tuberculosis during HIV-treatment.

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Modulation of Macrophage Signaling and Apoptotic Cell Vacuole Trafficking by Complement Protein C1q during the Uptake of Apoptotic Cells

Marie E . Benoit, Andrea J. Tenner
Department of Molecular Biology and Biochemistry, Institute for Immunology, University of California Irvine, Irvine, CA

The complement system is a powerful effector mechanism of the innate immune system critical for host defense through its capacity to recognize pathogens and upon activation to mediate their lysis and removal. The initial component of the classical complement pathway, C1q, independent of the rest of the complement components, is an activation ligand for phagocytosis, rapidly triggering enhanced phagocytic activity when presented to phagocytes immobilized on a surface or bound to the particle to be ingested such as apoptotic cells. While the capacity of C1q to enhance uptake of apoptotic cells has been reported for many years, the mechanisms and signaling cascades associated with this enhancement of uptake as well as the consequences on the maturation of the apoptotic cell vacuole are not well characterized. Here, we provide direct evidence that C1q enhances the uptake of apoptotic cells by promoting macropinocytosis in human-monocyte derived macrophages (HMDMs). Significantly more FITC-Dextran, a soluble fluorescent molecule that can be ingested only by macropinocytosis, co-localizes with C1q-bound apoptotic cells ingested by HMDMs than with ingested apoptotic cells not bound to C1q. In addition, C1q increases the activation of Akt, implicating PI3K activation which is required at multiple steps of particle uptake and subsequent vacuole trafficking. Finally, C1q accelerates the maturation of the apoptotic cell vacuole since C1q-bound apoptotic cell vacuoles acquire the late endosomal marker LAMP-1 more rapidly than vacuoles with apoptotic cells not bound to C1q. In summary, these results show that C1q directly modulates early steps in the uptake process in macrophages, regulating the activation of the PI3K-Akt pathway and the subsequent maturation of the apoptotic cell vacuole. Further investigation using specific PI3K inhibitors will determine whether these pathways stimulated by C1q are involved in the subsequent modulation of inflammation during the macrophage clearance of apoptotic cells in human macrophages.

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Distinct Subpopulations of Activated Macrophages Accumulate in the Liver during Acetaminophen-Induced Hepatotoxicity

Ana-Cristina Dragomir[1], Richard Sun[1], Hyejeong Choi[1], Jeffrey D. Laskin[2], Debra L. Laskin[1]
[1]Department of Pharmacology and Toxicology, Rutgers University; [2]Department of Environmental and Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ

Activated macrophages have been implicated in hepatotoxicity induced by the analgesic, acetaminophen (APAP). However, the exact contribution of these cells to the pathogenic process depends on their timing of appearance and their phenotype. In these studies we characterized the phenotype of macrophage subpopulations accumulating in the liver following APAP intoxication. Treatment of wild type mice with a hepatotoxic dose of APAP (300 mg/kg, i.p.) resulted in increases in serum transaminases, beginning within 6 h, and histological evidence of hepatic centrilobular necrosis, which persisted for 24-48 h. This was associated with a time-related increase in the number of $CD11b^+$ macrophages in the liver. Flow cytometry/cell sorting revealed that $CD11b^+$ cells consisted of two distinct subpopulations, which expressed high or low levels of the monocyte/macrophage activation marker Ly6C. $CD11b^+/Ly6C^{hi}$ macrophages exhibited a classically activated proinflammatory phenotype characterized by increased expression of TNF-α, CCR2, inducible nitric oxide synthase (iNOS), and the proinflammatory lectin, Gal-3. In contrast, $CD11b^+/Ly6C^{lo}$ macrophages were alternatively activated, expressing high levels of the anti-inflammatory cytokine, IL-10. Immunostaining indicated that Ly6C-positive macrophages were distinct from F4/80-positive Kupffer cells. Treatment of mice with liposome-encapsulated clodronate prior to APAP resulted in exaggerated hepatotoxicity, as measured by increases in serum transaminase levels relative to mice pretreated with empty liposomes. This was associated with increased numbers of $Ly6C^{hi}$ macrophages in the liver, increased Gal-3 expression, and a decrease in F4/80-positive macrophages. These results indicate that both classically and alternatively activated macrophages accumulate in the liver following APAP intoxication; moreover, clodronate-containing liposomes exert multiple effects on activated macrophage subpopulations in APAP-injured livers. Supported by NIH grants GM034310, ES004738, CA132624, AR055073 and ES005022.

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Generation of Macrophage-EGFP Reporter Rats

Clare Pridans, Simon Lillico, David A. Hume
The Roslin Institute and Royal (Dick) School of Veterinary Studies

Differentiation of BM progenitors into macrophages requires Csf1 which signals via its receptor Csf1r (whose expression is limited to trophoblasts and cells of the macrophage lineage). MacGreen mice were created previously by placing EGFP expression under the control of the Csf1r proximal promoter. These mice have consistent expression of EGFP in the same locations as the endogenous gene and provide a valuable tool for those interested in macrophage biology. Our laboratory is taking advantage of the recent breakthroughs in rat transgenesis to generate macrophage-EGFP reporter rats via a lentiviral vector. As the successful use of lentivirus' are dependent on the size of the construct (i.e.: max 6-7kb between the two LTRs), we have produced a 'cut down' version of the Csf1r-EGFP construct used to produce the MacGreen mice. This lentivirus has been used to successfully transduce both mouse and rat macrophages and the specificity has been tested by transducing rat bone marrow cells. The production of these MacGreen rats is currently underway and the progress will be presented and discussed.

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AMP-Activated Protein Kinase Regulates Myeloid APC Activity

Kelly C. Carroll, Jill Suttles
University of Louisville

AMP-activated protein kinase, AMPK, is a serine/threonine kinase that regulates energy homeostasis and metabolic stress in eukaryotes. Previous work from our laboratory demonstrated a role of AMPK as a negative regulator of inflammatory activity in macrophage cell lines. With use of AMPK-deficient mice, we have further demonstrated that absence of AMPK expression in both primary dendritic cells and macrophages results in heightened inflammatory function and an enhanced capacity for antigen presentation. AMPK-deficient dendritic cells and macrophages produced higher levels of the pro-inflammatory cytokines IL-6 and TNF-α and decreased production of the anti-inflammatory cytokine IL-10 in response to LPS as compared to wild-type cells. Additionally, deficiency of AMPK in myeloid APC increased their capacity to promote Th1 and Th17 responses in antigen presentation assays. A comparison of the role of AMPK in myeloid APC versus T cells revealed a significant contribution of AMPK in promoting a pro-inflammatory response in both cell types, with the strongest drive towards Th1 and Th17 function occurring when both APC and T cells lacked AMPK. Since the CD40-CD154 interaction is crucial to effective APC activity we examined the influence of AMPK on CD40 signal transduction. Stimulation of AMPK-deficient dendritic cells with a soluble, multimeric CD154 resulted in increased production of IL-6 and decreased production of IL-10. Investigation of CD40-activated signaling pathways showed that CD154-stimulation resulted in increased phosphorylation of ERK1/2, p38, and NF-κB p65 and decreased Akt, GSK3β, and CREB activity in dendritic cells deficient for AMPK. Overall, our data indicate that AMPK serves to attenuate pro-inflammatory activity of myeloid APC and that AMPK activity in both APC and T cells contributes to Th cell functional polarization during antigen presentation.

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HIV-1 Baits and Exploits Cross-Reactive CTL to Promote Dysfunctional Programming of Pro-inflammatory Dendritic Cells

Robbie B. Mailliard[1], Kellie N. Smith[1], Ronald J. Fecek[1], Simon C. Watkins[1], James I. Mullins[2], Charles R. Rinaldo[1]
[1]University of Pittsburgh; [2]University of Washington

The ability of HIV-1 to survive rapidly accumulating mutations provides the virus with an effective means of escaping CD8+ cytotoxic T lymphocyte (CTL) responses. Here we describe how subtle alterations in CTL epitopes expressed by naturally occurring HIV-1 variants can result in an incomplete escape from CTL recognition, providing the virus with a selective advantage. Rather than paralyzing the CTL response, these epitope modifications selectively induce CTL "helper" activity while inhibiting their killing capacity. Importantly, instead of dampening the immune response through CTL elimination of variant antigen-expressing immature dendritic cells (iDC), a positive CTL-to-DC immune feedback loop dominates whereby CTL program the iDC to differentiate into mature DC (mDC). However, unlike standard mature which typically have a diminished capacity to produce cytokines, the CTL-programmed mDC have an enhanced capacity to produce a number of proinflammatory factors, including IL-12, IL-6, CXCL10 and CCL5. In addition, these CTL-programmed mDC are uniquely capable of forming long, interconnected nanotubes as determined by confocal and scanning electron microscopy. This discordant induction of CTL "helper" activity in the absence of killing, resulting from altered peptide presentation, likely contributes to the chronic immune activation associated with HIV-1 infection, and could be utilized by HIV-1 to promote viral spread and persistence.

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Differential ISG12 Family Gene Expression Is Associated with Monocyte Apoptosis Sensitivity in High HIV-1 Viremic Patients versus Apoptotic Resistance in Low Viremic Patients

Sean C. Patro[1, 2], Bethsebah N. Gekonge[1], Ravi Gupta[1], Sharmistha Pal[1], Ramana Davuluri[1], Jay Kostman[3], Karam Mounzer[4], Luis J. Montaner[1]
[1]The Wistar Institute, Philadelphia, PA; [2]Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA; [3]Hospital of the University of Pennsylvania, Philadelphia PA; [4]Philadelphia Field Initiating Group for HIV-1 Trails (FIGHT), Philadelphia, PA

While T-cells of HIV-1 patients show increased apoptotic sensitivity, our group has described resistance to induced apoptosis in monocytes during chronic stage HIV-1 infection. We have proposed survival mechanisms including zinc content, metallothionein gene expression, p53/Rb1 protein activity, and a role for CCR5 signaling. However, others have described increased apoptosis sensitivity in monocytes upon HIV-1/inflammatory mediator treatment in vitro and increased monocyte turnover associated with disease progression in SIV disease in vivo, suggesting inflammatory burden or late stage disease may alter monocyte apoptosis beyond chronic disease. We addressed chronic and late stage disease in HIV-1 patients using HiSeq Deep RNA sequencing for in vivo gene expression paired with apoptosis induction ex vivo in both monocytes and T-cells. Patients were stratified by viral load and compared to uninfected donors [uninfected (n=2), low viral load (LVL), <100, 000 copies/mL (n=2), high viral load (HVL), >100, 000 copies/mL (n=2)]. Interestingly, HVL (mean VL 294, 975 copies/mL) patients had higher induced apoptosis than LVL (mean VL 22, 646 copies/mL) patients, but not higher than uninfected controls. Additional studies (n=20 donors) revealed monocyte apoptosis sensitivity was positively associated with HIV-1 viral load (Figure 1). RNA-Seq results showed a strong type I interferon (IFN) gene signature was selectively present in HVL monocytes relative to LVL and uninfected monocytes. Among differentially expressed genes, we focused analysis on ISG12 family genes that were highly expressed in HVL monocytes and are known to regulate apoptosis. IFI27 (ISG12a), a pro-apoptotic regulator, was expressed ~2000-fold over uninfected (1962.0) in HVL monocytes and ~50-fold (46.9) in LVL monocytes. By contrast, IFI6 (G1P3), an anti-apoptotic member, was expressed ~20-fold (20.9) in HVL

monocytes and ~6-fold (5.8) in LVL monocytes. Differential ISG12 family expression in HVL patients may result from conditioning of the immune environment during sustained high viral load in vivo, as treatment of uninfected monocytes with IFN-α in vitro induces dose- and time-dependent up-regulation of ISG12 genes. We propose that differential ISG12 family expression may contribute to overcoming established mechanisms of apoptosis resistance in chronic stage infection, thereby increasing monocyte apoptosis (or turnover) in association with HIV-1 disease progression.

Figure 1. Monocyte Apoptosis is Associated with HIV-1 Viral Load. Monocytes from HIV-1 donors were incubated with 20 μM CdCl2 for 24 hours to induce apoptosis. Spearman correlation of HIV-1 viral load (Log10) (x-axis) by induced apoptosis sensitivity assayed by active caspase-3 staining (y-axis).

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Intravenously-Infused Mesenchymal Stem Cells Promote Survival of Corneal Allograft by Reducing the Activation and Migration of CCR7-Expressing Dendritic Cells and Macrophages

Joo Youn Oh

Ophthalmology, Seoul National University Hospital

Mesenchymal stem/progenitor cells (MSCs) were reported to enhance the survival of cellular and organ transplants. However, their mode of action was not established. We here demonstrated that peri-transplant intravenous (IV) infusion of human MSCs (hMSCs) decreased the number of MHC II+ dendritic cells (DCs) and M1 macrophages in the cornea. In draining lymph nodes (DLNs; cervical LNs ipsilateral to the transplanted eye), the proportions of DCs, both MHC II^+ $CD11b^+$ $CD11c^+$ cells and MHC II^+ $CD11b^-$ $CD11c^+$ cells, were significantly decreased in mice treated with hMSCs. In addition to DCs, the proportion of MHC II^+ $CD11b^-$ $CD11c^+$ cells representing macrophages was significantly decreased in the group treated with IV hMSCs. Especially, IV hMSCs decreased the expression of chemokine (C-C motif) receptor 7 (CCR7) and its ligand CCL21 in the cornea and DLNs, key homing molecules for LC trafficking from the inflamed cornea to DLNs. Collectively, data demonstrated that IV hMSCs reduced the activation of antigen presenting cells in the cornea and DLNs. Therefore, the afferent limb of the alloimmune response was inhibited and subsequently, immune rejection was decreased. The observations may account for the favorable effects of MSCs seen previously in models of solid organ and cellular transplantation. Moreover, the data provide a basis for using MSCs to improve the survival of transplants of the cornea and possibly other organs.

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PEGylation of FVIII Results in Reduced Endocytosis by Dendritic Cells and Reduced Activation of FVIII-Specific T cells

Pedro E. Paz[1], Jinger Xie[1], Lorenz Fuelle[2], Fred Aswad[1]

[1]Biologics Research/Bayer Healthcare, San Francisco, USA; [2]Rheinische Friedrich-Wilhelms-Universität Bonn, Germany; [3]Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

An immune response to Factor VIII (FVIII), which results in the development of neutralizing antibodies, is the most significant complication in Hemophilia patients receiving FVIII replacement therapy. BAY 94-9027 is a PEGylated B-domain-deleted FVIII (PEG-FVIII) that exhibits coagulation activity equivalent to non-PEGylated full length FVIII but displays a longer circulating half-life and, consequently, extended efficacy in preclinical bleeding models. We evaluated the immunogenicity of PEG-FVIII in mice and human cells pre-clinically.

In vivo studies in hemophilia A (HemA) mice reveal reduced immunogenicity for PEG-FVIII as evidenced by a lower frequency of anti-FVIII antibody-positive mice and lower anti-FVIII antibody titers compared with full length FVIII treated mice. Ex vivo analysis of the activity of T cells isolated from PEG-FVIII treated mice also showed a correspondingly reduced FVIII-induced T cell response. The weak T cell activity, and ultimately the reduced antibody response against PEG-FVIII, correlated with poor antigen uptake as splenic dendritic cells (DC) from naive HemA mice failed to internalize the PEG-FVIII protein. Extending this study to human cells, PEG-FVIII also exhibited reduced DC uptake, which was manifested in an abrogated proliferative and cytokine response of FVIII-specific T cell lines generated from inhibitor positive hemophilia patients. FVIII has been shown to be internalized via the mannose receptor (MR, CD206) by human DC. Interestingly, FVIII uptake via the mannose receptorappears to be donor dependent as some donor DC was insensitive to mannan inhibition.

Originally rationalized as an approach for extending protein half-life in vivo, PEGylation of FVIII appears to provide the added benefit of reducing immunogenicity by presumably evading DC uptake for antigen presentation. Whether a reduction in DC uptake through PEG modification universally leads to reduced immunogenicity for other biotherapeutics is unclear. Furthermore, it remains to be seen what specific steps in the MHC processing pathway the PEG modification has exerted its de-immunizing effect on FVIII. Nonetheless, the observation of reducedPEG-FVIII immunogenicity in vitro with human cells could have significant implications if it is reproduced in clinical practice.

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Dectin-1 Triggered Recruitment of LC3 to Phagosomes Facilitates MHC Class II Presentation of Fungal-Derived Antigens

Jun Ma[1], Courtney Becker[1], Clifford A. Lowell[2], David M. Underhill[1, 3]

[1]1Division of Immunology, Cedars-Sinai Medical Center, Los Angeles, CA 90048; [2]Department of Laboratory Medicine, University of California, San Francisco, 94143 ; [3]3Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048

Dectin-1 is a pattern recognition receptor that is important for innate immune responses against fungi in humans and mice. Dectin-1 binds to β-glucans in fungal cell walls and triggers phagocytosis, production of reactive oxygen by the NADPH oxidase, and inflammatory cytokine production which all contribute to host immune responses against fungi. Although the autophagy pathway was originally characterized for its role in the formation of double-membrane compartments engulfing cytosolic organelles and debris, recent studies have suggested that components of the autophagy

pathway may also participate in traditional phagocytosis. In this study, we show that Dectin-1 signaling in macrophages and bone marrow-derived dendritic cells triggers formation of LC3II, a major component of the autophagy machinery. Further, Dectin-1 directs the recruitment of LC3II to phagosomes, and this requires Syk, activation of reactive oxygen production by the NADPH oxidase, and ATG5. Using LC3-deficient dendritic cells we show that while LC3 recruitment to phagosomes is not important for triggering phagocytosis, killing or Dectin-1-mediated inflammatory cytokine production, it facilitates recruitment of MHC class II molecules to phagosomes and promotes presentation of fungal-derived antigens to CD4 T cells

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Impact of Protective KIR/HLA Genotypes on NK Cell and T Cell Function in HIV-1 Infected Controllers

Luis J. Montaner[1], Costin Tomescu[1], Fuh-Mei Duh[2], Rebecca Hoh[3], Anne Viviani[3], Kara Harvill[3], Maureen P. Martin[2], Mary Carrington[2], Steven G. Deeks[3]

[1]The Wistar Institute; [2]NCI Frederick; [3]The University of California, San Francisco

Both protective T cell genotypes (ex: HLA-B*57) and Natural Killer cell genotypes (ex: KIR3DL1*h/*y) have been shown to be co-inherited in HIV-1 infected subjects who limit viral replication in absence of antiretroviral therapy (Controllers). Likewise, the combined genotype of HLA-B*57 with KIR3DL1*h/*y confers more protection from HIV-1 disease progression than either genotype alone suggesting that a synergistic response between the adaptive and innate immune compartment may work in concert to limit viral replication. However, a comparative analysis of the genotype and function of the innate and adaptive immune compartments in HIV-1 infected controller subjects has been understudied to date. Here, we simultaneously tested NK and T cell function in controllers to investigate the mechanism(s) that might account for host-immune control over HIV-1 replication. We measured $CD8^+$ T cell responses against HIV-1 utilizing overlapping 15-mer peptides spanning the HIV-1 Consensus Clade B Gag protein and tested NK cell degranulation and cytokine secretion against tumor target cells in the presence or absence of IFN-alpha stimulation. Among a cohort of 37 controllers, the presence of protective MHC-Class I HLA alleles was not correlated with HIV-1 Gag-specific $CD8^+$ responses. In contrast, the inheritance of a protective KIR3DL1*h/*y receptor genotype along with it's corresponding HLA-Bw4*80I ligand was associated with significantly heightened target cell-induced NK cell degranulation and cytokine production following IFN-alpha stimulation. Interestingly, we observed a significant inverse association between target cell-induced NK activity and HIV-1 Gag-specific $CD8^+$ T cell responses among elite controllers. Together, these results suggest that heightened NK responses can be evidenced independently of HIV-specific T cell responses in HIV-1 infected controllers and support the potential regulatory role of NK cells in modulating anti-viral CD8 responses during chronic HIV-1 infection.

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Notch-RBP-J Signaling Regulates IRF8 to Promote Inflammatory Macrophage Polarization

Sinead M. Smith[1], Haixia Xu[1], Jimmy Zhu[1], Julia Foldi[2], Baohong Zhao[1], Allen Y. Chung[1], Hasina Outtz[3], Jan Kitajewski[3], Chao Shi[2], Silvio Weber[4], Paul Saftig[4], Yeuming Li[5], Keiko Ozato[6], Carl Blobel[1], Lionel B. Ivashkiv[1, 2], Xiaoyu Hu[1, 7]

[1]Hospital for Special Surgery, New York, NY, USA; [2]Weill Cornell Graduate School of Medical Sciences, New York, NY, USA; [3]Columbia University, New York, NY, USA; [4]Kiel University, Kiel, Germany; [5]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [6]National Institute of Health, Bethesda, MD, USA; [7]Weill Cornell Medical College, New York, NY, USA

Introduction: The selective transcription of functionally related subsets of genes in response to environmental stimuli is important for achieving appropriate immune responses. Macrophages can assume a spectrum of activation states in response to environmental cues, ranging from classically activated M1 inflammatory macrophages to various alternatively activated macrophages involved in immunoregulation and tissue repair. Emerging evidence has linked specific transcription factors to functional macrophage phenotypes. The Notch signaling pathway regulates cell differentiation, proliferation, survival and development. Ligation of Notch receptors by their ligands results in the release of the Notch intracellular domain (NICD), which translocates to the nucleus where it interacts with the DNA-binding protein RBP-J to modulate target gene expression. This study aimed to elucidate the role of the Notch-RBP-J signaling pathway in macrophage polarization. **Results:** By comparing wild-type and RBP-J-deficient macrophages, we found that RBP-J was required for the expression of a subset of TLR4-inducible genes, including the M1 genes encoding IL-12 and iNOS. Following endotoxin challenge, mice with myeloid-specific deletion of RBP-J had significantly lower serum concentrations of IL12p40 than their wild-type littermates. In addition, RBP-J-deficient mice were more susceptible to infection with the intracellular pathogen *Listeria monocytogenes*. Further, we demonstrated that RBP-J is required for rapid LPS-mediated protein synthesis of the transcription factor IRF8 and recruitment of IRF8 to the *Il12b* promoter. Using a gain-of-function approach, we showed that constitutive expression of NICD1 in macrophages augments IRF8 expression and LPS-mediated M1 gene induction. IRF8 reconstitution in RBP-J-deficient macrophages by retroviral transduction restored TLR4-mediated *Il12* expression. Increased LPS-induced IRF8 protein expression was not due to upregulation of *Irf8* mRNA expression at the corresponding time-points, suggesting the involvement of post-transcriptional regulatory mechanisms. Indeed, RBP-J promoted IRF8 protein synthesis by selectively controlling expression of the proximal TLR signaling component IRAK2 and TLR4-mediated activation of the kinase MNK1 and it's downstream target, eIF4E that controls the initiation of translation. **Conclusion:** Our findings provide a functional link between the Notch-RBP-J pathway and the IRF family of transcription factors, and identify a mechanism by which RBP-J and TLR4 signaling are integrated to induce translation of IRF8 to selectively regulate genes important for inflammatory macrophage polarization.

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microRNA Expression Regulation during Monocyte-to-Macrophage Differentiation and Activation

Joel W. . Graff[1, 2], Renee L. Eigsti[2], Mary E. Wilson[1, 2]
[1]Iowa City VA Medical Center; [2]University of Iowa Carver College of Medicine

MicroRNAs (miRNAs) are regulatory molecules that often form feedback loops to enhance or antagonize signaling pathways involved in activation of monocytic cells including monocytes and macrophages. We hypothesized that the characterization of miRNAs with regulated expression during monocytic cell activation would identify therapeutic targets for controlling the function of monocytic cells. Using quantitive RT-PCR (RT-qPCR), we confirmed that the addition of LPS augmented the expression of miR-125a-5p, miR-146a, and miR-155 in primary human monocytes purified using CD14 microbeads. In contrast, IFNbeta and IFNgamma inhibited the accumulation of several miRNAs and antagonized LPS-mediated induction of miR-125a and miR-146a. While monitoring the effects of polarizing stimuli on miRNA expression, we found that the expression of all miRNAs examined to date increased in human monocytes during ex vivo culture in the absence of activating stimuli. The increase in basal level of miRNAs during monocyte-to-macrophage differentiation resulted in small amplitude of miRNA expression changes in fully differentiated, strongly polarized monocyte-derived macrophages. It was previously reported that Dicer protein levels accumulate dramatically during macrophage differentiation. However, we did not detect increased Dicer abundance in immunoblots using samples derived from two standard monocyte-to-macrophage differentiation methods, purification of adherent cells from PBMC cultures and M-CSF-stimulated differentiation of purified monocytes. We hypothesized that, rather than increased miRNA processing capability, the accumulation of miRNAs during monocyte-to-macrophage differentiation could be regulated at the transcriptional level. We used RT-qPCR to study the abundance of primary miRNAs, the transcripts that are processed to yield mature miRNAs, in human monocytes and macrophages for the abundance of primary miRNAs. As was seen with the mature miRNA forms, pri-miR-125a and pri-miR-222 had elevated expression in macrophages compared to freshly isolated monocytes. These results are consistent with increased transcription or stability of pri-miRNAs as a mechanism for miRNA accumulation during monocyte-to-macrophage differentiation. Other potential contributing factors to increased miRNA abundance during monocyte-to-macrophage differentiation include increased miRNA processing efficiency and increased miRNA stability. The accumulation of miRNAs may have an important role in regulating the monocyte-to-macrophage differentiation process. Furthermore, these differences could impact miRNA-based therapeutic strategies since overexpression of miRNAs may result in a more potent phenotype in monocytes compared to macrophages due to the inherent differences in basal miRNA expression.

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Identification of Interleukin-1 Receptor-Associated Kinase 1 as a Critical Component That Induces Post-transcriptional Activation of IκB-ζ

Tatsushi Muta, Tomoyuki Ohba, Yujiro Ariga, Takashi Maruyama
Laboratory of Cell Recognition and Response, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

IκB-ζ, an essential inflammatory regulator, is specifically induced by Toll-like receptor (TLR) ligands or interleukin (IL)-1β by post-transcriptional activation mediated via a 165-nt element in IκB-ζ mRNA. Here, we analyzed the TLR/IL-1 receptor signaling components involved in the post-transcriptional regulation of IκB-ζ with mutated estrogen receptor (ER(T2))-fusion proteins. Upon 4-hydroxytamoxifen treatment, the ER(T2)-fusion proteins with IRAK1 and IRAK4 elicited specific activation of a reporter gene for the post-transcriptional regulation of IκB-ζ. The TRAF6-ER(T2) protein activated nuclear factor-κB, but not the post-transcriptional regulation, indicating that the activation of IRAK1/4, but not that of TRAF6, are sufficient to activate the 165-nt element-mediated post-transcriptional mechanism. Interestingly, the post-transcriptional mechanism was not activated in TRAF6-deficient cells, indicating an essential role for TRAF6. Thus, the signaling pathway leading to nuclear factor-κB activation and the post-transcriptional activation bifurcates at IRAK1, suggesting a new pathway activated by IRAK1.

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microRNA-21 Targets the Vitamin D-Dependent Antimicrobial Pathway in Leprosy

Philip T. Liu[1, 2], Matthew Wheelwright[2], Rosane Teles[2], Evangelia Komisopoulou[3], Kristina Edfeldt[2], Benjamin Ferguson[2], Manali D. Mehta[4], Aria Vazirnia[4], Thomas H. Rea[5], Euzenir N. Sarno[6], Thomas G. Graeber[3], Robert L. Modlin[2, 4]
[1]Orthopaedic Hospital Research Center, University of California at Los Angeles, CA 90095, USA; [2]Division of Dermatology, Department of Medicine, David Geffen School of Medicine at University of California at Los Angeles, CA 90095, USA; [3]Crump Institute for Molecular Imaging, Institute for Molecular Medicine, Jonsson Comprehensive Cancer Center, California NanoSystems Institute, Department of Molecular and Medical Pharmacology, University of California, Los Angeles, CA 90095, USA; [4]Department of Microbiology, Immunology and Molecular Genetics, at University of California at Los Angeles, CA 90095, USA; [5]Department of Dermatology, University of Southern California School of Medicine, Los Angeles, CA; [6]Leprosy Laboratory Institute Oswaldo Cruz, Rio de Janeiro, Brazil.

Leprosy provides a model to investigate mechanisms of immune regulation in humans, given that the disease forms a clinical-immunological spectrum. Here, we identified 13 miRNAs that were differentially expressed in the lesions of patients with progressive lepromatous (L-lep) vs. the self-limited tuberculoid (T-lep) disease. Bioinformatic analysis including the Kolmogorov-Smirnov-based permutation test revealed a significant enrichment of L-lep-specific miRNAs that preferentially target key immune genes downregulated in L-lep vs. T-lep lesions. The most differentially

expressed miRNA in L-lep lesions, hsa-mir-21, was upregulated in *M. leprae*-infected monocytes and induced by the mycobacterial virulence factor phenolic glycolipid-I (PGL-I). Hsa-mir-21, by downregulating Toll-like receptor 2/1-induced CYP27B1 and IL1B as well as upregulating IL-10, inhibited the vitamin D-dependent antimicrobial peptides, CAMP and DEFB4, gene expression. Conversely, knockdown of hsa-mir-21 in M. leprae-infected monocytes enhanced expression of CAMP and DEFB4. Introduction of hsa-mir-21 was sufficient to block TLR2/1-induced antimicrobial responses against *M. tuberculosis* infection, whereas, silencing of hsa-mir-21 induction restored TLR2/1-mediated antimicrobial activity against *M. leprae*. Therefore, the ability of *M. leprae* to upregulate hsa-mir-21 targets multiple genes associated with the immunologically localized disease form, providing an effective mechanism to escape from the vitamin D-dependent antimicrobial pathway.

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The Role of microRNAs in Macrophage Responses to Contrasting Stimuli

Erik B. Wendlandt[1, 2], Joel W. Graff[1, 2], Theresa L . Gionannini[1, 2], Anton P. McCaffrey[3], Mary E. Wilson[1, 2]
[1]University of Iowa Iowa City, IA; [2]Veterans Affairs Iowa City, IA; [3]TriLink BioTechnologies

Macrophages are sentinels that help shape the immune response. Classically activated (M1) macrophages acquire microbicidal competence that can lead to clearance of intracellular microorganisms. Macrophages can also undergo a non-classical (M2) activation, resulting in different polar phenotypes. Treatment of macrophages with the toll-like receptor 4 (TLR4) agonist LPS plus interferon-gamma induces a strong M1 polarization. In contrast, we showed that infection with the protozoan Leishmania leads to a non-classical polarization state which results in a pro-survival environment for the parasites. We explored the role of microRNAs (miRNAs) in these opposing activation phenotypes. Several adaptor molecules involved in TLR signaling were identified as putative targets of miR-200a, miR-200b and miR-200c. MiR-200b and miR-200c, but not miR-200a, were found to antagonize TLR4 signaling and NF-kappaB activation in both HEK293-TLR4 cells and differentiated THP-1 cells. This can be explained, at least in part, by miR-200b and miR-200c targeting and repressing the expression of the adaptor molecule MyD88. The involvement of miRNAs in macrophage activation prompted an investigation of miRNA involvement in the macrophage M2-type activation state induced by Leishmania parasites. Human macrophages were infected with either virulent or avirulent Leishmania parasites. MiRNA expression profiling was performed and identified 11 miRNAs differentially regulated during infection. Of particular interest, miR-200b was downregulated during infection with avirulent but not virulent parasites, leading us to speculate that TLR pathway suppression through miR-200b is important for Leishmania survival. We also note miR-125a-3p, a miRNA that increases during M1 activation, was significantly suppressed during infection with avirulent parasites. These results suggest that some miRNAs that are augmented by LPS-induced M1-type macrophage activation are significantly suppressed by Leishmania infection. We hypothesize that miRNA suppression contributes to the global changes in macrophage polarization toward the M2-like phenotype observed during Leishmania infection.

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Semaphorin7A Promotes Monocyte Migration and a Pro-tumorgenic Immune Shift in Breast Cancer

Ramon A. Garcia-Areas[1], Stephania Libreros[1], Lillian Onwuha-Ekpete[1], Kathy Schilling[2], Vijaya Iragavarapu-Charyulu[1]
[1]Florida Atlantic University, Schmidt College of Medicine, Boca Raton, USA; [2]Boca Raton Regional Hospital, Boca Raton, USA

The purpose of this study is to explore the novel expression of axonal guidance molecule Semaphorin7A (Sema7A) in breast cancer and the possible hijacking of monocytes by Sema7A to promote tumor growth. Our laboratory recently identified the expression and secretion of axonal guidance molecule Sema7A in breast cancer. No published studies have yet elucidated a role for Sema7A in cancer. We have evidence that in addition to be being expressed by breast cancer tumor cells, Sema7A is expressed at high levels in bone marrow monocytes and splenic macrophages of mice bearing 4T1 mammary carcinomas. Sema7A was originally discovered for its chemotactic activity in neurogenesis, but it is also a immune modulator via α1β1integrins. Sema7A mediates chemotaxis of peripheral blood monocytes and induces the production of the proinflammatory cytokines: IL-1β, IL-6, TNF-α, and IL-8. Murine macrophages treated with Sema7A significantly increased their expression of CXCL2/MIP-2 an angiogenic murine chemokine that is a homologue of human IL-8. This proinflammatory chemokine is known to promote leukocyte-mediated angiogenesis and tumor growth. In our studies, we find that tumor derived Sema7A not only serves as a direct monocyte chemoattractant, but it also up-regulates the expression of Chemokine (C-C motif) ligand 2 (CCL2) also known as monocyte chemotactic protein-1 (MCP-1). We postulate that tumor-derived Sema7A may serve as a monocyte chemoattractant and skew monocytes to a pro-tumorgenic phenotype. We are further investigating the expression of Sema7A in leukocytes of breast cancer patients of varying degrees of disease progression. A putative relationship between tumor-derived Sema7A and monocytes could prove valuable in establishing new research avenues towards unraveling important tumor-host immune interactions in breast cancer patients.

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Lithium Treatment Reduced Microglia Activation and Inflammation after Irradiation to the Immature Brain

Changlian Zhu[2], Cuicui Xie[1], Kai Zhou[1], Klas Blomgren[1]
[1]Center for Brain Repair and Rehabilitation, University of Gothenburg, Sweden; [2]Department of Pediatrics, the Third Affiliated Hospital, Zhengzhou University, China

To evaluate the effects of lithium on microglia activation and inflammation after irradiation to the immature brain, eight-day-old male mice were injected 2 mmol/kg lithium chloride i.p. on postnatal day 7 (P7), additional lithium injections, 1 mmol/kg, were administered at 24 h intervals. Pups were subjected to whole brain 6Gy irradiation on P11. The animals were sacrificed

at 6h and 24h after IR. Microglia activation at 6 hours after IR to the brain can be detected by counting their numbers, their size, engulfment of cell debris or by the production of chemokines and cytokines. Microglia were stained using the marker Iba-1, revealing the presence of these cells throughout the brain under normal conditions, and apparently higher numbers and sizes after IR, particularly in areas where cell death occurred. Quantification of Iba-1+ cells in the GCL of DG showed a 136.7 % increase after IR ($p<0.0001$) but a 26.8 % lower increase in the lithium-treated brains. Phagocytosis of dead cells and cellular debris could be detected through the appearance of TUNEL+ chromatin inside Iba-1+ cells. Amoeboid and phagocytosing microglia are larger than the ramified ones, and measuring the total area of the Iba-1+ cells showed an increase after IR, but a 9.7 % lower increase in the lithium-treated brains. The concentration of MCP-1, IL-1α, IL-1β, GRO/KC in the hippocampus were increased significantly at 6h after IR compared with non-irradiation control. Lithium treatment significantly inhibited the increase. There was no significantly difference at 24h after IR. However, there was no significant reduction in the SVZ area after lithium treatment at both 6h and 24h. Lithium can specifically reduce inflammation through GSK3-ß inhibition or modified microglia activation.

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Chloride Transport in Functionally Active Phagosomes Isolated from Human Neutrophils

Guoshun Wang

Dept. of Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center

Chloride anion is critical for hypochlorous acid production and microbial killing in neutrophil phagosomes. However, the molecular mechanism by which this anion is transported to the organelle is poorly understood. In the current report, membrane-enclosed and functionally active phagosomes were isolated from human neutrophils by using opsonized paramagnetic latex microspheres and a rapid magnetic separation method. The phagosomes recovered were highly enriched for specific protein markers associated with this organelle such as lysosomal-associated membrane protein-1 (LAMP-1), myeloperoxidase (MPO), lactoferrin (LF) and NADPH oxidase. When FITC-dextran was included in the phagocytosis medium, the majority of the isolated phagosomes retained the fluorescent label after isolation, indicative of intact membrane structure. Flow cytometric measurement of acridine orange, a fluorescent pH indicator, in the purified phagosomes demonstrated that the organelle in its isolated state was capable of transporting protons to the phagosomal lumen via the V-ATPase proton pump. The isolated phagosomes also showed active MPO-mediated halogenation. When hydrogen peroxide and radioactive iodide were provided, pronounced iodination of the proteins covalently conjugated to the phagocytosed beads was detected. The phagosomal uptake of iodide was significantly blocked by chloride channel inhibitors including ethacrynic acid (EA), niflumic acid (NA) and CFTRinh-172. Further experiments determined that the V-ATPase-driving proton flux into the isolated phagosomes required chloride co-transport and the cAMP-activated CFTR chloride channel was a major contributor for the chloride transport. Taken together, the data suggest that the phagosomal preparation described herein retains ion transport properties, and multiple chloride channels including CFTR are responsible for chloride supply to neutrophil phagosomes.

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The Role of SNAP-23 in Sepsis-Induced Neutrophil Respiratory Burst

Jianwen Bai[1], Joanne Neira. Lomas[2], Chun-Shiang Chung[2], Yaping Chen[2], Alfred Ayala[2], Silvia Uriarte[3]

[1]Dept. of Emergency Medicine and Critical Care, Shanghai East Hospital, Tong Ji University, Shanghai, China; [2]Dept. of Surgery, Brown University/RI Hospital, Providence, Rhode Island, USA; [3]Dept. of Medicine, University of Louisville, Louisville, Kentucky, USA

Sepsis is a potentially deadly medical condition that is characterized by a whole-body inflammatory state and the presence of a known or suspected infection. Neutrophils are crucial components of the innate immune response during sepsis. Sepsis-primed neutrophils migrate to sites of inflammation and release reactive oxygen species and lytic granules to kill invading pathogens, this process is called respiratory burst. Studies have shown that synaptosome-associated protein (SNAP)-23 can modulate human neutrophil exocytosis, demonstrating that vesicle SNAP receptor-target SNAP receptor (v-SNARE- t-SNARE) interactions modulate neutrophil secretion. However, the role of SNAP-23 in neutrophil respiratory burst during sepsis has not been elucidated. The objective of this study was to investigate whether SNAP-23 plays a role in regulating neutrophil respiratory burst induced by sepsis. Male C57BL/6 mice (8-10weeks) were used for all experiments. Sepsis was induced via cecal ligation and puncture (CLP). Neutrophils were isolated from whole blood of naïve, CLP or CLP-sham treated mice and respiratory burst was measured over 60 minutes at dual wave length 550 & 630 and 37ºC. Neutrophils isolated from peripheral blood of naïve mice were used to optimize dosage of SNAP-23 to suppress respiratory burst in vitro. Cells were incubated with 0.4μg/ml, 0.6μg/ml or 1.0μg/ml SNAP-23 at 37ºC for 10 minutes then stimulated with TNF-α. Respiratory burst capacity was measured following incubation with PMA. For in vivo study, CLP and sham-CLP mice were given 12μg (20 times in vitro dosage) SNAP-23 in 200ul HBSS by i.v. tail injection immediately after surgery. Serum, blood neutrophils and lung were collected 4 hours post surgery. Respiratory burst capacity was measured in blood neutrophils; cytokine levels in the lung and serum, and lung myeloperoxidase (MPO) were assessed. Results showed that SNAP-23 at the concentration of 0.6μg/ml significantly decreased the neutrophil respiratory burst by 45% compared to the positive control in vitro. At the dose of 12μg/mouse in vivo, the respiratory burst of isolated peripheral blood neutrophils and lung MPO activity from SNAP-23 treated CLP was significantly inhibited compared to PBS treated CLP mice, while no effect was observed on shams. While serum levels of KC, MIP-2, IL-6, TNF-alpha, MCP-1 and IL-10 were not affected by SNAP-23 treatment in our septic mouse, our studies show that SNAP-23 suppresses the respiratory burst capacity of sepsis-primed neutrophils as well as lung MPO activity during sepsis.(Funding support provided in part by NIH HL073525 to A.A. and HL087924 to S.U.)

ABSTRACTS

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IRAK-4 Is Essential for Phytohaemagglutinin to Upregulate the Expression of a Costimulatory Molecule CD80 as well as TNF-α Production in Human Monocytes

Soichi Haraguchi[1], Nutthapong Tangsinmankong[2], John W. Sleasman[2]
[1]Department of Immunology, Tampa Bay Research Institute; [2]Department of Pediatrics, University of South Florida

Engagement of Toll-like receptors (TLRs) by their ligands triggers intracellular signaling pathways, resulting in the induction of variety responses including production of proinflammatory cytokines and upregulation of the expression of costimulatory molecules. Those TLR-mediated events control adaptive immune responses. Interleukin (IL)-1 receptor-associated kinase 4 (IRAK-4) is an intracellular molecule that functions as a central mediator in the TLR signaling pathway. Accordingly, blood cells of patients with IRAK-4 deficiency fail to produce cytokines in response to TLR agonists. In the present study, we show that monocytes from our IRAK-4-deficient patient fail to upregulate the expression of a costimulatory molecule CD80 as well as intracellular TNF-α production in response to a plant lectin phytohaemagglutinin (PHA), as assessed by flow cyyometry. Monocytes from two healthy subjects expressed TNF-α in response to PHA (17.2 and17.3%) and PMA plus ionomycin (10.4 and 27.1%). In contrast, the patient's monocytes failed to express TNF-α in response to PHA (0.2%), while 11.6% of the monocytes expressed TNF-α upon activation of bypass signaling pathways with PMA plus ionomycin. In response to PHA, 27.4 and 27.7% of monocytes from two healthy controls expressed CD80. However, PHA-induced expression of CD80 on the patient's monocytes was significantly impaired (3.1%). CD80 expression on the patient's monocytes upon stimulation with IFN-γ was comparable with control monocytes (patient, 64.2%; controls, 48.1 and 62.0%). It is well known that PHA binds to the T-cell receptor (TCR) and activates T cells in a macrophage-dependent manner. Our results suggest that PHA may interact with a TLR(s) on human monocytes/macrophages, leading to the upregulation of costimulatory molecules as well as cytokines, crucial events for T-cell activation, through an IRAK-4 dependent manner. This "experiment of nature" provides a novel TCR triggering mechanism in which PHA may cross-link the innate immune receptor TLR with the adaptive immune receptor TCR.

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The GPR43 Agonists Phenylacetamide 1 and 2 Induce Granules Release in Bovine Neutrophils

Rafael A. Burgos, María D. Carretta, Iván Conejeros, Pablo Alarcón, Claudia Concha, Luis Molina, María A. Hidalgo
Institute of Pharmacology, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, CHILE

Free fatty acid receptor 2 (FFA2; GPR43) and Free Fatty Acid receptor 3 (FFA3; GPR41) are G protein-coupled receptors for short-chain fatty acids (SCFAs). Several SCFAs are implicated in inflammatory and metabolic disorders. GPR43 is activated by SCFAs such as propionate, which is produced in high concentrations by microbial fermentation in the rumen of cattle. We previously demonstrated that propionate induces granules release from bovine neutrophils, but remains unclear if this effect occurs through the direct actions of this SCFA on the GPR43 receptor. Recently, phenylacetamide-1 (PH-1) and phenylacetamide-2 (PH-2) have been described as potent GPR43 agonists.

In this study, we quantified the expression levels of bGPR43 and bGPR41 by Real-Time PCR. Afterward, the effect of 0, 1-10 micromolar of PH-1 and PH-2 on granules release in bovine neutrophils was assessed. Myeloperoxidase (MPO; primary granules), Lactoferrin (secondary granule), and matrix metalloproteinase 9 (MMP-9, tertiary granules), was measured by colorimetric assay, ELISA and zymography, respectively.

We observed that the expression of bGP43 mRNA was higher than bGPR41 mRNA. In Fura 2/AM loaded neutrophils, PH-1 and PH-2 stimulated calcium mobilization in a concentration-dependent manner. The calcium flux elicited by agonists were strongly inhibited with U73122, a PLC inhibitor, and 2-APB a SOCE blocker. This response was partially reduced with EGTA an extracellular calcium chelator. Either PH-1 or PH-2 induced lactoferrin and MMP-9 release, but not increased the MPO release. These results suggest that SCFA can mediate secondary and tertiary release in bovine neutrophils through GPR43 receptor.

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Linoleic Acid Increases Chemotaxis, Intracellular Calcium Mobilization, Granules Release and Gene Expression in Bovine Neutrophils

Maria A. Hidalgo, Carolina Manosalva, Ruben Ramirez, Jaqueline Mena, Lhia Chandia, Daniel Carroza, Maria D. Carretta, Rafael A. Burgos
Institute of Pharmacology, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, CHILE.

Linoleic acid is a long chain fatty acid released during lipomobilization in periparturient cows. This period is characterized by an immunosuppression and high prevalence of infectious diseases. Linoleic acid has been described as a ligand of the GPCR FFA1 (GPR40) in human pancreatic cells and induces superoxide production in human and rat neutrophils. Previously, we demonstrated that FFA1 is expressed in bovine neutrophils, therefore, in this study we analyzed the effect of linoleic acid on chemotaxis, intracellular calcium mobilization, granules release and gene expression.

Neutrophils were isolated by hypotonic lysis method from blood samples of healthy heifers. The purity and viability was greater than 95%. Chemotaxis was analyzed in a 3 μm-pore transwell system, intracellular calcium mobilization was studied by spectrofluorimetry using Fluo4-AM-loaded neutrophils, metalloproteinase-9 (MMP-9) release was detected by zymography, CD11b surface expression was assessed by flow cytometry, and COX-2 and IL-8 mRNA expression by real time PCR. We observed that linoleic acid (100 μM) significantly increased the chemotaxis of neutrophils, compared with the vehicle. The intracellular calcium mobilization was stimulated by linoleic acid, with a significant increase of the

area under curve at 50 and 100 μM. The analysis of granules release, measured as MMP-9 activity and CD11b expression, showed an increase of MMP-9 release at 50 and 100 μM of linoleic acid, and the stimulation of CD11b surface expression at 100 μM. Linoleic acid also induced the COX-2 and IL-8 mRNAexpression in neutrophils compared with the vehicle.

In conclusion, the results showed that linoleic acid activates bovine neutrophils, increasing the intracellular calcium mobilization, chemotaxis, release of MMP-9 and CD11b surface expression and induces gene expression.

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Reactive Oxygen Species and Estradiol in Regulating Acute Inflammation

Alexandra Stubelius[1], Annica Andersson[1], Rikard Holmdahl[2], Ulrika Islander[1], Hans Carlsten[1]

[1]Centre for Bone and Arthritis Research (CBAR), Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, ; [2]Medical Inflammation Research, Karolinska Institutet3, Stockholm, Sweden

Estradiol (E2) and reactive oxygen species (ROS) can both regulate inflammatory responses. E2 has a direct pro-inflammatory effect on macrophages in LPS triggered inflammation, but can also act as an anti-inflammatory agent, a dichotomy that needs to be further studied. ROS are used for microbial killing but can also have a regulatory role in inflammation. In models of rheumatoid arthritis (RA), we have shown that ovariectomy (ovx) increases cell infiltration in the joints and aggravates arthritis, whereas E2 treatment down regulates disease.

Common denominators for inflammatory conditions such as a flare in RA, acute inflammation and cardiac ischemia are the destructive processes from excess oxygen radicals (ROS). These conditions are known to be regulated by sex steroids. The aim of this study was to investigate a possible connection between E2 and ROS, using the LPS triggered air-pouch model of acute inflammation, analyzing cell infiltration into a confined space.

Female mice defective in ROS production through a homozygous mutation in the Ncf1 gene in B10.Q mice (Ncf1*/*) was used in this study, together with wild-type B10.Q (WT) mice. The mice were either sham operated or ovx, and an air pouch was induced. Ovx-mice were administered E2 or vehicle, while sham operated mice were administered vehicle, two days prior to LPS challenge. Mice were sacrificed 6 hours after challenge, and the air pouch exudates were analyzed for leukocyte content by flow cytometry. Cytokines and chemokines were analyzed using cytometric bead array while surrounding tissues were analyzed by immunohistochemistry.

The majority of cells collected from the pouch were neutrophils and macrophages, however lymphocytes were also present.

In WT mice, ovx-veh led to a pro-inflammatory profile, with massive neutrophil infiltration and a decrease in monocytes/macrophages (MO), compared to WT sham and WT ovx-E2 treated mice. In Ncf1*/* ovx-veh mice, neutrophil infiltration increased compared to sham mice, but in ovx+E2 treated Ncf1*/* mice, the neutrophil fraction did not decrease as in WT mice, but increased further.

The MO's expressed differences in their innate MO profile (TLR-activated). CD200R was down-regulated by ovx in both genotypes. However, Tim4 was up-regulated in wt-ovx mice, but down-regulated in Ncf1*/* ovx-veh mice. IL-6, MCP-1 and TNF increased in both WT ovx-veh treated mice and Ncf1*/* ovx-E2 treated mice. In addition, gap-junction (Connexin 43), RAGE and HMGB1 in the surrounding tissues are involved in the process.

In this model of local LPS induced acute inflammation, E2 showed anti-inflammatory properties only in the presence of ROS, by reducing infiltration of neutrophils. In the absence of ROS, E2 instead increased infiltration of neutrophils, suggesting that ROS and E2 cooperate in regulating inflammation. More studies are needed to explore the exact mechanism for such modulation of inflammatory processes in order to develop new treatment strategies.

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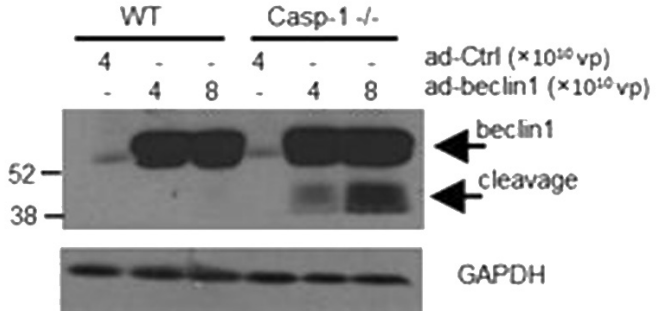
Caspase-1 Reduces Liver Cell Death and Mitochondrial ROS by Upregulating Beclin1 after Hemorrhagic Shock

Qian Sun[1, 2], Timothy Billiar[2], Melanie Scott[2]

[1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA, 15213.; [2]Department of Surgery, University of Pittsburgh, Pittsburgh, PA, 15213

Activation of the inflammasome by reactive oxygen species (ROS) produced after cellular oxidative stress leads to maturation of caspase1 and release of proinflammatory cytokines IL1β/IL18 leading to a potentially damaging inflammatory response. However, our previous data showed that caspase-1 was activated and protective after hemorrhagic shock with resuscitation (HS/R) in the liver. Similarly, caspase-1 protected isolated hepatocytes from increased cell death after hypoxia with reoxygenation in vitro. We have also previously shown that caspase1 is protective in our models by upregulating mitochondrial autophagy and decreasing levels of mitochondrial reactive oxygen species (ROS) in hepatocytes. Here we further investigated the mechanism by which caspase-1 regulates mitochondrial autophagy. There was no difference in levels of autophagy proteins Atg3, Atg12-Atg5 conjugate or Atg7 between WT and caspase1-/- hepatocytes after hypoxia. Protein and mRNA levels of beclin1, an initiator of autophagy, were significantly increased in WT but not caspase1-/- hepatocytes after hypoxia, or liver after HS/R. Furthermore, fragmented beclin1 was detected by western blot in caspase1-/- hepatocytes after hypoxia but not in WT cells, suggesting caspase1 protects beclin1 from being cleaved. To clarify the protective role of beclin1, we generated adenovirus encoding full length mouse beclin1 (ad-beclin1), or ad-GFP as control, injected i.v.48h prior to HS/R. Beclin1 was similarly cleaved in caspase1-/- livers but not in WT livers (Figure), suggesting caspase1 protects beclin1 from being cleaved. Beclin1 overexpression was hepatoprotective in caspase1-/- mice after HS/R, but did not confer additional protection in WT mice that have endogenously upregulated beclin1. Additionally, beclin1-overexpression reduced oxidative stress and cytochrome c release in caspase1-/- liver further suggesting

prevention of mitochondrial-triggered cell death. Taken together these data suggest that caspase1 plays a novel role in upregulating beclin1 levels to initiate autophagy in hepatocytes and reduce excessive mitochondrial ROS production during oxidative stress.
Figure:



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Enzymatic Activity-Dependent Induction of MCF-7 Cell Aggregation by a Neutrophil Serine Protease Cathepsin G

Riyo Morimoto-Kamata, Satoru Yui
Department of Parma-Science, Laboratory of Host Defense, Teikyo University

Introduction: The infiltrating neutrophils in tumor tissues affect on proliferation and metastasis of the cancer cells via secretion of cytokines, proteases and growth factors. We have revealed that cathepsin G (CG), which is a chymotrypsin-type serine protease secreted from neutrophils, facilitates cell motility and induces the formation of E-cadherin-dependent homotypic 3-D multicellular aggregates of a human breast cancer cell line MCF-7. The cellular spheroids morphologically resemble the lymphovascular emboli of inflammatory breast carcinoma. These findings suggest that CG regulates the dissemination of cancer cells during metastasis, however, the precious molecular mechanisms of the induction of cell aggregation by CG were unresolved. In the present study, we aimed to elucidate the mechanism by focusing of the enzymatic activity of CG. **Methods:** MCF-7 cells were cultured overnight with CG and the cell aggregation-inducing activity was estimated by the quantification of the cells that were tightly attached to the culture plate after staining with crystal violet. Recombinant CG was prepared from rat mast cell/basophilic cell line RBL-2H3 transfected with the CG gene. **Results:** We compared the cell aggregation-inducing activity of CG with that of chymotrypsin. CG induced MCF-7 cell aggregation at ~ 0.8 nM in a linear dose-dependent manner, whereas chymotrypsin was effective more than at 80 nM. The addition of serine protease inhibitors attenuated the cell aggregation-inducing activity of the CG. The cell lysate containing recombinant wild-type CG-overexpressing RBL-2H3 cells exhibited the cell aggregation-inducing activity, while the lysate of cells containing enzymatically inactive mutant S195G did not. These results suggest that the enzymatic activity of CG is required for the induction of the cell aggregation. If CG transduces the cell aggregation-inducing signal, CG should bind to a receptor-like specific partner. We examined the binding characters of 125I-labeled CG to MCF-7 cells. As the results, 125I- CG but not 125I-chymotrypsin bound to MCF-7 cells, and the 125I-CG binding was inhibited by addition of excess cold CG. However, the binding of recombinant wild-type CG and enzymatic inactive S195G CG was detected on MCF-7 cells by immunostaining using anti-CG antibody. Hence, it was shown that CG binding to the surface of MCF-7 cells is independent on its catalytic site. **Conclusion:** CG might bind the cell surface molecule(s) to transduce the cell aggregation-inducing signal by cleavage it or other target molecules of MCF-7 cells. In conclusion, we propose that CG induces MCF-7 cell aggregation via 2-step mechanism: catalytic site-independent binding to the cell surface and enzymatic activity-dependent induction of the cell aggregation.

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Lung and Abdominal Fat Inflammatory Macrophages Show Different Responses to a Systemic Anti-inflammatory in Obese Mice

Betty L. Herndon, Hamza Alshami, Tim Quinn, Anthony Accurso
Univ. MO-Kansas City School of Medicine, Kansas City, MO, USA

Background: Macrophages (mφ), essential innate immunity components, also contribute to metabolic activity. By M2-to-M1 polarization they characterize the chronic mild inflammatory state of adipose tissue that occurs in obesity. Aberrant pulmonary function is common in gravely obese humans, associated with, like the OB/OB mouse model, moderately elevated TNFα. To evaluate the input of obesity-related inflammation to the lung and the ability to reduce it, genetically obese mice (leptin k/o, OB/OB) were treated with TNFα receptor blocker or saline and inflammatory mφ in lung and fat were evaluated.
Hypothesis and Methods: We proposed that the TNFα receptor (TNFα-r) blocker would limit inflammation of M1 mφ in fat and lung of the obese mice, allowing improvement in lung inflammatory signs over the saline treated group. OB/OB mice received either 3 mg/kg TNFα-r blocker 2x weekly for 8 weeks (n=10) or saline control (n=10). At necropsy, alveolar lavage (BAL), sera and tissue were harvested for analysis. Sera were measured for TNFα and IL-6 (ELISA). Both lung and abdominal fat were stained for total macrophages (H&E) and inflammatory macrophages (F4/80). Nine high power fields were counted on each section and counts were analyzed using Statistica software.
Results: Total tissue macrophage counts in lungs and fat of untreated vs TNFα-r blocker are shown, cells per high power field. TNFα-r blocker decreased mφ count significantly ($p<0.001$) in fat only. Both total macrophage counts and the inflammatory (F4/80 stain) cell counts in fat decreased.
BAL control counts showed 21.9 total mφ, 5.8 lymphocytes, 0.8 PMN average count per field while the TNFα-r blocker BALs had 10.4 total mφ, 3.2 lymphocytes and 0.5 PMN, a small effect by blocker treatment. Serum TNFα and IL-6 showed the expected significant decreases in the TNFα-r block group.
Summary: Treatment of OB/OB mice with TNFα-r blocker significantly decreased –in abdominal fat—the number of inflammatory (F4/80 staining) mφ. The lungs of the same animals, however, failed to show a significant decrease in the inflammatory mφ count although BAL demonstrated a general decrease in cellularity in the treatment group. It appears the mφs resident in adipose tissue of these obese mice may be amenable to treatment,

but those F4/80 cells that have migrated to/polarized in the lung are not as responsive.

From the Geldmacher Pulmonary Research Program

	Abdominal Fat		Lung tissue	
group	Total mϕ H&E	Inflam. Mϕ F4/80	Total mϕ H&E	Inflam. Mϕ F4/80
controls	18.2 ± 0.8	21.2 ± 3.8	33.4 ± 21.2	38.7 ± 34.4
TNFα-r block	10.1 ± 3.1	9.5 ± 1.0	11.3 ± 9.5	28.8 ± 21.6

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Transient Receptor Potential Vanilloid-1 (TRPV1) Signaling Promotes the Development of Inflammation-Associated Cancer

Petrus R. De Jong, Naoki Takahashi, Samuel Bertin, James Jeffries, Michael Jung, Jen Duong, Amy I. Triano, David S. Herdman, Hui Dong, Lars Eckmann, Maripat Corr, Eyal Raz

Department of Medicine, University of California, San Diego, La Jolla, CA, USA

Introduction: The mammalian host is equipped with molecular sensors that are able to respond to physical and chemical irritants in the environment. These interactions mostly occur at epithelial surfaces, e.g. in the skin, lungs and the gut, and are mediated by ion channels that belong to the Transient receptor potential (TRP) family. TRPV1 is a prominent family member that can detect heat, oxidative substrates, lipid mediators and protons, commonly associated with tissue damage and inflammation, but this receptor can also respond to pungent food components (e.g. capsaicin) and alcohol. TRPV1 channels are typically expressed by sensory neurons, although their expression has also been identified in immune and epithelial cells. However, the physiological role of TRPV1 in non-neuronal cells is still unclear. We aimed to evaluate the expression of TRPV1 by intestinal epithelial cells and its potential role in gut inflammation and inflammation-associated cancer.

Approach: The expression of TRPV1 in intestinal epithelial cells (IEC) was assessed using real-time PCR, Western blotting, immunohistochemistry, flow cytometry, as well as functional assays. WT and *Trpv1*$^{-/-}$ mice were compared in a model of acute colitis by the administration of dextran sulphate sodium (DSS) in drinking water. Similarly, WT and *Trpv1*$^{-/-}$ mice were compared in a model of inflammation-associated cancer by injection of azoxymethane (AOM) followed by three cycles of DSS (AOM/DSS).

Main Findings: The expression of *Trpv1* mRNA was confirmed in primary murine IEC isolated from WT, but not *Trpv1*$^{-/-}$ mice, as well as in various murine and human IEC lines. TRPV1 expression by IEC at the protein level was confirmed in primary murine and human intestinal tissues and cell lines. Functional TRPV1 activity in IEC lines was confirmed by Ca^{2+} imaging. In the acute DSS-colitis model, *Trpv1*$^{-/-}$ mice showed significantly reduced barrier loss and less mucosal injury compared to WT mice. In line with these findings, *Trpv1*$^{-/-}$ mice developed dramatically less inflammation-associated colonic tumors after AOM/DSS treatment. Mechanistically, we found a direct molecular link between epithelial TRPV1 signaling and growth factor receptor activity. Thus, epithelial TRPV1 triggering affects epithelial restitution, inflammatory responses and inflammation-associated tumorigenesis in the gut.

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Induction of Breast Cancer Metastasis by Chitinase-3-Like-1 (CHI3L1) Protein and Mechanisms of CHI3L1—Chitin Microparticle Interactions to Reduced Metastasis

Stephania Libreros[1], Ramon Garcia-Areas[1], Cody Jackson[2], Lillian Onwuha-Ekpete[1], Vijaya Iragavarapu-Charyulu[1]

[1]Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL.; [2]Dept. of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL.

Breast cancer is the most common cancer inwomen in the US. Metastasis is responsible for a majority of breast cancerdeaths in spite of significant improvements in diagnosis and treatments. Recently, it was reported that expression of chitinase-3-like-1 protein (CHI3L1/YKL-40)is associated with poor prognosis in metastatic breast cancer patients. We have shown that there are elevated levelsof CHI3L1 in circulation with increased production of metastatic mediatorsCCL2, CXCL2 and MMP-9 in mice bearing mammary tumors. However, the direct role of CHI3L1 inpromoting metastasis has not been clearly delineated. To test the hypothesis that CHI3L1 isdirectly involved in promoting metastasis, we either overexpressed or silencedCHI3L1 gene in 4T1 mammary tumor cells. These cells were then implanted intoBALB/c mice and the effect on tumor growth and metastasis to the lung wasdetermined. Our recent publication has shown that in vivo administration ofchitin microparticles, the ligand for CHI3L1 reduced both tumor volume andmetastasis. To understand the role ofchitin microparticles in reducing tumor metastasis, signaling studies and theinteraction between chitin microparticles and CHI3L1 was assessed. Thesestudies provide a greater understanding of the role of CHI3L1 in enhancingmetastasis and the mechanisms by which metastasis is inhibited by chitinmicroparticles.

LATE-BREAKING ABSTRACTS

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The Complement C3a Receptor Mediates Protection from Intestinal Ischemia Reperfusion Injuries by Impairing Neutrophil Mobilization

Trent M. Woodruff[1], Mike C.L. Wu[1], Faith H. Brennan[1], Rick A. Wetsel[2], Marc J. Ruitenberg[1], Stephen M. Taylor[1]
[1]School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia, 4072; [2]Research Center for Immunology and Autoimmune Diseases, The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases, University of Texas Health Science Center at Houston, Houston, Texas 77030, USA

C3a is a key complement activation fragment, yet its neutrophil-expressed C3a receptor (C3aR) still has no clear role ascribed to it. In this study, we utilized a neutrophil-dependent animal model of intestinal ischemia-reperfusion (IR) injury to explore the role of C3aR in acute tissue injuries. C3aR deficiency significantly worsened injury outcomes with $C3aR^{-/-}$ mice displaying increased intestinal damage, hemorrhaging and apoptotic cell death alongside a significant increase in the number of infiltrating neutrophils. The circulating neutrophil count was dramatically increased in $C3aR^{-/-}$ mice as compared to wild-type mice, alongside known factors that influence mobilization such as G-CSF and SDF-1, indicating a specific role for C3aR in constraining neutrophil mobilization in response to intestinal injury. In support of this, $C3aR^{-/-}$ mice reconstituted with wild-type bone marrow reversed IR pathology back to wild-type levels. C5aR antagonism in $C3aR^{-/-}$ mice also reversed the worsened pathology after intestinal IR, but had no effect on circulating neutrophils, highlighting the divergent and opposing roles for C3a and C5a in disease pathogenesis. In contrast to C3a-C3aR blockade, generic mobilization of leukocytes by blocking the SDF-1-CXCR4 axis with AMD3100 failed to worsen intestinal injury after IR. Finally, we demonstrated that using a potent C3a agonist to activate C3aR in vivo reduced neutrophil mobilisation and ameliorated intestinal IR pathology in wild-type but not $C3aR^{-/-}$ mice. This study identifies a novel role for C3aR in regulating neutrophil mobilization following acute intestinal injury and highlights C3aR agonism as a novel treatment option for acute neutrophil-driven pathologies.

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IRF5 Induction and Localization in Polarized Macrophages

Melanie Wiesel, Gisbert Weckbecker, Helmut Sparrer
Novartis Institute for Biomedical Research, Autoimmunity, Transplantation & Inflammation, Basel, Switzerland

Differentiation and polarization of macrophages depends on the activation of critical transcription factors. We throughly characterized macrophage and DC populations generated under well-established differentiation protocols with regard to morphology, surface markers, gene expression and cytokine profiles after stimulation. Especially, we looked at expression and activation of IRF5, which has been postulated to be a master transcription factor for M1 differentiation. We found that IFNγ is the strongest inducer of IRF5 when compared to M-CSF, GM-CSF, IL-12, IL-23, IL-1, TNFα+IL-17 or type-I IFN. We correlated IRF5 expression levels with functional readouts for M1/M2 polarization and performed polarization switch experiments to test the importance of IRF5 in the turnover to a M1 phenotype. We confirmed that IRF5 induction via the inducer IFNγ has a dominant influence on M1 markers after 24h, even after prepolarization to M2 in the presence of IL-4. Finally, we followed the localization of the cytoplasmic IRF5 pool by imaging (confocal, microscopy, ImageStream) and CHIP experiments to learn about IRF5 nuclear trafficking. We detected membrane accumulation of IRF5 in IFNγ -polarized M1 macrophages and found only a small amount of IRF5 translocated to the nucleus upon TLR stimulation.

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Generation of Macrophages from Cynomolgus Bone Marrow as a Model to Estimate Effects of Drugs on Innate Immunity

Nianyu Li, Sue Ludmann, Ching He, Lisa Anest, Padma Narayanan
Discovery Toxicology, Amgen

Macrophages are important components of the innate immune system and play important roles in clearing infectious agents, neoplastic cells and xenobiotics as well as initiation of specific defense mechanisms of lymphocyte-mediated adaptive immunity to respond to pathogens. Macrophage function assays are often useful for understanding mechanisms of drugs in development. However, tissue macrophages are often difficult to collect in large animals and humans in a non-invasive manner. Therefore, in vitro differentiated macrophages are important tools to facilitate cross-species analysis of macrophage function. Various protocols exist for the differentiation of monocytes and bone marrow stem cells to tissue macrophages but they cannot be applied uniformly across species. In rodents, macrophages can be easily differentiated from bone marrow. However, for humans, differentiation of macrophages from bone marrow in vitro is difficult. Most commonly, human macrophages are differentiated from monocytes. Cynomolgus monkey is an important non-rodent species for pre-clinical drug development. To date, in vitro differentiation of cynomolgus macrophages have been poorly characterized. In the current study, we undertook several different methodologies to generate cynomolgus macrophages in vitro. Interestingly, in contrast to human, cynomolgus macrophages can be readily generated by culturing bone marrow cells with human colony stimulating factor 1 (CSF-1) for 5-7 days. Our results indicate that cynomolgus bone marrow-derived macrophages express classical macrophage surface markers including: CD14, CD32, CD64, CD86, CD80, and CD11, while not expressing markers of dendritic cells such as CD83. More importantly, these in vitro differentiated macrophages demonstrate phagocytic as well as bacterial killing activities. They also responded to LPS to induce cytokine release. In summary, we have established a novel methodology to derive macrophages from cynomolgus monkey bone marrow that can be used to investigate the effect of drugs on cynomolgus macrophage function in vitro.

LATE-BREAKING ABSTRACTS

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Influence of Alpha-Toxin (Hla) Expression by Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) on Immune Cell Integrity and Cytokine Expression during Infection of Human Blood

Tyler K. Nygaard, Kyler B . Pallister, Oliwia W. Zurek, Jovanka M . Voyich
Montana State University

This investigation examines the influence of alpha-toxin (Hla) expression by community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) on immune cell integrity and cytokine expression during infection of human blood. Cytokine expression analysis identified pro-inflammatory cytokines that are up-regulated during infection of human blood by CA-MRSA pulse-field gel electrophoresis type USA300 and underscored cytokines with reduced expression during infection with an isogenic deletion mutant of hla in USA300 (USA300Δhla). Transcription analysis further indicated a strong up-regulation of pro-inflammatory cytokine transcripts following infection of human blood by USA300 and USA300Δhla. Interestingly, an Hla-dependent decrease in the abundance of transcripts encoding cytokines expressed by monocytes was observed. Flow cytometry analysis demonstrated that Hla increases plasma membrane permeability of CD14+ peripheral blood mononuclear cells (PBMCs) and played a role reducing numbers of these cell types during USA300 infection of human blood. Additionally, increased numbers of FITC-labeled USA300Δhla appeared to be associated with CD14+ PBMCs relative to FITC-labeled USA300. Collectively these findings demonstrate expression of Hla by USA300 reduces the number of CD14+ PBMCs during infection of human blood while also promoting the expression of specific pro-inflammatory cytokines

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Oral Neutrophil Quantification in Uncooperative Patients with Special Needs

Anita Moosani[1, 2], Michael J. Sigal[1, 2], Michael Glogauer[1], Michael Goldberg[1, 2], Herenia P. Lawrence[1], Howard C. Tenenbaum[1, 2]
[1] Department of Pediatric Dentistry, Faculty of Dentistry, University of Toronto; [2] Department of Dentistry, Mount Sinai Hospital, Toronto, Ontario, Canada

Background: Patients with special needs have a high prevalence of oral disease, which can be difficult to diagnose with conventional clinical measures due to poor cooperation. The lack of appropriate diagnoses prevents the delivery of optimal treatment. This is especially relevant when recognizing oral health as an important component of general health and well being.
Purpose: To validate and assess the feasibility of using a swab assay of oral neutrophils (PMNs) to quantitatively measure periodontal inflammation in uncooperative patients with special needs.
Methods: Traditional periodontal probing, record of multiple indices, and PMN counts derived from oral swabs of the gingival tissues were obtained on patients with special needs having comprehensive dental treatment under general anesthesia (GA) at Toronto's Mount Sinai Hospital Dental Program for Persons with Disabilities. The conventional periodontal measurements were compared to PMN levels while the patient was under GA, and later at the patient's recall examination. Neutrophil counts were measured using the FLUOstar Optima microplate reader. Spearman's correlation (r_s) allowed for comparisons between periodontal parameters and PMN counts. A Wilcoxon Signed Ranks Test was used to compare the differences between parameters attained pre- and post-treatment.
Results: Forty-nine patients were assessed under GA and 30 (61%) returned for recall examination. The mean age of patients that were assessed under GA was 31.3 ± 11.3 years, consisting of 31 (63%) males and 18 (37%) females. Positive correlations were found for mean probing depths ($r_s = 0.286$, $p < 0.05$), modified gingival index ($r_s = 0.285$, $p < 0.05$), calculus index ($r_s = 0.385$, $p < 0.01$), and mobility ($r_s = 0.467$, $p < 0.01$). Despite limitations due to cooperation, it was possible to acquire PMNs from oral swabs for all patients that presented for recall examination in an ambulatory dental clinic for patients with special needs. All of the periodontal parameters improved significantly after treatment ($p < 0.05$), except for the plaque index ($p = 0.584$), despite prolonged recall intervals. Significantly lower PMN levels ($p < 0.05$) were also revealed, indicating a lower inflammatory load after periodontal treatment.
Conclusions: Oral PMN counts derived from swabs correlated significantly with conventional parameters of gingival inflammation and may serve as a standardized method of clinical assessment that would finally allow routine and reliable measurements for diagnosis and management of periodontal diseases in the special needs population.

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Local and Systemic Occurrences of HMGB1 in Gnotobiotic Piglets Infected with *E. coli* O55 Are Related to Bacterial Translocation and Inflammatory Cytokines

Alla Splichalova, Igor Splichal
Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, 549 22 Novy Hradek, Czech Republic

Background: High mobility group box 1 (HMGB1), a nuclear protein, can be secreted by stimulated cells or released from damaged cells. It is recognized as a late mediator of sepsis, but its extracellular occurrence has primarily been studied on the systemic level. Acute and chronic diseases of the gastrointestinal tract, however, have usually been connected with immediate local cell damage.
Hypothesis: Local and systemic occurrences of HMGB1 in gnotobiotic piglets infected with *E. coli* O55 are related to bacterial translocation and inflammatory cytokines.
Material and Methods: Germ-free (GF) colostrum-deprived miniature piglets were reared in isolators and fed to satiety 5-6 times a day with a diet of autoclave-sterilized milk. One-week-old gnotobiotic piglets were divided into three groups: i) GF (n = 5), ii) enteropathogenic *E. coli* O55-infected (1×10^8 CFU, n = 14), and iii) non-pathogenic *E. coli* O86-colonized piglets (1×10^8 CFU, n = 5). The group of O55-infected piglets was later divided post-hoc into the $O55^{THR}$ piglets (n = 6) that thrived and the $O55^{SUF}$ piglets (n = 8) that suffered from infection (anorexia, fever, diarrhea) 20-24 hours after the challenge. Samples of peripheral blood, lavages of

the small intestine, and homogenized mesenteric lymph nodes and lungs were cultured on MacConkey agar. A cut spleen and liver were imprinted on the agar. HMGB1, IL-1 beta, IL-6, IL-8, IL-12p40, and TNF-alpha were measured by ELISA. The differences in the bacterial CFUs or levels of HMGB1 and the cytokines (means ± SEM) were compared by one-way ANOVA with Dunnett's multiple comparison post-hoc test.

Results: Both O55 groups showed similar log CFU/g in mesenteric lymph nodes, but O86 showed significantly lower counts ($P<0.01$). The $O55^{SUF}$ piglets displayed significantly higher bacteremia ($p<0.01$), but no bacteremia was caused by *E. coli* O86. Bacteria-positive imprints of spleen and liver were found in the $O55^{SUF}$ piglets only. Levels of HMGB1, IL-6, IL-8, TNF-alpha (all $p<0.01$), IL-1-beta and IL-10 (both $p<0.05$) were significantly increased in the plasma of $O55^{SUF}$ piglets, as compared to $O55^{THR}$ piglets. IL-6, IL-8, IL-12/23 p40 and TNF-alpha (all $p<0.01$) levels increased in $O55^{SUF}$ piglets.

Discussion and Conclusions: The high levels of HMGB1 in the small intestine and its relation to the high levels of HMGB1 in plasma of the piglets infected with *E. coli* O55 that suffered from infection ($O55^{SUF}$) correlated with high levels of the inflammatory cytokines and bacterial translocation. These levels were higher than the HMGB1 levels in the piglets with mild clinical symptoms ($O55^{THR}$), the asymptomatic piglets colonized with non-virulent *E. coli* O86, or the germ-free piglets.

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Experimental Intraamniotic Infection of Pig Fetuses

Igor Splichal, Alla Splichalova, Tomas Hrncir

Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic

Background: Mammalian embryonic and fetal development takes place in the mother's uterus under sterile conditions. Despite decades of prematurity research, the incidence of preterm birth (< 37 weeks' gestation) is the leading cause of mortality and morbidity in newborn infants. One of reasons of the preterm births is intraamniotic infections. Suitable animal models can help to elucidate control mechanisms of preterm birth.

Hypothesis: Bacteria inducing a fetal inflammatory response syndrome (FIRS) are rapidly propagated in the fetus mainly via fetal breathing movements and swallowing of infected amniotic fluid. *E. coli* injected into amniotic fluid infects fetal lungs and intestine and induces a production of inflammatory mediators.

Methods: 10^6 CFU of enteropathogenic *E. coli* O55 were injected into amniotic fluid of pig fetuses around 80 days of gestation. The amniotic fluid was aspirated ten hours later and bacterial translocation was determined by cultivation method - samples of umbilical cord blood, lavages of the small intestine and lungs were cultured on MacConkey agar. Their distribution on the amniotic membrane, lungs and the small intestine was documented by FISH. Levels of inflammatory cytokines IL-8, IL-10 and TNF-alpha were measured by ELISA.

Results: The bacteria covered amniotic membrane epithelia and lung alveoli. No or limited bacteria were found in the small intestine. Inflammatory cytokines in amniotic fluid and plasma were significantly increased in the infected fetuses compare to sham-operated controls.

Discussion and Conclusions: The infection of the lungs was very fast probably due to fetal breathing movements that flush the lungs by infected amniotic fluid. In contrast, limited peristaltic movements and presence of a meconium in the lumen of the intestine probably prevented rapid passage of bacteria through the fetal gastrointestinal tract. The presence of high levels of inflammatory mediators in the amniotic fluid can trigger the preterm birth.

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IRAK4 Kinase Activity Is Not Required for Induction of Endotoxin Tolerance but Significantly Contributes to TLR2-Mediated Tolerance

Andrei Medvedev, Yanbao Xiong, Meghan Pennini, Stefanie Vogel

Department of Microbiology and Immunology, University of Maryland School of Medicine, 685 West Baltimore St., HSF-I Bldg., Rm. 380, Baltimore, MD 21201

Prior exposure to LPS induces "endotoxin tolerance" that reprograms TLR4 responses to subsequent LPS challenge by altering expression of inflammatory mediators. Endotoxin tolerance is thought to limit the excessive cytokine storm and prevent tissue damage during sepsis, but renders the host immunocompromised and susceptible to secondary infections. Tolerance initiated via one TLR can affect cellular responses to challenge via the same TLR ("homotolerance") or through different TLRs ("heterotolerance"). IRAK4, an essential component of the MyD88-dependent pathway, functions as both a kinase and an adapter, activating subsets of divergent signaling pathways. In this study, we addressed mechanistically the role of IRAK4 kinase activity in the induction of TLR4 and TLR2 tolerance using macrophages from wild-type (WT) vs. IRAK4 kinase-dead knock-in (IRAK4KDKI) mice. While IRAK4 kinase deficiency decreased LPS-induced signaling, pretreatment of WT and IRAK4KDKI macrophages with LPS similarly inhibited LPS-induced degradation of IRAK1 and IκB-α, MAPK phosphorylation, expression of proinflammatory cytokines, and increased expression of TLR negative regulators, A20 and IRAK-M. Pretreatment of WT macrophages with Pam3Cys, a TLR2-TLR1 agonist, ablated p38 and JNK phosphorylation in response to challenge with Pam3Cys or LPS. In contrast, IRAK4KDKI macrophages exhibited attenuated TLR2-elicited homo- and heterotolerance at the level p38 and JNK activation. Thus, IRAK4 kinase activity is not required for the induction of endotoxin tolerance, but significantly contributes to TLR2-elicited homo- and heterotolerance.

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***Moringa oleifera* Tea Abrogates Neutrophil Influx but not TNF-alpha Production**

Radiah Minor, Jenora Waterman, Jabria Allen, Donja Smith, Steven Hurley
North Carolina Agricultural and Technical State University

Inhaled Lipopolysaccaride (LPS) is also an important cause of environmentally induced airway disease in occupations where exposure to bacteria-contaminated organic dusts (bioaerosols) is common. In humans and animals, lung aerosol challenge or LPS instillation causes a neutrophil-rich inflammatory response. *Moringa oleifera Lam* is a tree that is nutritionally packed with vitamins, minerals and antioxidants and used because of its anti-inflammatory properties. The purpose of this study was to investigate whether tea brewed from dried Moringa leaves would abrogate inflammation in the lung after installation of LPS or extracts made from dust collected from a swine confinement facility (DE). In this study, mice were allowed to drink water or Moringa tea for seven days. There were no significant differences in weight gain, food consumption between the two groups, control (water) and experimental (Moringa tea) groups. Significant differences in liquid (water and tea) consumption were observed on days 1 and 6 where tea consumption was greater than that of water. On the seventh day, mice in both groups were dosed intranasally with PBS (vehicle) (PBS), LPS [10ug/ml] or DE [10%]. Necropsy of animals 24 h post intranasal installation revealed that while upper respiratory challenge with LPS or 10% DE induced inflammatory responses within the lung as compared to the animals challenged with PBS there was a significant difference in the inflammatory responses observed in the control group as compared to the experimental group. Flow cytometric analysis of cells within the brochoaveolar lavage (BAL) fluid showed significant decreases in the percent of neutrophils that influxed into the lung 2.5 for LPS exposed ($p<0.05$) and 4 fold for DE ($p<0.001$). Moreover, mice that consumed Moringa tea had significantly lower levels of total protein ($p<0.05$) within the BAL. Interestingly however, ELISA analysis of TNF-α revealed that mice that consumed Moringa Tea and exposed to 10% DE had greater levels of TNF-α as compared to mice that consumed water. Taken together these results demonstrate that leaves from the *Moringa oleifera* plant exert anti-inflammatory properties through a mechanism that involves regulation of neutrophils.

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Intranasal Immunization with Recombinant *Orientia tsutsugamushi* Outer-Membrane Protein Enhances Ag-Specific Antibody Responses, Cellular Immune Responses and Protective Immunity

Hyuk Chu[1], Sung-Moo Park[2], Kyu-Jam Hwang[1], Young Min Son[2], In Su Cheon[2], Han Wool Kim[2], Woon Hee Jeung[2], Hang-Jin Jeong[1], Byoung-Cheol Gil[1], Mi-Yeoun Park[1], Young-Ran Ju[1], Cheol-Heui Yun[2]
[1]Korea Centers for Disease Control & Prevention; [2]Seoul National University, ROK

Scrub typhus is an acute, febrile, infectious illness caused by *Orientia tsutsugamushi* transmitted by trombiculid mites. Humans are infected through bite of the larva of definite species of the thrombidium palladium mites in Korea. There is no vaccine to prevent the scrub typhus; only antibiotics are used as a common treatment. We need the vaccine study due to the overuse of antibiotics and the emergences of mutants.

In the present study, the immune responses of BALB/c mice immunized via intranasal (I.N.) route with recombinant *O. tsutsugamushi* outer-membrane proteins plus mucosal adjuvant have been examined. The mice immunized with I.N. recombinant proteins plus cholera toxin produced significant higher Ag-specific antibody responses in serum than thoses in mice treated with recombinant proteins alone. The percentage of IFN-r, TNF-a, IL-2 and IL-17 producube T cells were higher in recombinant proteins with cholera toxin immunized mice than in control mice. More importantly, I.N. immunization with recombinant proteins plus cholera toxin fully protected against intraperitoneal (I.P.) challenge with lethal dose of *O. tsutsugamushi* Boryong strain, whereas the survival rate of the control group was low.

These results showed that I.N. immunization with recombinant *O. tsutsugamushi* outer-membrane proteins induce antigen specific humoral, cellualr immune responses and protective immunity against systemic infectious disease.

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TRPM2 Is Not Required for Airway Inflammation in OVA-Induced Allergic Asthma in Mice

Adriana Sumoza-Toledo[1, 2], Andrea Fleig[1, 2], Reinhold Penner[1, 2]
[1]Laboratory for Cellular and Molecular Signaling, Center for Biomedical Research at The Queen's Medical Center; [2]John A. Burns School of Medicine, University of Hawai'i, Honolulu, HI USA 96813

Airway inflammation and asthma have been linked to oxidative stress and the melastatin-related transient receptor potential cation channel, member 2 (TRPM2) has emerged as a potential therapeutic target for inflammatory diseases. TRPM2 channels are non-selective calcium-permeable cation channels, which allow calcium (Ca^{2+}) entry into neutrophils and monocytes and lysosomal Ca^{2+} release in mouse bone marrow derived-dendritic cells (BMDC). TRPM2 channel is co-activated by intracellular adenosine diphosphate ribose (ADPR) and Ca^{2+}, but can also be activated by reactive oxygen species (ROS). TRPM2 plays a protective role in lipopolysaccharide (LPS)-induced lung inflammation mouse model by preventing ROS production in neutrophils. It can also control the severity of chronic colitis in mouse by affecting the production of chemokine (C-X-C motif) ligand 2 in monocytes and subsequent infiltration of neutrophils. Contrariwise, a recent study in chronic obstructive pulmonary disease showed no role for TRPM2 in airway inflammation in mice exposed to ozone, LPS and tobacco smoke. In the present study, we investigated whether TRPM2 ion channel plays a role in the pathophysiology of allergic asthma in mouse. Allergic asthma was initiated in wild type (WT) and deficient ($TRPM2^{-/-}$) mice by repeated sensitization with ovoalbumin (OVA)/aluminum hydroxide on Days 0, 7 and 14, followed by intranasal challenge on Days 21 and 23. Our results showed that the airway resistance in OVA-treated WT and $TRPM2^{-/-}$ mice were strongly increased with no significant differences between the mouse strains,

when assessing responses to increasing doses of acetylcholine, compared to saline-challenged mice. Histologic examination of PAS-stained lungs from OVA- and saline-challenged WT and TRPM2$^{-/-}$ mice also showed similar severity of goblet cell hyperplasia. Likewise, the number of eosinophils, macrophages, lymphocytes, and neutrophils in the BAL fluid and mast cell counts in the lung tissue of OVA-challenged WT and TRPM2$^{-/-}$ mice were equivalent. Also, OVA challenge evoked significant and comparable increases in the levels of IL-6, IL-10, TGFβ1 and IL-13 cytokines in WT and TRPM2$^{-/-}$ mice. Finally, depletion of TRPM2 did not affect allergen-induced production of IgE. In summary, our data indicate that TRPM2 channels are not involved in the acute inflammatory response and airway responsiveness in OVA-induced allergic asthma in mice. It also suggest that TRPM2 might not be a suitable therapeutic target for airway inflammation caused by allergens in humans.

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Oral Neutrophils Express T-Cell Receptors and Display a Site-Specific Phenotype

Flavia Lakschevitz[1, 2, 3], Michael Glogauer[1, 2, 3]

[1]University of Toronto; [2]Faculty of Dentistry; [3]Matrix Dynamics Group

Background: Neutrophils, key cells of the innate immune system, are mistakenly described as terminally differentiated cells with a highly condensed nucleus, incapable of altering their gene expression following differentiation and maturation in the bone marrow. Only recently it has been shown that neutrophils carry out rapid and complex changes in gene expression during inflammatory responses. Previous work from our lab has demonstrated differences in reactive oxygen species (ROS) production between oral and peripheral blood neutrophils isolated from periodontitis patients, suggesting that oral neutrophils present with a distinctive oral phenotype when compared with blood neutrophils. Understanding differences in the neutrophil transcriptome following transit from circulation into the site of inflammation will give us new insights into how these innate immune cells function during inflammation.

Methods: Venous blood and oral rinse samples were obtained from healthy subjects. Blood neutrophils were isolated using a standard gradient method. Oral neutrophils were isolated through nylon mesh filters of different pore sizes. After isolation of neutrophils from both samples and further RNA purification, gene expression analysis was assessed by microarray technology and confirmed by qRT-PCR. Additionally, immuno-fluorescence microscopy and Cytokine Array were performed.

Results and Discussion: We were able to optimize oral neutrophil isolation which we show is critical when analyzing gene expression as isolation through 40μM alone does not purify the sample, and results in epithelial cells contamination. We also show that oral neutrophils present with a significant increase in T cell receptor expression compared to circulating neutrophils, suggesting a role for oral neutrophils in cross-talk between innate and adaptive immune system in the mouth.

Conclusions: Our goal was to use a modified method to isolate highly pure and viable neutrophils from oral rinse samples in order to characterize OPMN using a transcriptome approach. With this method we compared the oral neutrophil phenotype to the circulating neutrophil phenotype using microarray analysis and qRT-PCR. Using a bioinformatics approach for clustering neutrophil gene expression, we found that oral neutrophils present with a unique transcriptome compared to circulating neutrophils. In addition we show for the first time that oral neutrophils express T-Cell receptors indicating that these cells may play a key role in linking the innate and adaptive arms of the oral immune system.

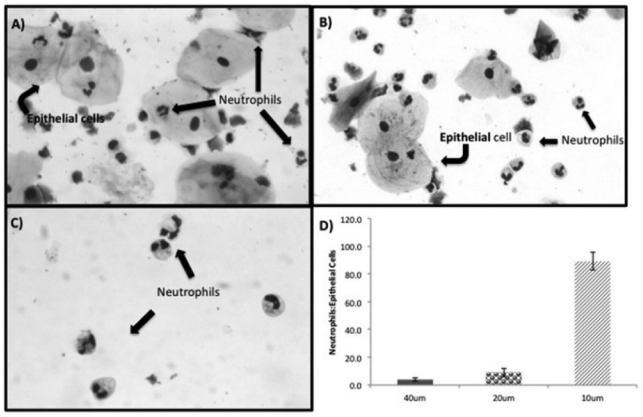

Figure 1 – Filtration is required to isolate oral neutrophils form rinse samples: Oral Neutrophils were isolated by sequential filtration and cytospins were made and stained with Diff-Quick. **(A)** Oral samples after 40um filtration only **(B)** Isolated PMN after 20um filtration **(C)** and after additional 10um mesh. Original magnification, X20. **(D)** Ratio of neutrophils to epithelial cells were calculated by counting all cells contained in 5 different fields.

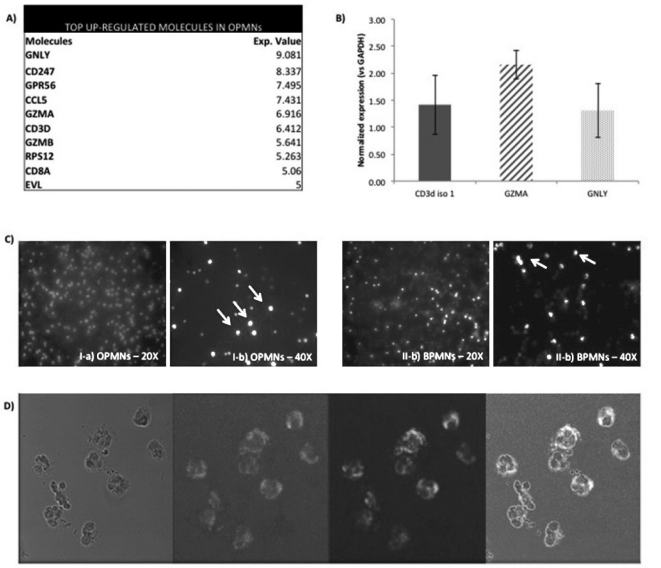

Figure 2 – Functional confirmation of microarray expression data with qRT-PCR and Immunocytochemistry: (A) List of the top up-regulated molecules in oral neutrophils selected by IPA software. **(B)** qRT-PCR was used to quantify gene expression of T-cell receptors in OPMNs. Results are expressed as fold changes vs BPMNs expression used as internal control. Gene expression was normalized with GAPDH as a reference gene. **(C)** Shown are cytospins of merged images of Cd11b (green) and CD3d (red): I) OPMNs and II) BPMNs from the same subject a) Original magnification, X20 and b) Original magnification, X40. White arrows shows PMNs stained for CD3d **(D)** Confocal microscopy demonstrating TCR localization in the cell membrane of oral neutrophils: (I) bright field and stained for (II) CD11b, (III) CD3d (IV) merged image.

LATE-BREAKING ABSTRACTS

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Dectin-1-Mediated Immunity to Commensal Fungi Influences Colitis

Iliyan D. Iliev, Vincent Funari, Christopher Reyes, Courtney Becker, David Underhill

Inflammatory Bowel & Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Interactions between commensal microbiota and the immune system are critical for establishing a proper balance between immune host defense mechanisms and tissue health. Changes in the composition of gut bacterial communities have been associated with intestinal inflammation and obesity. Recent studies have begun to note that a fraction of mucosa-associated microorganisms are not bacterial. Mucosal fungal infections are relatively common in Crohn's Disease patients, and antibodies against fungal antigens (ASCA) are a widely accepted clinical marker for disease severity.

What fungi populate the intestine and how immunity to them might play a role in inflammatory disease is currently unknown. Fungi are sensed by number of innate immune receptors among which Dectin-1 has emerged as the main innate immune receptor for recognition, phagocytosis, and killing of fungi by myeloid phagocytes. We found that mice lacking Dectin-1, recognizing fungal cell wall β-glucan, are more susceptible to experimental colitis characterized by increased infiltration of Th17 and Th1 cells in the colon. Interestingly this pathology was driven by intestinal fungus, and antifungal therapy ameliorated colitis severity in knockout mice. Deep sequencing analysis of the fungal mycobiome revealed fungal species that are overrepresented in the gut during experimental colitis. When Dectin-1-/- mice were supplemented with Candida tropicalis, a specific commensal fungus found in the intestine during colitis, they experienced more severe intestinal inflammation and augmented Th17 mucosal responses in absence of Dectin-1. Consistently, intestinal dendritic cells (DCs) from Dectin-1-/- mice, but not WT DCs, showed reduced ability to kill fungi. Therefore the data suggest that an inability of Dectin-1-/- mice to mount effective immune responses to specific intestinal fungi creates conditions that promote inflammation.

Since the mouse model suggested that Dectin-1 is involved in contributing to the severity of colonic disease, we focused on the severity of ulcerative colitis (UC), the form of IBD that always affects the colon. In particular we focused on severe UC, termed medically refractory UC (MRUC), consisting of patients requiring colectomy as a result of lack of response to medication. We found that a specific variant of the gene for Dectin-1 is strongly associated with a severe form of ulcerative colitis requiring colectomy.

Together our findings reveal a novel eukaryotic fungal community in the gut and show that altered interactions between the fungal microflora and the host intestinal phagocytes can profoundly influence intestinal pathology.

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IL1 Receptor Signaling in Determining the Life Span of Neutrophils after in Vivo Transmigration

Karin Christenson, Lena I. Björkman, Claes Dahlgren, Johan Bylund

Department of Rheumatology and Inflammation Research, University of Gothenburg, Sweden

Neutrophils are a very important part of our innate immunity and they leave the circulation to perform their major tasks in the tissue. The inflammatory sites, into which the neutrophils transmigrate, are filled with a diversity of substances with either pro- or anti-inflammatory qualities. The exudates from aseptic inflammatory reactions are for example enriched in the pro-inflammatory cytokine interleukin-1β (IL1β) as well as the anti-inflammatory factor interleukin-1 receptor antagonist (IL1Ra). Neutrophils are short-lived cells that die rather swiftly by apoptosis, and since several factors found at inflammatory sites have been shown to either enhance or delay neutrophil apoptosis in vitro, we next wanted to investigate the role of IL1β and IL1Ra regarding regulation of the neutrophil life-span

Using an established neutrophil apoptosis model we show that IL1β had an anti-apoptotic effect on neutrophils from peripheral blood and that this effect was reversed by the receptor antagonist IL1Ra. In addition tissue neutrophils isolated from different types of inflammatory sites produced different amounts of IL1β. These data suggest that the balance between the IL1R agonist and antagonist may influence life and death of transmigrated neutrophils. Accordingly, we found that neutrophils isolated from inflammatory sites with elevated levels of IL1β were totally resistant to anti-apoptotic stimulation, whereas it still was possible to postpone apoptosis of neutrophils that had transmigrated to an inflammatory site with lower levels of IL1β. The level of IL1Ra was similar at the two inflammatory settings. This indicates that the ratio between IL1β and IL1Ra at an inflammatory site can be of importance for neutrophil survival and may also be an explanation to why neutrophils from different inflammatory settings respond differently to anti-apoptotic stimulation.

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miR-132/212 Induction Is Involved in M1 Differentiation through an Ahr-Dependent Mechanism

Nam T. Nguyen, Taisuke Nakahama, Hamza Hanieh, Ichino Chinen, Tadamitsu Kishimoto

WPI-Immunology Frontier Research Center, Osaka University, JAPAN

Molecular mechanisms underlying macrophage differentiation from bone marrow (BM) cells into M1 and M2 macrophages remain largely undefined. M1 are essential for clearing bacterial, viral and fungal infections while M2 have an important role in responses to parasite infection, tissue remodeling, and tumor progression. M1 and M2 are characterized by the expression of such molecules as arginase-1 and the levels of inflammatory cytokines under TLR activation. Recently, there is increasing evidence that microRNAs (miRs) play a crucial role in immune regulation via interacting with various transcription factors. Therefore, we asked whether

miRs and aryl hydrocarbon receptor (Ahr) are involved in the differentiation of M1 and M2. BM cells (M0) were cultured for six days in the presence of GM-CSF for harvesting M1 or cultured for five days in the presence of M-CSF for harvesting M2. In some certain experiments, M0 cells are treated with TCDD or FICZ at the first day of culture. The expression of selected miRs in M1 and M2 was examined and compared to those in M0 cells by quantitative real time PCR (qPCR). We found that the expression of miR-132/212 cluster which is little known function in immune responses was highly up-regulated in M1 cells but not M2 compared to M0. Interestingly, the miR-132/212 is significantly suppressed in Ahr-KO- compare to WT M1. In addition, TCDD and FICZ can induce the expression of miR-132/212 in WT- but not in Ahr-KO-M1 cells. Therefore, the expression of miR-132/212 regulated by Ahr may be crucial for M1 cell differentiation. Furthermore, Ahr activation is critical for LPS- and CpG-induced miR-132/212 expression in M1. Importantly, we found that increased miR-212 expression decreases both mRNA and protein levels of Smad4, a key protein in TGF-β signaling. Smad4, thus, is a potential target of miR-132/212 cluster in M1 differentiation.

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Ethanol Concentration-Dependent Alterations in Gene Expression in the HIV-1 Transgenic Rat during Acute Binge Drinking

Sulie L. Chang[1, 2], Sraboni Sarkar[1]

[1]Institute of NeuroImmune Pharmacology, Seton Hall University, South Orange, NJ, USA; [2]Department of Biological Sciences, Seton Hall University, South Orange, NJ, USA

Binge drinking of high ethanol (EtOH) concentration beverages is common among young adults, and can be a risk factor for exposure to sexually transmitted diseases, including HIV-1. We used a novel non-infectious HIV-1 transgenic (HIV-1Tg) rat model that mimics HIV-1 patients in terms of altered immune responses and deficits in cognitive learning and memory to investigate EtOH concentration-dependent effects on 48 alcohol-modulated genes during binge EtOH administration. HIV-1Tg and control F344 rats were administered water, 8% EtOH, or 52% EtOH by gavage (i.g.) for 3 d (2.0 g/kg per d). Two hrs after final treatment, blood, liver, and spleen were collected from each animal. Serum blood EtOH concentration (BEC) was measured, and gene expression in the liver and spleen was determined using a specifically-designed PCR array. The BEC was significantly higher in the 52% EtOH-treated HIV-1Tg rats compared to the 8% EtOH group; however, the BEC was higher in the 8% EtOH-treated control rats compared to the 52% EtOH group. There was no change in expression of the EtOH metabolism related genes, Adh1, Adh4, and Cyp2e1, in either the 8% or 52% EtOH-treated HIV-1Tg rats, whereas expression of those genes was significantly higher in the liver of the 52% EtOH control rats, but not in the 8% EtOH group. In the HIV-1Tg rats, expression of the GABAA, metabotropic-glutamate, and dopamine neurotransmitter receptor genes was significantly increased in the spleen of the 52% EtOH group, but not in the 8% group, whereas no change was observed in those genes in either of the control groups. Our data indicate that, in the presence of HIV-1 infection, EtOH concentration-dependent binge drinking can have significantly different molecular effects (NIH K02 DA016149 & RC2 AA019415 to SLC).

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Engagement of Siglec-7 Receptor Induces a Pro-Inflammatory Response Selectively in Monocytes

Domenico Mavilio[1], Stefania Varchetta[2], Enrico Brunetta[1], Alessandra Roberto[1], Joanna Mikulak[1], Kelly L. Hudspeth[1], Mario U. Mondelli[2]

[1]Unit of Clinical and Experimental Immunology, Istituto Clinico Humanitas, Rozzano, Milano, Italy ; [2]Department of Infectious Diseases, Research Laboratories, IRCCS, Fondazione San Matteo and University of Pavia, Italy

Sialic acid binding immunoglobulin-like lectin-7 (Siglec-7) is a trans-membrane receptor carrying immunoreceptor tyrosine based inhibitory motifs (ITIMs) and delivering inhibitory signals upon ligation with sialylated glycans. This inhibitory function can be also targeted by several pathogens that have evolved to express sialic acids on their surface to escape host immune responses. Here, we demonstrate that cross-linking of Siglec-7 by a specific monoclonal antibody (mAb) induces a remarkably high production of IL-6, IL-1α, CCL4/MIP-1β, IL-8 and TNF-α. Among the three immune cell subsets known to constitutively express Siglec-7, the production of these pro-inflammatory cytokines and chemokines selectively occurs in monocytes and not in Natural Killer or T lymphocytes. This Siglec-7-mediated activating function is associated with the phosphorylation of the extracellular signal-regulated kinase (ERK) pathway. The present study also shows that sialic acid-free Zymosan yeast particles are able to bind Siglec-7 on monocytes and that this interaction mimics the ability of the anti Siglec-7 mAb to induce the production of pro-inflammatory mediators. Indeed, blocking or silencing Siglec-7 in primary monocytes greatly reduced the production of inflammatory cytokines and chemokines in response to Zymosan, thus confirming that Siglec-7 participates in generating a monocyte-mediated inflammatory outcome following pathogen recognition. The presence of an activating form of Siglec-7 in monocytes provides the host with a new and alternative mechanism to encounter pathogens not expressing sialylated glycans.

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Novel Role of a C-Type Lectin Receptor in Host Defense against Gram Negative Pneumonia

Anthony L. Steichen, Brandilyn J. Binstock, Bibhuti B. Mishra, Jyotika Sharma

University of North Dakota

C-type lectin family of receptors play critical role in a variety of homeostatic and immune responses. Clec4d is an orphan C-type lectin receptor whose ligand/s and physiological function is unknown. Here we report that, in a murine inhalation model for pneumonia and sepsis, pulmonary infection with Gram –ve bacterium Klebsiella pneumoniae (KPn) induces kinetic upregulation of Clec4d in lungs of mice. Furthermore, Clec4d-/- (KO) mice are highly susceptible to respiratory infection with KPn and exhibit significantly higher mortality as compared to Clec4d+/+ wild-type (WT) mice. Temporal kinetic analysis of the

infection process revealed that KPn infected WT and KO mice exhibited similar bacterial burden and inflammatory cytokines at initial phase of infection. In contrast, while the WT mice were able to resolve the infection as indicated by reduction in bacterial counts in systemic organs and decrease in levels of inflammatory cytokines, the KO mice displayed a progressive increase in these parameters and ultimately succumbed to infection. This was consistent with progressively severe lung pathology accompanied by massive increase in accumulation neutrophils and neutrophil associated immune mediators in infected KO mice. In contrast, the WT mice displayed a moderate and transient increase in neutrophil accumulation. These results suggest that Clec4d plays an important role in resolution of inflammation by way of facilitating neutrophil turnover in lungs following pulmonary infection with KPn. This is the first report depicting physiological function of Clec4d and may have implications in understanding its role in the pathophysiology of acute respiratory infections.

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p53 Cooperates with MAP Kinase and NFkB Signal Transduction Pathways to Potentiate Human Immune/ Inflammatory Response

Maria Shatz[1], Igor Shats[2], Daniel Menendez[1], Michael A. Resnick[1]
[1]Chromosomes Stability Section, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences (NIEHS), NIH, Research Triangle Park, NC 27709; [2]Duke Institute for Genome Sciences and Policy, Duke University, Durham, NC 27710

The p53 tumor suppressor regulates transcription of genes associated with cellular functions including apoptosis, growth arrest, DNA repair, differentiation and glycolysis. Recently, we extended this list to include Toll-like receptor (TLR) human innate immunity genes. TLRs are highly conserved integral membrane glycoproteins that recognize a variety of chemically distinct pathogen-associated molecular patterns (PAMPs). Upon stimulation, TLRs recruit adaptor molecules that lead to activation of NFκB, interferon responsive factors (IRFs) and MAP kinases, resulting in distinct patterns of gene expression essential to immune/ inflammatory responses and elimination of pathogens.

To determine the extent to which p53 dependent up-regulation of the TLR receptors could influence downstream response, we utilized a pair of isogenic cell lines with very different levels of p53: wild type p53 MCF7 breast adenocarcinoma cells or MCF7 cells stably transfected with p53 shRNA. Cells were pre-treated with Nutlin-3 to activate p53 and subsequent induction of TLR receptors expression (Nutlin-3 blocks the p53 inhibitor MDM2 and stabilizes p53 protein) and then exposed to the TLR5 ligand flagellin. Gene expression analysis revealed over 200 genes that were synergistically increased by the combination of p53 induction and flagellin treatment. Genes associated with Gene Ontology terms such as immune, defense and inflammatory response and also having NFkB binding sites in their promoters were significantly (p-value <0.0001) over-represented and included, among others, interleukin 6, interleukin 8 and urokinase-type plasminogen activator that are tightly associated with inflammation and immunity.

The p53-dependent increase in transcriptional response to flagellin was accompanied by enhanced phosphorylation of p38 MAP kinase and was partially reversed by p38 inhibitor SB203580, suggesting an important role for this kinase in the p53 associated inflammatory responses. Interestingly, Nutlin-3 also increased cytokine expression in response to TNFalpha. Since both TNFalpha and TLR signaling are transduced via MAP kinase and NFkB pathways, it appears that p53 can have broad interactions with signal transduction pathways that lead to immune/inflammatory response. These findings are expected to increase opportunities in TLR-based cancer treatments, especially since therapies may include both DNA damaging agents and increased levels of p53.

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Complement-Altered Human Red Blood Cells Induce IL-6 Release in Circulating T Cells through an ATP-Dependent Mechanism

Vasileios Kyttaris, Andrew Fraser, Ourania Kampagianni
Beth Israel Deaconess Medical Center, Harvard Medical School

Human red blood cells (RBCs) have critical, non-redundant roles in creating and maintaining a non-inflammatory intravascular environment by capturing C3b- and C4b-opsonized particles, and delivering them to macrophages in the liver and spleen through a process, called "immune adherence clearance. The restricted co-expression of complement receptor 1 (CR1) and glycophorin A (GPA) solely on human RBC membranes makes them preferentially targeted by C3b and C4b fragments generated during both immune adherence-clearance and excessive complement activation. In systemic lupus erythematosus (SLE), dysregulated complement activation generates excessive amounts of soluble C3b/C4b fragments that will engage GPA irreversibly, through their thioester groups. Recently, in active SLE a profound functional alteration of circulating T cells due to the presence of excessive intravascular ATP was documented, although the source(s) of the extracellular ATP has yet to be identified. On the present study, we focused on the mechanisms responsible for the complement-mediated ATP release in circulating RBC from SLE patients, and their functional consequence on the circulating T cells. We observed that in SLE, complement-positive circulating RBC show increased amounts of reactive oxygen species (ROS), which in turn are responsible for activation of cytosolic pro-caspase 3. We have found the ROS-mediated activation of PKC (protein kinase C) promotes a MRP (multi-drug resistance associated protein)- and CFTR (cystic fibrosis transmembrane conductance regulator)-dependent RBC ATP release, whereas caspase-3 dependent proteolysis of β-spectrin significantly decreases RBC membrane deformability. Co-culture experiments using isolated RBC from SLE patients or healthy controls revealed an ATP-dependent manner interleukin-6 (IL-6), a cytokine that plays a role in the generation of Th17 pathogenic responses in SLE. The production of IL-6 was inhibited following incubation of T cells with apyrase, a soluble ATP-diphosphohydrolas, or P2X7 specific inhibitors.

Our new data challenge the canonical role of RBCs as strictly non-inflammatory cells, revealing that in SLE, complement fragments convert circulating RBCs into effector cells that continuously release significant and unregulated amounts of ATP, which could be responsible for the generation of Th17 pathogenic responses in SLE.

LATE-BREAKING ABSTRACTS

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Revamping Neutrophil Degranulation: Inhibition Studies Using TAT-VAMP-Fusion Proteins

Maria G. Gutierrez[1], Junyi Le[2], Lisandra Rodriguez-Hernandez[2], Kylie McNeil[1], Silvia M. Uriarte[1, 2]
[1]Department of Microbiology and Immunology/University of Louisville; [2]Department of Medicine/University of Louisville

Neutrophils are powerful combatants against invading microorganisms, but dysregulated neutrophil activation and release of granule components can cause severe tissue damage during inflammation. Neutrophils have four different types of granules which are hierarchically released during inflammation. Members of the soluble-N-ethylmaleimide-sensitive-factor accessory-protein receptor (SNARE) superfamily, including members of the vesicle-associated membrane proteins (VAMP), are involved in neutrophil granule exocytosis. The ability to selectively inhibit the different neutrophil granule subtypes will be a valuable tool to study their role and contribution during inflammation. In this study, we tested the hypothesis that blocking different VAMP proteins will result in a differential inhibition of neutrophil granule exocytosis. Fusion proteins containing the TAT cell-penetrating sequence and the synaptobrevin domain of either VAMP-3 (TAT-VAMP-3) or VAMP-8 (TAT-VAMP-8) were generated. Exocytosis of secretory vesicle, specific granules and azurophil granules was determined by flow cytometry measuring the increase plasma membrane expression of CD35, CD66b, and CD63 respectively. Neutrophils were untreated, or stimulated with the bacterial peptide N-formylmethionine leucine phenylalanine (fMLF) in the presence of increasing concentrations of either TAT-VAMP-3 or TAT-VAMP-8 (0.1, 0.5, 0.8 or 1.0 μg/ml). TAT-VAMP-3 inhibited secretory vesicle exocytosis in a dose dependent manner, with a 93% inhibition at 1.0 μg/ml, only a 29% inhibition of specific granules, and a 34% inhibition of azurophil granules at that same concentration; but had no effect on exocytosis of gelatinase granules measured by ELISA. TAT-VAMP-8 inhibited secretory vesicle exocytosis to the same extent as TAT-VAMP-3, however this fusion protein had a significant inhibition of specific granules (78% at 1.0 μg/ml), only a 20% inhibition of azurophil granules; but had no effect on gelatinase granule exocytosis. In conclusion, the results demonstrate that VAMP-3 and VAMP-8 are key SNAREs involved in exocytosis of secretory vesicle but not in gelatinase granules. VAMP-8 is involved in specific granule exocytosis. TAT-VAMP-3 had a more significant inhibitory effect on azurophil granule exocytosis than TAT-VAMP-8 indicating that VAMP-3 might play a more relevant role in azurophil granule release. Further studies will test the effect of other TAT-VAMP fusion proteins on neutrophil degranulation.

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Neutrophils Contribute to Antigen Presenting Cell Defects Post-Injury

Carol Miller-Graziano, Paul Bankey, Gautam Bandyopadhyay
Department of Surgery, University of Rochester Medical Center, Rochester, NY

Severely injured trauma patients demonstrated an impaired immune response. A contributing mechanism may be the reduced capability of monocytes (MO) to differentiate to dendritic cells (DC) or MO differentiation to a dysfunctional DC subset. DC expression of CD16 has been linked with a impaired immune responsiveness. The role of activated neutrophils (PMN) in MO to DC differentiation is inadequately characterized. We hypothesize that activated PMN may direct MO differentiation and signal expression of CD16 on DC. In this study we tested whether Interferon-gamma (IFN) activated PMN can alter MO differentiation, DC CD16 expression, and subsequent DC phenotype and function. To test this MO from healthy donors were co-cultured 3 days with IFN treated or control autologous PMN and then the MO were re-isolated using magnetic beads [MO: PMN = 1:10]. PMN were tested for viability and apoptosis as we have previously shown that IFN treatment delays PMN apoptosis. (Fig. 1D) Re-isolated MOs were then in vitro differentiated to DC using Interleukin-4+GM-Colony Stimulating Factor over 5 days. DC receptor expression and T cell stimulatory capacity were then compared between treatment groups (DC=No PMN-MO co-culture; PMN-DC=Control PMN-MO co-culture; IFN PMN-DC= Activated PMN co-culture)
Our results demonstrate the following: first, PMN co-cultured in vitro with autologous MO have decreased apoptosis and remain viable (Fig.1C); second, DC differentiated from PMN co-cultured MOs express increased CD16 (Fig. 1E); third, these increased CD16 expressing DC demonstrate depressed T cell stimulatory capability (Fig. 1F). Similarly, immune impaired or dysfunctional DC from trauma patients express increased CD16 and depressed T cell stimulatory capacity (Fig. 1A&B). Our data suggest that activated PMN can alter MO to DC differentiation, increase expression of CD16, and result in a suppressed, defective DC subset.

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Role of PGE-2 in Immuno-Editing of Tumor-Associated Macrophages

Dmitriy Gromov
Kansas City University of Medicine and Biosciences

Tumor microenvironment has a strong impact on immune surveillance and effector properties of tumor-associated macrophages (TAMs). TAMs are “immunoedited” and switch their function properties from anti-tumor M1 (IL-12high, IL-23high, and IL-10low) to M2-like (IL-12low, IL-23low, and IL-10high) phenotype promoting the tumorigenesis. Tumor-derived prostaglandin E2 (PGE2) has been implicated as a critical regulator of M1/M2 balance in TAMs. In this regard, poor prognosis in various human carcinomas correlates with the extent of PGE2 production by the tumors. The extraordinary intricacy of PGE2 effects on macrophages (Mφ) and TAMs is complicated by multiple subtypes of PGE2 receptor (EP1-4) co-expressed in the same cell. Although EP2 and EP4 are strongly implemented in regulation of Mφ activities, the role of each EP subtype in immunoediting of TAMs remains unclear. Therefore, we hypothesized that tumor cells secreting PGE2 modulate an M2-like phenotype in

TAMs in an EP2-dependent manner. To test our experimental hypothesis, we generated human Mφ (PMA-treated THP-1 cells) stably transfected with shRNA lentiviral vector plasmids capable of effective inhibition of EP2 gene. The EP2low phenotype of THP-1 Mφ obtained was validated by a confocal microscopy using a monoclonal rabbit anti-EP2 antibody. To reproduce tumor microenvironment in vitro, EP2low THP-1 Mφ were co-cultured with cyclooxygenase-2 (COX2)-positive and PGE2-producing HCA-7 human carcinoma cells. TAMs obtained were then purified using CD14-specific immuno-magnetic beads. Protein levels of cytokines and chemokines produced by EP2low TAMs in co-culture with HCA-7 cells were analyzed with Proteome ProfilerTM Human Cytokines and Chemokines Array and compared to that of untransfected wild-type TAMs. Our data strongly indicate that tumor-derived PGE2 may suppress M1 inflammatory response in TAMs in an EP2-dependent manner. Specifically, EP2low TAMs showed an increased production of M1-type anti-tumor cytokines and chemokines including IL-12, IL-23, IL-27, IFN-γ, IP10, and RANTES. Moreover, EP2low TAMs also exhibited suppressed production of pro-tumor IL-17, IL-32, macrophage migration inhibitory factor (MIF), serpin peptidase inhibitor (SERPINE1), and stromal cell-derived factor-1 (SDF-1). Anti-tumor apoptosis-inducing properties of EP2low TAMs were further validated by analyses of apoptotic responses in cocultured HCA-7 cells by using a Proteome ProfilerTM Human Apoptosis Array, CellTiter 96® AQ Proliferation Assay, and FITC-labeled annexin V. Our data show a strong enhancement of apoptosis-inducing activities of EP2low TAMs which were confirmed by activation of the extrinsic and intrinsic apoptotic pathways in tumor cells. Collectively, tumor-derived PGE2 may suppress M1 inflammatory response in TAMs in an EP2-dependent manner suggesting potential efficacy of selective EP2 antagonists in treatment of Cox-2+ tumors.

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Preclinical Assessment of iNKT Cell Modulating Monoclonal Antibodies

Felix Scheuplein, Katherine Schlieper, Robert Mashal, Alem Truneh

NKT Therapeutics, Inc.

Invariant Natural killer T (iNKT) cells are a subset of T lymphocytes that share surface markers and functional characteristics with both conventional T cells and natural killer (NK) cells. Unlike other T cells, they recognize glycolipid antigens rather than peptide antigens by the MHC class-I-like protein CD1d. In contrast to most T cell subpopulations, which have diverse sequences for their T Cell Receptors (TCRs), iNKT cells express a uniquely rearranged, highly conserved, semi-invariant TCR-α chain (Vα24-Jα18 in humans and Vα14-Jα18 in mice), which preferentially pairs with specific TCR-β chains (Vβ11 in humans or Vβ8.2, Vβ7 and Vβ2 in mice). iNKT cells are rapid-onset cells with a universal receptor. They also share properties of T cells in that they require thymic positive selection and recognition of antigen presented on the MHC-I like molecule CD1d. As such, they serve as a bridge between the two systems where they can play both pro- inflammatory or immuno-regulatory roles either to enhance or attenuate developing immune responses, respectively. iNKT cells have been shown to be involved in mediating tissue injury and inflammation following ischemia reperfusion injury (IRI) in multiple organ systems. There is a growing understanding that chronic IRI is associated with the pathophysiology of Sickle cell disease (SCD). The role of iNKT cell activation in the pathology of SCD is supported by studies in a mouse model of sickle cell disease (Wallace et al. Blood 114:667-676, 2009). These data suggest that iNKT cell depletion would be of effective in reducing the inflammatory IRI state in the SCD patient. In addition, the activation of iNKT cell function has been considered for the treatment of cancer (Metelitsa, Clinical Immunology 140:119-129, 2011). NKT Therapeutics has developed fully humanized monoclonal antibodies directed to the human invariant TCR that specifically and exclusively either deplete (NKTT120) or activate (NKTT320) human invariant NKT cells. Employing human invariant TCR transgenic mice we assessed the pharmacodynamic profile of these antibodies. We found that NKTT120 can potently and rapidly deplete iNKT cells in vivo with higher doses needed for tissue depletion than peripheral depletion. iNKT cells in the transgenic mice recovered within weeks of complete depletion. We found that NKTT320 can potently activate iNKT cells in vivo, indicated by IFN-γ secretion, up regulation of CD69 and CD25 as well as their proliferation measured by BRDU-incorporation. While iNKT cell super-agonistic glycolipid α-Galactosylceramide induces anergy of iNKT cells after activation, NKTT320 was able to activate iNKT cells in consecutive treatments. Overall, NKTT120 and NKTT320 are promising candidate mAbs for manipulation of iNKT cell function in vivo.

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Does miRNA98 Regulate Anti-tumor Activities of Macrophages?

Mark Lee, Dax H. Demaree, Dmitriy Gromov, Ron Bean, Brett Calka, Alex Shnyra

Kansas City University for Medicine and Biosciences

Macrophages (Mφ) recruited to solid tumors, a.k.a. tumor-associated Mφ (TAMs), show an M2-like phenotype (IL-12low, IL-23low, and IL-10high) of alternatively-activated cells and promote tumor growth and the metastasis. Therefore, immunotherapies aimed at the switching M1/M2 balance towards an M1 classically activated Mφ (IL-12high, IL-23high, and IL-10low) represent a valid strategy for the development of novel immuno-modulatory approaches for treatment of cancer patients. Regulation of M1/M2 functional state occurs at multiple levels including miRNA, a class of small noncoding RNAs which bind to mRNAs leading to either mRNA degradation or inhibition of translation. Recently, it was found that the overexpression of miR-98 suppressed production of IL-10 in LPS-challenged Mφ suggesting that miR-98 may serve as a major regulator of M1 versus M2 responses. Recently, we identified two miRNA species, i.e. miR21 and miR155, which were overexpressed in Mφ co-cultured with HCA-7 human carcinoma cells. Therefore, we hypothesized that the overexpression of miR-98 alone or accompanied by the targeted inhibition of miR21 and miR155 may potentiate the anti-tumor activities of TAMs. To test our experimental hypothesis we used Mφ (PMA-treated THP-1 cells) cocultured with HCA-7 cells. Mφ were transiently transfected with miR-98 mimics alone or with miR21 or miR155

antagomir or in a combination of both before coculturing with HCA-7 tumor cells. In order to evaluate Mφ-induced apoptosis, HCA-7 cells were then isolated by a negative selection using CD14-specific immuno-magnetic beads. Induction of apoptosis in carcinoma cells was assessed by using a Proteome ProfilerTM Human Apoptosis Array, CellTiter 96® AQ Proliferation Assay, and FITC-labeled annexin V. Our data show a strong enhancement of apoptosis-inducing potential of Mφ overexpressing miR98 which were validated by activation of the extrinsic and intrinsic apoptotic pathways in tumor cells. Specifically, increased protein levels of pro-apoptotic Bad, Bax, pro-caspase 3, cleaved caspases 3, and SMAC/Diablo were detected in HCA-7 cells. Apoptosis in HCA-7 cells was further confirmed by a confocal microscopy using FITC-labeled annexin V and PE-labeled anti-CD14 antibody used for counterstaining of miR98high Mφ. Moreover, cotransfection with either miR21 or miR155 antagomir further potentiated the apoptosis-inducing properties of miR98-overexpressing Mφ predominantly augmenting the intrinsic apoptosis in HCA-7 cells. Unexpectedly, co-transfection with a combination of miR21 and miR155 antagomirs inhibited anti-tumor properties of miR98high Mφ in a co-culture with HCA-7 cells. Taken together, our findings suggest that miRNAs regulating M1/M2 immuno-inflammatory properties of Mφ may represent legitimate pharmacological targets for the development of novel immunotherapeutic modalities for treatment of solid tumors.

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Mesenchymal Stem Cell Treatment Diminishes Pulmonary Inflammation in Mice Exposed to Binge Alcohol and Burn Injury

Jill A. Ippolito[1, 2, 3], Luis Ramirez[2, 3], Jessica L. Palmer[2, 3], Anita Zahs[2, 3], Kovacs J. Elizabeth[1, 2, 3]

[1]Program of Integrative Cell Biology; [2]Burn & Shock Trauma Institute; [3]Loyola University Chicago, Stritch School of Medicine, Maywood, IL 60153

Binge alcohol drinking is practiced by an estimated 38 million adults in the United States and factors into more than 50 different injuries or diseases, including burn injury. Clinical evidence reveals that 50% of burn patients are under the influence of alcohol at the time of hospital admission and that the combined insult of alcohol and burn injury causes increased risk of pulmonary complications leading to multiple organ failure and death. Our previous work demonstrated that a mouse model of alcohol and burn injury resulted in prolonged pulmonary inflammation characterized by amplified neutrophil accumulation and dramatic increases in pro-inflammatory cytokines and chemokines relative to burn injury and sham groups. Successfully controlling this excessive inflammatory response is essential to reducing morbidity and mortality rates in patients with combined injury. Since mesenchymal stem cells (MSCs) have been shown to attenuate acute lung inflammation, we explored exogenous MSCs as a post-injury therapy in our model of multi-day (episodic) binge alcohol exposure and burn injury. Eight week-old C57BL/6 male mice were subjected to episodic binge alcohol exposure (3 days of ethanol, 4 days off, and 3 days of ethanol). Thirty minutes following the final ethanol exposure, mice were given a 15% total body surface area dorsal scald injury. One hour after injury, groups of mice were given bone marrow-derived MSCs ($5x10^5$ i.v.). At 24 hours post injury, examination of lung tissue revealed that mice exposed to the combined insult had increased pulmonary edema and leukocyte accumulation relative to controls, while mice that were treated with MSCs showed a notable decrease in alveolar wall thickening and cellularity. In parallel to the decrease in cellularity observed histologically, flow cytometry of whole lung cell isolates from mice subjected to ethanol and burn showed a 2-fold increase in CD45+ cells in comparison to sham groups ($p<0.001$), while combined injury mice treated with MSCs had a 40% reduction in these cells ($p<0.01$). Moreover, MSC given to combined injury mice also reduced neutrophil chemoattractant KC (CXCL1) levels in lung tissue by 40%, in comparison to combined injury treated with vehicle only, suggesting a potential mechanism for the decline in leukocyte accumulation. Additionally, ethanol exposed, burn injury mice treated with MSC had a 60% reduction in lung interleukin-6 levels in comparison to their respective controls ($p<0.01$). Together, these data demonstrate that exogenous administration of MSC to mice after binge ethanol and burn injury lessens the over exuberant pulmonary inflammatory response seen after combined injury. Further research to elucidate how MSCs attenuate inflammation in this model may uncover novel therapeutic targets which will help to improve survival in all burn patients. [This work was supported by R01AA012034 (EJK), T32 AA013527 (EJK), F31 AA019913 (AZ), Ralph and Marian C. Falk Medical Research Trust (EJK)].

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Kinase Activity of Phosphoinositide 3-Kinase Gamma (PI3Kγ) Is Needed for T Cell Development, Activation And Chemotaxis

Nadia Ladygina, Wai-Ping Fung-Leung

Immunology, Janssen Research & Development, L.L.C., San Diego, CA92121, USA

Phosphoinositide 3-kinase gamma (PI3Kγ) catalyzes the production of phosphatidylinositol-3, 4, 5-triphosphate by phosphorylating phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4, 5-bisphosphate (PIP2). Catalytic subunit of PI3Kγ is activated by βγ subunits of heterotrimeric G proteins in response to chemokines, growth factors and hormones, which in turn coordinates cell migration, cell growth, cell cycle entry, and cell survival. PI3Kγ has been implicated in T cell function, but direct effect of its kinase function has not been evaluated. Using mice expressing a catalytically inactive mutant of PI3Kγ(PI3KγKD/KD) we were able to demonstrate that defect in kinase activity leads to T cell developmental defect in the thymus and results in decreased peripheral T cell numbers. PI3KγKD/KD peripheral CD4 and CD8 T cells demonstrated impaired Ag-induced activation, reduced chemokine driven chemotaxis and impaired T cell differentiation. Thus, our study demonstrated that kinase activity of PI3Kγ amend status of T cell response by contributing to thymic development, T cell activation and differentiation as well as lymphocyte chemotaxis.

AUTHOR INDEX

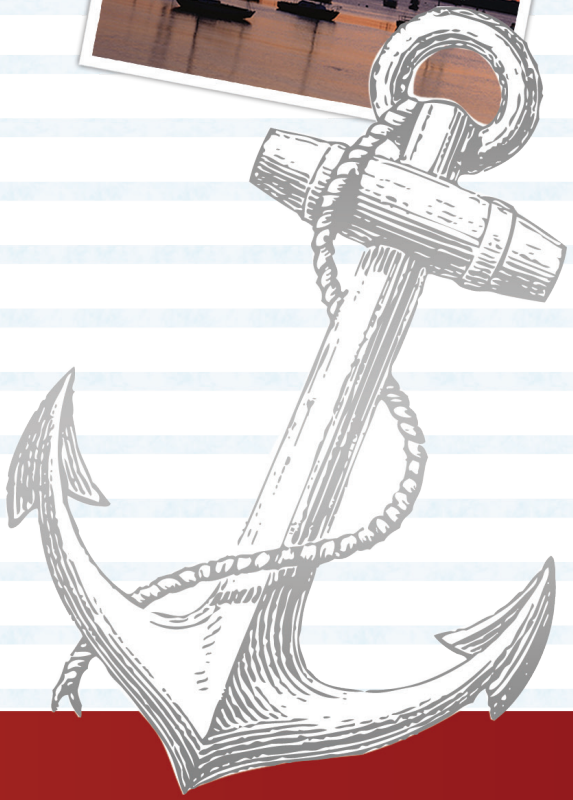
The number following each name refers to the abstract number.

October 20-22, 2013
Newport Marriott, Newport, Rhode Island

46th Annual Meeting of the
Society for Leukocyte Biology
Regulators of Innate Cell Plasticity

Plenary Sessions

1. Unique approaches to Dissecting Innate Cell Microbial Interactions
   – Christian Stehlik, Leonard Shultz & Fiona Powrie
2. Myeloid Subset as Contributors to Pathology
   – Liwu Li, Lisa Coussens, & Alberto Mantovani
3. Life at the Leukocyte: Epi-/Endo-thelial Cell Interface
   – Mark Miller, Paul Kubes & Claire Doerschuk
4. Novel Prospective on Co-Inhibitor Function and Modulation in Inflammation and Disease
   – Andrew Lichtman, Al Ayala, & Carol Miller-Graziano
5. Leukocyte Function/Significance in the Aging Host
   – Ruth Montgomery, Elizabeth Kovacs, & Carlos Orihuela



Concurrent Sessions

1. Lymphocyte roles in Innate Immunity – Dana Philpott
2. Under Appreciated Leukocyte Roles in Immunity – Joel Ernst
3. Novel Leukocyte regulators – Bonnie Dittel
4. Negative signaling regulators of Lymphocyte and Leukocyte Differentiation – Francisco Quintana
5. Regulatory Mechanisms in Leukocyte Trafficking – Minsoo Kim
6. Leukocyte Mediators in Inflammation/Infection – Dan Remick
7. Novel Adjuvants/Activators of Leukocyte Function
   – Jonathan Reichner
8. Mechanisms of Immune Privilege and/or Tolerance
   – Rachel Caspi

Visit **www.leukocytebiology.org** for more information

Agenda-At-A-Glance

SATURDAY, OCTOBER 27TH

8:00 am-2:00 pm
Satellite Symposium
Alcohol and Immunology: Receptors and Signal Transduction
LOCATION: Plumeria

SUNDAY, OCTOBER 28TH

7:00-8:15 am
Networking Breakfast
LOCATION: Haleakala Gardens

7:00-8:15 am
Street Smarts of Science: A Survival Guide for Graduate Students
LOCATION: Plumeria

8:15-8:30 am
Welcome
LOCATION: Haleakala 4/5

8:30-10:00 am
Plenary I: Innate Immune Recognition Mechanisms and Host Defense
LOCATION: Haleakala 4/5

10:00 am-5:00 pm
Exhibits Open
LOCATION: Haleakala Gardens

10:00-10:30 am
Coffee Break
LOCATION: Haleakala Gardens

10:30 am-12:30 pm
Concurrent Sessions

CS 1: Host-Pathogen Interactions
LOCATION: Haleakala 4/5

CS 2: B lymphocytes in Immune Regulation and Autoimmunity
LOCATION: Haleakala 2

12:30-1:30 pm
Lunch on Your Own

1:30-3:00 pm
SLB Presidential Nominees
LOCATION: Haleakala 4/5

3:00–4:00 pm
Poster Session A / Coffee Break
LOCATION: Haleakala 1 and Gardens

4:00-5:00 pm
Senior Level Awards
LOCATION: Haleakala 4/5

5:00-6:00 pm
Keynote Lecture
LOCATION: Haleakala 4/5

6:00 pm-7:00 pm
Opening Reception
LOCATION: Haleakala Gardens

MONDAY, OCTOBER 29TH

7:00-8:30 am
Networking Breakfast
LOCATION: Haleakala Gardens

7:00 am-5:00 pm
Exhibits Open
LOCATION: Haleakala Gardens

7:00-8:30 am
How to Write Your First Grant Application
LOCATION: Plumeria

8:30-10:00 am
Plenary II: Inflammasomes
LOCATION: Haleakala 4/5

10:00-11:00 am
Poster Session B / Coffee Break
LOCATION: Haleakala 1 and Gardens

11:00 am-1:00 pm
Concurrent Sessions

CS 3: Cytokine Signaling and Inflammation I
LOCATION: Haleakala 4/5

CS 4: Dendritic Cell and Macrophage Regulation
LOCATION: Haleakala 2

1:00-2:00 pm
Lunch on Your Own

2:00–3:30 pm
Plenary III: Myeloid Cell Populations in Cancer: The Link with Inflammation
LOCATION: Haleakala 4/5

3:30-4:00 pm
Member Business Meeting and Presidential Award Announcements
LOCATION: Haleakala 4/5

4:00-5:00 pm
Poster Session C / Coffee Break
LOCATION: Haleakala 1 and Gardens

5:00-7:00 pm
Concurrent Sessions

CS 5: Treg and Th17 Cells in Host Defense and Autoimmunity and γδT, NK, NKT cells
LOCATION: Haleakala 4/5

CS 6: Cytokine Signaling and Inflammation II
LOCATION: Haleakala 2

7:00 -9:00 pm
Social Mixer
Chapel Lawn

TUESDAY, OCTOBER 30TH

7:00 am-8:30 am
Networking Breakfast
LOCATION: Haleakala Gardens

7:00 am-2:00 pm
Exhibits Open
LOCATION: Haleakala Gardens

7:00-8:30 am
Women and Diversity Workshop
LOCATION: Plumeria

8:30-10:00 am
Plenary IV: Epithelium: The Interplay Between Innate and Th2 Immunity
LOCATION: Haleakala 4/5

10:00-11:00 am
Poster Session D / Coffee Break
LOCATION: Haleakala 1 and Gardens

11:00-1:00 pm
Concurrent Sessions

CS 7: Micro RNA and Post-transcriptional Regulation in Inflammatory Response
LOCATION: Haleakala 4/5

CS 8: Neuroimmunology
LOCATION: Haleakala 2

1:00-2:00 pm
Lunch on Your Own

2:00-4:00 pm
Plenary V: Metabolism in Immune Regulation
LOCATION: Haleakala 4/5