20th International Conference

July 20th –July 21st 2018

Temple Medical School

Philadelphia, PA

Meeting Program & Abstract Book

Welcome Letter from the Meeting Organizers

July 20th 2018

Dear Colleagues,

On behalf of the Inflammation Research Association (IRA), we are excited to introduce the 20th international conference of the IRA, jointly organized with the Temple University School of Medicine on July 20-21 of 2018 at Philadelphia, PA. Every two years, the Inflammation Research Association (IRA) organizes an International Conference that attracts 200-300 pre-clinical and clinical research scientists working in the pharmaceutical and biotechnology industries, and in academia. For more than 40 years the IRA has worked to bring scientists together for the sharing of scientific and technological advances, as well as collegial interaction. The scientific program of the 20th International Conference is designed to reflect the mission of the IRA.

The Inflammation Research Association is a non-profit organization dedicated to engaging and encouraging scientists, researchers, students, teachers and clinicians with an interest in inflammation biology. Our mission is to foster and facilitate a more informed, connected and collaborative community whose goal is to serve and promote scientific and technological advances towards developing new therapeutic strategies for complex and diverse inflammation-related diseases.

Besides an outstanding scientific program, our meeting will provide opportunities for participants to meet colleagues, build networks, and establish future collaborations. There will be an opening reception on the first night of the meeting following the poster session. We are looking forward to collegial and professional networking, in addition to vigorous discussion of cutting-edge science that integrate basic, translational and industry research on inflammation biology.

Finally, we express our sincere thanks and appreciation to all our sponsors and exhibitors including Pfizer, Biocytogen, Biomodels, BolderBioPath, Calvert Labs, Cell Biologics, Eurofins, Focus Biomolecules, Inflammatory Markers Laboratory, Myriad RBM, Nanostring, and Proteintech- whose generous support helps make this program possible.

Sincerely

Liwu Li, Hong Wang, Xiaofeng Yang, Lisa Schopf

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Conference Program

Friday, July 20, 2018

9:00 am – 4:00 pm	REGISTRATION
9:00 am – 4:00 pm	EXHIBITION OPEN
9:00 am – 9:10 pm	President's Welcome
9:10 am -12 noon	**SYMPOSIUM 1 Non-resolving inflammation in chronic diseases**
9:10 am-9:45am	Dr. Edimara Reis/John Lambris, University of Pennsylvania *Complement Therapeutics for Inflammatory Diseases*
9:45 am-10:20 am	Dr. Glen Barber, U Miami
10:20 am -11:05am	Dr. Dmitry Gabrilovich, The Wistar Institute
11:05 am- 11:50	Dr. Hong Wang, Temple Medical School *Immune cell subsets and pathophysiological network in metabolic disorders*

Afternoon Session
Friday, July 20, 2018

12:00noon-1:00pm	IRA BOARD MEETING/LUNCH
12:00- 1:00pm	Lunch, exhibitor visit
1:00 pm – 3:00 pm	**Concurrent symposium 1 - Cellular and molecular mechanisms for inflammation** **-chair: Dr XiaoFeng Yang, Dr. Jun Yu,Temple Medicine**
1:00pm - 1:25 pm	Dr. XiaoFeng Yang, Temple Medicine *Endothelial cells are innate immune cells*
1:25 pm - 1:50 pm	Dr. Mohsin Khan, Temple Medicine *Stem cells, exosomes and microRNAs in cardiac wound healing*
1:50 pm - 2:05 pm	Dr. Jun Yu, Temple Medicine
2:05 pm - 2:30 pm	COFFEE BREAK (with vendors)
2:30 pm - 2:45 pm	Dr. Katya Koltsova, Fox Chase Cancer Institute
2:45 pm - 3:00 pm	Dr. Mike Autieri, Temple Medicine

1:00 pm – 3:00 pm	**Concurrent symposium 2 - Inflammation in Autoimmunity** **-chair: Dr Stefania Gallucci, Temple Medicine**
1:00pm - 1:20pm	Dr. Stefania Gallucci, Dept. Micro-Immuno, LKSOM, Temple University Title: "*Role of Type I Interferons in Systemic Lupus Erythematosus*"
1:20 pm -1:40pm	Dr. Sharlene Velichko, Eurofins DiscoverX, Title: "*Phenotypic comparison of approved anti-IL-17 therapeutics using the BioMAP® human cell-based profiling platform"*
1:40 pm- 2:00pm	Dr. Philip L. Cohen, Dept. Medicine, LKSOM, Temple University Title: "*The TAM Kinases: Key Regulators of Autoimmunity and Inflammation*"
2:00pm -2: 15pm	COFFEE BREAK (with vendors)
2:15 pm-2:30 pm	Connie C. Qiu, Dept. Micro-Immuno, LKSOM, Temple University Title: "*Bacterial amyloids from biofilms induce type I interferon response in plasmacytoid dendritic cells.*
2:30 pm-2:45 pm	Mona A. Bawazeer, Tufts University, Title: "*IL-33 Stimulating Human Mast Cell Chemokine, and Inhibition by Tetramethoxyluteolin".*
2:45 pm-3:00 pm	Ryan Pachucki, Dept. Medicine LKSOM, Temple University Title: "*Bacterial biofilm product Curli/eDNA induces NETs and serum anti-Curli/eDNA levels correlate with bacteriuria and lupus activity*".
1:00 pm – 3:00 pm	**Concurrent symposium 3 - Inflammation and Cancer** **-chair: Dr Mike McQueney, Oncoveda**
1:00pm -1:10 pm	Dr. Mike McQueney, Oncoveda opening remarks
1:10 pm-1:45 pm	Dr. Murali Gururajan, Bristol-Myers Squibb *Targeting inflammatory cells for anti-tumor immunity in pancreatic cancer*
1:45 pm-2:05 pm	Dr. Jason Trama (Institute for BioMarker Research) *Using Molecular Mammo Scan(TM) to Assess the Function of Drug Target Mutations*
2:05 pm-2:25 pm	COFFEE BREAK (with vendors)
2:25 pm-3:00 pm	Dr. Jeonghyun Ahn, University of Miamia *Colonic Inflammatory Disease and The STING Innate Immune Pathway*

3:30 pm – 5:00 pm	**Joint Van Arman and Junior Investigator Session** **-chair: Dr Mohsin Khan, Sponsored by Pfizer**
3:30pm - 3:35pm	Opening Remarks
3:35 pm-3:50 pm	Elaine Bradford *In vitro model of neutrophil swarming in a chronic, low-level inflammatory state*
3:50 pm-4:05 pm	Fu Fang *Severe Hyperhomocysteinemia Potentiates Inflammatory Monocyte Differentiation and Vascular Dysfunction in T2DM*
4:05 pm-4:20 pm	M.H. Lee *Bacterial amyloids from biofilms break tolerance in lupus by simultaneous BCR/TLR signaling*
4:20 pm-4:35 pm	Gayani Nanayakkara *Lysophosphatidylinositol and endothelial activation*
4:35 pm-4:50 pm	Raj Putatunda *Adult Neurogenic Deficits in HIV-1 Tg26 Transgenic Mice*
4:50pm -5:05 pm	Bharat Behl Mo*lecular determinants of noncanonical inflammasome activation by LPS*
5:05 pm - 6:30 pm	POSTER PRESENTATION AND COMPETITION
6:30 pm – 9:00 pm	Dinner and Reception

Saturday, July 21, 2018

9:00 am – 4:00 pm	REGISTRATION, EXHIBITION OPEN
9:00 am -12 noon	**SYMPOSIUM 2** **Intervention of systems inflammation and chronic diseases**
9:00 am-9:45 am	Dr. Lynn Soong, UTMB *Pathogenic Mechanisms of Endothelial Damage in Severe Scrub Typhus*
9:45 am-10:20 am	Dr. Filip Swirski, Harvard University
10:20am -11:05am	Dr. Liwu Li, Virginia Tech *Dynamics of innate immune memory in health and disease*
11:05 am-11:50	Dr. Erica Stone, Wistar Institute *Further investigating mechanisms of action of anti-CTLA4 therapy*

Afternoon Session
Saturday, July 21, 2018

12:00noon-1:00pm	IRA BOARD MEETING/LUNCH/Exhibitor visit
1:00 pm – 3:05 pm	**Concurrent symposium 4 – Microbiome, Novel technology in systems inflammation** **-chair: Dr Wenhui Hu, Co-chair: Cagla Tukel, Temple Medicine**
1:00pm - 1:18pm	Dr. Cagla Tukel, Temple University
1:18 pm - 1:36 pm	Dr. Vicent Tam, Temple University *Resolution of Inflammation during Influenza Impacts the Anti-Bacterial Host Response*
1:36 pm - 1:54 pm	Dr. Wenhui Hu, Temple University *Genome editing in inflammation*
1:54 pm -2:12 pm	Dr. Yan Zhou, Fox Chase Cancer Center
	2:12pm- 2:20pm Coffee break
2:35 pm - 2:50 pm	Dr. S Maktar, CUNY *Anti-inflammatory action of statins on chemokine induction and survival in severe peritonitis*
2:50 pm - 3:05 pm	Jeremy Drees, MD Biosciences *Diet-induced animal models of Non-alcoholic steatohepatitis*
3:05 pm – 3:25 pm	Hangfei Fu, Temple Medicine *Anti-inflammatory cytokine IL-35 inhibits ischemia/hypoxia-induced angiogenesis*

1:00 pm – 3:05 pm	**Concurrent symposium 5 - Neuro-inflammation** **-chair: Matthew Buczynski**
1:00pm -1:25 pm	Dr. Matthew Buczynski *Integrated Overview of the Neuroinflammation Session*
1:25 pm-1:50 pm	Dr. Ann Gregus *Inhibition of spinal 15-LOX-1 attenuates TLR4-dependent, NSAID-unresponsive hyperalgesia*
1:50 pm-2:20 pm	COFFEE BREAK (with vendors)
2:20 pm-2:45 pm	Dr. Alicia Pickrell *PINK1 and Parkin Influence Cell Cycle by Sequestering Active TBK1 to Damaged Mitochondria During Mitosis*

2:45 pm-3:10 pm	Dr.Georgia Hodes *Immune mechanisms of individual differences in the behavioral response to social defeat stress*
3:10 pm-3:25 pm	Dr. Yow-Pin Lim *Inter-alpha Inhibitors as a Novel Immunomodulator in Neonatal Hypoxic-Ischemic Brain Injury and Ischemic Stroke*
3:25 pm - 3:50 pm	Award and closing ceremony

Poster Session Abstracts

A101

Immune inflammation in seemingly unaffected areas of IPF lungs

Sergei P. Atamas*, Nevins W. Todd, Irina G. Luzina
University of Maryland School of Medicine, Baltimore, MD, USA

In gross examination of the lungs of patients with idiopathic pulmonary fibrosis (IPF), peripheral and basilar areas appear overtly scarred (IPFs), whereas central areas often appear normal (IPFn), although microscopic foci of organizing pneumonia and cellular non-specific interstitial pneumonia are widespread in such areas. We used an RNASeq approach to compare the transcriptomes of 10 IPFn and 8 IPFs lung tissue samples from explant lungs of 3 IPF patients as well as 8 healthy control (HC) lung tissue samples from 3 healthy donors. Pronounced elevations in the expression of extracellular matrix-, immunity-, and inflammation-related mRNAs were observed in IPFn and IPFs compared with HC samples, whereas IPFs had elevated expression of epithelial mucociliary mRNAs compared with IPFn samples. There were minimal transcriptomic differences between primary fibroblast cultures derived from IPFn and IPFs samples. Thus, despite the macro- and microscopic heterogeneity of IPF lung tissues, there is a great deal of transcriptomic homogeneity, which includes elevated expression of genes related to immune and inflammatory responses in seemingly disease-spared areas. These findings support the notion of immune and inflammatory mechanism contributing to lung fibrosis.

A102

Exposure to Bisphenol A or Bisphenol S does not affect dendritic cell activation

A. Atencio*, C. Qiu, S.Gallucci
Department of Microbiology & Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140

Bisphenol A (BPA) and Bisphenol S (BPS) are polycarbonate plastic components with negative implications on human health. Their roles in autoimmunity are not well understood. We treated murine bone marrow-derived dendritic cell (DC) cultures containing conventional dendritic cells (cDCs) and enriched for plasmacytoid dendritic cells (pDCs) with BPA and BPS at varying doses. Activation of cDCs and pDCs were evaluated by flow cytometry analysis of surface costimulatory markers (CD86 and CD40). Culture supernatants were assayed for IL-6, a pro-inflammatory cytokine, and CXCL10, an important chemokine under control of type I IFN. Our data shows no significant upregulation of either surface costimulatory markers or cytokines and chemokines after 24h of exposure to 0.1nM, 500uM, and 1uM BPA or BPS. These results suggest that BPA and BPS do not interfere with pDC and cDC activation *in vitro*. The negative results do not support the present alarm for the use of BPA and BPS in food containers but more experiments, especially in vivo, are required to confirm the safety of these compounds.

A103

IL-33 Stimulating Human Mast Cell Chemokine, and Inhibition by Tetramethoxyluteolin

Mona A. Bawazeer*[1], Theoharis C. Theoharides PhD, MD[1,2]

[1]Graduate Program in Pharmacology and Experimental Therapeutics, Sackler School of Graduate Biomedical Sciences and [2]Department of Immunology, Tufts University School of Medicine, Boston, MA 02111, USA

Mast cells (MCs) are hematopoietic cells that mature in all tissues and are known to be stimulated by allergic triggers such as IgE/anti-IgE, which is augmented by the cytokine IL-33. Upon stimulation, MCs release pre-formed mediators (histamine), as well as newly synthesized cytokines (TNF) and chemokines [CXCL8 (IL-8), CCL5 (RANTES), and CCL2 (MCP-1)]. SP and IL-33 synergistically stimulate TNF release from human cultured MCs with IL-33 having the most potent effect. This work is intent to investigate the action of IL-33 on chemokine secretion in human LAD2 MCs. IL-33 (10ng/ml) stimulates LAD2 via MAPK activation and leads to release of CXCL8 (5,800pg/ml), CCL5 (400pg/ml), CCL4 (50,000pg/ml), CCL3 (8,000pg/ml), and CCL2 (3,800pg/ml), and in corresponding the gene expression. Inhibition of phosphorylated p38 and JNK, by compounds known to inhibit each, inhibited chemokine secretion. IL-33 stimulated responses are significantly inhibited by the flavonoid, tetramethoxyluteolin (methlut) (10, 50, 100) μM with 30%-70% inhibition for all the investigated chemokine except CXCL8. The inhibition is not via MAP kinases inhibition and further experiment will be conducted looking at methlut effect on AP-1 transcription and its localization in LAD2 MCs. This work will lead to understand chemokine regulation in human MC and find a novel anti-allergic and anti-inflammatory treatment.

A104

Molecular determinants of noncanonical inflammasome activation by LPS

Bharat Behl[1*], Sivapriya Kailasan Vanaja[1] and Vijay Rathinam[1]

[1]Department of Immunology, UConn Health School of Medicine, 263 Farmington Avenue, Farmington CT 06030, USA

Septic shock is the leading cause of morbidity and mortality in intensive care units worldwide. Gram-negative bacteria constitute one of the most common causes of sepsis that results in high fatality by initiating an excessive and uncontrolled host inflammatory response. At the epicenter of this response is the innate immune detection of lipopolysaccharides (LPS). In addition to TLR4 recognition of LPS, recent studies revealed a new LPS sensing mechanism in the cytosol. Inflammatory caspases, such as caspase-11, detect LPS to execute pyroptosis, an inflammatory form of cell death, and caspase-1 activation. A prerequisite for the activation of noncanonical inflammasome is the transcriptional induction of caspase-11, which is mediated by TLR4-TRIF, type I interferon, and complement signaling. Additionally, guanylate binding proteins (GBPs), interferon regulatory factor 1, and IRGB10 orchestrate the release of LPS from the vacuolar bacterial pathogens into the cytosol. While the host factors involved in LPS activation of caspase-11 are fairly characterized, the role of bacterial factors in this process is not clear. Our new findings from this study delineate the bacterial components necessary for the optimal engagement of the cytosolic LPS sensing pathway and eliciting noncanonical inflammasome responses.

A105

Proposed *in vitro* model of neutrophil swarming in a chronic, low-level inflammatory state

Elaine Bradford*, Liwu Li.

Dept. of Biology, Virginia Tech, Virginia.

Chronic, low-grade inflammation is an underlying condition across a globally increasing number of debilitating diseases. These diseases include obesity, atherosclerosis, and diabetes and their resultant low-grade inflammation can be effectivity modeled with low dose stimulants such as

lipopolysaccharide (LPS). While the innate immunity plays a significant role in fighting infectious disease, an initial exposure to low dose LPS hinders secondary infection clearance and pre-disposes murine models for fatal sepsis. Neutrophils are the most prevalent circulating innate immune cell and their homotypic aggregation, or swarming, is a key mechanism in clearing pathogens greater than 20 μm in size. We hypothesize that neutrophil swarming ability is altered when in a low dose LPS primed state; potentially leading to an overall altered innate immune response in the face of infection. However, an *in vitro* model does not currently exist to reliably quantify and compare neutrophil swarms across treatment groups. Here we propose a novel model utilizing fungal zymosan coated beads as a uniform target to which neutrophils may swarm.

A106

Homocysteine-mediated expression changes link to energy metabolism in endothelial cells dysfunction

Michael Jan, Ramon Cueto*, Xioahua Jiang, Xinyu Xiong, Justine E. Yu, Hung Pham, Xiao-Feng Yang, Hong Wang. Center for Metabolic Diseae Research, Temple Medical School

Homocysteine (Hcy) is a non-traditional risk factor for CVD whose pathological mechanisms remain to be fully elucidated. Hcy has been shown to inhibit endothelial cell (EC) growth and proliferation through modulation of specific molecules and pathways. In addition, Hcy have been strongly associated with energy metabolism including glycolysis and mitochondrial, given the relatively close relationship between Hcy metabolic pathway and this pathways. The purpose of this study is to examine the effects of Hcy on global expression patterns of microRNA (miRNA) and mRNA in EC as well as target pathway analyses. Significantly differentially expressed (SDE) miRNA and mRNA were entered into experimentally-verified databases to determine associated mRNA and miRNA, respectively. To determine global functional significance, Ingenuity Pathway Analysis and Geen Set enrichment Analysis (GSEA) was applied to miRNA-mRNA pairs with inversely correlated expression changes from microarray data. In addition, specific fuel pathways including glycolysis, TCA cycle, Fatty acid and mitochondrial electron transport chain (ETC) was analyzed. Our finding showed that SDE were involved mechanisms of CVD and functions of the cell cycle and lysosomes. In the other hand, the energy pathway analyses shows indication of hyper-metabolism. This data may provide potential target interventions as well as new knowledge to the understanding for contributory pathways and mechanisms of EC dysfunction and CVD.

A107

Diet-induced animal models of Non-alcoholic steatohepatitis

Jeremy Drees*, Takashi Kangas, Weiyu Zhang, Britnie James, Department of Preclinical Services, MD Biosciences

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in the Western world, and its more advanced manifestation, Non-alcoholic steatohepatitis (NASH), has emerged as an important area of therapeutic potential. To model NASH development, two experimental diets (Diet 1 and 2) were fed to wild type male C57BL/6 mice for 4-9 weeks. Disease progression was observed longitudinally for both Diet 1 and Diet 2 via significant increases in serum alanine aminotransferase and aspartate aminotransferase levels in experimental diet-fed animals compared to standard diet-fed controls. Liver samples from mice collected at various time points also demonstrated pathological findings consistent with NAFLD and NASH development, including varying levels of steatosis, inflammation, and fibrosis. While both experimental diets induced liver disease over time, they varied in their timing and severity.

Additionally, animals fed Diet 1 showed a significant reduction in body weight, which was alleviated in mice fed Diet 2. These results demonstrate successful NASH modeling in wild-type animals and illustrate the potential value of diet-induced mouse models to assist with therapeutic development in this disease area.

A108

Canonical and non-canonical inflammasomes may sense hyperlipidemic DAMPs and initiate liver inflammation in NAFLD *Drummer IV, C*., Patel, D., Johnson, C., Wang, H., Yang, X.F. Center for Metabolic Disease Research, Cardiovascular Research Center, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140*

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function in countries with western-style, high-fat diets. While the role of the canonical inflammasome pathway in the pathogenesis of NAFLD is well studied, the non-canonical inflammasome pathway is not well studied. We hypothesized that the non-canonical pathway has its own upstream and downstream mediators, which may be independent of that associated with the canonical pathway. To test our hypothesis, we performed experimental data mining in various NAFLD and macrophage GEO microarray datasets. Our analysis revealed the following: 1) Canonical and non-canonical inflammasome pathway genes are differentially expressed between patients with hepatosteatosis and NASH, and 2) canonical and non-canonical inflammasome pathway genes are highly differentially expressed in human M1 macrophages. These findings have provided insights on differential regulatory mechanisms of canonical and non-canonical inflammasomes in macrophages related to the pathogenesis of NAFLD.

A109

Specialized pro-resolving mediators and transcriptomic signatures in bleomycin-induced lung fibrosis

M. Dubourdeau*, G. Chêne, V. Baillif, C. Guigné, E.Wanecq, E. Van Goethem
Ambiotis SAS – Toulouse – France

Idiopathic pulmonary fibrosis (IPF) is characterized by the apparition of collagen fibers into the lung parenchym, leading to an excessive and irreversible healing of the tissue associated with a loss of its function. We investigated the potential involvement of specialized mediators of inflammation resolution (SPMs) in the control of fibrosis using an animal model of pulmonary fibrosis. Mice were inoculated with bleomycin and lipid mediators of inflammation and resolution were analyzed using mass spectrometry. RNA expression was evaluated thanks to a dynamic array.

We have shown an increase of pro-inflammatory lipids (PGE2, TxB2,…), intermediate lipids to SPMs (15HETE, 18HEPE; 17HDOHE). We also noticed an overexpression of inflammatory genes (COX1, IL6, Ccl1, MCP-1) and genes involved in the remodeling of the extracellular matrix (col1, Fn1, mmp2, Timp1). Resolvin D2 was the only detected SPM. These results suggest that fibrosis context is associated with an inflammatory status that did not seem counterbalanced by the production of SPMs. These results might thus draw the first evidence of a defect during fibrosis to produce a complete network of SPMs.

A110

Severe Hyperhomocysteinemia Potentiates Inflammatory Monocyte Differentiation and Vascular Dysfunction in T2DM

Pu Fang*, Huimin Shan, Xinyuan Li, Xiao-Feng Yang, Hong Wang

Center for Metabolic Disease Research, Department of Pharmacology, Lewis Kats School of Medicine, Temple University, Philadelphia, PA

***Background*:** Hyperhomocysteinemia (HHcy) is associated with increased diabetic cardiovascular diseases. Inflammatory monocyte (MC) has been implicated in type 1 diabetes mellitus (T1DM) and severe hyperhomocysteinemia (HHcy)-related cardiovascular complications. However, what's its role in type 2 diabetes mellitus (T2DM) and HHcy compound disease is unknown.

***Material and Methods*:** db/db mouse was used as a T2DM model in which a spontaneous mutation in leptin receptor gene (blood glucose 493 mg/dL). Severe HHcy (>100 μM) was induced by a high fat+high methionine diet (HF+HM diet, 8 weeks) in control (db/+) and T2DM (db/db) mice (129 μM and 180 μM, respectively), and rescued by vitamin supplement (HF+HM+HV diet) to 42 μM and 87 μM (moderate HHcy, 20-100 μM), respectively. Inflammation monocyte (MC) differentiation was assessed by examining MC subsets in using flow cytometry analysis. Vessel reactivity to acetylcholine (ACh) was determined in mouse aorta. Metabolic parameters were assessed by metabolic cage, plasma lipid/blood/insulin levels, and major organ weights.

Results: Severe HHcy potentiated inflammatory MC ($CD45^+CD11b^+Ly6C^+$ MC) in aorta isolated from T2DM mice. HHcy- and T2DM-induced mononuclear cells (MNC), $CD11b^+$ MC, $CD11b^+Ly6C^{middle+high}$ inflammatory MC and M1 macrophages (MØ) were potentiated by the combination of HHcy and T2DM in mouse bone marrow (BM), peripheral blood, and spleen. These effects are independent of lipids, since we also observed similar results in T2DM mice on HM diet (no HF). Folate based Hcy-lowering therapy (HF+HM+HV) reversed systemic MNC, MC, inflammatory MC and MØ and aortic inflammatory MC increases in T2DM mice. Moreover, severe HHcy aggravated T2DM-impaired endothelial-dependent vessel relaxation to ACh, which was abolished by endothelial nitric oxide synthase (eNOS) inhibitor NG-nitro-L-arginine methyl ester. Finally, bone marrow transplantation (BMT) using lentivirus-Ly6C shRNA transduced BM cells, which decreased blood $Ly6C^+$ inflammatory MC, resulted in ameliorated endothelial-dependent vessel relaxation. In conclusion, our data suggested that severe HHcy accelerated T2DM-induced systemic inflammatory MC and MØ differentiation, aortic inflammatory MC infiltration, and vascular dysfunction.

***Conclusion*:** Severe HHcy potentiated systemic and vessel wall inflammation, and vascular dysfunction via inducing inflammatory MC subset (Ly6C+) in T2DM mice. Ly6C may be a therapeutic strategy for cardiovascular complications in HHcy and DM including T1DM and T2DM.

A111

Severe Hyperhomocysteinemia Potentiates Inflammatory Monocyte Differentiation and Vascular Dysfunction in T2DM

Pu Fang*, Huimin Shan, Xinyuan Li, Xiao-Feng Yang, Hong Wang

Center for Metabolic Disease Research, Department of Pharmacology, Lewis Kats School of Medicine, Temple University, Philadelphia, PA

***Background*:** Hyperhomocysteinemia (HHcy) is associated with increased diabetic cardiovascular diseases. Inflammatory monocyte (MC) has been implicated in type 1 diabetes mellitus (T1DM) and severe hyperhomocysteinemia (HHcy)-related cardiovascular complications. However, what's its role in type 2 diabetes mellitus (T2DM) and HHcy compound disease is unknown.

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(db/db) mice (129 μM and 180 μM, respectively), and rescued by vitamin supplement (HF+HM+HV diet) to 42 μM and 87 μM (moderate HHcy, 20-100 μM), respectively. Inflammation monocyte (MC) differentiation was assessed by examining MC subsets in using flow cytometry analysis. Vessel reactivity to acetylcholine (ACh) was determined in mouse aorta. Metabolic parameters were assessed by metabolic cage, plasma lipid/blood/insulin levels, and major organ weights.

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***Conclusion*:** Severe HHcy potentiated systemic and vessel wall inflammation, and vascular dysfunction via inducing inflammatory MC subset (Ly6C+) in T2DM mice. Ly6C may be a therapeutic strategy for cardiovascular complications in HHcy and DM including T1DM and T2DM.

A112

Anti-inflammatory cytokine IL-35 inhibits ischemia/hypoxia-induced angiogenesis

Hangfei Fu*, Jun Yu, Eric T. Choi, Hong Wang, Xiaofeng Yang
Center for Metabolic Disease Research, Cardiovascular Research, Thrombosis Research, Department of Pharmacology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140

Current poor understanding of the roles of inflammation in angiogenesis prevents new therapies for improving post-ischemia angiogenesis. In our study, we made the following findings of IL-35 in angiogenesis: ***1)*** hyperlipidemia weakens hind-limb (HL) ischemia-induced angiogenesis in $ApoE^{-/-}$ mice, and in tube formation assay with human microvascular endothelial cells (HMVECs); ***2)*** IL-35 subunits are upregulated in ischemic HL muscle and hypoxic HMVECs; ***3)*** IL-35 receptor subunit Il12rb2 is induced in ischemic HL muscle while the other subunit Il6st is reduced in both ischemic muscle and hypoxic HMVECs, suggesting Il12rb2 homodimer may be a dominant IL-35 receptor; ***4)*** IL-35 cytokine therapy inhibits HLI-induced angiogenesis, and $Il12rb2^{-/-}/ApoE^{-/-}$ DKO mice rescue impaired blood perfusion in $ApoE^{-/-}$ mice; ***5)*** IL-35 inhibits FGF2-induced angiogenesis in Matrigel plug assay *in vivo*; and ***6)*** IL-35 inhibits pro-angiogenic proteins FGF-2, PGF, and CCL2, and promotes anti-angiogenic proteins PAI-1, PEDF, and MASPIN in HMVECs. These results suggest that IL-35 inhibits ischemia/hypoxia-induced angiogenesis.

A113

Inhibition of spinal 15-LOX-1 attenuates TLR4-dependent, NSAID-unresponsive hyperalgesia

Ann M. Gregus*, Matthew W. Buczynski, Darren S. Dumlao, Paul C. Norris, Ganesha Rai, Anton Simeonov, David J. Maloney, Ajit Jadhav, Qinghao Xu, Spencer C. Wei, Bethany L. Fitzsimmons, Edward A. Dennis and Tony L. Yaksh
Virginia Tech School of Neuroscience, Blacksburg, VA

In the current study, direct activation of spinal Toll-like 4 receptors (TLR4) by the intrathecal (IT) administration of KDO2 lipid A (KLA), the active component of lipopolysaccharide (LPS), elicits a robust tactile allodynia that is unresponsive to cyclooxygenase (COX) inhibition, despite elevated expression of COX metabolites in the spinal cord. IT KLA increases 12-lipoxygenase-mediated hepoxilin production in the lumbar spinal cord, concurrent with expression of the tactile allodynia. The TLR4-induced increased expression of the 12/15-lipoxygenase enzyme 15-LOX-1 and hepoxilin production primary spinal microglia. Finally, chemical inhibitors ML127 and ML351 both reduced activity of the rat homolog of 15-LOX-1 heterologously expressed in HEK-293T cells and completely abrogated NSAID-unresponsive allodynia in vivo following IT KLA. These findings suggest that spinal TLR4-mediated hyperpathic state is mediated through microglial 15-LOX-1.

A114

Immune mechanisms of individual differences in the behavioral response to social defeat stress

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Repeated social defeat stress (RSDS), an animal model of stress related disorders, induces a spectrum of depression and anxiety -like behaviors in stress susceptible animals, whereas resilient animals behave more akin to controls. Using RSDS we demonstrate a functional role for the peripheral immune system in contributing to the stress response. Animals that became stress susceptible had a pre-defeat phenotype defined by increased white blood cells that were more prone to release interlukin-6 (IL-6) in response to ex-vivo stimulation. Bone marrow transplants from stress susceptible donors increased social avoidance behavior in hosts exposed to a sub-threshold stress. Animals that received transplants from IL-$6^{-/-}$ mice displayed a resilient phenotype following 10 days of RSDS. Injections of an IL-6 monoclonal antibody, that neutralized the cytokine in the periphery, was protective from RSDS. Additional studies determined that stress susceptible animals had a more permeable blood brain barrier following RSDS that allows IL-6 into the brain and is necessary for RSDS induced changes in post-synaptic plasticity of reward area associated circuitry.

A115

Mechanistic Study of Four Co-Morbid Hyperlipidemia-related Pathologies Reveals NFκB and STAT3-Mediated miR-155 and miR-221 Potentially Serve as Master Regulators, and Elucidates Metabolically Healthy Obese Model

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Hyperlipidemia afflicts more than 70 million Americans and is commonly linked with atherosclerosis, obesity, non-alcoholic fatty liver disease (NAFLD) and type II diabetes. In

recent years, microRNAs (miRNAs) have been firmly established as important regulators in these hyperlipidemia-related pathologies. However, much remains to be understood regarding miRNAs' overlap and function within these hyperlipidemia-related pathologies. Our recent *JBC* paper (PMID: 27856635) has shown that miRNA-155 is significantly increased in atherosclerosis but reduced in high-fat diet-induced obesity. We published the findings that global miR-155 deficiency in an atherosclerotic mouse background yielded a novel metabolically healthy obese mouse model, which exhibits improved atherosclerosis but resulted in obesity, NAFLD, and hyperinsulinemia without insulin resistance. Such a finding implied 1) a potent role of a single miRNA to differentially affect hyperlipidemia-related diseases such that miRNAs achieve master gene candidacy status; and 2) a differential expression of downstream targets of miRNA in different cell types. In our current study, we sought to expand our understanding of the various miRNAs in these hyperlipidemia-related conditions, specifically in regards to their expression direction, regulation and function. We hypothesized that miRNAs function as a potent master regulator that directs pathogenesis of hyperlipidemia-related diseases where only a few miRNAs are shared between two or more hyperlipidemia-related conditions (e.g., atherosclerosis, obesity, insulin resistance/type II diabetes and NAFLD). Using microarray and other experimentally verified-based datamining approaches, we found that, among nearly 150 miRNAs, miR-155 and miR-221 are significantly modulated in all four hyperlipidemia-related diseases. Our results also show a significant role for NFκB and STAT3 as upstream regulators for these and other miRNAs. Our datamining results identified mRNA targets that could contribute to the disease manifestations. Additionally, we identified groups of miRNAs that are unique to one hyperlipidemia-related disease type as well as miRNAs that were modulated in comorbidities, which may be useful as future disease biomarkers in differentiation, diagnosis and prognosis. Taken together, we are first to show that NFκB- and STAT3-mediated miR-155 and miR-221 potentially serve as master regulators for four co-morbid hyperlipidemia-related diseases. Word count: 310. Funding source: NIH-NHLBI

A116

Low intensity ultrasound inhibit inflammation and induce anti-tumorigenic effects via modulating the expressions of cell death regulators

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Low intensity ultrasound (LIUS) has been extensively used in anti-inflammatory therapies and anti-tumor therapies. However, the molecular mechanisms underlying the effects of LIUS in suppressing inflammations and tumorigenesis remain poorly determined. We hypothesized that LIUS fulfill its anti-inflammatory and anti-tumorigenesis effects via modulating the expression of cell death regulators. To examine this novel hypothesis, we determined the mRNA expression of 301 regulators involved in all the 13 types of cell death characterized so far in the three microarray experimental data, which were deposited in the NIH/NCBI-Geo Datasets database. We make the following significant findings: *1)* LIUS induced 25 gene expression changes (13 gene upregulation and 12 gene downregulation) in lymphoma cells; and 40 gene changes (9 gene

upregulation and 31 gene downregulation) in non-cancer cells; *2)* the Ingenuity Pathway Analysis showed that LIUS-induced most of the top pathways regulating cell death regulators in lymphoma are not shared with that in non-cancer cell lines. LIUS induced more cell death related pathways in lymphoma cells but decreased several inflammation signaling pathways including NF-KB signaling in non-cancer cells; *3)* since LIUS may fulfill its therapeutic effects by inducing the effects of mild hyperthermia and mechanical force, we also examined the expression changes of cell death regulators in mild-hyperthermia-treated cells and mechanical force-treated cells. The IPA analysis revealed that some of the top 10 pathways activated in LIUS-treated cells, hyperthermia-treated cells and mechanical force-treated cells are shared; *4)* LIUS also induced the expression changes of 13 out of 70 DNA binding proteins, suggesting that LIUS may induce gene expression changes by inducing chromosome conformation changes; and *5)* LIUS-modulated cell death regulators have a list of long-range gene interaction in the human chromosomes. Our finding have demonstrated for the first time that LIUS induce therapeutic effects in suppressing tumorigenesis and inhibiting inflammation by modulating the expressions of cell death regulators. These insights may lead to novel therapeutic targets for treating cancers and inflammation.

A117

Bacterial amyloids from biofilms break tolerance in lupus by simultaneous BCR/TLR signaling

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Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease, in which infections are considered to play a pathogenic role, but the culprits remain unknown. Bacterial biofilms are multicellular bacterial communities important in the establishment of chronic infection. We have previously shown that systemic administration of curli/DNA complexes, functional bacterial amyloids, accelerated autoimmunity in lupus-prone mice and induced autoantibody production in wild-type mice, suggesting curli/DNA complexes as novel players in SLE pathogenesis. We present evidence that curli/DNA complexes polyclonally activate B cells *in vivo* and *in vitro* in wildtype and 3H9 mice, the latter expressing an anti-DNA Ig heavy chain. Interestingly, curli/DNA complexes also induce isotype switching and *aicda*, the master regulator of class switch recombination, in the absence of T cells help *in vitro*. This suggests that the fibrillar structure of curli/DNA complexes can cross-link BCR, some recognizing DNA. Our results suggest that curli/DNA complexes may induce anti-DNA antibody production by simultaneous BCR/TLR signaling, which leads to B cell antibody production in the absence of T cell help.

A118

Proatherogenic lysophosphatidylcholine induces the upregulation of numerous circular RNAs in activated human aortic endothelial cells

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Human aortic endothelial cell (HAEC) activation is an initial step for recruitment of

inflammatory cells into arteries and atherogenesis. Circular RNAs (circRNAs) are unique non-conding RNAs that form covalently closed continuous loops and often act as gene regulators. We hypothesize that proatherogenic lysophosphatidylcholine (LPC) induces a set of circRNAs in HAEC activation. We characterized circRNAs from LPC-activated HAEC RNA-Seq and found: LPC induces significant modulation of 77 cirRNAs, of which 47 (61%) are upregulated; 34 (72%) of these are upregulated when the corresponding mRNAs are downregulated, suggesting many circRNAs are upregulated via LPC-induced "abnormal splicing" while canonical mRNA splicing is suppressed; and upregulation of 47 circRNAs is associated with LPC-upregulated cholesterol synthesis-SREBP2 and LPC-downregulated TGF-β pathways. Thus, a set of LPC-induced circRNAs may contribute to proatherogenic LPC-induced HAEC activation. These novel insights may help identify new targets for metabolic and inflammatory disease.

A119

Inter-alpha Inhibitors as a Novel Immunomodulator in Neonatal Hypoxic-Ischemic Brain Injury and Ischemic Stroke

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Inter-alpha Inhibitor Proteins (IAIP) are human plasma derived proteins that have been shown to broadly control the host immune response by down-regulating pro-inflammatory cytokines, inhibiting serine proteases, blocking complement activation, binding damage signals (extracellular histones) and additionally by facilitating tissue repair in acute severe inflammation. Recently, we have obtained further evidence that these therapeutic proteins exert neuroprotective effects. Our results demonstrated that IAIP treatment has significant beneficial effects in established models of neonatal Hypoxic-Ischemic brain injury in fetal sheep and newborn rats as well as in the adult stroke model (Middle Cerebral Artery Occlusion) in young and aging mice. IAIP treatment significantly reduced neuroanatomical injury in the brain of experimental animals, and improved long-term behavioral indices of learning and memory tasks. Taken together, IAIP have significant potential to reduce systemic and neuro-inflammation resulting in neuroprotection and improved outcomes.

A120

Cystathionine attenuates hyper-homocysteinemia-induced hepatic cell death and neonatal lethality in cystathionine β synthase (Cbs) knockout mice

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In this study, we showed that Cbs-/- mice have extremely high levels of homocysteine, whereas cystathionine were undetectable. On the other hand, Tg hCBS Cbs-/- mice have high levels of homocysteine and cystathionine. We demonstrated that in Tg hCBS Cbs-/- mice, there is detectable hCBS expression and CBS. On the other hand, although Cse expression in Tg hCBS Cbs-/- and Tg hCBS Cbs+/+ mice were comparable, Cse activity is inhibited by homocysteine. As evidence of, (1) liver Cse activity is lower in Tg hCBS Cbs-/- mice compared to Tg hCBS Cbs+/+ mice; (2) liver Cse activity is inhibited by Hcy in a dose dependent manner; (3) Cse activity of Hcy treated hepatocytes is lower than that of untreated. Therefore, the high levels of cystathionine in Tg hCBS Cbs-/- mice can be explained by the combined effect of expression of hCBS and inhibition of Cse. We further hypothesize that cystathionine protects HHcy mice from neonatal death. We showed that cystathionine treatment in Cbs-/- mice, prolonged lifespan for an

average of 12 days. Cystathionine treatment inhibited Hcy- induced mitochondrial ROS production in mice hepatocytes. Furthermore, Hcy- induced apoptosis in hepatocytes was also attenuated by cystathionine treatment.

A121

Role of Programmed Cell Death Pathways in the Resolution of Inflammation during Influenza/*Staphylococcus aureus* super-infection

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An inflammatory response is induced when the immune system senses microbial pathogens. The ensuing resolution, an active process of returning to homeostasis, is equally important. Eicosanoids and related bioactive lipids mediate the induction and resolution of inflammation. Cytochrome P450 (CYP450) metabolites, a group of anti-inflammatory lipid mediators, are significantly increased during influenza/*S. aureus* super-infection. These metabolites activate the nuclear receptor and transcription factor, PPAR-alpha, which enhances necroptosis and leads to increased mortality and morbidity. To investigate how the activation of PPAR-alpha influences different programmed cell death pathways, apoptosis (anti-inflammatory/pro-resolution) or necroptosis (pro-inflammatory) was induced in mouse derived macrophages (C57BL/6, *Rip3*$^{-/-}$). Addition of WY14643, a PPAR-alpha agonist, exacerbates necroptotic cell death during stimulating conditions inducing necroptosis. Interestingly, macrophages undergo apoptosis when stimulated solely with WY14643. These data suggest that PPAR-alpha plays a major role in programmed cell death thus affecting the resolution of inflammation.

A122

Anti-inflammatory action of statins on chemokine induction and survival in severe peritonitis

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Earlier studies showed that excessive systemic levels of blood chemokines prevent neutrophil recruitment from the circulation to the site of infection, leading to an inhibition of bacterial clearance and increased mortality in a mouse model of severe peritonitis. The effect of two statins (Rosuvastatin and Atorvastatin), on the induction of neutrophil recruiting chemokines MIP-2 and KC by mouse peritoneal cells in response to LPS as well as 2 different strains of *E. coli* was studied. Both statins significantly inhibited the induction of MIP-2 and KC *in vitro* in response to LPS and *E. coli*. We therefore tested the effect of the statins in severe peritonitis induced by an i.p injection of a sub-lethal dose of *E. coli*. The administration of statin led to decreased induction of MIP-2 and KC in circulation in the infected mice, and slightly improved survival of statin treated mice; however results were not statistically significant. Similar results in survival studies were obtained in mice treated with anti-inflammatory modified aspirin (NOSH-aspirin). Thus the anti-inflammatory effect of statins or NOSH-aspirin did not improve survival in severe *E. coli* peritonitis, whereas they significantly reduced chemokine induction by peritoneal cells in response to LPS and *E. coli*. (PSC-CUNY Award 67742-0045 to S. Metkar)

A123

Oral infection of S. Typhimurium leads to *in vivo* expression of curli and generation of anti-dsDNA autoantibodies

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Amyloids are proteins with a cross-beta sheet structure that fold into a quaternary fibrillar structure. They are found within the organs and tissues throughout the human body and have been linked to the development of a variety of diseases. Like humans, bacteria also produce amyloids. It is estimated that 40% of bacterial species produce amyloids and these proteins are major structural components of biofilms. Members of the Enterobacteriaceae family including *Salmonella enterica* serovar Typhimurium and Escherichia coli produce a beta amyloid called curli. Curli, encoded by two operons known as *csgBAC* and *csgDEFG*. Curli production can be induced *in vitro* at temperatures lower than 30°C, suggesting that the expression of the fibers are limited to environmental conditions and not *in vivo* during infection at 37°C. Previous reports suggest that intraperitoneal injection of purified curli or *S.* Typhimurium, that were triggered to express curli by growing the bacteria at 28°C, lead to the generation of anti-dsDNA and anti-chromatin autoantibodies. However, it is not known whether curli is expressed during natural *S.* Typhimurium infection, and whether this expression would be enough to trigger autoantibody generation. We used Nramp^{+} CBA and 129/SvJ mice that are resistant to *S.* Typhimurium infection to investigate curli expression *in vivo.* We found that curli is expressed in the cecum and colon of mice and this expression led to the generation of anti-dsDNA autoantibodies. In addition, we performed similar experiments in Nramp^{-} C57BL/6 mice that are susceptible to S. Typhimurium and found *in vivo* expression of curli throughout the gastrointestinal tract.

A124

Lysophosphatidylinositol and endothelial activation

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Our metabolomic studies indicated that a metabolite named lysophosphatidylinositol (LPI) is significantly increased in atherosclerotic ApoE mouse aortas. LPI treatment increased cytokine production and expression of adhesion molecules, which indicate endothelial cell (EC) activation. We identified via knock down assays that GPR55 (G-protein coupled receptor) as a potential mediator of LPI effects in ECs. This was confirmed by intravital microscopy in hyperlipidemic GPR55-/-/ApoE -/- (DKO) mice, which showed less leukocytes adherence to endothelium than controls. Further bioinformatics studies revealed that metabolite ADMA level was significantly correlated with GPR55 mRNA level and this was consolidated by our metabolomics study which indicated that ApoE mice had high level of ADMA in the plasma. PRMT, an enzyme that is responsible for ADMA production was significantly upregulated in hyperlipidemic ApoE mice, but not in DKO. Knockdown of PRMT significantly reduced EC activation in vitro. Conclusion: LPI induce EC activation via GPR55-PRMT signaling pathway.

A125

Activation of Autoimmune Response by Amyloid Curli

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Salmonella enterica serotype Typhimurium is a Gram negative, motile bacterium that causes infection via the fecal oral route. These bacteria, as well as other enterobacteriaceae, produce amyloid proteins called curli as a major proteinaceous component of their biofilm. I hypothesize that the pathogenic curli/DNA complexes activate cytosolic sensors like the NLRP3 inflammasome leading to cell death and the creation of anti-dsDNA autoantibodies which

contribute to the autoimmune disease phenotype in diseases such as SLE. Recently, I determined the ability to manipulate the amount of DNA in the curli complexes. Being able to control the variable of high and low amounts of DNA within the curli would be invaluable in future studies to delineate the role of eDNA within the curli complexes. To test this idea, mice will be treated with high DNA and low DNA curli preps at the same concentration and determine autoantibody production. We expect that the autoantibody production will correlate with the amount of DNA present in the preps. Additionally, we are investigating the role of the microbiome and inflammation in the immune response. More specifically the presence of *Helicobacter spp*. are known to dampen TLR signaling and therefore would decrease the autoimmune response when present in mice.

A126

PINK1 and Parkin Influence Cell Cycle by Sequestering Active TBK1 to Damaged Mitochondria During Inhibiting Mitosis

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The mitochondrial-targeted kinase PINK1 and E3 ubiquitin ligase Parkin are established mediators of mitophagy; the selective removal of damaged mitochondria by autophagy. PINK1 and Parkin have also been proposed to act as tumor suppressors. However, it is unclear how both PINK1 and Parkin act in coordination to regulate cell cycle. We found PINK1 and Parkin genetically interact with proteins involved in cell cycle regulation in *Drosophila* and that loss of these proteins accelerates cell growth. PINK1-Parkin-mediated activation of Tank binding kinase 1 (TBK1), an innate immunity signaling protein, at the mitochondria during mitophagy leading to a block in mitosis due to the sequestration of active TBK1 from its physiological role at the centrosomes during mitosis. These data support a global cellular response aimed at arresting cell cycle during mitochondrial quality control.

A127

In vitro study of macrophage polarization induced by chronic exposure to sub-clinical low dose lipopolysaccharide (s-LPS) and/or oxidized-low density lipoprotein (oxLDL)

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Chronic inflammatory diseases such as atherosclerosis, diabetes, and age-related neurological disorders are some of the major health risks highlighted in recent studies. Macrophages, the key immune cells are primarily affected during chronic inflammation. Despite rigorous research, the underlying pathogenesis behind these diseases is not clearly understood. We focus on two culprits leading to aggravated chronic inflammatory diseases: Sub-clinical low dose lipopolysaccharide (s-LPS) and Oxidized-Low Density Lipoproteins (OxLDL). Our recent studies have confirmed that s-LPS polarizes macrophages into a prolonged non-resolving pro-inflammatory state by inducing the expression of Subset 1 and Subset 2 pro-inflammatory cytokines via phosphorylation of STAT1 and activation of Ox-CamKII and NFkB, respectively. This prolonged inflammatory state is further sustained via suppression of anti-inflammatory genes (Subset 3) by inhibiting NRF2 functions. On the contrary, oxLDL does not inhibit Nrf2 functions. In addition, oxLDL does not induce STAT-1 phosphorylation. However, it mildly induces the expression of Subset 2 genes by activating Ox-CamKII and NFkB, suggesting that s-

LPS and oxLDL may select different pathways to induce inflammatory responses. This study highlights s-LPS and oxLDL as significant modulators leading to macrophage polarization. However, further investigation is essential to understand the selective molecular pathways, and to explore potential complex association between these causative agents in chronic inflammation.

A128

Adult Neurogenic Deficits in HIV-1 Tg26 Transgenic Mice

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Even in the antiretroviral treatment (ART) era, HIV-1-infected patients suffer from milder forms of HIV-1-associated neurocognitive disorders (HAND). Recent studies point to the possibility that chronic HIV-1 induced neuroinflammation negatively impacts adult neurogenesis, thus contributing to the evolution of HAND. While the viral proteins Tat and gp120 have been shown to individually affect the neurogenic process, no studies have characterized the effects of all the combined viral proteins on adult neurogenesis. To this end, we conducted *in vitro* and *in vivo* neurogenic studies on HIV-1 Tg26 transgenic mice and their wild-type (WT) littermates. Our studies demonstrate that chronic HIV-1 infection impairs neural stem cell (NSC) proliferation, as well as the early differentiation process from NSCs to neural progenitor cells (NPCs). Lineage differentiation studies revealed that Tg26 NSCs were unable to differentiate as efficiently towards a neural lineage, and generated more astrocytes. Finally, newborn dentate granule neurons in Tg26 mice have less dendritic complexity and dendritic spine density. These results demonstrate that low-level HIV-1 infection can contribute to neurogenic deficits, which could possibly lead to cognitive decline.

A129

Bacterial amyloids from biofilms induce type I interferon response in plasmacytoid dendritic cells

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An increased expression of type I interferon (IFN) regulated genes is a hallmark of systemic lupus erythematosus. Medically, bacterial biofilms are important for the establishment of persistent infections, an important environmental trigger for lupus flares and contributor to morbidity and mortality. To strengthen the extracellular matrix of their biofilms, bacteria produce amyloids. We have reported that *Salmonella* and *E. coli* biofilms contain the functional amyloid protein curli. Systemic administration of curli-DNA complexes purified from biofilms accelerated autoimmunity in lupus-prone mice, and triggered production of autoantibodies in wildtype mice. We present here for the first time that bacterial amyloid curli activates plasmacytoid dendritic cells (pDCs), the primary producer of type I IFNs. Additionally, we show that curli is capable of inducing expression of interferon-stimulated genes and ifnα. In addition, curli induces *in vivo* activation of DCs. Our results identify bacterial amyloid curli as a novel activator of pDCs and suggest that curli may accelerate lupus by directly activating pDCs and their powerful type I IFN activity.

A130

GATA3, HDAC6, and BCL6 Regulate FOXP3+ Treg Plasticity and Determine Treg Conversion into Either Novel Antigen-Presenting Cell-Like Treg or Th1-Treg

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We conducted an experimental database analysis to determine the expression of 61 CD4+ Th subset regulators in human and murine tissues, cells, and in T-regulatory cells(Treg) in physiological and pathological conditions. We made the following significant findings: (1) adipose tissues of diabetic patients with insulin resistance upregulated various Th effector subset regulators; (2) in skin biopsy from patients with psoriasis,
and in blood cells from patients with lupus, effector Th subset regulators were more upregulated than downregulated; (3) in rosiglitazone induced failing hearts in ApoE-deficient (KO) mice, various Th subset regulators were upregulated rather than downregulated; (4) aortic endothelial cells activated by proatherogenic stimuli secrete several Th subset-promoting cytokines; (5) in Treg from follicular Th (Tfh)-transcription factor(TF) Bcl6 KO mice, various Th subset regulators were upregulated; whereas in Treg from Th2-TF GATA3 KO mice and HDAC6 KO mice, various Th subset regulators were downregulated, suggesting that Bcl6 inhibits, GATA3 and HDAC6 promote, Treg plasticity; and (6) GATA3 KO, and Bcl6 KO Treg upregulated MHC II molecules and T cell co-stimulation receptors, suggesting that GATA3 and BCL6 inhibit Treg from becoming
novel APC-Treg. Our data implies that while HDAC6 and Bcl6 are important regulators of Treg plasticity, GATA3 determine the fate of plastic Tregby controlling whether it will convert in to either Th1-Treg or APC-T-reg. Our results have provided novel insights on Treg plasticity into APC-Treg and Th1-Treg, and new therapeutic targets in metabolic diseases, autoimmune diseases, and inflammatory disorders.

A131

Characteristics of CD4+Foxp3+ regulatory T cells in mice with atherosclerosis

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CD4+Foxp3+ regulatory T cells (Tregs) are an essential part in maintaining immune homeostasis and have indispensable functions in suppressing vascular inflammation and controlling the development of atherosclerosis. It remains unclear how Tregs carry out their immunosuppressive functions upon atherogenic stimulation. In this study, we detected key functional markers of Tregs by using flow cytometry and applied RNA sequencing for purified Tregs from atherogenic mice. We found: 1) Tregs are significantly increased in spleen from high fat feeding APOE-/- mice compared with wide type mice, but not in peripheral blood; 2) Tregs in spleen from APOE-/- mice have stronger expression of suppressive markers, such as CTLA-4 and PD-1, compared with Tregs from wide type control; and 3) RNAseq analysis illustrate

regulatory patterns in signaling networks of Tregs from atherogenic environment. These findings suggested that atherosclerosis-induced expansion of Tregs confer more suppressive signaling. The novel results provide more promising therapeutic targets for treatment of atherosclerosis.

A132

Identification of SAH-responsive genes and tissue expression profile

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S-adenosylhomocysteine (SAH) is an intermediate metabolite of homocysteine metabolism and has been involved in cardiovascular disease and stroke. However, the molecular mechanism regulating SAH production in specific tissues remains unknown. We perform tissue expression profile of 107 SAH metabolic genes, 89 glucose metabolic genes and 46 lipid metabolic genes in 21 human and 20 mouse tissues. We perform correlation analysis of these genes with Hcy-related metabolites in 6 mouse tissues. We found that these genes were differentially distributed between tissues and species. Also, we identified 35 genes whose tissue mRNA levels were positively correlated with SAH concentrations were named as SAH-responsive genes, including 16 SAH metabolic genes, 9 glucose metabolic genes, 10 lipid metabolic genes. These data provide new insight of how SAH is been regulated in different tissues and interplay of this metabolite with energy metabolism pathways.

A133

Pathogenic Mechanisms of Endothelial Damage in Severe Scrub Typhus

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We have recently established lethal and sublethal *Orientia tsutsugamushi* infection models in C57BL/6 mice, which mimic clinical/pathologic features of human scrub typhus and are valuable for future mechanistic studies. A hallmark of lethal *Orientia* infection is Th1-skewed, but Th2-impared, immune responses, accompanied with severe endothelial damage and multi-organ failure, especially in the lungs. Importantly, while tissue bacteria reach peaks at day 6 (the onset of disease) in mice, neutrophil and CD8 T cell influx/activation reach peaks in the lungs at day 10 (the severe disease stage prior to host death). We hypothesize a two-stage-dysfunction loop for severe scrub typhus: 1) *Orientia* replication in phagocytes and endothelial cells (EC) triggers inflammatory responses and host defense machinery; 2) infection-mediated release of damage-associated molecular patterns (DAMPs) exacerbates vascular dysfunction, even after the control of bacterial replication. This hypothesis was supported at the tissue level by immunofluorescent co-staining of bacteria with leukocyte-, EC-, and platelet-specific markers, Western blot, and qRT-PCR. Form days 0, 2, 6, and 10 of infection, the progressive loss of vasculature and tight junctions (occludin, VE-cad, lectin positivity) and vascular function (reduced angiopoietin 1, Tie2, and pTie2 levels), as well as reduced $CD41^+$ platelets in the lungs, were positively linked to the frequency/number/activation of neutrophils and CD8 T cells, rather than body bacterial loads. The mechanisms of immune dysregulation were further examined by using infected

human endothelial cell cultures and mouse bone marrow-derived macrophages and neutrophils. While *Orientia* infection can directly alter the Ang-Tie2 axis in EC, IL-33/IL-36-like DAMPs (released from/processed by leukocytes) can contribute to vascular damage and tissue damage. Our studies provide new insights into immune dysregulation and pathogenesis of severe scrub typhus. A better understanding of infection- versus immune-mediated dysregulation and prognostic biomarkers will help the control of this neglected tropical disease.

A134

Lysophosphatidylcholines promotes aortic endothelial cell activation via H3K14 acetylation-genomic DNA long range interaction mechanisms

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The genomic/transcriptomic programs control aortic endothelial cell activation remain poorly characterized. After treatment of human aortic endothelial cells (HAECs) with lysophosphatidylcholines (LPC) followed by chromatin immunoprecipitation with antibody to histone 3 lysine 14 acetylation (H3K14ac)-DNA sequencing (CHIP-Seq) and RNA sequencing (RNA-Seq), we performed the new correlation analyses on the data of LPC-increased H3K14ac bindings on HAEC genomic DNAs with LPC-modulated HAEC RNA transcriptome. We found: 1) LPC-induced H3K14ac increases the transcription of 24 mRNAs encoding adhesion molecules, cytokines and other biochemical process regulators; 2) LPC-induced H3K14ac increases 12 chromosome insulators controlling DNA long range contacts; 3) LPC-induced H3K14ac allows long range genomic DNA interaction from the target genes. Our findings on genomic/transcriptomic programs of LPC-induced HAEC activation have provided new therapeutic targets for treating cardiovascular diseases and other inflammations.

A135

Human anti-amyloid monoclonal antibodies reduce biofilm formation of *Salmonella* Typhimurium by targeting curli

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Bacteria form multicellular communities, termed biofilms, in many diverse niches. Biofilms protect bacteria from immune mechanisms and antimicrobials and associated with 65% of human infections. Thus, new anti-biofilm strategies necessary. The extracellular matrix of a biofilm is composed of proteins, DNA, and polysaccharides, including amyloids. Curli is the best characterized bacterial amyloid. We used monoclonal antibodies (mAbs) that exhibit pan host amyloid properties to inhibit biofilm formation by targeting curli of *Salmonella* Typhimurium (STM) biofilms. mAbs reduce biofilm mass and curli content of the biofilm matrix. mAb treated biofilms exhibited a more diffuse architecture with an increased recovery of bacteria and enhanced macrophage phagocytosis. As biofilms are a significant complication associated with implanted medical devices, we explored the ability of the mAb to be utilized as an anti-biofilm therapeutic *in vivo*. Catheters colonized with STM were implanted into the back flanks of mice. mAbs were efficacious against *in vivo* STM biofilms. As amyloids are produced by bacteria across four phyla, these studies will provide a potential therapy strategy to prevent biofilm formation by targeting the amyloid using amyloid specific mAbs.

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Phenotypic comparison of approved anti-IL-17 therapeutics using the BioMAP® human cell-based profiling platform

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Aberrant IL-17 signaling has been implicated in the pathogenesis of a number of autoimmune diseases. Anti-IL-17 therapeutic antibodies represent a compelling new treatment regimen for patients that are refractory to traditional DMARD treatment or to other targeted therapeutics. Two new biological therapies have been developed to block this key cytokine and its downstream effects; secukinumab (Cosentyx™) and ixekizumab (Taltz™) are fully humanized, monoclonal anti-IL-17A antibodies approved for the treatment of moderate to severe plaque psoriasis. In randomized clinical trials, both agents have shown superiority to placebo, etanercept and ustekinumab. However, ixekizumab has been classified as "best-in-class" by achieving higher 90% improvement in Psoriasis Area and Severity Index (PASI 90) and PASI 100 responses at week 12 compared with secukinumab. In the absence of head-to-head clinical trials, direct comparison using a phenotypic profiling approach can evaluate potential biomarker differences between these 2 treatments and provide insights that can help inform on clinical and economic decisions.

Ixekizumab and secukinumab were evaluated using an *in vitro* human primary cell based phenotypic profiling platform (BioMAP) in assays modeling immune cell biology. In addition to the expected decrease in sIL-17A, both agents decreased sIL-6 consistent with the inhibition of Th17-type inflammation. Differences in clinical performance may relate to the stronger effects of ixekizumab versus secukinumab over a range of concentrations from 20000 to 20 ng/ml. Phenotypic profiling can identify common activities of drugs and development candidates that could serve as clinical response biomarkers as well as differentiating activities that can inform on potential difference in clinical efficacy and guide therapeutic strategies.

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GATA3, HDAC6, and BCL6 Regulate FOXP3+ Treg Plasticity and Determine Treg Conversion into Either Novel Antigen-Presenting Cell-Like Treg or Th1-Treg

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We conducted an experimental database analysis to determine the expression of 61 CD4+ Th subset regulators in human and murine tissues, cells, and in T-regulatory cells(Treg) in physiological and pathological conditions. We made the following significant findings: (1) adipose tissues of diabetic patients with insulin resistance upregulated various Th effector subset regulators; (2) in skin biopsy from patients with psoriasis,
and in blood cells from patients with lupus, effector Th subset regulators were more upregulated than downregulated; (3) in rosiglitazone induced failing hearts in ApoE-deficient (KO) mice, various Th subset regulators were upregulated rather than downregulated; (4) aortic endothelial cells activated by proatherogenic stimuli secrete several Th subset-promoting cytokines; (5) in Treg from follicular Th (Tfh)-transcription factor(TF) Bcl6 KO mice, various Th subset regulators were upregulated; whereas in Treg from Th2-TF GATA3 KO mice and HDAC6 KO

mice, various Th subset regulators were downregulated, suggesting that Bcl6 inhibits, GATA3 and HDAC6 promote, Treg plasticity; and (6) GATA3 KO, and Bcl6 KO Treg upregulated MHC II molecules and T cell co-stimulation receptors, suggesting that GATA3 and BCL6 inhibit Treg from becoming
novel APC-Treg. Our data implies that while HDAC6 and Bcl6 are important regulators of Treg plasticity, GATA3 determine the fate of plastic Tregby controlling whether it will convert in to either Th1-Treg or APC-T-reg. Our results have provided novel insights on Treg plasticity into APC-Treg and Th1-Treg, and new therapeutic targets in metabolic diseases, autoimmune diseases, and inflammatory disorders.

A138

Hyperhomocysteinemia Induces Endothelial Lipase Expression via Hypomethylation Related Mechanism

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Hyper-homocysteinemia (HHcy) is an independent risk factors of cardiovascular diseases (CVD). HHcy is associated with reduced HDL-cholesterol and decreased large HDL particle in CVD. Here, we investigated its underlying mechanism.

We examined protein/gene expression, activities of four HDL-degradation related lipases including endothelial lipase (EL), lipoprotein lipase (LPL), hepatic lipase (HL), and pancreatic lipase (PL). Severe HHcy (98.4±22 μM) was established in cystathionine betasynthase-gene mutant mice (*Cbs*$^{+/-}$), moderate HHcy (23.5±5 μM) was also developed in *Cbs*$^{+/+}$ mice, both fed a high methionine (HM) diet for 8-week. Moderate and severe HHcy increased protein levels of EL in aorta, lung, and decreased LPL in aorta(%), lung(%), spleen(%). HL and PL protein levels were not changed by HHcy. Activities of overall lipases were increased in moderate and severe HHcy in aorta (204.1%, 283.5%), plasma (157.6%, 310%), adipose (328.1%, 470.8%) and liver (108.2%, 186.2%) versus control. Similar results were obtained from survivable genetic model without diet intervention (Tg hCBS/*Cbs*$^{-/-}$). EL increased in aorta, lung, and overall lipases activities were increased. Furthermore, lipases levels in isolated EC from the long were detected by flow cytometer, EL was selectively increased in HHcy Tg-hCBS/*Cbs*$^{-/-}$ mouse (148%). To confirm it, Human umbilical vein endothelial cells (HUVECs) were treated with L-Hcy (0.5 μmol/L) for 24hr. Both of EL gene and protein expression was increased under Hcy treatment. It suggests EL gene induction is dependent on Hcy level.

Tissue expression profile of Hcy degradation enzymes were established by database mining analysis. The profiles were done correlative analysis with the levels of Hcy, and its metabolites (SAM, SAH) in heart, lung, brain, spleen, kidney and liver from wild type mice. We found that mRNA levels of EL, HL, and PL were positively correlated with Hcy and negatively correlated with SAM/SAH ratio, which indicated of methylation status. We identified a large CpG island with 113 CG dinucleotide pairs in human EL promoter region, where four HDL-related single nucleotide polymorphisms (SNPs) was overlapping with CpG containing transcription factor consensus element. Finally, the hypomethylation status induced by Hcy on defined sites were confirmed by sequencing-based DNA methylation analysis.
We conclude that HHcy may induce EL gene induction/activatioin via DNA hypomethylation, which causes HDL degradation.

A139

CAROM, a novel gene suppressing endothelial cell migration

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***Background*:** Hyperhomocysteinemia (HHcy), a syndrome displayed by high concentration of homocysteine (Hcy) in blood has been demonstrated as a significant risk factor for cardiovascular disease (CVD). We have shown that Hcy leaded to endothelial cells (EC) injuries through inhibition of proliferation and migration. However, the comprehensive disclosure of mechanisms accounted for Hcy mediated EC injuries are still lacking up to date. It's compelling to discover more molecules that participate in the pathogenesis of diseases involving HHcy

***Material and Methods*:** Through differential display screening of cDNA from Hcy stimulated cultured EC, we discovered a highly induced gene and it was identified as *CAROM*, which was also called *FCHSD2* (FCH and double SH3 domains 2). Four major studies were focused: 1) Genomic alignment among species and prediction of interacting partners through bioinformatics; 2) Mass Spectrometry (MS) analysis for discovering interacting proteins; 3) Characterizing its role of regulating EC function; 4) Cytokine profiling is performed in EC to determine the potential cytokine(s) regulated by CAROM.

Results: CAROM protein belongs to Bin-Amphiphysin-Rvs (BAR) superfamily and specifically it is a FBAR protein since it displays Fes/CIP homology (FCH) domain on N-terminus. This gene displays highly conservative homology among mammalian species, with 96.5% identity of amino acids between human and mouse homologue. Information collected from multiple interactome databases showed CAROM could bind to more than thirty proteins, although most of them haven't been experimentally verified. We used samples from CAROM overexpression EC for MS analysis. Our data disclosed that the interacting protein of CAROM manly function in regulating endocytosis, vesicle trafficking and cytoskeleton dynamics. These partners included EEA1(Early Endosome Antigen 1), Filamin, Vinculin, Importin-5, Dynein et al, suggesting it could regulate motility, migration and other cell behaviors. CAROM expression was reduced more than 80% by siRNA targeted at different regions of its transcript. Our preliminary data showed that inhibition on EC migration by Hcy were partially reversed after CAROM downregulation, indicating its interference with EC repair after vascular injury. Cytokine array analysis of cultured human EC infected with CAROM adenovirus showed the releases of several chemokines into cultured medium, including CXCL10, CXCL11 and CCL5 were upregulated. Quantitative PCR analysis also confirmed the transcript level of these chemokines are increased in these EC, which were consistent with Hcy stimulation.

***Conclusion*:** As a significantly induced gene in HHcy, CAROM could have multiple roles of regulating EC function. Carom inhibits EC migration and this suggests its role of inducing vascular injury. Structure and MS analysis show evidence that it could regulates endocytosis and other cellular trafficking events, which are tightly connected with normal EC function. The stimulation of chemokines indicates its EC regulation could be related to inflammatory response. The underlying molecular mechanisms of these regulations in EC and vessels need further investigations.

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GA TA3, HDAC 6, and BCL 6 Regulate FOXP3+ Treg Plasticity and Determine Treg Conversion into Either Novel Antigen-Presenting Cell-Like Treg or Th1-Treg

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We conducted an experimental database analysis to determine the expression of 61 CD4+ Th subset regulators in human and murine tissues, cells, and in T-regulatory cells (Treg) in

physiological and pathological conditions. We made the following significant findings: in Treg from follicular Th (Tfh)-transcription factor(TF) Bcl6 KO mice, various Th subset regulators were upregulated; whereas in Treg from Th2-TF GATA3 KO mice and HDAC6 KO mice, various Th subset regulators were downregulated, suggesting that Bcl6 inhibits, GATA3 and HDAC6 promote, Treg plasticity;andGATA3 KO, and Bcl6 KO Treg upregulated MHC II molecules and T cell co-stimulation receptors, suggesting that GATA3 and BCL6 inhibit Treg from becoming novel APC-Treg. Our data implies that while HDAC6 and Bcl6 are important regulators of Treg plasticity, GATA3 determine the fate of plastic Tregby controlling whether it will convert in to either Th1-Treg or APC-T-reg. Our results have provided novel insights on Treg plasticity into APC-Treg and Th1-Treg, and new therapeutic targets in metabolic diseases, autoimmune diseases, and inflammatory disorders.

A141

Novel Molecular Pathways May Mediate Low-Intensity Ultrasound-Induced Anti-inflammatory Effects

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Low-intensity ultrasound (LIUS) was shown to be beneficial in mitigating inflammation and facilitating tissue repair in various pathologies, but the underlying molecular mechanisms remain poorly characterized. We conducted cutting-edge database mining approaches to determine the anti-inflammatory mechanisms exerted by LIUS. Our data revealed following interesting findings: (1) LIUS anti-inflammatory effects are mediated by upregulating anti-inflammatory gene expression; (2) LIUS induces the upregulation of the markers and master regulators of immunosuppressor cells including MDSCs (myeloid-derived suppressor cells), MSCs (mesenchymal stem cells), B1-B cells and Treg (regulatory T cells); (3) LIUS can make use of natural membrane vesicles as small as exosomes derived from immunosuppressor cells as a novel mechanism to fulfill its anti-inflammatory effects; (4) LIUS upregulates the expression of extracellular vesicle/exosome biogenesis mediators and docking mediators; and (5) Exosome-carried anti-inflammatory cytokines and anti-inflammatory microRNAs inhibit inflammation of target cells via multiple shared and specific pathways, suggesting exosome-mediated anti-inflammatory effect of LIUS feasible. Our results have provided novel insights into the mechanisms underlying anti-inflammatory effects of LIUS.

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DNA checkpoint and repair factors and high genome risk in severe burns and trauma

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DNA damage response factors (DDCF) and repair factors (DDRF) play a vital role in managing genomic integrity. However, how DDCFs and DDRFs are modulated under burns and severe trauma are not known. We took an experimental database analysis to determine the expression of 26 DDCFs and 42 DDRFs in patients with severe burns and trauma in comparison to that in healthy controls. Our findings are as follows: (1) 11 out of 26 DDCFs are selectively modulated, 2 are upregulated while 9 are downregulated in leukocytes. (2) 18 out of 42 DDRFs are selectively modulated, 4 are upregulated and 14 are downregulated. (3) Ingenuity Pathway

Analysis indicated that upregulated DDCFs and DDRFs are associated with pancreatic adenocarcinoma and ovarian cancer signaling, suggesting that severe burns and trauma can have high genomic risk. Our results show that severe burns and trauma increase genomic instability, therefore can be classified as pathologies with high genomic risks and increase the susceptibility to develop cancers.

A143

Nogo-B regulates hyperglycemia induced endothelial dysfunction by modulating mitochondria function.

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Background:

As an important part of the vasculature, the endothelium plays multiple functions in maintenance of vascular homeostasis. Dysfunction of endothelial cell is associated with several pathologies, including diabetes, hypertension and atherosclerosis. Emerging evidences suggest that mitochondria have a profound impact on endothelial cell function. Hyperglycemia leaded mitochondrial defects are associated with endothelial dysfunction and pathogenesis of diabetes related microvascular and macrovascular diseases. However, the mechanism by which hyperglycemia causes mitochondrial dysfunction are not fully elaborated.

Methods and Results:

Here we examined the role of Nogo-B, a regulator of mitochondria-endoplasmic reticulum unit, in mitochondrial dysfunction that triggered by hyperglycemia. We found that endothelial Nogo-B expression is high in diabetic mouse and high glucose treated cells. Depletion of Nogo-B enhances the ability of high glucose to cause mitochondrial fission and inhibition of angiogenesis. Suppressed mitochondrial membrane potential and respiration rate were observed in Nogo-B depleted cells. Further results show that Nogo-B deficiency results in elevated JNK phosphorylation, which can lead to MFN2 degradation and cell apoptosis.

Conclusion:

Taken together, our findings provide novel insight into the unexpected role of Nogo-B. Nogo-B is a regulator of mitochondrial morphology and function under high glucose treatment in endothelial cells. The results from this study deepen our understanding on the mechanism of diabetes related endothelium dysfunction.

A144

Idiopathic pulmonary fibrosis and systemic sclerosis in mice

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Idiopathic pulmonary fibrosis (IPF) and systemic sclerosis (SSc) are fibrotic diseases with poorly understood pathophysiology and minimal treatment options for patients, warranting the need for better preclinical models to help drive drug development. While IPF only effects the lungs, SSc can affect the skin, lungs and other internal organs and both diseases can have poor prognoses after lung function begins to decline. To model these disease in mice, bleomycin (BLM) can be instilled locally to the lung for IPF or systemically through a slow release mechanism for SSc. In the IPF model, similar to humans, significant increases in total protein, pro-fibrotic TGF- β and lymphocyte accumulation into the lungs were observed and accompanied by distorted lung architecture, severe thickening of alveolar walls, and fibrotic nodule development. In the SSc model, dermal thickness and lung toxicity was observed in systemically BLM-induced mice

compared to control animals. These data suggest that BLM induced fibrosis, either via local or systemic adminstration, is an attractive model to analyze the underlying mechanism of fibrosis and test the efficacy of potential therapies.

A145

Mitochondrial-mediated NLRP3 inflammasome activation promotes cardiomyocyte death in myocardiac infarction in hyperhomosysteinemia mice

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Introduction: Elevated level of serum homocysteine (Hcy) has been identified as a risk factor for accelerating progression of cardiovascular disease. The underlying mechanism by which NLRP3 inflammasomal activation accelerates cardiomyocyte death through damaged mitochondrial is unclear.

Methods and Results: Acute myocardial infarction (AMI) procedure was performed on hCBS/m*cbs* knockout mice at the age of 10weeks. Severe HHcy remarkably aggravated infarction size, cardiomyocyte area, and interstitial fibrosis 6 weeks after MI from 22.7 ± 4.3%, 340 ± 54μm^2, 13.9 ± 1.9% in control mice (Hcy, 7-10μM) to 33.6 ± 6.5%, 485 ± 65μm^2, 26 ± 4.5%, respectively, (P=0.035, P= 0.041, P=0.002). Increased LV cavity dilatation and dysfunction were more significant as compared with control mice by echocardiography (5.9 ± 0.2 vs. 4.2 ± 0.4mm, P=0.039; LV ejection fraction, 22.6 ± 3.9% vs. 36.8 ± 4.0%, P=0.011). Cardiomyocyte apoptosis and ROS production were increased in post-MI HHcy mice as well. Cleavaged IL-1 β and caspase1, and ASC oligomerization were observed at the early stage of post-MI in severe HHcy mice. Cultured neonatal mouse ventricular myocyte (NMVM) treated with the combination of DL-Hcy (500μM, 48h) and hypoxia (0.5% O2) showed that increased NLRP3 and caspase1 expression and activity. Mitochondrial ROS (mtROS), dissipation of mitochondrial membrane potential, and mitochondrial permeabilization were increased. Caspase-1 activity and cardiomyocyte death were lessened as NMVMs were administrated with mitochondria-targeted antioxidants Mito-temple and SOD2, whereas caspase1 inhibition was not able to fully rescue damaged mitochondria. Cardiac function and apoptosis were mitigated in hCBS/m*cbs* $^{-/-}$ Caspase 1$^{-/-}$ mice.

Conclusion: AMI initiates an intense inflammatory response in myocardium that promotes cardiomyocyte death, ventricular remodeling, and cardiac dysfunction. Hcy accelerates cardiomyocyte death post-MI in part through mitochondrial-mediated NLRP3 inflammasome activation and inflammation, which contributes to adverse cardiac remodeling, ventricular dysfunction, and heart failure.