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Felix Baumgartner preparing his LifeMonitor for the world record-smashing Red Bull Stratos skydive in 2012.

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2016 APS Conference
Inflammation, Immunity, and Cardiovascular Disease

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Acknowledgements

The Conference Organizers and The American Physiological Society gratefully recognize the generous financial support from the following:

American Heart Association’s Council on Hypertension and the Kidney in CV Disease
Data Sciences International
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*Funding for this conference was made possible (in part) by 1R13HL134271-01 from the National Institutes of Health. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.
### 2016 APS Conference: Inflammation, Immunity and Cardiovascular Disease

#### Week-at-a-Glance Schedule

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<td>3:00-8:00 PM: Registration</td>
<td>7:00 AM-7:30 PM: Registration</td>
<td>7:30 AM-7:30 PM: Registration</td>
<td>7:30 AM-4:30 PM: Registration</td>
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<tr>
<td>6:00-8:00 PM: Welcome and Opening Reception</td>
<td>8:00-10:00 AM: Symposia I: Basic Aspects of Innate Immune Cells Chairs: Cornelia Weyand and Tomasz Guzik</td>
<td>8:00-10:00 AM: Symposia V: CV Disease in Inflammatory and Autoimmune Disease Chair: Mike Ryan</td>
<td>8:00-10:00 AM: Symposia VIII: Inflammation, Hypertension, and End-Organ Damage Chairs: David Mattson and Jens Titze</td>
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<td>8:00-8:15 PM: Opening Comments Speaker: David G. Harrison</td>
<td>10:00-10:30 AM: Symposia I (Continued)</td>
<td>10:00-10:30 AM: Symposia VI: Inflammation and Hypertension During Pregnancy and Gender Differences (with Selected Oral Presentations) Chair: Kathryn Sandberg</td>
<td>10:00-10:30 AM: Break</td>
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<td>8:15-9:15 PM: Plenary Lecture: The Endothelium and Vascular Immunity Speaker: Jordan Pober</td>
<td>10:00-10:30 AM: Break</td>
<td>10:00-10:30 AM: Break</td>
<td>10:00-10:30 AM: Break</td>
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<tr>
<td>12:30-2:00 PM: Poster Session I and Lunch</td>
<td>12:45-2:30 PM: Poster Session II and Lunch</td>
<td>12:30-1:30 PM: Lunch</td>
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<td>2:00-4:00 PM: Symposia II: Basic Aspects of T Cells Chair: Jorg Goronzy</td>
<td>2:30-3:30 PM: Career Workshop: Get a Job: Build the Skills that Employers Want!</td>
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<tr>
<td>4:00-7:00 PM: Free Time</td>
<td>3:30-7:00 PM: Free Time</td>
<td>2:30-4:30 PM: Symposia IX: Inflammation and Atherosclerosis Chair: Elena Galkina</td>
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<tr>
<td>7:00-8:30 PM Symposia III: Basic Aspects of B Cells Chair: Amy Major</td>
<td>7:00-8:30 PM: Symposia VII: Inflammation, Immunity, Intestinal Flora and the Metabolic Syndrome Chair: Sean Davies</td>
<td>4:30-7:00 PM: Free Time</td>
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<tr>
<td>8:45-10:30 PM Symposia IV: Basic Aspects of Vascular Cells with Immune Function Chair: Meena Madhur</td>
<td>8:45-10:00 PM: Symposia VII: Inflammation, Immunity, Intestinal Flora and the Metabolic Syndrome (Continued and with Selected Oral Presentations) Chair: Sean Davies</td>
<td>7:00-9:30 PM: Closing Banquet and Awards Ceremony</td>
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**Location:**
The 2016 APS Conference: Inflammation, Immunity, and Cardiovascular Disease will be held August 24—27, 2016 at the Westin Westminster Hotel located at: 10600 Westminster Blvd., Westminster, CO 80020, telephone (303) 410-5000.

**Onsite Registration Hours:**
- Wednesday, August 24………..3:00—8:00 PM
- Thursday, August 25…………..7:00 AM—4:00 PM
- …………………………………………6:30—7:30 PM
- Friday, August 26………………...7:30 AM—4:00 PM
- …………………………………………6:30—7:30 PM
- Saturday, August 27……………...7:30 AM—4:30 PM

**On-Site Registration Fees:**
- APS Member.............................................$850
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- APS Postdoctoral Member............................ $650
- Postdoctoral Nonmember.............................. $750
- APS Student Member................................. $550
- Student Nonmember................................. $650

**Payment Information:**
Registrants may pay by institutional or personal check, traveler’s check, MasterCard, VISA or American Express or in United States Dollars. Checks must be payable to “The American Physiological Society” and drawn on a United States bank payable in US dollars.

**Student Registration:**
Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Nonmember students who register onsite must provide a valid university student ID card. APS student members should present their current APS membership card indicating their student category status.

**Postdoctoral Registration:**
Any person who has received a Ph.D. degree in physiology or related field, **within four years** of this conference, as attested to by the department head is eligible to register at the postdoctoral fee. A **statement signed by the department head must accompany the registration form and remittance when registering.**

**Included in your Registration:**
Your registration to this conference includes entry into all scientific sessions, program book, opening reception, poster sessions, networking socials, and the closing banquet meal. **There are no substitutions or refunds.** You must pay the registration fee regardless of whether you are not able to stay for the entire conference or partake in any of the meals during the conference program. Guests of attendees are not permitted in the scientific sessions.

**Press Registration:**
Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

**Photograph/Video Recording:**
The photographing and/or the video recording of any of the conference sessions for personal or private use is strictly prohibited.

**Code of Conduct:**
APS is committed to providing a friendly, safe, and welcoming environment for all, regardless of gender, sexual orientation, disability, race, ethnicity, religion, national origin, or other protected characteristics. We expect all attendees, media, speakers, volunteers, organizers, venue staff, guests, and exhibitors to help us ensure a safe and positive workshop experience for everyone. Alert the APS Registration Desk if you notice a dangerous situation, someone in distress, or violations of this Code of Conduct.

**Program Objective:**
The purpose of this conference is to review fundamentals of innate immunity and how various cardiovascular diseases affect these functions. Particular areas of interest include monocyte/macrophages, complement and reactive oxygen species. As well as review fundamentals of adaptive immunity, and in particular discuss T cell function and why many facets of this might be altered in diseases like atherosclerosis and hypertension. Function of various T cell subtypes, such as CD4+, CD8+, T regulatory cells and TH17 cells will be highlighted. To review role of B cells and humoral immunity in cardiovascular diseases and to discuss gender differences in cardiovascular inflammation. We will discuss the propensity for cardiovascular disease among individuals with autoimmune disease as well as encourage presentation of preliminary data from young investigators regarding inflammation and cardiovascular disease.

**Target Audience:**
This conference is intended for all professionals involved in teaching, research and clinical fields related to inflammation, immunity and cardio-vascular diseases.

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**Did you register early?**

_The drawing for the iPad Pro will be held on Saturday, August 27 during the Closing Banquet and Awards Ceremony_

*Winner must be in attendance to receive prize*
DAILY SCHEDULE

WEDNESDAY, AUGUST 24, 2016

Welcome
1.0 WELCOME AND OPENING COMMENTS
Wednes., 8:00—8:15 PM, Westminster III.

8:00 PM 1.1 Welcome and Opening Comments.
David Harrison, Vanderbilt Univ., and
David Mattson, Med. Coll. of Wisconsin,
Milwaukee.

Plenary Lecture
2.0 PLENARY LECTURE
Wednes., 8:15—9:15 PM, Westminster III.


THURSDAY, AUGUST 25, 2016

Symposia I
3.0 BASIC ASPECTS OF INNATE IMMUNE CELLS
Thurs., 8:00 AM—12:30 PM, Westminster III.

Chairs: Cornelia Weyand, Stanford Univ.
        Tomasz Guzik, Univ. of Glasgow, UK.

8:00 AM 3.1 Tissue-resident Intimal Macrophages Contribute to Atherosclerotic Lesion Initiation and Plaque Progression. Jesse Williams. Washington Univ., St. Louis.

8:30 AM 3.2 IL-13 Signaling in Tissue Repair and Fibrosis. Thomas A. Wynn. NIH, NIAID.

9:00 AM 3.3 The Regulation of Macrophage Activation by Endogenous Secretory Products. David M. Mosser. Univ. of Maryland, College Park.


10:00 AM Break

10:30 AM 3.5 DAMPS & PAMPS. Karen Newell-Rogers. Texas A&M Univ.

11:00 AM 3.6 Interleukins and Innate Immunity. Charles Dinarello. Univ. of Colorado, Denver.

11:30 AM 3.7 MHC and Antigen Presentation. Elizabeth Mellins. Stanford Univ.

12:00 Noon 3.8 Neuro-immune Reflex Arc. Kevin J. Tracey. Hofstra Univ, North Shore-LIJ.

Poster Session I
4.0 POSTER SESSION I
Thurs., 12:30—2:00 PM, Westminster IV.

Poster Board


4.2 Aortic and Microvascular Endothelial Cells Differentially Influence Inflammatory and Vascular Responses to Engineered Nanomaterial. V. Minarchick, J. Shannah, E. Sabolsky, and J. Brown. Univ. of Colorado Denver, and West Virginia Univ.


4.5 Mechanisms of Increased Complement Activation in Placental Ischemia-induced Hypertension in the Rat. J. Regal, C. Wing, N. F. Nieto, J. Gilbert, and S. Fleming. Univ. of Minnesota Med. School, and Kansas State Univ. (9.4)

4.6 Platelet-P2Y12 Receptor is Important for the Cellular Immune Response in Erosive Arthritis. C. A. Barrero, A. E. Garcia, W. Shao, M. Amin, M. F. Barbe, O. M. Perez-Leal, and M. C. Rico. Temple Univ. (7.3)

4.7 Interleukin-19: A Novel Sexually Dimorphic Cardiac Cytokine. D. Bruns, S. Thoemmes, A. Ghincea, C. Ghincea, and L. Walker. Univ. of Colorado, Denver. (9.5)

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<th>Increased Pro-inflammatory T cells and HMGB1 are Associated with Vascular Dysfunction in Male SHR. E. Gillis, J. Mussalli, and J. Sullivan. Augusta Univ. (9.6)</th>
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<td>HIV-1, Inflammation and Endothelial Dysfunction. R. Fay, B. Weil, J. Greiner, B. Stauffer, E. Connick, and C. DeSouza. Univ. of Colorado, Boulder, Univ. of Arizona, Tucson, and Univ. of Colorado Denver. (15.4)</td>
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<tr>
<td>21</td>
<td>4.21</td>
<td>Targeted UPLC-MS/MS Analysis of Oxylipins: From Profiling to Quantification for Translational Research Studies. B. Molloy, and A. Peek. Waters Corp., Wilslow, UK, and Waters Corp., Milford, MA.</td>
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<td>22</td>
<td>4.22</td>
<td>Ly6CHI Monocytic Clearance of Dying Cardiomyocytes by CD36 Activates NR4a1 and is Required for Transition to Reparative Inflammation Following Myocardial Infarction. S. Dehn, and E. Thorp. Northwestern Univ. (15.5)</td>
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<td>23</td>
<td>4.23</td>
<td>Interaction of IL-6 and TNF-α Contributes to Endothelial Dysfunction in Type 2 Diabetic Mice Heart. J. Lee, S. Lee, C. Zhang, and Y. Park. Univ. of Houston, and Univ. of Missouri, Columbia.</td>
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Symposia II
5.0 BASIC ASPECTS OF T CELLS
Thurs., 2:00—4:00 PM, Westminster III.
Chair: Jorg J. Goronzy, Stanford Univ.
2:00 PM 5.1 T Cell Subsets and Immune Responses. John O’Shea. NIH, NIAMS.
2:30 PM 5.2 T Cell Trafficking and Memory. Jorg J. Goronzy. Stanford Univ.
3:00 PM 5.3 T Cell Trafficking and Memory. Eric Clambey. Univ. of Colorado, Denver.

Symposia III
6.0 BASIC ASPECTS OF B CELLS
Thurs., 7:00—8:30 PM, Westminster III.
7:00 PM 6.1 B Cell Subsets and Immune Responses. Amy Major. Vanderbilt Univ. Med. Ctr.
8:00 PM 6.3 B Cells and Cardiovascular Disease. Myra Lipes. Harvard Univ. Med. Sch.

Symposia IV
7.0 BASIC ASPECTS OF VASCULAR CELLS WITH IMMUNE FUNCTION
Thurs., 8:45—10:30 PM, Westminster III.
8:45 PM 7.1 CD4+ T cells in atherosclerosis. Dennis Wolf. La Jolla Inst. for Allegy and Immunology.
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<td>9:15 PM</td>
<td>7.2</td>
<td>IL1β Promotes Athero-Protective Changes in Late Stage Atherosclerotic Lesions.</td>
<td>Gary Owens.</td>
<td>Univ. of Virginia Sch. of Med.</td>
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<td>10:00 PM</td>
<td>7.4</td>
<td>Role of Smooth Muscle Cell Specific iPLA2β in Vascular Inflammation and Neointimal Formation in a Murine Femoral Artery Wire Injury Model.</td>
<td>Shu Liu.</td>
<td>Univ. of Kentucky.</td>
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**Friday, August 26, 2016**

**Symposia V**

**8.0** CV DISEASE IN INFLAMMATORY AND AUTOIMMUNE DISEASE

*Fri., 8:00—10:00 AM, Westminster III.*

Chair: Mike Ryan, Univ. of Mississippi Med. Ctr.

8:00 AM 8.1 Utilizing A Human Model of Disease to Study Inflammatory Atherosclerosis. Nehal Mehta. NIH, NHLBI.

8:25 AM 8.2 Periodontal Inflammation and CV Disease. Tomasz Guzik. Univ. of Glasgow; UK.


9:30 AM 8.5 Vascular Inflammation/Arteritis. Cornelia Weyand. Stanford Univ.

10:00 AM Break.

**Symposia VI**

**9.0** INFLAMMATION AND HYPERTENSION DURING PREGNANCY AND GENDER DIFFERENCES

*Fri., 10:30 AM—12:45 PM, Westminster III.*

Chair: Kathryn Sandberg, Georgetown Univ.

10:30 AM 9.1 Adaptive Immunity and Gestational Hypertension During Pregnancy. Babette LaMarca. Univ. of Mississippi Med. Ctr.


11:30 AM 9.3 Gender Differences in Inflammation and Hypertension. Kathryn Sandberg. Georgetown Univ.


12:15 PM 9.5 Interleukin-19: A Novel Sexually Dimorphic Cardiac Cytokine. Danielle Bruns. Univ. of Colorado, Denver.

12:30 AM 9.6 Increased Pro-inflammatory T Cells and HMGB1 are Associated with Vascular Dysfunction in Male SHR. Ellen Gillis. Augusta Univ.

**Poster Session II**

**10.0** POSTER SESSION II

*Fri., 12:45—2:45 PM, Westminster IV.*

**Poster Board**


2 10.2 Obesity and Circulating Inflammation-Related microRNAs. J. Hijmans, T. Bammert, P. Kavlich, G. Linenberg, K. Diehl, J. Greiner, B. Stauffer, and C. DeSouza. Univ. of Colorado, Boulder; and Univ. of Colorado, Denver. (12.5)

3 10.3 Characterization of Factors that Pre-dispose to the Metabolic Syndrome and Adipose Tissue Inflammation in Aged Male and Female Mice. H. Ahnstedt, A. Chauhan, M. Roy-O'Reilly, and L. D. McCullough. Univ. of Texas Hlth. Sci. Ctr., Houston. (12.6)
10.4 Effects of an 8-Week Paleo Dietary Intervention on Inflammatory Cytokines. C. Dolan, A. Carrillo, N. Davies, and M. Markofski. Univ. of Houston, and Chatham Univ., Pittsburgh.

10.5 Perivascular Adipose Tissue Macrophages are Responsible for Endothelial Dysfunction in the Obese Microvasculature. J. Candela, and C. White. Rosalind Franklin Univ., Chicago. (12.7)

10.6 Cullin3 Regulates Endothelial Function by Modulating eNOS Activity. J. Wu, K. Lu, L. Agbor, X. Liu, M. Mukohda, A. Nair, and C. Sigmund. Univ. of Iowa, Iowa City.

10.7 Renal and Splenic Cytokines are Altered in Early Life Stressed (ELS) Adult Male Rats. I. Obi, C. De Miguel, D. Ho, A. Loria, and J. Pollock. Univ. of Alabama at Birmingham, Tripler Army Med. Ctr., Honolulu, HI, and Univ. of Kentucky.


10.13 Infiltrating Macrophages Promote Vascular Sympathetic Hyperinnervation


10.15 CARD9 Knockout Ameliorates Fibrosis and Hypertrophy in a TAC Pressure-overload Model. M. Peterson, S. Haller, K. Wilson, D. P. Thomas, and G. He. Univ. of Wyoming, Laramie. (14.2)

10.16 Cytotoxic CD8+ T-cells Play a Role in Hypertension-Associated Inflammatory Responses in Female Dahl Rats. A. V. Pai, A. Souza, C. A. West, and K. Sandberg. Georgetown Univ.


10.19 Loss of ET1 Receptor Function Activates NOD-like Receptor and Inflammasome Signaling Pathways in Renal Outer Medulla During Type 1 Diabetes Through an ER Stress-independent Mechanism. C. De Miguel, W. C. Hamrick, D. M. Pollock, and J. S. Pollock. Univ. of Alabama at Birmingham.

10.20 A High Fat Diet Increases Blood Pressure and Leads to a Proinflammatory Immune Cell and Cytokine Profile in the Aortae of Female Dahl Salt-Sensitive Rats (DSS). L. Taylor, B. Baban, and J. Sullivan. Augusta Univ.
21 10.21 Induction of AP-1, NF-κB, miR-21 Expression, and Angiogenesis by Tungsten Carbide-Cobalt Nanoparticles Involves ROS-Mediated MAPK Pathways and Results in Transformation of JB6 and BEAS-2B Cells. T. Barber, J. Aldinger, L. Bowman, T. Meighan, and M. Ding. CDC, NIOSH, Morgantown, WV.


23 10.23 Induction of Ap-1 Signaling and DNA Damage by Copper Oxide Nanoparticles Involve ROS-mediated MAPK Pathways. T. Barber, L. Bowman, J. Aldinger, and M. Ding. CDC, NIOSH, Morgantown, WV.


28 10.28 Interleukin 21 Promotes Hypertension and End-Organ Dysfunction. B. L.
DAILY SCHEDULE

Career Workshop
11.0 CAREER WORKSHOP
Fri., 2:30—3:30 PM, Westminster III.


Symposia VII
12.0 INFLAMMATION AND HYPERTENSION DURING PREGNANCY AND GENDER DIFFERENCES
Fri., 7:00—8:30 PM, Westminster III.

Chair: Sean Davies, Vanderbilt Univ. Med. Sch.

7:00 PM 12.1 TMAO as a Mediator and Therapeutic Target in Cardiovascular Disease. Stan Hazen. Cleveland Clinic.


8:00 PM 12.3 Metabolic Syndrome. Kamal Rahmouni. Univ. of Iowa, Iowa City.

8:30 PM 12.4 Altering the Microbiota for Weight Control. Sean Davies. Vanderbilt Univ. Med. Ctr.

9:00 PM Break.

9:15 PM 12.5 Obesity and Circulating Inflammation-related microRNAs. Jamie Hijmans. Univ. of Colorado, Boulder. (10.2)

9:30 PM 12.6 Characterization of Factors that Pre-dispose to the Metabolic Syndrome and Adipose Tissue Inflammation in Aged Male and Female Mice. Hilda Ahmstedt. Univ. of Texas Hlth. Sci. Ctr. (10.3)

9:45 PM 12.7 Perivascular Adipose Tissue Macrophages are Responsible for Endothelial Dysfunction in the Obese Microvasculature. Carl White. Rosalind Franklin Univ. (10.5)

PAD PRO DRAWING will be held at the Closing Banquet and Award Ceremony on Saturday, August 27. You must be in attendance to win!

SATURDAY, AUGUST 27, 2016

Symposia VIII
13.0 INFLAMMATION, HYPERTENSION, AND END-ORGAN DAMAGE
Sat., 8:00 AM—12:30 PM, Westminster III.


10:00 AM Break.


Selected Oral Presentations
14.0 SELECTED ORAL PRESENTATIONS
Sat., 1:30-2:30 PM, Westminster III.


1:45 PM 14.2 CARD9 Knockout Ameliorates Fibrosis and Hypertrophy in a TAC Pressure-
overload Model. Matt Peterson, Univ. of Wyoming. (10.15)

2:00 PM 14.3 Dynamic Vascular T Cell-antigen Presenting Cell Interactions During Hypertension. Antony Vinh, Monash Univ., Australia. (10.18)


Symposia IX

15.0 INFLAMMATION AND ATHEROSCLEROSIS
Sat., 2:30—4:30 PM, Westminster III.

Chair: Elena Galkina, Eastern Virginia Med. Sch., Norfolk.


3:00 PM 15.2 B Cell Subsets in Atherosclerosis. Coleen McNamara, Univ. of Virginia, Charlottesville.


4:00 PM 15.4 HIV-1, Inflammation, and Endothelial Dysfunction. Jared Greiner, Univ. of Colorado, Boulder. (4.16)

4:15 PM 15.5 Ly6CHI Monocytic Clearance of Dying Cardiomyocytes by CD36 Activities NR4a1 and is Required for Transition to Reparative Inflammation Following Myocardial Infarction. Shirley Dehn, Northwestern Univ. (4.22)

NOTES
2016 APS Conference
Inflammation, Immunity, and Cardiovascular Disease

Abstracts of Invited and Contributed Presentations

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THE ENDOTHELIUM AND VASCULAR IMMUNITY

Jordan S. Pober*

1Immunobiology, Yale Sch. of Med., 10 Amistad St., Rm. 401D, New Haven, CT, 06519.

Endothelial cells (EC) line the entire vascular system but display characteristics that vary among tissues and among vascular segments within tissues. Post-capillary venular EC typically regulate the recruitment and activation of circulating immune cells at sites of inflammation. EC are typically activated to recruit leukocytes by cytokines of innate immunity (TNF or IL-1) that increase expression of proteins that capture and activate the migration of myeloid and lymphoid cells. EC additionally respond to cytokines of adaptive immunity (IFN-g and IL-4) by expressing proteins that selectively recruit specialized immune effector cells. Surprisingly, EC are unresponsive to IL-17, which instead acts on pericytes. Human microvascular EC basally express both class I and class II MHC molecules (signal 1) and a key costimulator for memory T cells (CD58, signal 2) and inductively express additional costimulators as well as cytokines (signal 3) that enable effective antigen presentation to circulating effector memory T cells. Antigen presentation induces effector memory T cells to undergo diapedesis by a process that differs from chemokine-based recruitment in that T cells round up instead of spreading and push their MTOC in front of their nuclei instead of trailing behind their nuclei in a uropod. CD4+ T cells recruited by antigen use the lateral border recycling compartment of EC to transmigrate and partly degranulate during this process, releasing granzyme A, whereas CD8+ T cells do neither. Antigen-recruited T cells shut off expression of i-NOS, proliferate and produce effector cytokines whereas chemokine-recruited T cells express i-NOS but not cytokines. IFN-g further increases EC expression of immune-genic signals (MHC molecules and cytokines) but also induces inhibitory signals (PD-L1 and PD-L2). In some settings, e.g. transplantation, graft MHC molecules not only activate host T cells but also trigger B cells to produce anti-donor antibodies that can activate complement, and through novel signaling pathways activated by membrane attack complex, further enhance immune functions of EC. These immune functions of EC present new targets for therapeutic intervention. [Grants from NIH (HL36003, HL51034, and HL109455), AbbVie and Alexion]. Refs: Pober and Tellides, Trends Immunol 2011; 33:49-57; Pober and Sessa, Cold Spring Harb Persp Biol. 2104; 7 piai016345; Abrahimi, Liu and Pober Am J Transplant 2015; 15:1748-1754.
macrophages early lipid deposition in the aortic arch is ablated. They remain a component of plaque macrophages at all stages we examined and seem to localize particularly to plaque borders. Finally, we have developed isolation approaches to characterization gene expression profiles of resident aortic macrophages before and following high fat diet exposure relative to monocyte-derived macrophages. Future studies will determine whether these macrophages have a distinct role and regulation in atherosclerosis compared with macrophages derived from other origins.

3.2 IL-13 SIGNALING IN TISSUE REPAIR AND FIBROSIS

Richard L. Gieseck III1,2, Thirumalai R. Ramalingam1, Kevin M. Hart1, Kevin M. Vannella1, David A. Cantu1, Wei-Yu Lu3, Sofia Ferreira-González3, Stuart J. Forbes3, Ludovic Vallier2,4, and Thomas A. Wynn1


Fibroproliferative diseases are a major cause of morbidity and mortality and affect nearly every organ system in the body. Recent studies have suggested that type-2 cytokine responses (Interleukin-4/13) are critically involved in tissue repair; however, the mechanisms that regulate tissue repair versus pathological fibrosis are not well understood. Here, we show that the type-2 effector cytokine interleukin-13 simultaneously, yet independently, directs hepatic fibrosis and the compensatory proliferation of hepatocytes and biliary cells. Using conditional knockout mice with interleukin-13 signaling disrupted in hepatocytes, biliary cells, or resident tissue fibroblasts, we reveal direct and distinct roles for interleukin-13 in fibrosis, steatosis, cholestasis, and hepatobiliary proliferation. Together, these studies show that these mechanisms are simultaneously controlled but distinctly regulated by interleukin-13 signaling. Thus, IL-13 could be exploited in the clinic to promote tissue repair without generating pathological fibrosis.

3.3 THE REGULATION OF MACROPHAGE ACTIVATION BY ENDOGENOUS SECRETORY PRODUCTS

Kajal Hamidzadeh1, Elizabeth Dalby1, Stephen Christensen1, and David Mosser1

1Cell Biology and Molecular Genetics, Univ. of Maryland, Rm. 3102 BRB, College Park, MD, 20742.

Genome-wide transcriptional profiling was performed to examine the resolution of the macrophage activation response over time following stimulation with the TLR ligand, LPS. As expected, the initial response to this stimulus included the upregulation of transcripts encoding inflammatory cytokines, chemokines, and mediators. TLR stimulation also rapidly induced transcripts encoding receptors for purinergic nucleotides and prostaglandins. Over time many of the cytokines and chemokines that were initially produced were turned off, and replaced by an upregulation in transcripts encoding growth and angiogenic factors and anti-inflammatory cytokines. The induction of these new regulatory transcripts depended on the initial stimulus-induced upregulation of receptors for purinergic nucleotides and prostaglandins. We hypothesize that human macrophages maintain homeostasis by the stimulus-dependent induction of receptors for adenosine and prostaglandin. The upregulation of receptor expression increases their sensitivity to the endogenous production of purinergic nucleotides and prostaglandin E2. We propose that this feedback loop is necessary for macrophages to actively terminate inflammatory responses and initiate the process of wound healing. This work was supported by NIH R01 GM 102589 to DMM.

3.4 IMMUNOGENETICS OF NATURAL KILLER CELLS

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Natural killer (NK) cells are lymphocytes of innate immunity that have many of the same effector functions as the CD4 and CD8 T cells of adaptive immunity. Notably, they include cellular cytotoxicity and cytokine production. The development, also called education, and immune response of NK cells are regulated by interactions between NK cell receptors and major histocompatibility complex (MHC) class I ligands. Whereas T-cell receptor diversity is created by rearranging genes encoding its component polypeptides, NK cell receptor diversity is the result of a variety of receptors, each encoded by a conventional non-rearranging gene. The MHC class I receptors of human NK cells are membrane glycoproteins of two structural very different types: the CD94:NKG2A/C receptors with binding sites similar to those of C-type lectins and the killer cell immunoglobulin-like receptors (KIR) that have binding sites made from two or three immunoglobulin-like domains. The ligand for CD94:NKG2A/C receptors is the complex of HLA-E, a conserved MHC class I mol-
ecule, and a nonamer peptide that is cleaved from the leader sequences of HLA-A, HLA-B and HLA-C, the highly polymorphic MHC class I molecules. The ligands for the KIR receptors are a set of mutually exclusive epitopes of the HLA-A, -B and -C molecules. Although structurally different the lectin-like receptors and the immunoglobulin-like receptors are functionally analogous and work together in complementary fashion. A major difference is that the interactions between and HLA-A,-B and -C are highly diverse within human individuals and populations, whereas the interaction of HLA-E and CD94:NKG2A/C is conserved.

3.5
DAMP’S AND PAMP’S
M. Karen Newell Rogers

The innate immune response is the “front line” of defense against infection, damage, and injury, culminating in acute inflammation. An evolutionarily conserved protective strategy of the innate response is the host response to pathogen-associated molecular patterns (PAMPs), molecules with relatively conserved molecular patterns that are the products of pathogens, or damage/danger associated molecular patterns (DAMPs), host-derived molecules that induce a noninfectious inflammatory response. PAMPs are recognized by Toll-like receptors (TLR) in both plants and animals. PAMPs protect the host from infection by inducing an acute inflammatory response. Lipopolysaccharides (LPS) are released from bacteria, and as prototypical PAMPs are recognized by TLR4, while other PAMPs including CpG motifs are recognized by TLR9. Bacterial flagellin are recognized by TLR5, and virally associated nucleic acids, including double- or single-stranded RNA are recognized by TLR3. When PAMPs bind to the cellular TLR, adapter proteins within the cell are recruited to the complex and are then responsible for the activation of protein kinases that can lead to inflammation, the production of cytokines, proliferation, and survival. Proteins of the NOD family of receptors (NLR) detect a variety of stimuli and are central to an IL-1 response. TLR engagement can also facilitate the transition to an adaptive immune response. DAMPs are the products of damage or injury to the host and, like PAMPs, bind to host receptors that initiate and propagate signals that lead to inflammation, cytokine production, and survival signals. Following tissue damage, DNA can be released outside the cell where it serves as a danger signal. The characteristics of DAMPs vary between injured tissues. In addition to nucleic acids as DAMPs, protein DAMPs include heat-shock proteins, high-mobility group box 1 (HMGB1) proteins, extracellular matrix proteins, ATP, uric acid, heparin sulfate, and S100 proteins, a family of calcium modulate proteins. Receptors for HMGB1 include TLR2 and TLR4. HMGB1 can induce increased expression of costimulatory molecules and CD11c on dendritic cells, can induce production of IL-1, TNF-α, IL-6, and IL-8, and can upregulate expression of cell adhesion molecules on endothelial cells. Extracellular ATP can trigger mast cell degranulation via P2X7 receptor signaling and adenosine via P1 receptor signals. Thus, a system of either microbial or host derived molecules, the products of infection or injury, respectively, are centrally important in initiating the protection afforded by an innate immune response to the infection or the danger resulting from host injury. REFERENCES: Han J, Brown T, Beutler B. Endotoxin-responsive sequences control cachectin/TNF biosynthesis at the translational level. J Exp Med. 1990;171:465–475. Janeway C (September 1989). "Immunogenicity signals 1,2,3 ... and 0". Immunol. Today. 10 (9): 283–6. doi:10.1016/0167-5699(94)90081-9. PMID 2590379. Matzinger P (1994). "Tolerance, danger, and the extended family". Annu. Rev. Immunol. 12:991–1045. doi:10.1146/annurev.iy.12.040194.005015. PMID 8011301.

3.7
MHC AND ANTIGEN PRESENTATION
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T cells require that protein antigen be processed into peptides, typically inside antigen presenting cells (APC), such as dendritic cells or macrophages, and the peptides are then bound to major histocompatibility complex (MHC) molecules and these MHC/peptide complexes are presented on the APC surface. This paradigm applies to both CD4+ T and CD8+ T cells, with CD4+ T cells recognizing MHCII/peptide complexes and CD8+ T cells recognizing MHC1/peptide complexes. Peptide loading of MHC1 and MHCII molecules occurs in primarily in different intracellular compartments, with the involvement of distinct accessory molecules. Both canonical and alternative peptide-loading pathways have been described, presumably broadening self and foreign peptide presentation for tolerance and host defense, respectively. These pathways also contribute to immune consequences associated with the atherosclerotic lesion. Reference: Mellins, E.D., 2016. Ligand Selection and Trafficking for MHC II. In: Ratcliffe, M.J.H. (Editor in Chief), Encyclopedia of Immunobiology, Vol. 2, pp. 247–254. Oxford: Academic Press.

4.0: POSTER SESSION I
4.1 KLF6-BCL6 SIGNALING AXIS REGULATES MACROPHAGE-MEDIATED INFLAMMATION
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The macrophages are the predominant innate immune cells recruited to tissues following injury or infection. These early-responding pro-inflammatory macrophages play an essential role in the amplification of inflammation. However, macrophage pro-inflammatory gene expression should be tightly regulated to avert host tissue damage. In this study, we identify KLF6-BCL6 signaling axis as a novel regulator of macrophage inflammatory gene expression and function. Utilizing complementary gain- and loss-of-function studies, we observed KLF6 is essential for macrophage motility under ex vivo and in vivo conditions. Concordant with these observations, myeloid specific deficiency of KLF6 significantly attenuate macrophage pro-inflammatory gene expression, recruitment and progression of inflammation. At the molecular level, KLF6 suppress Bcl6 expression by elevating PRDM1 levels in macrophages. Interestingly, pharmacological or genetic inhibition of BCL6 in KLF6-deficient macrophages completely abrogated attenuation of pro-inflammatory cytokine/chemokine expression and cellular motility. Collectively, our observations reveal that KLF6 repress BCL6 to enhance macrophage inflammatory gene expression and function.

4.2 AORTIC AND MICROVASCULAR ENDOTHELIAL CELLS DIFFERENTIALLY INFLUENCE INFLAMMATORY AND VASCULAR RESPONSES TO ENGINEERED NANO-MATERIALS
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Exposure to cerium dioxide nanoparticles (CeO2) has been shown to cause vascular dysfunction in animal models. However, the mechanisms and target cell types of vascular dysfunction are unknown. We hypothesized that phenotypic differences in aortic and microvascular endothelial cells will influence responses to CeO2 exposure and ultimately impact vascular dysfunction. Rat aortic endothelial cells (RAEC) or rat microvascular endothelial cells (RMEC) were exposed to CeO2 (0-100 µg/ml). Cytotoxicity, alterations in inflammatory (IL-6) and surface adhesion marker (ICAM and VCAM) mRNA expression, and reactive oxygen species (ROS) generation were assessed following exposure. To assess potential influences on vascular function, isolated naive arterioles were intraluminally exposed to the supernatant from CeO2 exposed RAEC and RMEC. Endothelium-dependent and – independent reactivity was assessed with acetylcholine (ACh, 10-9-10-4 M), and spermine NONOate (10-9-10-4 M). Finally, enhanced darkfield microscopy and flow cytometry was used to determine CeO2 cellular uptake in vitro. Following exposure to 25 µg/ml CeO2, RAEC had an increase in nitric oxide synthase (NOS, 2.5-fold) and VCAM (3.3-fold) mRNA expression. RMEC also had an increase VCAM (2.8-fold) which was similar to the RAEC; however, NOS mRNA expression (35.7-fold) significantly higher than the RAEC. Lastly, CeO2 exposure did not elicit ROS production in RAEC but was increased 23±1.3% in RMECs as compared to control following 25 µg/ml treatment. Taken together, these results indicate that the aortic and microvascular endothelium respond uniquely to CeO2. Furthermore, these distinct responses may have differential impacts on vascular function. R01-ES019311 (JMB), K99-ES024392 (JHS).

4.3 LYMPHATICS-THE KEY COMPONENT OF THE IMMUNOCULAR SYSTEM
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The lymphatic vascular system is part of the cardiovascular system responsible for the return of fluid and macromolecules extravasated from the exchange blood vessels into the interstitium back to the venous blood. In this role it accounts for the removal of ~ 4 liters of fluid from the interstitium daily in the form of lymph. Lymphatics are known to play key roles in the development and resolution of inflammation in many body tissues. Eff ere nt lymph also carries macromolecules, lipids, antigens, cytokines, parenchymal cell degradation products, infectious and inflammatory agents, immune cells and other immune relevant signals from the interstitium to the node. Eff ere nt lymph then carries the unique response elements elicited from the nodal cells into the lymph inside the node back to the blood. Understanding the role of the lymphatics in the regulation and integration of fluid and immune homeostasis if of critical importance towards a
full understanding of edema, inflammation and immunity. Over the last decade we have integrated our studies of the role of lymphatics in body fluid homeostasis with their roles in inflammation, tolerance and immune response. This work has lead us to identify and characterize the intimate interactions of the muscularized prenodal lymphatics with a number of immune cells that exist in and around these lymphatics and are responsive to inflammatory and/or tolerogenic signals from the parenchyma. These interactions lead to the modulation of lymph transport as a means to alter immune signal delivery to the lymph node and to changes in immune cell function. We identified a unique resident population of MHCII+ cells that live on and within the wall of the muscularized lymphatics that can rapidly sense, capture and process antigens carried in lymph. These cells are variably attracted to the lymphatics under different conditions, can then modulate lymphatic function and be quickly transported to the node to integrate rapid remote immune responses. These and other local and resident immune cells have been shown to interact with each other, and to local and distant parenchymal inflammatory/immune signals carried in lymph to modulate lymphatic transport and immune functions. Thus the lymphatics form the critical information path of the immunovascular system responsible for linking the fluid and immune status of the parenchymal environment to inflammation, immune response and cardiovascular function.

4.4 PLASMA LEVELS OF ANGIOPOIETIN-1, ANGIOPOIETIN-2 AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN SICKLE CELL ANAEMIA PATIENTS WITH OR WITHOUT COMPLICATIONS IN GHANA: A CASE-CONTROL STUDY

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Sickle Cell Disease (SCD) results in ongoing vasculopathy and end organ damage. Angiogenesis have been implicated as a key contributing factor to ongoing vascular mediated tissue injury in SCD. The relative plasma levels of angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) greatly influence angiogenesis. Dysregulation of these growth factors leading to a pro-angiogenic state have been found in blood samples of Non-Ghanaian SCD. Ghana has one of the highest prevalence of SCD where 2% of all births are burdened with this disease. To date, there is no data on the levels of angiogenic factors in Ghanaian SCD patients. The aim of this study was to assess plasma levels of Ang-1, Ang-2 and VEGF in homozygous (HbSS) SCD patients with or without complications and healthy controls (HbAA) in Ghana. The study was a case-control study involving 544 participants conducted at the Sickle Cell Clinic Accra, Ghana. The plasma levels of Ang-1, Ang-2 and VEGF were measured with a double sandwich enzyme-linked immunosorbent assay (ELISA) technique and Complete Blood Count with an autoanalyser. The mean plasma Ang-1, Ang-2 and VEGF were significantly higher in HbSS SCD patient with or without complications than healthy controls (p<0.001). The Ang-1/Ang-2 ratio was significantly higher in the controls than the HbSS patients (p<0.001). Ang-2/Ang-1 ratio was higher in the HbSS patients with complications especially, leg ulcer patients than healthy controls (p<0.001). There were higher plasma levels of Ang-1, Ang-2 and VEGF in HbSS patients with complications than those without complications and healthy controls. In conclusion, there was an overall evidence of angiogenesis, which was highest in HbSS patients with complications, especially those with leg ulcer. The study provides first report on plasma levels of Ang-1, Ang-2 and VEGF HbSS SCD patients in Ghana. Funded by ORID University of Ghana and University of Ghana-Carnegie Next Generation of Academics in Africa.
Preeclampsia is characterized by newly diagnosed hypertension and reduced placental perfusion. Previous studies in a rat model of placental ischemia-induced hypertension demonstrated that inhibiting complement activation attenuated increased maternal blood pressure, indicating that complement is a promising therapeutic target. Given that both natural IgM and antigen antibody complexes can initiate complement activation, we hypothesized that placental ischemia exposes neoepitopes recognized by IgM to cause complement activation and hypertension. Alternatively, we considered that autoantibody to angiotensin II Type 1 receptor (AT1-AA) interacts with AT1 receptors to cause complement activation. Since complement activation occurs in kidney and placenta in preeclampsia, we used immunohistochemistry to determine IgM deposition and local complement activation in each organ (C3 deposition), and qRTPCR to quantitate mRNA for endogenous regulators of complement activation CD55, CD59 and Crry. On gestation day (GD)14, timed pregnant Sprague Dawley rats underwent Sham surgery or placement of clips on inferior abdominal aorta and ovarian arteries to create placental ischemia (RUPP). As previously reported, RUPP increased mean arterial pressure and circulating C3a on GD19. In placenta, IgM and C3 deposition increased in RUPP vs Sham animals (p<0.05), whereas mRNA for complement regulators Crry and CD59 decreased in RUPP compared to Sham (Crry, 0.85±0.03; CD59, 0.79±0.10 fold; p<0.05). In kidney, IgM deposition increased in RUPP vs Sham (p<0.05) without a significant change in C3 deposition. Kidney mRNA for complement regulators increased in RUPP compared to Sham (CD55, 1.21±0.09, p=0.07; CD59, 1.19±0.04 fold, p<0.05). The AT1 receptor antagonist losartan is known to prevent AT1-AA interaction with AT1 receptors. Losartan treatment prevented placental ischemia-induced hypertension without affecting complement activation as measured by circulating C3a or placental C3 deposition. Overall, these data suggest that increased complement regulators limit local complement activation in kidney, and decreased complement regulators in placenta are consistent with increased C3 deposition with placental ischemia. Importantly, our studies indicate that complement activation following placental ischemia is not due to AT1-AA but is associated with IgM deposition, suggesting a role for natural antibodies interacting with neoepitopes following placental ischemia. Funding: NIH HL109843.

4.6 PLATELET-P2Y12 RECEPTOR IS IMPORTANT FOR THE CELLULAR IMMUNE RESPONSE IN EROSIIVE ARTHRITIS

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Rheumatoid arthritis (RA) is an immune-mediated inflammatory disease with a complex pathogenesis, which comprises activation of humoral and cellular immune responses. There is increasing evidence of the role of platelets in RA. Platelet-derived microparticles, platelet-leukocyte interaction and activated platelets are related to RA development. Therefore, inhibiting platelet activity might benefit RA patients. Adenosine diphosphate activates platelets through the Gq-couple receptor P2Y1 and the Gi-couple receptor P2Y12. P2Y12 receptor has been targeted to inhibit platelet activity using thienopyridine pro-drugs such as clopidogrel and prasugrel. Our previous studies using a rat model demonstrated aggravation of arthritis after administration of thienopyridine compounds. Therefore, we evaluate the role of the P2Y12 receptor in arthritis by using a P2Y12 null mice. In contrast to the results obtained with P2Y12 pharmacological inhibition, the induction of arthritis by administration of collagen antibodies in the P2Y12 KO mice was mild. We found decrease in synoviocyte hyperplasia, articular leukocyte infiltration, pannus formation, and pro-inflammatory cytokine production including I-L6 and TNFα when compared to WT. This conundrum in results could be explained by the difference of the mouse and rat arthritis models. The rat model generates a cellular mediated immune response against the joints of the rat while the mouse model generates an arthritogenic effect due to the humoral immune response of auto-antibodies against the collagen epitope. These suggest that the P2Y12 receptor is a selective regulator of the platelet-mediated cellular immune response in RA, and might have potential for the development of new therapies against RA.

4.7 INTERLEUKIN-19: A NOVEL SEXUALLY DIMORPHIC CARDIAC CYTOKINE

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Heart failure (HF), the leading cause of mortality within the United States, is a sexually dimorphic disease. Al-
though the prevalence of HF is lower in women compared to men, treatment and survival outcomes for female patients are poorer with females having disproportionately higher morbidity and mortality. The deleterious effects of inflammatory cytokines in the context of HF are well documented as elevated circulating levels of cytokines predict adverse outcomes. Few studies, however, have examined sex-specific differences in cytokine expression, though sparse data indicate inflammatory profiles may be sexually dimorphic. Interleukin-19 (IL-19) is a recently described member of the anti-inflammatory IL-10 cytokine family. There is significant controversy surrounding the role of IL-19 in cellular signaling as it has been shown to be both pro- and anti-inflammatory depending on cellular context. To date, the role of IL-19 in the heart has not been characterized. We now show that IL-19 is expressed in the rodent and human heart, with increased expression in a female-dominant model of HF, the dominant-negative CREB (dnCREB) transgenic mouse. Further, we show that the relative expression of the two IL-19 receptor isoforms IL-20Ra/β, known to have divergent influences on cell phenotype, manifest differently in the heart by sex and by disease in both our murine HF model and in cardiac tissue from human HF patients. To test the hypothesis that IL-19 promotes cardiac dysfunction in dnCREB female mice we generated a double transgenic (DTG) mouse of IL-19 knockout and dnCREB. Surprisingly, survival analyses demonstrate that female DTG were not protected and that male DTG mice had accelerated mortality, as their corresponding dnCREB cohort. To understand the mechanism(s) of IL-19 signaling in the male and female heart, we treated mouse cardiac myocytes and fibroblasts with recombinant IL-19 and show that IL-19 treatment activates STAT3, a canonical downstream IL-19 signaling cascade. Together, these data suggest IL-19 is an important cytokine in sex-specific cardiac dysfunction. Ongoing investigations will elucidate the mechanism(s) of sex-specific IL-19 mediated cardiac remodeling.

4.8 PRIOR PREECLAMPSIA RESULTS IN PERSISTENT IMMUNE ACTIVATION FOLLOWING PREGNANCY IN THE DAHL SALT SENSITIVE RAT
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Preeclampsia is a risk factor for later cardiovascular disease resulting in an increased risk of stroke, heart attack, hypertension, and chronic kidney disease 5-15 years postpartum. While it is known that immune activation is involved in the pathogenesis of preeclampsia and that T cells contribute to hypertension and end organ damage in male experimental animals, the role of T cells in the progression of cardiorenal disease following preeclampsia has not been examined. We hypothesized that prior preeclamptic pregnancy would result in postpartum activation of T cells and production of the inflammatory cytokine tumor necrosis factor (TNF)-α. Dahl salt-sensitive rats on a 0.3% salt diet (previously characterized by our lab as a model of superimposed preeclampsia) who experienced 2 pregnancies (at 12 and 17 weeks of age) and virgin littermate controls were allowed to age to 6 months. Rats were implanted with telemetry transmitters (DSI), mean arterial pressure (MAP) was recorded, and rats were placed in metabolic cages for 24 hour urine collection prior to tissue harvest. Prior pregnancy did not result in a further increase in MAP at 6 months (virgin: 185±6.9 mm Hg, prior pregnancy: 184.6±6.6 mm Hg, n=8-10); however, despite similar blood pressure, rats who experienced prior preeclamptic pregnancy had significantly greater renal injury compared to virgin littermates. Urinary excretion of protein (96±20 vs 195±45 mg/day), nephrin (0.6±0.4 vs 3.1±1.2 µg/day), and podocalyxin (4.9±1.0 vs 21.0±7.6 µg/day) was higher in the postpartum group (p<0.05, Bradford assay and Exocell ELISA). These measures of renal injury were corroborated by histological examination as kidneys from rats that experienced preeclampsia (2.9±0.3) demonstrated greater glomerular sclerosis compared to virgin littermates (2.5±0.3, p=0.05). Flow cytometry analysis revealed that CD3+ T cells (10±3 vs 19±3%, p<0.05) and specifically CD3+CD4+ T helper cells (10±4 vs 17±2%, p<0.05) are greater in the kidney following preeclamptic pregnancy. Furthermore, we observed greater plasma levels of the pro-inflammatory cytokine TNF-α (0.7±0.3 vs 1.5±0.1 pg/ml) and lower levels of the anti-inflammatory cytokine interleukin-10 (12.0±1.0 vs 8.8±1.4 pg/ml, p<0.05), suggesting that there may be an imbalance in effector T cells vs regulatory T cells. These data support the hypothesis that activation of immune cells could link the maternal syndrome of preeclampsia to the increased postpartum risk of cardiovascular and renal disease.

4.9 INCREASED PRO-INFLAMMATORY T CELLS AND HMGB1 ARE ASSOCIATED
WITH VASCULAR DYSFUNCTION IN MALE SHR
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Dysfunctional inflammatory responses have been implicated in the development of hypertension, however, the mechanisms that trigger these inflammatory processes are not yet well understood. High mobility group box 1 protein (HMGB1) is a damage-associated molecular pattern released by dying cells that can then trigger an inflammatory response and cause vascular dysfunction. Recently, our lab studied vascular reactivity in the aortas of male and female Spontaneously Hypertensive Rats (SHR) and normotensive Wistar Kyoto (WKY) rats. Male rats had enhanced contractile and diminished vascular relaxation, compared to female rats, in both strains. Furthermore, SHR males had the greatest contractile response and most diminished vasorelaxation compared to all other groups. We hypothesized that hypertension in the SHR increases HMGB1 to promote a pro-inflammatory T cell profile in the aorta. Male and female SHR and WKY rats (n=6 per group) were sacrificed at 13 weeks of age, and aortas were collected for flow cytometric analysis. Anti-inflammatory Tregs were lower in both male (p<0.001) and female (p=0.001) SHR compared to the normotensive WKY controls, with male SHRs having the lowest Treg population (Table). Pro-inflammatory TH17+ cells and HMGB1+ cells were greater in the SHRs relative to the WKY rats (Table). No sex difference was observed in TH17+ cells in the WKY rats, but male SHRs did have significantly greater TH17+ cells compared to the female SHR (p=0.02). Interestingly, a sex difference in HMGB1 levels was observed in both strains, with male SHR (p<0.001) and WKY (p=0.02) having greater expression of HMGB1 compared to their female controls. These data suggest that enhanced aortic vascular dysfunction in male SHR is associated with greater increases in pro-inflammatory T cells and HMGB1 compared to all other groups. Future studies will address the ability of HMGB1 to activate T cells and induce vascular dysfunction.

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<th>Tregs</th>
<th>TH17+</th>
<th>HMGB1</th>
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<td>5.0±0.8</td>
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<tr>
<td>Female WKY</td>
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<tr>
<td>Male WKY</td>
<td>6.2±0.5</td>
<td>2.3±0.3</td>
<td>2.5±0.3</td>
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*p<0.05 vs. male, same strain, "p<0.05 vs. WKY, same sex.

4.10 MITOCHONDRIAL ROS MEDIATES PKM2 KINASE ACTIVITY TO PROMOTE PRO-INFLAMMATORY ACTIVITY OF HUMAN MACROPHAGES IN CORONARY ARTERY DISEASE
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Inflammatory macrophages are key drivers of the tissue inflammatory response typical for coronary artery disease (CAD). Reactive oxygen species (ROS) are produced in macrophages by cytoplasmic and mitochondrial sources and regulate macrophage function through a multitude of pathways. Processes underlying the production of pro-inflammatory cytokines by macrophages in CAD are insufficiently understood. Here, we have examined how metabolic activity of such macrophages is mechanistically connected to their cytokine release. We found that LPS stimulated macrophages from coronary artery disease (CAD) patients overexpress the glycolytic master regulators, e-Myc and HIF-1α as well as genes involved in glucose transport and its glycolytic breakdown (PKM2, GLUT3, PDK1, FPK FB3, HK2, PGK1, PFK1, and GLUT1). To understand the contribution of the glycolytic machinery to cytokine production, we quantified and manipulated the metabolic activity of these macrophages. Compared to healthy macrophages, CAD macrophage had over 20% higher rate of glucose absorption and utilization. Their mitochondrial respiration and membrane potential indicated higher mitochondrial activity. Using [U-¹³C₅] glutamine isotope tracing we analyzed glutamine utilization as an indicator of mitochondrial activity and confirmed intensified mitochondrial metabolism. ¹³C enrichments of the Krebs cycle-associated metabolites malate, aspartate, and glutamate increased by approximately two-fold in CAD macrophages. We found that increased metabolic activity augmented mitochondrial superoxide and hydrogen peroxide production. These mitochondrial ROS targeted the glycolytic enzyme pyruvate kinase M2 (PKM2) and induced its dimerization. Dimerized PKM2 increased mitochondrial ROS, which regulated IL-1α and IL-6 release by macrophages. Inhibiting ROS thereby decreased cytokine production, suggesting that increased mitochondrial ROS may be an important regulator of the inflammatory response in CAD.
PKM2 tetramerization (ML265) or limited ROS production (mitoTempo, Tempol) prevented PKM2 dimerization, abrogated translocation to the nucleus, suppressed STAT3 phosphorylation and corrected the proinflammatory CAD macrophage phenotype. Taken together, our study shows that the production of the key pro-inflammatory cytokines IL-6 and IL-1β in CAD is driven by metabolic reprogramming and depends on a pathway involving oxidative modification of a cytoplasmic enzyme to sustain nuclear STAT3 activation.

4.11 IDENTIFICATION OF AN ADDITIONAL ROLE FOR APOC-III MODULATES BASAL T CELL PROFILE IN MICE

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Apolipoprotein C-III (apoC-III) is a component protein of triglyceride-rich lipoproteins and an independent predictor of cardiovascular disease (CVD) in humans. ApoC-III functions in the blood and liver to increase plasma triglyceride levels, but in the intestine it functions to delay dietary fat absorption. Therefore, apoC-III may have differing roles in promoting CVD, depending upon the circumstances of its expression and secretion. Since the immune system plays such a large role in atherogenesis, we asked whether apoC-III is a potential regulator of inflammation and immune profile, which has not yet been fully elucidated. We characterized the circulating CD3+CD4+T cell subsets and CD4+CD68+ monocyte subsets in mice that overexpress the human apoC-III gene (apoC-III Tg) compared with their wild-type C57Bl/6J littermates. Whole blood was isolated from the tail of each group and prepared for flow cytometric analysis. Samples were lysed, stained for surface antigens, fixed in 2% paraformaldehyde, and permeabilized for intracellular staining. Flow cytometric analysis was performed using BD LSR II-B (Becton-Dickinson) after appropriate fluorescence compensation, and subsets were gated using FlowJo software (Treestar). Interestingly, total CD19+ B cell count did not vary between the groups but total CD3+ T cells, CD4+ T cells, CD4+CD25+FoxP3+ regulatory T cells, and CD4+CXCR4+ helper T cells decreased significantly in the apoC-III transgenic mice. Overall, significant increases were seen in the CD8α+ cytotoxic T cell and CD4+CD44hiCD62Lhi central memory T cell populations. In the myeloid cell fraction, significant increases were seen in the Ly-6ChiM1-like macrophage population while CD68Ly-6C+ monocytes significantly decreased in apoC-III Tg. We also characterized the cytokine profile in these mice to confirm the phenotype of these cells. Interestingly, these studies were completed in the absence of high fat diet feeding or LPS stimulation, suggesting that apoC-III itself, or its causative role in hypertriglyceridemia, results in changes to the circulating immune profile in mice. These results show a significant trend towards a pro-inflammatory profile of apoC-III Tg at baseline and suggest that apoC-III may play a previously unknown role in stimulating CD8α+T-cells to modulate atherogenesis.

4.12 ROLE OF SMOOTH MUSCLE CELL SPECIFIC iPLA2β IN VASCULAR INFLAMMATION AND NEOINTIMAL FORMATION IN A MURINE FEMORAL ARTERY WIRE INJURY MODEL

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Coronary heart disease is the single leading cause of death in the United States. Coronary revascularization, including coronary artery bypass graft and percutaneous coronary intervention (PCI) is the most common modality in patients with coronary diseases. However, it is also among the most costly and is often associated with a high incidence of restenosis. Thus, there is an urgent need to identify new therapeutic targets for coronary heart disease. Calcium independent phospholipase A2β (iPLA2β) is a member of the phospholipase A2 superfamily that acts on phospholipids to produce a free fatty acid and a lysophospholipid. iPLA2β is ubiquitously expressed and is implicated in many human diseases. To investigate whether targeting smooth muscle-specific iPLA2β is sufficient to reduce vascular inflammation and neointima formation, we established a femoral artery injury model that better mimics PCI and developed a novel tamoxifen-inducible smooth muscle-specific iPLA2β knockout mouse model (SM-iPLA2β-iKO). By using genomic PCR with smooth muscle-specific Cre-mediated genomic DNA recombination, we demonstrated that iPLA2β was specifically deleted in smooth muscle tissues such as aorta, carotid artery, mesenteric artery, and bladder, but not that in others, including heart, kidney, lung, brain, liver, and skeletal muscle. Smooth muscle cell specific deletion of iPLA2β was verified by real-time PCR, Western blot, and iPLA2 enzymatic assay. Interestingly, SM-iPLA2β-iKO mice, unlike global iPLA2β knockout mice, did not have
iPLAγ, iPLAζ, and cPLAα genetic compensatory up-regulation. iPLAβ protein was upregulated by wire injury and was predominantly detected in neointima area in a time dependent manner. Using a femoral artery injury model, we illustrated that wire-injury-induced neointima formation was markedly decreased in SM-iPLAβ-iKO mice, which was temporally correlated with a remarkable reduction of neutrophil infiltration into neointima in SM-iPLAβ-iKO mice. These data demonstrate a critical role of smooth muscle cell iPLAβ in neointima formation and inflammation in a femoral artery injury model, suggest that smooth muscle cell iPLAβ participates in the initiation and early progression of vascular inflammation and neointima formation, and indicate that iPLAβ may represent a novel therapeutic target for treatment of coronary heart disease. This work was supported by VA Merit Award I01BX002141, NIH grants HL088389, HL106843, and HL125228.

4.13
ROLE OF NEUREGULIN-1β IN THE ACUTE INFLAMMATORY RESPONSE TO CORONARY ARTERY BYPASS GRAFTING (CABG)
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Neuregulin-1β (NRG) is a cardioactive growth factor required for cardiac morphogenesis and maintenance. NRG interacts with tyrosine kinase receptors ERBB2, ERBB3, and ERBB4 to activate intracellular signaling cascades regulating proliferation, apoptosis, protein synthesis, cell motility and survival. Our previous work has demonstrated a cardioprotective effect of recombinant NRG therapy has already shown promise as a safe and effective therapy for heart failure. Given that recombinant NRG therapy has already shown promise as a safe and effective therapy for heart failure, we will be able to determine whether circulating NRG status correlates with the inflammatory response to CABG. These studies are of high translational potential given that recombinant NRG therapy has already shown promise as a safe and effective therapy for heart failure. Funded by the Osher Research Fund at Maine Medical Ctr.

4.14
THE ROLE OF PSGL-1-EXPRESSING CD4+ T CELLS IN THE ATHEROSCLEROTIC DEVELOPMENT AND PLAQUE INSTABILITY
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Background: Many inflammatory infiltrates, such as leukocytes are observed in the atherosclerotic plaque. Adhesion of circulating leukocytes to the endothelial cells and subsequent trans-endothelial migration are important steps in the development of atherosclerosis. Recently, we found PSGL-1+CD4 T cells contribute to the pathogenesis of acute coronary syndrome. Methods and Results: In this study, we examined the role of PSGL-1+CD4 T cells for the atherosclerotic development and plaque instability. We constructed PSGL-1+Apoe−/− mouse fed with high-fat diet (PSGL-1+Apoe−/−) and compared to apolipoprotein E−/− mice (Apoe−/−) fed with high-fat diet (HFD). Immunohistochemistry of aortic sinus showed many integrinβ2 CD4 T cells, IFNγ CD4 T cells (Th1), and IL17 CD4 T cells (Th17) were infiltrated into the atherosclerotic...
plaque in ApoE−/−; however, these inflammatory infiltrates were less in PSGL-1−/−ApoE−/−. Furthermore, we examined the expression of PSGL-1 ligands, P-selectin and E-selectin in atherosclerotic plaque. P-selectin and E-selectin expression on endothelial cells and vascular smooth muscle cells were strongly increased in ApoE−/−. In contrast, those in PSGL-1−/−ApoE−/− were inhibited. Finally, TUNEL positive apoptotic cells in atherosclerotic plaque were strongly suppressed in PSGL-1−/−ApoE−/−, contributing more stabilized morphologic features. **Conclusion:** We concluded that PSGL-1 expressing CD4−T cells participate directly in the development of atherosclerosis and plaque instability.

**4.15 INFLUENCE OF HIV-1 ON CIRCULATING VASCULAR-RELATED MICRO-RNAs**

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Over 1.2 million adults in the United States are living with human immunodeficiency virus (HIV)-1. Alarmingly, cardiovascular disease (CVD) is the leading cause of morbidity and mortality in adults with HIV-1 in the United States. MicroRNAs (miRs) are short (~22 nt), non-coding RNAs that regulate gene expression post-transcriptionally. miRs are transported in the circulation by argonaute2 complexes, lipoproteins and microvesicles. Extracellular miRs are abundant and stable in blood and have been linked with vascular disease and numerous CVD risk factors such as dyslipidemia and hypertension. As a result, it has been suggested that circulating miRs may be a useful biomarker for vascular risk and disease. The aim of this study was to determine whether the expression of specific circulating endovascular-related miRs (miR-17, miR-92a, and miR-126) are dysregulated in HIV-1 seropositive adults. Thirty-eight adults were studied: 18 HIV-1 seronegative (12M/6F; age 40±3 yr) and 20 HIV-1 seropositive (13M/7F; 38±2 yr) on stable efavirenz-based antiretroviral therapy. All subjects were non-obese, normotensive, normolipidemic and free of overt cardiometabolic disease. Circulating expression of miRs was determined in plasma using standard RT-PCR techniques with miR primers of interest. Expression was normalized to exogenous C. elegans miR-39 and reported as relative expression in arbitrary units (AU). There was no significant difference in circulating expression of miR-17 in the HIV-1 seronegative (0.10±0.03 AU) and seropositive (0.11±0.04 AU) groups. In contrast, circulating expression of miR-92a was ~70% higher (2.96±0.47 vs 1.71±0.35 AU; P<0.05) and miR-126 was ~90% higher (0.58±0.11 vs 0.30±0.04 AU) in HIV-1 seropositive compared with seronegative adults. Differential expression of miR-92a may be involved with various pathologies associated with HIV-1. For example, overexpression of miR-92a leads to downregulation of endothelial nitric oxide-synthase and, in turn, nitric oxide bioavailability. In contrast, miR-126 is highly enriched in endothelial cells and considered to be vasculoprotective as it regulates endothelial cell angiogenic activity, cell adhesion and, in turn, vascular permeability. Upregulation of miR-126 with HIV-1 may represent an endothelial protective mechanism. Expression profile of circulating vascular-related miRs may provide mechanistic insight into the increased CVD risk associated with HIV-1 infection and deserves further study.

**4.16 HIV-1, INFLAMMATION AND ENDOTHELIAL DYSFUNCTION**

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Cardiovascular disease morbidity and mortality is a burgeoning problem in human immunodeficiency virus (HIV)-1-infected adults. Mechanisms underlying the increased cardiovascular risk are not completely understood. Local and systemic inflammation and oxidative stress play an important role in the initiation and progression of atherosclerotic vascular disease. HIV-1 infection and various types of antiretroviral therapy have the potential to adversely influence oxidative stress and inflammatory burden. In an ongoing study, we are testing the hypothesis that increased inflammatory burden and oxidative stress may contribute to endothelial dysfunction in HIV-1-infected adults. To date, 10 HIV-1-seronegative (9M/1F; age 36±3 yr; BMI: 24.0±0.7 kg/m²) and 10 HIV-1-seropositive adults on stable antiretroviral therapy (9M/1F; 41±2 yr; 25.1±1.1 kg/m²) have been studied. All subjects were sedentary, normotensive, normolipidemic and free of overt cardiovascular disease. Forearm blood flow (FBF) responses to intrabrachial infusions of acetylcholine (Ach: 8.0-32.0 µg/min) and sodium nitroprusside (NTP: 2.0-8.0 µg/min) were measured by venous occlusion plethysmography. Plasma concentrations of C-reac-
cardiovascular disease in this population. HIV-1-infected adults, and, in turn, the increased risk of impairment in endothelium-dependent vasodilation in addition to Ach. Increased inflammation may contribute to the

**Results:**

- Plasma concentrations of CRP (1.1±0.2 vs. 0.5±0.1 mg/L), IL-6 (1.7±0.2 vs. 0.9±0.1 pg/mL), and IL-18 (304.6±30.4 vs. 194.2±14.1 pg/mL) were significantly higher (~50%–100%) in the HIV-1-seropositive than HIV-1-seronegative adults. There were no significant differences in plasma concentrations of ox-LDL (73.1±11.8 vs 49.9±6.6 U/L), MPO (114.7±22.7 vs 104.4±21.2 mg/L), and TNF-α (1.7±0.4 vs. 1.6±0.3 pg/mL) between the groups. Plasma concentrations of CRP (r=-0.68, P<0.05) and IL-18 (r=-0.56, P<0.05) were inversely related to peak vasodilation to Ach. Increased inflammation may contribute to the impairment in endothelium-dependent vasodilation in HIV-1-infected adults and, in turn, the increased risk of cardiovascular disease in this population.

**4.17**

**THE ALPHA7 NICOTINE ACETYLCHOLINE RECEPTOR AGONIST AZ6983 REDUCES ATHEROSCLEROSIS IN APOE-/- MICE AND TNF PRODUCTION IN HUMAN CELLS**

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**Background and objective:** Autonomic nerve signaling via the alpha 7 nicotinic acetylcholine receptor (α7nAChR) is known to modulate inflammation. We have previously shown that the α7nAChR is expressed by human atherosclerotic plaques, however, little is known about the functional role of the α7nAChR in human cells. The aim of the current study is to investigate the functional role of the α7nAChR in human cells and to compare the effects of the α7nAChR with other nicotinic receptors expressed by human blood cells.

**Methods and results:** Atherosclerosis-prone ApoE-/- mice on a high fat diet were treated with AZ6983 or vehicle mixed in food for 12 weeks, or infused by osmotic minipumps (50 μmol/kg/day) for 8 weeks. Quantification of atherosclerotic lesions in thoracic aorta and aortic root revealed that AZ6983 reduced atherosclerosis by 49% after 12 weeks of oral treatment and 37% after 8 weeks of minipump treatment. AZ6983 treatment did not influence cholesterol levels, nor CD3+ T cells or CD68+ macrophages in the aortic root, quantified by immunostaining. There was no difference in immune cell markers in the aorta, investigated by real-time PCR. In addition, AZ6983 treatment in LPS-stimulated human and murine whole blood, reduced pro-inflammatory cytokine TNF in a dose-dependent manner in vitro (p<0.01 and p<0.001, respectively). **Conclusion:** Treatment with AZ6983 decreases the progression of atherosclerosis in ApoE-/- mice, and additionally inhibits pro-inflammatory cytokine production in human whole blood, thus suggesting that stimulation of the α7nAChR may be a novel anti-inflammatory treatment strategy for atherosclerosis. Funding sources: The Swedish Heart and Lung Association, The Åke Wiberg Foundation and The Wilhelm and Martina Lundgren Foundation.

**4.18**

**THE ANTI-INFLAMMATORY ROLE OF α7NACHR AND α4β2NACHR IN HUMAN BLOOD SAMPLES**

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**Background and Aim:** Autonomic nerve signaling via the alpha 7 nicotinic acetylcholine receptor (α7nAChR) is known to modulate inflammation. We have previously shown that the α7nAChR is expressed by CD68+ macrophages and CD3+ T cells in human atherosclerotic plaques, however, little is known about the functional role of the α7nAChR in human cells. The aim of the current study is to investigate the functional role of the α7nAChR in human cells and to compare the effects of the α7nAChR with other nicotinic receptors expressed by human blood cells.

**Methods:** To evaluate the role of the α7nAChR in patients with atherosclerosis, blood samples from patients and healthy donors were stimulated with lipopolysaccharide (LPS, 10ng/ml) and subsequently treated with nicotine (1000 μmol/L). After 4 h of incubation, samples were centrifuged and supernatant was saved for analysis. Further, to characterize the specific effects of α7nAChR stimulation, human blood samples from healthy donors were incubated with LPS and treated with different concentrations (0.001-1000 μmol/L) of selective agonists for the α7nAChR (GTS-21 or PHA 568487) and α4β2nAChR (RJR-2403 or A85380), as described above. In part of the experiments, blocking with alpha-bungarotoxin, a specific α7nAChR antagonist, was included. From the collected supernatant, TNFα production was measured using ELISA.

**Results:** In both blood samples...
from patients with atherosclerosis and healthy donors, nicotine reduced the TNF-a, IL-6 and IL1-β levels. When further characterizing the α7nAChR-specific effects in whole blood stimulations from healthy donors, both α7nAChR agonists decreased TNF-a cytokine production. After blocking the α7nAChR with alpha-bungarotoxin, only the PHA 568487 agonist lost its ability to decrease the TNF-a levels. Further, both α4β2nAChR agonists were also capable of reducing the TNF-a production in the highest concentration. **Conclusions:** This study shows that stimulation of nicotinic acetylcholine receptors decreases pro-inflammatory TNF-a production, partly via the α7nAChR. However, alpha-bungarotoxin did not abolish the TNF-a response when using GTS-21, raising the possibility of GTS-21 acting on other nicotinic receptors. Interestingly, α4β2nAChR agonists also reduced the TNF-a response. Thus, both α7nAChR and α4β2nAChR may have an immune modulating role. Funding source: Swedish Heart-Lung foundation.

### 4.19 THE ASSOCIATION BETWEEN AUTONOMIC DYSFUNCTION, INFLAMMATION AND PREVALENT CARDIOVASCULAR DISEASE IN SUBJECTS WITH ATHEROSCLEROSIS

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**Background:** Cardiovascular diseases, including atherosclerosis, are associated with autonomic dysfunction. However, the mechanism linking autonomic dysfunction to cardiovascular disease is not known. We investigated the role of inflammation as the link between autonomic dysfunction and presence of atherosclerosis, and how it is associated with prevalent cardiovascular disease, defined as stroke or myocardial infarction. **Methods:** We examined 226 subjects with carotid atherosclerosis, determined by duplex ultrasound imaging. Autonomic function was assessed by measuring heart rate variability (HRV SDNN) and baroreflex sensitivity (BRS Slope), and inflammatory markers were determined (white blood cell count; WBCC, C-reactive protein; CRP). Risk factors including age, gender, smoking, body mass index (BMI), hypertension, lipids and diabetes were assessed. **Results:** Subjects with prevalent CVDs showed a larger carotid plaque area (p<0.01), higher WBCC (p<0.05), and reduced BRS Slope (p<0.01) compared to subjects with subclinical atherosclerosis. Further, BRS Slope was inversely associated with carotid plaque area (p<0.05). In a mediator analysis, adjusting for WBCC, the association between BRS Slope and carotid plaque area was decreased with 29%, indicating that WBCC may link autonomic tone to atherosclerosis. In subsequent analysis, BRS Slope and HRV SDNN were both inversely associated with WBCC (p<0.01), but not with CRP. Further, WBCC was independently associated with carotid plaque area (p<0.05), after adjusting and controlling for confounders including gender, age, BMI, medication and cardiovascular risk factors. **Conclusions:** Reduced BRS is associated with increased carotid plaque area, at least partly mediated via WBCC. This suggests that autonomic dysfunction is associated with the extent of atherosclerosis, possibly mediated via increased systemic inflammation. Funding source: The Swedish Heart-Lung Foundation.
jected in vivo, oxidized LDL specifically induced non-classical monocyte localization to the endothelial wall and was preferentially taken up via the scavenger receptor CD36. By live imaging of blood vessels, we found that nonclassical monocytes from CD36−/− mice showed reduced uptake of oxLDL and failed to patrol. Nonclassical monocytes produced CCL5 in response to atherogenic diet feeding. In summary, nonclassical monocytes are activated early in atherogenesis to patrol the vasculature in response to oxidized LDL signaling and this is dependent on CD36. Thus, targeting this monocyte subset is important therapeutically for reducing the inflammation associated with cardiovascular disease.

4.21
TARGETED UPLC-MS/MS ANALYSIS OF OXYLIPINS: FROM PROFILING TO QUANTIFICATION FOR TRANSLATIONAL RESEARCH STUDIES
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Oxylipins are signaling molecules that play a role in the regulation of many key biological processes, most notably inflammation. Here, we describe targeted, quantitative ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) assays for the analysis of two subsets of oxy lipins. These subsets represent down-stream products from particular precursor lipids and metabolic pathways. Developing separate analytical methods for subsets of analytes is a more specific approach, giving superior results overall when compared to generic, low-fidelity profiling methods that compromise on performance. Matrix samples were prepared using mixed mode OASIS MAX μElution SPE and analyzed using an ACQUITY UPLC I-Class system interfaced to a Xevo TQS Micro tandem quadrupole mass spectrometer. We demonstrate these methods to be sensitive, selective, linear and precise and therefore suitable for use in quantitative translational research studies.

4.22
LY6CHI MONOCYTIC CLEARANCE OF DYING CARDIOMYOCYTES BY CD36 ACTIVATES NR4A1 AND IS REQUIRED FOR TRANSITION TO REPARATIVE INFLAMMATION FOLLOWING MYOCARDIAL INFARCTION
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Rationale. Efficient phagocytosis of dying cells is a prerequisite for inflammation resolution and tissue repair after myocardial infarction (MI). MI triggers the early recruitment of Ly6CHI monocytes, which subsequently require Nr4a1 to differentiate into Ly6CLO macrophages. Separately, Ly6CLO macrophages require MERTK for the clearance of myocardial apoptotic cells. The phagocytic contributions of Ly6CHI monocyte receptors to heart repair, which are first to encounter the necrotic myocardium, are unclear. Objective. To test causal requirements and consequences of Ly6CHI phagocytic receptors during cardiac wound repair and macrophage differentiation post MI. Results. Scavenger receptor CD36 was found to be heightened on Ly6CHI cardiac monocytes post experimental MI. Bone marrow-derived Cd36 was required for both necrotic cardiomyocyte clearance and containment of early infarct size, specifically before the emergence of Ly6CLO cardiac macrophages. Decreased engulfment was directly associated with suppressed monocytic Nr4a1 expression and an increased ratio of Ly6CHI:Ly6CLO myeloid cells. Chromatin immune-precipitation revealed direct Cd36-dependent binding of NR4A1 to MERTK, sequentially linking the two critical phagocytic receptors. Deficiency of both Cd36 and Mertk (i.e., double knockout mice) led to catastrophic defects in phagocytic clearance and an increased incidence of myocardial rupture. Conclusions. Taken together, these data identify a CD36 phagocytosis-initiated Nr4a1-Mertk axis during inflammatory repair of the infarcted heart.

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4.23
INTERACTION OF IL-6 AND TNF-α CONtributes to ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETIC MICE HEART
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Background/Aims: Although inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are known as the important contributors to endo-
thelial dysfunction in obesity and type 2 diabetes (T2D), their interactions established in coronary endothelial function remain unclear. The aim of this study is to elucidate the interactive effect of TNF-α and IL-6 in impairment of the coronary endothelial function in T2D. 

Methods: We used wild type (WT), diabetic db/db, db/db null for TNF (dbTNF−/dbTNF−), and db/db mice treated with neutralizing antibody to IL-6 (anti-IL-6). Endothelium-dependent (acetylcholine, ACh, or flow) vasodilation of isolated and pressurized coronary arterioles was measured by a concentration-dependent manner using ACh and sodium nitroprusside (SNP). Real-time PCR, Western blot, and immunofluorescence staining were utilized for mechanistic studies. Results: Dilation to ACh or flow was blunted in db/db mice and dbTNF−/dbTNF− + IL-6, whereas it was greater in dbTNF−/dbTNF− and db/db mice treated with anti-IL-6 compared with db/db mice. Immunofluorescence staining illustrated co-localization of IL-6 with endothelial cells. Protein and mRNA expression of IL-6 and superoxide (O2−) production were higher in db/db mice but they were reduced by anti-IL-6 treatment. Increased protein and mRNA expression of TNF-α in db/db mice were reduced by anti-IL-6 treatment and genetic depletion of TNF-α in db/db mice reduced the mRNA and protein expression of IL-6 suggesting that TNF-α and IL-6 regulated their expression in parallel and interacted with each other. Superoxide dismutase 2 (SOD2) and phosphorylated eNOS (p-eNOS) were lower in db/db mice coronary arterioles and they were restored in db/db + Anti-IL-6 and dbTNF−/dbTNF− mice. Conclusion: Our findings suggest that the endothelial dysfunction of coronary circulation in T2D mice results from the interactive effects of TNF-α and IL-6 through enhanced oxidative stress and reduced phosphorylation of eNOS.

4.24 INFILTRATING MACROPHAGES PROMOTE ADVERSE CARDIAC REMODELING DURING ACUTE AND CHRONIC PERSUE-OVERLOAD

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Recent evidence indicates that tissue-resident and infiltrating macrophages (Mφ) have different reparative and pro-inflammatory capacities in the heart. During the early stages of cardiac pressure overload (PO), cardiac Mφ may serve as important drivers of both subsequent left ventricular (LV) hypertrophy and the activation of T-cells that contribute importantly to the transition to heart failure. We hypothesized that cardiac Mφ are expanded during the early stages of PO, primarily due to an increase in infiltrating Mφ, and contribute to long-term T-cell activation in the chronically pressure-overloaded heart. Cardiac Mφ were characterized by flow cytometry as MerTK+CD64+F480+MHCII+ cells, with infiltrating Mφ populations identified as expressing C-C chemokine receptor 2 (CCR2). One week after the imposition of PO by transverse aortic constriction (TAC), C57Bl/6 mice exhibited significant (p < 0.05) increases in cardiac macrophages (3.15 ± 0.64 vs 1.09 ± 0.24%), specifically in the infiltrating CCR2+ population (2.8 ± 0.6 vs 0.9 ± 0.2%). TAC mice also exhibited higher circulating levels of Gr1+Ly6C+ monocytes (2.6 ± 0.5 vs 1.3 ± 0.2%) that are recruited to tissue via CCR2. During acute PO, treatment with a CCR2 antagonist in TAC mice significantly blunted LV hypertrophy when compared to vehicle-treated TAC animals (heart weight/tibia length 8.9 ± 0.3 vs 10.53 ± 0.3 mg/mm; LV Wall thickness 0.70 ± 0.01 vs 0.77 ± 0.02 mm) and suppressed cardiac infiltration of macrophages. To determine activation of the adaptive immune response during acute PO, we analyzed the T-cell population in mediastinal heart draining lymph nodes (MLNs). Expansion of CD3+ T-cells in MLNs was blunted in CCR2 antagonist-treated TAC mice when compared to vehicle controls (1.3 x 10^5 vs 2.6 x10^5 cells/MLN). To determine the effects of acute CCR2 blockade on chronic LV remodeling, we treated mice with a CCR2 antagonist for 3 days during the first week of PO, and assessed cardiac function 4 weeks post-TAC. Chronic TAC mice treated with the antagonist had significantly improved ejection fraction when compared to vehicle-treated TAC mice (42.0 ± 2.5 vs 33.5 ± 2.9%). We conclude that infiltrating CCR2+ Mφ are major contributors to PO-induced adverse cardiac remodeling and help drive systemic T-cell activation. Moreover, these results suggest targeting infiltrating Mφ during PO hypertrophy may have therapeutic potential for delaying or preventing the transition to heart failure.

4.25 THE ALARMIN COMPLEX S100A8/9 AS A BIOIMAGING AND THERAPEUTIC TARGET AFTER MYOCARDIAL INFARCTION

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Objective: Myocardial infarction (MI) is an ischemic injury of the myocardium. Excessive myocardial inflammation after infarction increases the risk of recurrent coro-
nary events and heart failure. One of the biological mediators involved in post-MI inflammatory processes, the alarmin complex S100A8/A9, is potently upregulated after MI in the heart tissue mainly due to a strong influx of neutrophils an subsequently monocytes. In turn, S100A8/A9 amplifies local inflammation by supporting further myeloid cell recruitment. We hypothesize that in-vivo visualization of this protein complex could provide important information on MI severity. Additionally, we aimed to test whether S100A8/A9 inhibition with the specific blocker ABR-238901 can reduce inflammation and lead to improved physiological performance of the heart. **Methods and Results:** We used coronary artery ligation to induce MI in C57Bl/6 WT mice. Using PET/CT and radio-labelled Cu64 anti-S100A9 Ab, the complex was visualized and strongly upregulated after MI compared to the sham operated animals. The S100A8/A9 positive signal was present in the walls of the left ventricle, reflecting the infarcted areas and the size of the respective injury. The PET/CT signal was still detectable in ex-vivo PET/CT imaged hearts, collected 8 days after MI. In a parallel experiment mice undergoing permanent coronary artery ligation were treated with 30mg/Kg ABR-238901 administered i.p. daily, and the performance of the heart was monitored using ultrasound. Two days after MI, ABR-238901-treated mice showed significantly higher ejection fraction (EF=55%) in a comparison with animals treated with buffer (EF=43%). Moreover, EF was higher both in the AMI and in the sham groups treated with ABR, suggesting beneficial effects of the compound. **Conclusion:** *In-vivo* PET/CT bioimaging of S100A8/A9 after MI provides useful information about the size and the location of the injury. Our data indicates that S100A8/A9 blockage using ABR-238901 has beneficial effects on reducing myocardial damage, reflected by improved ejection fraction in the immediate period after an MI.

4.26 **OXIDATIVE AND NITROSATIVE STRESS IN VALVE TISSUE OF PATIENTS WITH AORTIC VALVE DISEASE**

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**Background:** Calcific aortic valve disease is becoming the most frequent heart illness in aged people of developed countries. Although several lines of evidence suggest that the mechanism of aortic valve degeneration is similar to that of atherosclerosis, the true mechanism remains undefined. Here we have investigated the levels of oxidative and nitrosative stress in aortic valve tissue of patient with aortic valve disease. **Methods:** The explanted aortic valve leaflets of 56 consecutive patients aged between 18-90 years, with a LV ejection fraction>30%, with normal sinus rhythm and without coronary artery disease were taken at the time of surgery for aortic valve replacement. Those suffering from renal or liver disease were excluded. The leaflets were put in physiological serum and kept at 4°C, then the calcium deposits were removed and the remaining tissues were kept at -80°C until analysis. The tissues were homogenated and processed to determine the content of nitric oxide (Nitrated/NitriteColorimetricAssayKit), superoxide anion (by luminescence using coelenterezine) and nitrotyrosine (OxiSelect Nitrotyrosine ELISA Kit). Patients were divided by their valve pathology: (i) calcific aortic valve stenosis (transvalvular gradient≥30mmHg, n=30), (ii) non-calcific aortic valve regurgitation (regurgitation≥grade II, n=14) and (iii) mix aortic valve disease (transvalvular≥30mmHg and regurgitation≥grade II, n=12). Characteristic demographics and cardiovascular risk factors were recorded and the results expressed as median [IQR]. **Results:** Tissue valve contents of superoxide anion were significantly elevated by the increase with age (p<0.05). The valve tissues from patients with aortic valve stenosis and mixed disease (calcific valves) had also greater content in nitrotyrosine than in patients with pure aortic valve regurgitation (non calcific valves) (67.96[52.25-86.94] and 89.18[45.86-110.88] vs. 42.96[22.05-63.79], respectively; p<0.05). The presence of cardiovascular risk factors did not result in significant changes. **Conclusion:** Oxidative and nitrosative stress is an important component of calcific aortic valve disease and may play an important role in the degeneration of the valve, particularly in elderly people. The results support the use of antioxidants as a useful therapeutic strategy to stop or reduce the development of calcific aortic valve disease. **Support:** VHIR, Hospital Vall d’HebrónBarcelona, Proposal N°: 603049 TRANSLINK Project.

4.27 **INHIBITION OF INFLAMMATORY SERINE PROTEASES POTENTIATES B CELL DEVELOPMENT AND FUNCTION IN DIABETIC CARDIOMYOPATHY**

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Objective: To investigate the novel role of serine proteases on B cell development and growth and their reciprocal humoral response which may contribute to pathological cardiac remodeling during the development of diabetic cardiomyopathy (DCM). Methods and Results: 10 weeks old male mice with deletion of dipeptidyl peptidase-I (DPPI), an enzyme involved in maturation of inflammatory serine proteases (ISPs), and wild type (WT) mice were injected with streptozotocin (50 mg/kg for 5 days, intraperitoneally) and studied after 4 and 8 weeks after induction of diabetes mellitus (DM). Mice elevated glucose levels (>300mg/dL) were classified as DM. Induction of diabetes in WT mice resulted in an increase in DPPI expression and activity in hearts compared to shams that correlated with an increase in the activity of several ISPs (cathepsin G (CG), elastase, chymase). DPPI deletion had no effect on hyperglycemic state of the animals. However, DPPI deficiency reduced ISP activity, increased B cell expression and their anti-inflammatory cytokines as well as toll like receptors, attenuated cell apoptosis and improved cardiac function after STZ treatment compared to WT mice. To delineate the mechanisms involved, we found that diabetes increases IL-21 which mediates B cell apoptosis through down-regulation of BC12 and BCL-xL. Interestingly, DPPI deletion significantly decreased IL-21 expression and up-regulated the two anti-apoptotic members of the BC12 family compared to WT-diabetic mice. Conclusions: These findings emphasize the implication of inflammatory serine proteases in B cell development and growth alteration, myocyte loss and adverse cardiac remodeling observed in DCM. These insights might have useful implications on future studies utilizing neutrophil serine protease blockers to treat human DCM.

4.28 INFLAMMATORY SERINE PROTEASE INHIBITION ATTENUATES MYOCYTE APOPTOSIS AND CARDIAC DYSFUNCTION VIA INTERVENTION OF PPAR GAMMA-INDUCED LIPOPOTOXICITY AND INFLAMMATION IN HIGH FAT DIET-INDUCED DIABETIC CARDIOMYOPATHY

Kunal Sikder1, Amrita Sarkar1, Sanket Shukla1, Aimee Abbott1, Domenica Carrier1, Carlos Barbery1, Lifeng Tian2, Richard Pestell2, Christine Pham3, and Khadija Rafiq1

Background: A diet consisting of high percentage of fat induces lipotoxicity, leading to type II diabetes (T2D) and diabetic cardiomyopathy (DCM). Although high fat diet (HFD)-mediated lipotoxicity has been previously associated with hyperglycemia and myocyte apoptosis, the underlying mechanisms leading to myocardial death is not well understood. An emerging concept is that neutrophil inflammatory serine proteases (ISPs) released as a result of lipotoxicity might be linked to the alteration of nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) signaling. Altered PPAR-γ signaling then induces the maladaptive structural and functional cardiac pathology during the development of DCM via its downstream mitochondrial enzymes 3-hydroxy3-methylglutaryl-CoA synthase2 (HMGC2) and pyruvate dehydrogenase kinase 4 (PDK4). This study aimed to investigate the effect of ISP on PPAR-γ activation and its downstream effects on HFD-induced inflammation and apoptosis that occur in DCM. Methods and Results: Hearts from wild type (WT) diabetic mice (16 weeks HFD-fed) showed increased neutrophil infiltration, Cathepsin G activity and myocyte death. Immunoblot analysis and qPCR showed an increase in the expression of PPAR-γ. The latter was associated with an enhancement in mitochondrial lipogenic and ketogenic enzymes, such as HMGC2 and PDK4, indicating an increase in lipid metabolism and potential lipotoxicity. qPCR also revealed that PPAR-γ activation is involved in mTOR/AKT signaling downstream regulation and myocyte apoptosis in HFD-diabetic mice. Interestingly, ISP deletion in vivo using DiPeptidyl Peptidase I (DPPI) KO mice (mice that lacking major ISPs), diminished the expression of PPAR-γ, HMGC2 and PDK4. Additionally, apoptotic markers (Bax, Bcl2, Bad and cleaved Caspase3) were also significantly attenuated in HFD-fed DPPI-KO group in comparison to WT-HFD. Gene expressions of mTOR and AKT were increased in DPPI-KO group compared to WT group fed with HFD. Interestingly DPPI deletion did not affect high levels of lipids in diabetic mice, suggesting that ISPs independently affect myocyte survival. Conclusions: The findings from our study emphasize that DPPI inactivation enhances myocyte survival and improve cardiac remodeling through neutralization of major ISPs that upregulate the PPAR-γ mediated inflammatory cascade. Our data highlight DPPI and PPAR-γ as potential therapeutic targets for the preservation of cardiac structure and function in DCM.
4.29
PORPHYROMONAS GINGIVALIS LIPOPOLYSACCHARIDE ENHANCES THE RUP-TURE OF EXPERIMENTAL CEREBRAL ANEURYSMS BY INDUCING VASCULAR INFLAMMATION
Takeshi Miyamoto1, Keiko T. Kitazato1, Hidetsugu Makaw1, Kenji Yagi1, Kenji Shimada1, Yoshihito Tada1, Masaaki Kori1, Tadashi Yamaguchi1, Tomoya Kinouchi1, Yasuhisa Kanematsu1, Junichiro Satorari1, and Shinji Nagahiro1


Introduction. Subarachnoid hemorrhage (SAH) is a catastrophic event that results in high morbidity and a poor prognosis. To prevent SAH its pathogenesis must be understood. Bacterial infections have been suggested to have a role in the etiology of cardiovascular disease. Recently the evidence that dental infection could be a part of pathophysiology in intracranial aneurysm was reported. We hypothesized that periodontal pathogen enhances the formation and rupture of experimental cerebral aneurysms and studied it with our aneurysmal rat model.

Methods. Ten-week-old female Sprague-Dawley rats were subjected to aneurysmal induction surgery, which consisted of estrogen deficiency, renal hypertension and hemodynamic stress. Two weeks later, they were divided into 2 groups; rats treated with Porphyromonas gingivalis lipopolysaccharide (LPS) or a saline control (VC). The VC- (n=17) or LPS (n=13) rats received intraperitoneal injection weekly. We observed death or abnormal behavior in the course of 90 days after last operation and confirmed ruptured aneurysm. Results. 7 LPS rats (54%) and 6 VC rats (35%) suffered aneurysmal rupture during 90 days. Especially LPS rats (38%) had higher incidence of aneurysmal rupture than VC rats (6%) within 60 days. LPS promoted experimental aneurysmal rupture in our rat model. The administration of LPS increased the plasma level of IL-1β and MMP9 and gene expression of TLR2, pro-inflammatory cytokines and matrix degradation molecules in vascular wall in rat aneurysm model.

Conclusion. Our findings suggest that Porphyromonas gingivalis enhances the rupture of intracranial aneurysms through promotion of inflammatory response.

4.30
LOSS OF LYMPHOCYTE ADAPTOR PROTEIN LNK PROMOTES AORTIC DISSEC-TION
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Objective: Although inflammation likely contributes to aortic dissection and rupture, the role of the lymphocyte adaptor protein LNK in aortic dissection is unknown. Genome-wide association studies revealed that a polymorphism in the gene SH2B3 encoding LNK is associated with several autoimmune and cardiovascular diseases in humans. LNK is an intracellular adaptor protein that negatively regulates cytokine signaling. We studied the effects of LNK deletion on the development of aortic dissection and rupture in angiotensin II infused mice.

Approach and Results: WT and LNK−/− mice were infused with angiotensin II (Ang II; 1000 ng/kg/min) for 14 days in the absence or presence of an initial intraperitoneal injection of saline equal to 10% of the body weight. LNK deficiency predisposes to aortic dissection (50% survival in LNK−/− mice versus 100% in WT mice) and saline injection prior to Ang II infusion, accelerates the rate of aortic dissection or rupture compared to WT mice. Histological studies showed an increase in macrophages and T lymphocytes in the aorta of LNK−/− mice compared to WT mice. Aortic expression of the Th1 markers (T-bet and IFNγ) and MMP9 were increased, while the expression of the procollagens type I and III were reduced in LNK−/− mice. Furthermore, analyses by flow cytometry of the aortic draining lymph nodes reveals that, in the absence of LNK, the early phase of the disease (3 days after Ang II infusion) is characterized by an increase in the number of IFNγ producing CD4+ and CD8+ T cells compared to WT mice. In addition, when naïve splenic CD4+ T cells isolated from LNK−/− mice are cultured for 72 hours in the presence of Th1 polarizing cytokines, they express more T-bet and produce more IFNγ compared to naïve CD4+ T cells isolated from WT mice. In contrast, Th17 polarization is not affected by loss of LNK.

Conclusion: Deletion of LNK enhances Th1 polarization and promotes adverse aortic remodeling leading to the development of aortic dissection and rupture.
Diabetic cardiomyopathy (DCM) is characterized by ventricular remodeling and dysfunction independent of atherosclerosis, coronary heart disease, or hypertension. DCM is characterized by intramyocardial inflammation, cardiomyocytes apoptosis and cardiac fibrosis. The molecular mechanism that links inflammation to DCM is incompletely understood. An emerging concept is that inflammation might be linked to alteration in CXC chemokine receptor (CXCR) 4 signaling, thus leading to the maladaptive cardiac structural and functional consequences of DCM. Therefore we hypothesized that increased inflammatory proteases may contribute to CXCR4 downregulation that lead to the pathophysiological changes observed in the heart during the development of diabetic cardiomyopathy. **Methods and Results:** 10 weeks old mice with deletion of dipeptidyl peptidase I (DPPI), an enzyme involved in the maturation of major ISPs, and wild type (WT) mice controls were injected with streptozotocin (50 mg/kg for 5 days, intraperitoneally) and studied 4 weeks after induction of diabetes. Induction of diabetes in WT mice resulted in an increase in DPPI expression and activity in hearts compared to shams that correlated with an increase in the activity of several ISPs (cathepsin G (CG), elastase, chymase). DPPI deletion had no effect on hyperglycemic state of the animals. However, DPPI deficiency reduced ISPs activity, and attenuated CXCR4 signaling downregulation, reduced cell apoptosis and improved cardiac function after STZ treatment compared to WT mice. DPPI inhibition also enhanced insulin sensitivity and increased glucose uptake after STZ treatment compared to WT mice, suggesting that ISPs negatively regulate CXCR4 signaling. To delineate the mechanisms involved, we found that treatment of cardiomyocytes with neutrophil-derived protease CG impaired CXCR4 signaling and led to myocyte apoptosis. Incubation of myocytes in hyperglycemic conditions enhanced CXCR4 signaling deterioration and myocyte apoptosis induced by CG. **Conclusion:** These data suggest that ISPs are important regulators of CXCR4 signaling and myocyte death and may play an important role in regulating glucose metabolism during the development of DCM.

**4.32 NEUTROPHIL FUNCTIONAL CHANGES ARE ASSOCIATED WITH METABOLIC ALTERATIONS FOLLOWING TEN WEEKS OF HIGH INTENSITY INTERVAL EXERCISE TRAINING IN MEN AND WOMEN WITH PREDIABETES**

**Objective:** Diabetic cardiomyopathy (DCM) is characterized by ventricular remodeling and dysfunction independent of atherosclerosis, coronary heart disease, or hypertension. DCM is characterized by intramyocardial inflammation, cardiomyocytes apoptosis and cardiac fibrosis. The molecular mechanism that links inflammation to DCM is incompletely understood. An emerging concept is that inflammation might be linked to alteration in CXC chemokine receptor (CXCR) 4 signaling, thus leading to the maladaptive cardiac structural and functional consequences of DCM. Therefore we hypothesized that increased inflammatory proteases may contribute to CXCR4 downregulation that lead to the pathophysiological changes observed in the heart during the development of diabetic cardiomyopathy. **Methods and Results:** 10 weeks old mice with deletion of dipeptidyl peptidase I (DPPI), an enzyme involved in the maturation of major ISPs, and wild type (WT) mice controls were injected with streptozotocin (50 mg/kg for 5 days, intraperitoneally) and studied 4 weeks after induction of diabetes. Induction of diabetes in WT mice resulted in an increase in DPPI expression and activity in hearts compared to shams that correlated with an increase in the activity of several ISPs (cathepsin G (CG), elastase, chymase). DPPI deletion had no effect on hyperglycemic state of the animals. However, DPPI deficiency reduced ISPs activity, and attenuated CXCR4 signaling downregulation, reduced cell apoptosis and improved cardiac function after STZ treatment compared to WT mice. DPPI inhibition also enhanced insulin sensitivity and increased glucose uptake after STZ treatment compared to WT mice, suggesting that ISPs negatively regulate CXCR4 signaling. To delineate the mechanisms involved, we found that treatment of cardiomyocytes with neutrophil-derived protease CG impaired CXCR4 signaling and led to myocyte apoptosis. Incubation of myocytes in hyperglycemic conditions enhanced CXCR4 signaling deterioration and myocyte apoptosis induced by CG. **Conclusion:** These data suggest that ISPs are important regulators of CXCR4 signaling and myocyte death and may play an important role in regulating glucose metabolism during the development of DCM.

**Conclusion:** Ten weeks of interval training in older individuals at risk for diabetes improved aerobic fitness with no change in body composition. Improved neutrophil functions were associated with mitochondrial improvements on a per cell basis. Ten weeks of interval training in persons with prediabetes alters neutrophil metabolism which is associated with improved primary functions, potentially reducing the risk of infection and inflammatory insult which can enhance diabetes risk. This work was funded by an EU Marie Curie Outgoing Fellowship Grant (PIOF-GA-2013-629981).
Inflammation and dietary salt intake have been implicated in the pathogenesis of hypertension. We have previously shown that NADPH oxidase-dependent formation of immunogenic isoketal-protein adducts in dendritic cells (DCs) contributes to the development of experimental hypertension. Recently, it has been shown that salt can accumulate in the interstitial space and promote inflammation. In the current study, we tested the hypothesis that exposure to high salt activates DCs, leading to an NADPH oxidase-dependent production of immunogenic isoketals and the promotion of hypertension. To test this hypothesis, mouse splenic DCs were cultured in media with either normal salt (NS, 150 mM NaCl) or high salt (HS, 190 mM NaCl) for 24 hours. Exposure to HS caused a 2-fold increase in superoxide production in DCs compared to NS. This was NADPH oxidase-dependent since incubation with the gp91ds-tat peptide prevented the increase. Western blots revealed that all NADPH oxidase subunits (p47phox, p22phox, gp91phox and p67phox) were increased by exposure of cells to HS. Moreover, phosphorylation of the regulatory subunit, p47phox and assembly of NADPH oxidase were increased by HS. Exposure to HS also led to an increase in the activation markers CD80 and CD86, and doubled the number of DCs containing isoketal-protein adducts. These effects were prevented by inhibition of the serum-and-glucocorticoid-inducible kinase-1 (SGK1). In additional experiments, mice received adoptive transfer of splenic DCs that were cultured for 24 hours in either NS (n = 6); HS (n = 5); HS plus an SGK1 inhibitor (n = 5) or HS plus an isoketal scavenger (n=6). Mice were implanted with radiotelemeters to measure mean arterial pressure (MAP). Following recovery and two days of baseline, subcutaneous osmotic minipumps were implanted for administration of a generally subpressor dose of angiotensin II (140 ng/kg/min). This caused no increase in blood pressure in mice that received NS DCs, whereas MAP increased significantly (14 ± 4 mmHg, p<0.05) by one week of angiotensinogen II infusion in mice that received salt-activated DCs. The pro-hypertensive effect of salt on DCs was completely blocked by inhibition of SGK1 and scavenging of isoketals during salt exposure. These data indicate that DCs can be activated by exposure to a HS environment which can exist in vivo, and that this likely involves increased NADPH oxidase-mediated superoxide production and formation of isoketal-protein adducts. Moreover, high salt exposure can cause DCs to become pro-hypertensive. These studies define a new pathway linking salt to immune activation and identify a previously undefined role of SGK1 in this process.

5.0: BASIC ASPECTS OF T CELLS

5.3 T CELL TRAFFICKING AND MEMORY

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As cells of the adaptive immune system, T cells are characterized by exquisite antigen specificity. Naïve CD4 and CD8 T cells primarily circulate between blood, lymphatics and secondary lymphoid organs. In contrast, activated T cells acquire multiple properties including enhanced effector functions (e.g. cytokine production, cytolytic function) and altered trafficking patterns, to enter into non-lymphoid tissues where they are both influenced by the local microenvironment and further shape inflammation and immunity. Notably, a subset of activated T cells further achieves long-term survival to become memory T cells, which have altered sensitivity to stimulation and the capacity to respond to inflammatory cytokines in a bystander manner. In this talk, I will discuss how T cell trafficking and memory T cell responses can profoundly alter the local composition of lymphocytes in a tissue, in turn influencing the baseline responsiveness of these tissues to subsequent inflammatory cues and tissue damage. (Support: AHA #13SDG14510023 and CCFA #311295).

once the pathogen has been contained, necessitate tight regulation of antigen specific T cell functions. This type of control is accomplished, at least in part, via the activation and expansion of T regulatory cells (Tregs). The purpose of this overview is to review Tregs in terms of development (including naïve versus memory Tregs), function, subset characterization, and distribution. We will characterize their contributions to homeostatic control and maintenance of self-tolerance, and we will also explore the capacity of some Treg, or their absence, to contribute to pathologies ranging from cancer to autoimmune disease, respectively. We will review existing information on the phenotype, function, cytokines, and growth factors involved in Treg activation and function, discussing the best-characterized of the Treg sub-populations, including natural Tregs (nTreg) and inducible costimulator (ICOS)(+) Tregs, inducible/adaptive Tregs (iTreg), interleukin (IL)-10-producing type 1 Tregs (Tr1 cells), CD8(+) Tregs, and IL-17-producing Tregs. These cells share some common features, including expression of the transcription factor Foxp3 (except for Tr1 cells), and secretion of inhibitory cytokines, including IL-10 and/or TGF-β. As to their distribution, Foxp3(+)CD4(+) regulatory T cells circulate throughout lymphoid organs and appear to have counterparts that also function to suppress immune responses in parenchymal tissues. In this overview, we will explore proposed basic and molecular mechanisms by which the various Treg subsets exert their immune suppressive activities and we will discuss the implications for both the beneficial and deleterious consequences of their activation and expansion. References: Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Horis S, Jiang S, Kuchroo VK, Abbas AK, Bluestone JA, Campbell DJ, Ghosh S, Horis S, Jiang S, Kuchroo VK, Mathis D, Roncarolo MG, Rudensky A, Sakaguchi S, Shevach EM, Vignali, DAA*, Ziegler SF. Regulatory T cells: Recommendations to simplify the nomenclature. Nature Immunology. 2013 14 (4):307-8. Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, Qin FX, Gilliet M, Liu YJ: Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. Immunity. 2008, 28 (6): 870-880. 10.1016/j.immuni.2008.03.018. Noble A, Giorgini A, Leggat JA: Cytokine-induced IL-10-secreting CD8 T cells represent a phenotypically distinct suppressor T-cell lineage. Blood. 2006, 107 (11): 4475-4483. 10.1182/blood-2005-10-3994. Voo KS, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Litman DR, Liu YJ: Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A. 2009, 106 (12): 4793-4798. 10.1073/pnas.0900408106. Wu K, Bi Y, Sun K, Wang C: IL-10-producing type 1 regulatory T cells and allergy. Cell Mol Immunol. 2007, 4 (4): 269-275.

6.0: BASIC ASPECTS OF B CELLS

6.1 B CELL SUBSETS AND IMMUNE RESPONSES

Amy Major


B cells are lymphocytes that function in adaptive humoral immunity primarily by producing antigen-specific antibody. Throughout the years, the diversity of B cell function and phenotype has grown and it is now known that, in addition to antibody production, B cells can present antigen to T cells, secrete cytokines and down regulate inflammation. Under normal circumstances, B cells protect against bacterial and viral infections by producing antibodies that inhibit infection or clear pathogens. Some of these responses are dependent on T cell help (T-dependent) whereas responses to polysaccharides or other pathogen components are T-independent. In general, the function and phenotype of B cells is associated with where they reside in secondary lymphoid tissues and by their environmental cues. In patients with atherosclerosis, antibody titers against oxidized-forms of LDL (oxLDL) have been associated with increased plaque burden. In animal models atherosclerosis, B cells have been demonstrated to be protective and harmful. Results of these studies appear to be dependent on experimental system, and specific type of B cell examined. Here, we offer an overview of basic aspects of B cell subsets and responses in health and disease, briefly discussing their relevance to cardiovascular disease.

6.2 GENETIC RISK FACTORS AND LOSS OF B CELL ANERGY IN DEVELOPMENT OF AUTOIMMUNITY

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Stochastic use of germline variable region genes inevitably leads to generation of B cells that are reactive with self-antigens and must be censored to prevent autoimmunity. Studies using primarily reductionist immunoglobulin transgenic models have defined mechanisms of autoreactive B cell silencing in the mouse but little is known regarding mechanisms operative in autoreactive B cell silencing in the human. Autoreactive B cells have been found in multiple phenotypically distinct B cell compart-
ments in human peripheral blood but the functional status of these cells is largely unknown. To examine and compare the \textit{in vivo} lifestyles of autoreactive and naïve antigen-specific human B cells we devised nanoparticle adsorbent-based approaches to identify and isolate antigen reactive B cells, and study their status. This presentation will focus on results of analysis of cells reactive with insulin (autoantigen) and tetanus toxoid (exogenous antigen). Although it is well accepted that T cells function as the pancreatic beta cell executioners in Type 1 Diabetes (T1D), it is unclear what events precede and precipitate pancreatic beta cell executioners in Type 1 Diabetes (T1D), it is unclear what events precede and precipitate pancreatic beta cell executioners in Type 1 Diabetes (T1D), and how autoreactive B cells also contribute to this disease process, independent of autoantibody (AAb) production, and how cardiac AAb can serve as biomarkers of heart disease. Starting with the unexpected discovery that humanized DQ8+NOD mice – a model of type 1 diabetes (T1D) – develop premature death due to spontaneous myocarditis, we found that the earliest antigenic target of IgG AAb responses, α-myosin heavy chain (α-MyHC), was also the major target of pathogenic CD4 T cells. We further observed that the spreading of humoral immune responses - from α- to β-MyHC, and then to other cardiac antigens - paralleled disease progression from myocarditis to dilated cardiomyopathy. With this knowledge, and modeling on the success of “biochemical” islet AAb assays for detecting individuals at high risk for developing T1D, we developed a panel of similarly designed radiobinding assays to measure cardiac AAb in humans. We then showed that patients with acute myocarditis tested positive for multiple cardiac AAb, especially α- and β-MyHC, similar to DQ8+NOD myocarditis mice. In another body of studies, we discovered experimental and clinical evidence for a chronic post-myocardial infarction autoimmune syndrome in T1D and furthermore, that cardiac AAb profiling can differentiate between ischemic heart disease in T1D and T2D. We hypothesize that unrecognized autoimmune heart disease contributes to the poor CVD outcomes in T1D and that cardiac AAb profiling might eventually be used to identify the patients most likely to benefit from antigen-specific therapeutic approaches. \textbf{Support: NIH R01DK103609. References:} Lv H et al.: Impaired thymic tolerance to α-myosin directs autoimmune to the heart in mice and humans. \textit{The Journal of Clinical Investigation}, 2011; 121:1561–1573. doi:10.1172/JCI44583. Gottumukkala R et al.: Myocardial infarction triggers chronic cardiac autoimmunity in type 1 diabetes. \textit{Science Translational Medicine}, 2012; 4:138ra80. doi:10.1126/scitranslmed.3003551.

\textbf{6.3}  
\textbf{B CELLS AND CARDIOVASCULAR DISEASE}  
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The role of B cells, and antibodies in particular, in the pathogenesis of CVD is perhaps most widely recognized in rheumatic carditis, in which antibodies against streptococci cross-react with myocardial proteins, triggering inflammatory heart disease. However, the pathogenic role of antibodies in other forms of inflammatory cardiomyopathy is much less clear, with emerging data pointing to CD4 T cells as the critical mediators of myocarditis in both animal models and humans. Here, we will discuss how B cells also contribute to this disease process, independent of autoantibody (AAb) production, and how cardiac AAb can serve as biomarkers of heart disease. Starting with the unexpected discovery that humanized DQ8+NOD mice – a model of type 1 diabetes (T1D) – develop premature death due to spontaneous myocarditis, we found that the earliest antigenic target of IgG AAb responses, α-myosin heavy chain (α-MyHC), was also the major target of pathogenic CD4 T cells. We further observed that the spreading of humoral immune responses - from α- to β-MyHC, and then to other cardiac antigens - paralleled disease progression from myocarditis to dilated cardiomyopathy. With this knowledge, and modeling on the success of “biochemical” islet AAb assays for detecting individuals at high risk for developing T1D, we developed a panel of similarly designed radiobinding assays to measure cardiac AAb in humans. We then showed that patients with acute myocarditis tested positive for multiple cardiac AAb, especially α- and β-MyHC, similar to DQ8+NOD myocarditis mice. In another body of studies, we discovered experimental and clinical evidence for a chronic post-myocardial infarction autoimmune syndrome in T1D and furthermore, that cardiac AAb profiling can differentiate between ischemic heart disease in T1D and T2D. We hypothesize that unrecognized autoimmune heart disease contributes to the poor CVD outcomes in T1D and that cardiac AAb profiling might eventually be used to identify the patients most likely to benefit from antigen-specific therapeutic approaches. \textbf{Support: NIH R01DK103609. References:} Lv H et al.: Impaired thymic tolerance to α-myosin directs autoimmune to the heart in mice and humans. \textit{The Journal of Clinical Investigation}, 2011; 121:1561–1573. doi:10.1172/JCI44583. Gottumukkala R et al.: Myocardial infarction triggers chronic cardiac autoimmunity in type 1 diabetes. \textit{Science Translational Medicine}, 2012; 4:138ra80. doi:10.1126/scitranslmed.3003551.

\textbf{7.0:} \textbf{BASIC ASPECTS OF VASCULAR CELLS WITH IMMUNE FUNCTION}  

\textbf{7.1} \textbf{CD4 T CELLS IN ATHEROSCLEROSIS}  
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Autoreactive CD4+ T cells drive atherosclerosis. They respond to auto-antigens including ApoB-100, the main protein in low-density lipoprotein (LDL), and accumulate...
in atherosclerotic lesions. However, atherosclerosis-specific CD4⁺ T cells have not been identified in vivo. In particular, their origin and differentiation in the plaque is unknown. Using a new multimer of the MHC-II-restricted ApoB-100 auto-epitope P6 (P6:1-A*) that enabled detection of antigen-specific CD4⁺ T cells, we identified a novel population of auto-reactive CD4⁺ T cells in healthy, young C57BL/6 mice that is predominately differentiated into athero-protective T-regulatory cells (Treg). This repertoire of T cells is detectable in the thymus at birth and expands within 28 days in peripheral lymph nodes. In atherosclerotic Apoe⁻/⁻ mice, P6:1-A*⁺ CD4⁺ T cells are initially expanded, but their numbers decline during the natural course of disease. The fraction of these ApoB-100-specific T cells in peripheral lymph nodes correlates negatively to the extent of advanced atherosclerotic lesions, suggesting that these FoxP3⁺ auto-reactive T cells are protective. In contrast, we observed a distinct phenotype of T cells residing in the atherosclerotic plaque. In Apoe⁻/⁻ mice with mature atherosclerotic lesions after 5 months of high fat diet, most aortic T cells express a unique combination of transcription factors (FoxP3⁺ T-bet⁺). These cells are exclusively found in the aorta and para-aortic lymph nodes. In contrast to classical Treg in peripheral lymphoid tissue they do not suppress T-cell proliferation in vitro and are less potent in inhibiting cytokine secretion. Transcriptomic analysis shows that this subset of aortic T cells is closer to effector T cells than to Treg. They secrete interferon-γ, interleukin-10, and tumor necrosis factor and increase atherosclerosis after an adoptive transfer. These findings indicate that atherosclerosis-specific CD4⁺ T cells are protective FoxP3⁺ Treg before initiation of disease in lymphoid tissue, but acquire a pro-atherogenic phenotype in the aorta in later stages of disease. Indeed, in a Treg lineage tracing mouse, Treg specifically recognizing the ApoB-100 epitope P6 were more likely to lose FoxP3. In the plaque, many T cells can be identified as such previous Treg, suggesting that atherosclerosis-specific CD4⁺ T cells that homed to the aorta lost their regulatory capacity and instead further exacerbate atherosclerosis. Funding source: This work was supported by National Institutes of Health grants R01 HL115232 and HL121697 to K. Ley and from the Deutsche Forschungsgemeinschaft to D. Wolf.

7.2 IL-1β PROMOTES ATERO-PROTECTIVE CHANGES IN LATE STAGE ATEROSCLEROTIC LESIONS

D. Gomez¹, R. Baylis², A. Newman¹, B. Durgin¹, G. F. Alencar¹, S. Francis², E. Pintaux³, H. Gran⁴, P. Ridker⁵, M. Nahrendorf⁶, F. Swirski⁷, P. Libby⁸, and G. Owens⁹

¹Cardiovascular Res. Ctr., Univ. of Virginia Sch. of Med., 415 Lane Rd., P.O. Box 801394, Charlottesville, VA, 22908, ²Cardiovascular Res. Unit., Univ. of Sheffield, Sch. of Med. & Biomed. Sci., Sheffield, UK, ³Fac. of Life Sci, Univ. of Manchester, Manchester, UK, ⁴Immunology, Molecular Biology, Physiology, Novartis Inst. for Biomed. Res., 250 Massachusetts Ave., Cambridge, MA, 02139, ⁵Harvard Med. Sch., Brigham & Women's Hosp., 75 Francis St., Boston, MA, 02115, ⁶Ctr. for Systems Biology, Massachusetts Genl. Hosp., 185 Cambridge St., Boston, MA, 02114, ⁷Immunology, Massachusetts Genl. Hosp., 185 Cambridge St., Boston, MA, 02114. Despite decades of research, little is known regarding mechanisms and factors that control the pathogenesis of late stage atherosclerotic lesions and in particular the probability of plaque rupture that may lead to possible myocardial infarction or stroke. The widely accepted dogma is that plaques having a thin fibrous cap and a paucity of Acta2+ smooth muscle cells (SMC) relative to CD68+ macrophages have an increased risk of rupture. However, our recent rigorous lineage tracing studies have provided compelling evidence that SMC play a much more important role than has generally been appreciated with >80% of SMC derived cells failing to be detected using conventional SMC marker genes, and >30% of these expressing markers of macrophages or mesenchymal stem cells (Shankman et al. NME 2015). Of even greater significance, we have shown the SMC specific conditional knockout of the pluripotency genes Klf4 or Oct4 (Cherepanova et al. NME 2016) results in dramatic changes in lesion pathogenesis. However, contrary to dogma, we showed that they can play either a beneficial or detrimental role depending on the nature of their phenotypic transitions. Thus, there is a critical need to identify factors and mechanisms that promote beneficial changes in SMC phenotype including the ability to invest into the fibrous cap, to produce extracellular matrix (ECM). The pro-inflammatory Interleukin-1β (IL-1β) is believed to be a major driver of atherosclerotic plaque progression and neutralization of this key regulator of systemic inflammation is tested in an ongoing large-scale clinical trial (CANTOS). However, previous preclinical studies investigating the impact of genetic or pharmacological inhibition of the IL-1β pathway have provided ambiguous evidence of a beneficial effect of IL-1β neutralization. In this talk, I will present results of our recent studies in which we show that treatment of our SMC lineage tracing mice with the Novartis IL1β-neutralizing antibody after the establishment of advanced atherosclerosis (18 weeks of Western Diet feeding) resulted in multiple changes consistent with reduced plaque stability including marked reductions in the number of SMC-derived eYFP⁺ cells.
within the fibrous cap, enrichment in macrophages, as well as an inhibition of the beneficial outward remodeling of the brachiocephalic artery. Studies provide evidence that IL1β plays an unexpected protective role in maintenance of plaque stability in late stage atherosclerosis. Supported by NIH Grants R01 HL121008, HL087867, and HL057353 to GKO.

8.0: CV DISEASE IN INFLAMMATORY AND AUTOIMMUNE DISEASE

8.1 UTILIZING A HUMAN MODEL OF DISEASE TO STUDY INFLAMMATORY ATHEROGENESIS

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A growing body of evidence suggests that inflammation predisposes to atherosclerosis and cardiovascular events. The mechanisms by which inflammation predisposes to atherogenesis are not fully understood, and remain a topic of intense investigation with two large-scale, ongoing interventional studies (CIRT, CANTOS) testing the hypothesis that reduction in inflammation will reduce future CV events. While these trials will be highly informative to understand if reduction in inflammation by inflammasome or T-cell inhibition translates into clinical outcomes, they will not augment mechanistic understanding of how inflammation promotes atherogenesis. Our transdisciplinary research program at the Intramural Research Program at the National Heart Lung and Blood Institute utilizes a human inflammatory skin disease, psoriasis, to understand pathways involved in modulation of inflammatory cardiometabolic dysfunction. The talk will focus on our deep phenotyping efforts utilizing imaging, cellular phenotyping and lipoprotein characterization in our ongoing cohort study (NCT01778569) which will serve as a growing resource to understand inflammatory atherogenesis.

8.3 AUTOIMMUNITY: A CASUAL FACTOR IN THE PATHOGENESIS OF HYPERTENSION

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Autoimmune diseases including systemic lupus erythematosus (SLE) and rheumatoid arthritis are associated with a marked risk for developing hypertension. In addition, several studies report that essential hypertension is associated with increased circulating levels of autoanti-

bodies suggesting that a loss of immune tolerance and autoimmunity may be an important underlying mechanism in the pathogenesis of hypertension. Previous work from our laboratory demonstrated a role for renal inflammation, oxidative stress, and impaired renal hemodynamic function in SLE associated hypertension. More recently, we demonstrated that preventing autoimmunity in an experimental mouse model of SLE prevented the development of hypertension. Current work in the laboratory is related to testing the hypothesis that humoral immunity, specifically autoantibodies, mechanistically promote the development of hypertension during SLE. (BX002604-01A2, P20GM104357, P01HL051971).

8.4 DEFECTIVE INFLAMMATION RESOLUTION IN ATHEROSCLEROSIS: MECHANISMS AND THERAPEUTIC OPPORTUNITIES

Ira Tabas
The inflammatory response to acute and reversible infection or tissue damage, mediated by PAMPs and DAMPs, respectively, triggers an essential resolution response that curtails inflammation and restores tissue homeostasis. This resolution response is mediated by a panoply of lipid and protein mediators that activate specific cell surface receptors and signal transduction pathways to trigger resolution endpoints. However, when the inflammatory response is indolent and persistent, the resolution phase is often not engaged, leading to a chronic, low-grade inflammatory response that causes clinically serious tissue damage. We have proposed that the maladaptive inflammatory response in atherosclerosis is, in essence, a classic example of failed resolution. The initial inflammatory response is triggered by the subendothelial retention of apoB-containing lipoproteins, and resolution fails because this trigger is not only persistent but is actually amplified. This scenario leads to a vicious cycle of failed resolution, tissue damage-mediated DAMP formation, and amplified DAMP-mediated inflammation. This talk will review the features of atherosclerosis progression that are consistent with failed resolution; discuss in detail how clearance of apoptotic cells (efferocytosis) is part of the resolution program and how it goes awry in advanced atherosclerosis; reveal new molecular-cellular mechanisms of how certain resolution mediators and apoptotic cells activate resolution signaling pathways in macrophages, a key inflammatory cell type in atherosclerosis; and discuss and show data supporting the contention that inflammation resolution mediator therapy may be an ideal way to pre-
vent atherosclerosis progression in a manner that would not compromise host defense.

9.0: INFLAMMATION AND HYPERTENSION DURING PREGNANCY AND GENDER DIFFERENCES

9.1 ADAPTIVE IMMUNITY AND HYPERTENSION DURING PREGNANCY

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Preeclampsia (PE), new onset hypertension during pregnancy, is associated with a proinflammatory profile characterized by elevated CD4+ T cells, inflammatory cytokines and autoantibodies to the angiotensin II Type I receptor (AT1-AA), compared to women with normal pregnancy (NP). Previous studies from the Zenclussen lab showed that adoptive transfer of Th1-like splenocytes increased MAP, fetal rejection and inflammatory cytokines when transferred to NP mice, but the mechanisms causing the PE symptoms were not further investigated. We hypothesize that CD4+ T cells play an important role to cause much of the pathophysiology associated with PE and illicit a memory response for production of the AT1-AA, thereby contributing to cardiovascular disease later in life among previously PE women.

9.2 INNATE IMMUNITY AND GESTATIONAL HYPERTENSION

Brett M. Mitchell, Piyali Chatterjee, Valorie L. Chiasson, Kelsey R. Bounds, and Catalina A. Lopez


Gestational hypertension (GH), a disease that affects ~10% of all pregnancies, may result from over-activation of the maternal immune system and is characterized by excessive innate immune cell activation, inflammation, and injury at the vascular, renal, and placental level. We and others have demonstrated that activation of the innate immune system as well as the adaptive immune system in female animals is able to produce a pregnancy-dependent phenotype that mimics GH in women. Ligands that activate the pattern recognition receptors on innate immune cells are able to induce GH in animals, and animals lacking these receptors are resistant to the development of GH. Therefore, exploring how the innate immune system is involved in the development of GH and how it signals to the adaptive immune system is important, and how we can specifically prevent this will be discussed in this symposium.

10.0: POSTER SESSION II

10.1 THE IMPACT OF EXERCISE INTENSITY AND CYTOMEGALOVIRUS INFECTION ON IMMUNE CELL REDEPLOYMENT BY EXERCISE

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Exercise results in a well-characterized leukocytosis that is directly related to the intensity of the exercise, with
greater cell mobilizations observed as exercise intensity increases. High differentiated immune cells with effector functions are more exercise responsive. Factors driving the accumulation of the high differentiated cells, including infection with cytomegalovirus (CMV), significantly augment T cell mobilization by exercise. In contrast, CMV does not appear to impact the monocyte response to exercise, despite these cells being targeted by CMV infection. It is not known whether the impact of CMV on the exercise-induced redistribution of monocyte and T cell subsets varies with exercise intensity. Exercise is also reported to alter T cell cytokine expression. The relationship of these changes to exercise intensity has not yet been examined.

**Objectives:** 1) Determine if CMV affects T cell and monocyte redeployment across multiple exercise intensities, and 2) determine cellular expression of type I and type II cytokines at varying exercise intensities.

**Methods:** 17 cyclists completed three 30min cycling trials at -5, +5, and +15% of blood lactate threshold (LT). Monocyte and CD4+ and CD8+ T cell subsets (defined by CD14/CD16 and CD27/CD28 expression) and cytokine-expressing T cells (IL-2, IFN-γ, TNF-α, IL-6, IL-4, IL-10) present in the blood pre-, post-, and 1h post-exercise were characterized by flow cytometry. Effects of CMV and intensity on exercise-induced changes in cell subsets were analyzed with linear mixed models. Research conformed to the Declaration of Helsinki.

**Results:** All exercise intensities increased monocyte and T cell subsets present in the blood post-exercise. CMV+ participants had greater numbers of high differentiated CD4+ and CD8+ T cells compared to CMV- at all time points, independently of exercise intensity. The ingress of T cells into the blood post exercise, and their egress 1h post exercise, increased as exercise intensity increased, independently of CMV. CMV did not impact the monocyte response to exercise at any intensity. Exercise-induced increases in T cell expression of type I cytokines increased with intensity.

**Conclusions:** The mobilization of monocyte and T cell subsets by exercise was directly related to exercise intensity, as was exercise-induced changes in T cell cytokine expression. Although CMV+ mobilized more high differentiated T cells than CMV-, this occurred above and below LT. Therefore the augmenting effect of CMV on T cell mobilization is independent of exercise intensity.

### 10.2 OBESITY AND CIRCULATING INFLAMMATION-RELATED microRNAs

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MicroRNAs (miRs) are short single stranded noncoding RNAs that are involved in the regulation of a number of physiological and pathological processes. miRs down regulate target gene expression post-transcriptionally by degrading messenger RNA and/or by blocking translation. It is now recognized that miRs play a key role in regulating inflammation, vascular health and, in-turn, cardiovascular disease (CVD). For example, altered expression of specific miRs such as, miR-34a and miR-146a, have been linked with heightened vascular inflammation. Obesity is associated with increased inflammatory burden that is thought to contribute to greater CVD risk. The mechanisms underlying obesity-related inflammatory stress are not fully understood. It is currently unknown whether inflammation-related miRs are dysregulated with obesity. Accordingly, the aim of this study was to determine the influence obesity, independent of other risk factors, on circulating expression of miR-34a, miR -126, miR -146a and miR -150. To address this aim 30 sedentary, middle-aged (47-64 years) adults were studied: 15 normal weight (10M/5F; BMI: 22.9±0.5 kg/m2) and 15 obese (10M/5F; 31.4±0.5 kg/m2). All subjects were non-smokers, normotensive and free of overt cardiometabolic disease. Circulating expression of miRs was determined in plasma using standard RT-PCR techniques with miR primers of interest. Expression was normalized to exogenous C. elegans miR-39 and reported as relative expression in arbitrary units (AU). Circulating expression of miR-34a (0.024±0.015 vs 0.01±0.006 AU) was significantly higher (~240%); whereas miR-126 (0.182±0.037 vs 0.315±0.048 AU) and miR-146a (0.031±0.007 vs 0.057±0.010 AU) expression was significantly lower (~40%) in the obese compared with normal weight adults. There was no significant group difference in miR-150 (0.088±0.120 vs 0.133±0.025 AU). Higher expression of miR-34a and lower expression of both miR-126 and miR-146a is consistent with a proinflammatory phenotype. In summary, these data suggest that obesity, independent of other cardiometabolic risk factors, adversely influences key inflammation-related miRs. Dysregulation of miRs may contribute mechanistically to the heightened inflammatory state associated with obesity.
INFLAMMATION, IMMUNITY, AND CARDIOVASCULAR DISEASE
ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

MATION IN AGED MALE AND FEMALE MICE
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The metabolic syndrome is defined by the presence of multiple risk factors for diabetes, heart disease and stroke. Younger and middle aged women are more likely to have increased triglycerides, low HDL cholesterol, an epidemiology that shifts after the age of 65, when risk factors become similar between the sexes. Stroke incidence is disproportionately climbing in middle aged women, and rates increase after menopause. Elderly women are more likely to die after a stroke compared to men, and have worse functional recovery. In pre-clinical studies, young female mice have less histological injury after stroke compared to males (27% vs 43%), while in middle aged and aged cohorts this scenario was reversed, and greater damage was seen in females (43% vs 23%) [1]. The underlying mechanism for the “switch” to an ischemia damage was seen in females (43% vs 23%) [1]. The un-baseline differences in middle-aged mice that involve features of the metabolic syndrome. The current study characterized multiple factors involved in the metabolic syndrome in aged male and female mice including assessment of obesity, lipids and glucose. We also evaluated sex differences in white adipose tissue inflammation by flow cytometry. Body weights as well as abdominal white adipose tissue, liver and spleen weights were compared in 14-15 month old C57BL/6 mice (n=10 per group). Glucose and insulin tolerance tests were performed after 6 h fasting. Glucose, triglycerides and high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were determined in plasma from fasted animals. Aged males had higher body weights than females (49±1g vs 35±1g), as well as higher liver (2.45±0.3g vs 1.43±0.07g) and abdominal white adipose tissue weights (2.19±0.15g vs 1.43±0.07), while no difference were seen in spleen weights. Plasma glucose concentrations were not significantly different in males and females (132±8 mg/dL vs 137±4 mg/dL). Male mice were less sensitive to insulin compared to females but only marginal differences in glucose sensitivity were seen. Lipid profiling showed a trend to higher triglycerides in females (1.28±0.19 mM vs 1.79±0.42), no difference in LDL cholesterol while lower concentrations of HDL were seen in females (0.84±0.07 µg/µl vs 1.35±0.06 µg/µl). Flow cytometry on adipocyte-derived immune cells indicate that aged males and females have different subsets of T lymphocytes. Sex differences in adipose tissue may proportionally affect females and contribute to an enhanced pro-inflammatory milieu in aging females. References: 1. Manwani, B., et al. Exp Neurol, 2013. 249: p. 120-31.

10.4 EFFECTS OF AN 8-WEEK PALEO DIETARY INTERVENTION ON INFLAMMATORY CYTOKINES
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Introduction: The Paleolithic (Paleo) diet is promoted as a potential remedy to the supposed Western diet-induced “life-style” diseases of modern times. The purpose of this study was to determine the feasibility of transitioning from a traditional Western diet to a Paleo diet, and measure the effects of this diet on bio-markers of inflammation. Methods: Eight healthy individuals (age: 32.4 ± 12.0 years; body mass: 86.9 ± 34.3 kg; BMI: 29.9 ± 6.1 kg/m²) were recruited for this study. Prior to the 8-week Paleo diet intervention all participants were consuming a typical Western diet. Dietary counseling and Paleo menus were provided to assist in this dietary modification. Pre- and post-intervention testing included resting serum concentrations of IL-4, IL-10, IFN-γ, and TNF-α. Data are reported as percent change from pre-intervention (%Δ). Results: Resting IL-10 increased 35.5% and resting IFN-γ increased 66.7% after the intervention. TNF-α and IL-4 changed minimally. Relationships between pre-intervention IL-4 and IL-10, IFN-γ, and TNF-α were found (p=0.02, R²=0.88; p=0.02, R²=0.92; p≤0.001, R²=0.97, respectively). Additionally, pre-intervention IL-10 and IFN-γ were found to have a relationship with post-intervention TNF-α (p=0.03, R²=0.85; p=0.01, R²=0.95, respectively). Similarly, post-intervention relationships between IL-4 and IL-10, IFN-γ, and TNF-α were found (p=0.03, R²=0.85; p=0.02, R²=0.92; p=0.001, R²=0.97, respectively). Further, post-intervention IL-10 and IFN-γ were found to have a relationship with post-intervention TNF-α (p=0.07, R²=0.78; p=0.01, R²=0.95). Conclusion: The findings confirm that it is feasible to counsel research participants to transition from a typical Western diet to an ad-libitum Paleo diet when provided dietary counseling and a Paleo specific menu but not food. Furthermore, the Paleo diet induced weight loss (previously reported data) despite the ad-libitum intake and dietary counseling centric to individual levels of satiety not weight reduction. As a result of this preliminary feasibility project a research
study with a larger participant pool and longer duration is possible, and further examination of inflammation and the Paleo diet is warranted.

10.5 PERIVASCULAR ADIPOSE TISSUE MACROPHAGES ARE RESPONSIBLE FOR ENDOTHELIAL DYSFUNCTION IN THE OBESE MICROVASCULARIZATION
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The function of perivascular adipose tissue (PVAT) as an anti-contractile mediator is lost during obesity. Obesity results in inflammation of the PVAT that is paralleled by decreased levels of the vasorelaxant signaling molecule hydrogen sulfide (H2S). The current objective was to assess the role of proinflammatory macrophages in determining vascular [H2S] and defining how this impinged on vasodilatation. Mesenteric resistance arterioles isolated from 20 week-old lean and obese mice were loaded with the fluorescent H2S indicator SF7-AM, mounted in a pressure myography chamber, pressurized and imaged confocally to assess [H2S] in the smooth muscle and endothelial layers. Steady-state [H2S] was lower in both the smooth muscle and endothelium of obese compared with lean mouse vessels which correlated with impaired endothelial-dependent vasodilatation. To assess the role of macrophages in driving these phenotypes, vessels from lean and obese mice were cocultured overnight in the presence of macrophages purified by immunomagnetic separation, or macrophage-conditioned media, from either lean or obese mice. In vessels from lean mice, [H2S] in both smooth muscle and endothelium was decreased by exposure to macrophages from obese but not lean mice, which was observed in parallel with impaired vasodilatation. These effects were mediated by low molecular weight species (<3.5 kDa) and dependent on macrophage inducible nitric oxide synthase (iNOS) activity. Collectively, these data support a model in which iNOS activity of PVAT-resident proinflammatory macrophages promotes microvascular endothelial dysfunction by reducing the bioavailability of H2S, and suggest that vascular H2S depletion underpins the loss of PVAT anti-contractile function in obesity.

10.6 CULLIN3 REGULATES ENDOTHELIAL FUNCTION BY MODULATING eNOS ACTIVITY
Jing Wu1, Ko-Ting Lu1, Larry Agbor1, Xuebo Liu1, Masashi Mukohda1, Anand Nair1, and Curt Sigmund2
1Pharmacology, Univ. of Iowa, 51 Newton Rd., 2-340 BSB, Iowa City, IA, 52242, 2Pharmacology, Univ. of Iowa, 51 Newton Rd., 2-471B-1 BSB., Iowa City, IA, 52242, 3Univ. of Iowa Ctr. for Hypertension Res., 51 Newton Rd., 2-471B-1 BSB, Iowa City, IA, 52242. Pseudohypoaldosteronism type II (PHAII) patients expressing dominant negative cul3 mutations exhibit increased renal NaCl reabsorption and develop hyperkalaemia, metabolic acidosis and hypertension. It is unclear whether loss of cul3 function in extra-renal tissues contributes to the hypertensive phenotype. In the vasculature, endothelial Nrf2 stability is tightly regulated by cul3-based E3 ubiquitin ligase, which binds Nrf2 via the redox-sensitive adaptor Kelch-like ECH-associated protein 1 (Keap1). In the present study, we found that 24-hour treatment with a pan cullin inhibitor MLN4924 (1 μM) caused a 3-fold increase of Nrf2 protein in mouse lung endothelial cells (MLECs), while tert-butyl hydroperoxide (tBHP, 240 μM) had no effect on Nrf2 level. However, both MLN4924 and tBHP triggered time-dependent accumulation of Nrf2 in the nuclei, which peaked at 40 minutes following treatment. As a result, both treatments induced marked upregulation of antioxidant genes including NAD(P)H quinone oxidoreductase 1, heme oxygenase 1, glutamate cysteine ligase (rate-limiting enzyme in glutathione synthesis), and catalase both in MLECs and primary mouse aortic endothelial cells (MAECs). Of note, MLN4924 and tBHP suppressed the expression of Nox1 and Nox4, but both markedly increased intracellular superoxide as determined by dihydroethidium (DHE) staining in cultured MLECs. This was associated with decreases in intracellular and extracellular nitric oxide (NO) bioavailability with no changes in endothelial nitric oxide synthase (eNOS) messenger RNA. To determine whether this redox imbalance was due to changes in eNOS protein or its activation, western blot analysis was performed. Inhibition of total cul3 activity caused a 25% reduction in total eNOS and a 75% reduction in phosphorylated eNOS, while tBHP caused a 50% reduction in phosphorylated eNOS with no effect on total eNOS, suggesting that decreased eNOS activity contributed to the oxidative stress induced by these agents. To further verify whether cul3 regulates endothelial redox machinery, MAECs isolated from cul3flox/flox mice were treated with adenovirus expressing Cre recombinase for 48 hours to induce deletion of cul3. This lead to an 80% reduction in cul3 protein and significant decreases in total eNOS, phosphorylated eNOS and NO bioavailability, supporting a role of cul3 in regulating eNOS expression and activity. These data imply that deletion or suppression of cul3 in arterial endothelial cells may dampen endothelium-dependent vascular relaxation and contribute to the blood pres-
sure elevation observed in PHAII patients with global loss of cullin3 function. Although cullin3 also negatively regulates Nrf2-mediated antioxidant responses in vascular endothelial cells, this likely occurs as a compensatory mechanism.

10.7 RENAL AND SPLENIC CYTOKINES ARE ALTERED IN EARLY LIFE STRESSED (ELS) ADULT MALE RATS

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Substantial evidence indicates that inflammation contributes to the development of cardiovascular disease (CVD). Epidemiological observations showed that ELS is associated with elevated circulating markers of inflammation in adults. Thus, we hypothesized that ELS alters the inflammatory state of adult male rats. Maternal separation (MatSep), a rodent model of ELS, involves separating rat pups from the dam for 3hrs/day from postnatal day 2 (PD2) to PD14. Normally reared littermate rats were used as controls. Inflammation status was monitored by determinations of immune cell numbers and types as well as renal and splenic cytokine expression. Our results showed that renal mononuclear cells and T-cell numbers were similar in adults control and MatSep rats (control vs. MatSep; renal PBMC: 52.9 ± 9.2 vs. 56.5 ± 8.3 x 106 cells/kidney; renal T cells: 7.3 ± 1.2 vs. 6.7 ± 1.2 x 106 cells/kidney; p < 0.05). Renal expression of interferon gamma (IFNγ), interleukin 6 (IL-6), and interleukin 4 (IL-4) were similar in control and MatSep rats, while renal IFNγ expression, (2) interleukin 1 beta (IL-1β) was significantly increased in MatSep rats compared to control (control: 4.4 ± 0.5 pg/mg protein, MatSep: 7.9 ± 1.0 pg/mg protein; p<0.0186). This increased IL-1β immunoreactivity was localized in the renal distal tubular epithelial cells of MatSep rats compared to control rats. In contrast, splenic IL-4 and IFNγ were significantly decreased in MatSep rats compared to control rats (IL-4: control: 0.7 ± 0.05 pg/mg protein, MatSep: 0.5 ± 0.08 pg/mg protein; IFNγ: control: 9.2 ± 0.5 pg/mg protein, MatSep: 7.6 ± 0.3 pg/mg protein p<0.05). No difference in IL-1β and IL-6 levels was observed in the spleen of control and MatSep rats. In response to an immune challenge with low dose LPS (2mg/kg), no differences were observed in renal and splenic IL-1β, IL-6, IL-4, IFNγ and TNFα protein expression (control LPS vs. MatSep LPS). These findings indicate that MatSep induces differential cytokine expression in the kidneys and spleen of control and MatSep rats at baseline. Future studies will determine: (1) which distal tubular epithelial cells have elevated IL-1β expression, (2) the effects of renal tubular IL-1β expression on renal transport function, and (3) whether IL-1β promotes renal dysfunction. (Fundied by APS Porter Fellowship to IEO, T32DK007545 to CDM, K99/R00HL111354 to ASL, F32HL116145 to DHH, and P01HL69999 to JSP).

10.8 γ/δ T CELLS MEDIATE ANGIOTENSIN II-INDUCED HYPERTENSION AND VASCULAR INJURY

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Objective: Both innate antigen-presenting cells and the adaptive immune system have been shown to play a role in the development of hypertension. Nevertheless, the T cell subsets involved in the pathophysiology of hypertension remains unclear. There is a small subset of “innate-like” T cells expressing the γ/δ T cell receptor (TCR) rather than the α/β TCR that could play a role bridging between the innate and adaptive immune systems. However, it is unknown whether γ/δ T cells contribute to the development of hypertension. We hypothesized that angiotensin (Ang) II-induced hypertension and vascular injury would be blunted in Tcrδ−/− mice, which are devoid of γ/δ T cells. Design and method: Thirteen to 15-week old male C57BL/6 wild-type and Tcrδ−/− mice were infused or not with Ang II (490 ng/kg/min, SC) for 7 or 14 days. Telemetric blood pressure (BP), mesenteric artery endothelial function and vascular remodeling by pressurized myography, and spleen T cell profile by flow cytometry were evaluated. Results: Fourteen days of Ang II increased systolic BP by 42 mmHg (P<0.01) in wild-type compared to control mice. The frequency of γ/δ T cells (2.3-fold, P<0.05) and activated (CD69+) γ/δ T cells (1.6-fold) was increased after 7 days of Ang II, and 7 days later remained increased or rose further (2.4-fold) in wild-type compared to control mice. Ang II decreased mesenteric artery relaxation responses to acetylcholine by 42% (P<0.01) and increased media/lumen by 45% (P<0.01) in wild-type mice compared to controls. BP rise and all the above mentioned Ang II effects were abrogated in Tcrδ−/−
10.9 MACROPHAGES REGULATE THE EXPRESSION OF STROMAL CELL-DERIVED FACTOR 1 VIA INDOLEAMINE 2,3-DIOXYGENASE AFTER THE RENAL ACUTE ISCHEMIA-REPERFUSION INJURY

Xin Wan1, Feng Zhang1, and Changchun Cao1


Objective: To observe the expression of indoleamine 2,3-dioxygenase (IDO) and stromal cell-derived factor 1 (SDF-1) in the kidney after ischemic reperfusion injury (IRI) and explore the relationship between IDO, SDF-1 and macrophage by depleting macrophages before the IRI.

Methods: A total of 32 healthy C57BL/6 male mice were used to establish renal IRI model by clamping unilateral renal pedicle for 60 minutes followed by reperfusion. Kidney tissue samples were collected at indicated time points. Renal histological changes were estimated. The expression of SDF-1 and IDO were determined by immunohistochemistry, ELISA and real-time PCR. In LC group, after the liposomal clodronate was injected intra-peritoneally, the location of CD68 was observed by immunofluorescence. In 1-MT group, IDO was evaluated by immunofluorescence after injecting intraperitoneally with 1-methyl-tryptophan. Renal histology and protein expression of SDF-1 and IDO were also detected.

Results: Compared with sham-operated group, classical tubular damage was found in IRI group, accompanied by a lot of inflammatory cells infiltrate. The expression of total renal SDF-1 and IDO peaked on day 1 and decreased to normal levels after two weeks. IDO doesn’t express in healthy kidneys, while SDF-1 in healthy kidney was localized at cortex and expand to the other area of the kidney during IRI. Compared with IRI groups elimination of macrophage by injection of liposomal clodronate alleviated renal IRI and down-regulated the expressions of CD68 while up-regulating SDF-1. In 1-MT group, which IDO was depleted by using 1-MT, the expression of CD68 was normal while SDF-1 was up-regulated.

Conclusions: SDF-1 expression is up-regulated in IRI kidney and is associated with macrophages express with IDO. SDF-1 may play a role in the early phase of acute kidney injury and IDO inhibiter can be a new medicine in therapy of AKI.

Key Words: Reperfusion injury; Macrophages; Stromal cell-derived factor 1; Acute kidney injury; indoleamine 2,3-dioxygenase.

10.10 MODEL ESTABLISHING AND FACTOR ANALYSIS: AN INSIGHT INTO THE PREDICTION OF ACUTE KIDNEY INJURY AFTER HEART SURGERY IN CHINA

Xin Wan1, Jing Li1, and Changchun Cao1


Objective: To explore the overall prediction of different stages of cardiac surgery associated-acute kidney injury (CSA-AKI) and the changes in renal function after cardiac surgery, try to make further efforts into factor analysis and give ideas to both clinical practice and predictive model establishing in China.

Methods: Five years (2008-2012, n=2811) of retrospective data were collected in the Division of Thoracic and Cardiovascular Surgery, Nanjing First Hospital. The method of Logistic Regression was applied to establish three preliminary models for predicting different stages of CSA-AKI, and with both Logistic Regression and non-parametric statistical analysis were used to study the influences of different factors on renal function.

Results: The three models for different stages of CSA-AKI showed differences in both the selected covariates and accuracy of prediction (the AUC of ROC curve for model of stage 1 to stage 3: 0.676 vs. 0.759 vs. 0.813). Covariates selected (with different methods) include male, age, emergency surgery, aortic aneurysm, hypertension, diabetes mellitus, insulin controlled diabetes, preoperative renal function, diagnosed chronic kidney diseases, preoperative hemoglobin, erythrocytes transfusion, duration of mechanical ventilation, ejection fraction (EF), body mass index (BMI) and surgical manner. Wherein, emergency surgery and aortic aneurysm showed statistical significance in non-parametric test (P <0.05) while the result is the opposite in Logistic Regression. The rank sum of factors suggested that abnormal BMI, lower preoperative hemoglobin (<130g/L in male, <120g/L in female) and lower EF (<50%) may increase the risk of postoperative AKI and lower the postoperative renal benefits.

Conclusion: Both Logistic regression models and scoring model may have some defects in predicting CSA-AKI; the previous classification for some factors may need adjustment; adjustment of BMI, EF and hemoglobin before elective surgery may lower the incidence of CSA-AKI and improve the prognosis.
10.11 EXTRACELLULAR HISTONES IN RELATION TO ORGAN DYSFUNCTION AND INFLAMMATION DURING KIDNEY-LUNG CROSSTALK
Xin Wan¹, Yasser Gendoo¹, and Changchun Cao¹

Background: Mortality rates due to kidney-lung crosstalk have remained high despite advances in the management of AKI and ARDS. The inflammatory mediators believed to be responsible for this are yet unidentified. Extracellular histones are known to exacerbate inflammation and tissue injury. Here we investigate whether there is a clinical correlation between blood histone concentrations, kidney function, and lung function during AKI and ARDS. Methods: In a prospective cohort, blood samples were collected from 54 patients upon admission to our hospital who were diagnosed with AKI, ARDS, community acquired pneumonia, or a combination of these. Serum histone concentrations were measured by ELISA and plotted against PaO2/FiO2, eGFR, neutrophils, and CRP. Pearson correlation test was performed and p < 0.05 was considered significant. Results: The scattergraphs show histone concentrations against the four parameters. In the overall sample population, histone concentrations were significantly correlated with PaO2/FiO2 (P=0.0017, R²=0.1652); change of eGFR from baseline (P<0.0001, R²=0.3107); neutrophil counts (P=0.0174, R²=0.1021) and CRP (P=0.0004, R²=0.2207). However, when patients were divided according to diagnosis, there was no significant correlation between these same parameters. Conclusions: When viewed in light of the adverse effects of extracellular histones, the correlations reported in this study strongly suggest that extracellular histones may be potential mediators of kidney-lung crosstalk. Additional research is necessary to reveal the mechanisms of their involvement and whether they participate in or are merely a consequence of injury. Further endeavours to understand the pathophysiology of crosstalk will likely lead to improved treatment strategies and outcomes.

10.12 RISK FACTORS OF IN-HOSPITAL MORTALITY IN AKI ASSOCIATED WITH CARDIOPULMONARY BYPASS: A RETROSPECTIVE COHORT STUDY
Xin Wan¹, Xiaobin Ji¹, and Changchun Cao¹

Background: CSA-AKI is a common and serious complication which increases morbidity and mortality and remains without effective prevention strategies. Studies in recent years have focused on identifying predictors of CSA-AKI incidence while few have explored the risks contributing to mortality. This study aims to identify the risk factors which can affect mortality and which can potentially be adjusted. Study Design: Retrospective cohort study. Setting & Participants: Consecutively, 2833 patients underwent first documented cardiac surgery with cardiopulmonary bypass (CPB) between January 2008 and December 2012 in Nanjing first hospital, China. Of these, 843 developed CSA-AKI and were thus included in the present study. Risk Factors: All possible perioperative variables were collected and analyzed to identify the factors involved in the mortality related to CSA-AKI. Outcomes: In-hospital mortality post CSA-AKI. Results: Logistic regression was used to analyze the independent influence of factors on outcomes. Cohort consisted of 2833 patients with a mean age of 55.9 years, 54% men, 36.5% (n=843) with AKI. The overall in-hospital mortality rate was 2.1% (18 of 842). In the multivariate analysis, sex (OR=0.074, 95% CI: 0.015-0.358, P<0.001), DIALYSIS (OR=216.008, 95% CI: 30.873-1511.352, P<0.001), ICU duration (OR=0.896, 95% CI: 0.811-0.990, P=0.031), Red blood cells (RBC) transfused (OR=4.715, 95% CI: 2.257-9.851, P<0.001) were independent predictors of mortality. Limitation: Single center, retrospective cohort study. Conclusions: Multivariable logistic regression analysis of more than 20 variables showed that sex, CPB duration, dialysis and RBC transfused were independent risk factors of in-hospital mortality.

10.13 INFILTRATING MACROPHAGES PROMOTE VASCULAR SYMPATHETIC INNERVATION DURING EXPERIMENTAL HYPERTENSION
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The immune system has been implicated in the development of hypertension, although the pathophysiological contribution remains unclear. Hypertension is associated with elevated sympathetic innervation and activity that may promote vasoconstriction of blood vessels. Macrophages are known to produce nerve growth factor (NGF), a neurotrophin that induces sympathetic nerve growth, and may be an immune-mediated contribution to vascular innervation during experimental hypertension.
dysfunction and hypertension. The aim of this study was to determine the contribution of macrophages to vascular sympathetic nerve innervation during experimental hypertension. C57Bl/6j male mice were infused with either vehicle or angiotensin (Ang II; 490 ng/kg/day, s.c.) for 14 days. A subset of Ang II-infused mice was randomly assigned to receive liposomes containing PBS or clodronate (50 mg/kg, i.v.) every 3 days to deplete macrophages. To test the effect of lowering blood pressure alone, another group of Ang II-infused mice received hydralazine (25 mg/L) via drinking water. Blood pressures were measured using tail-cuff plethysmography. Following treatment, whole mount mesenteric artery immunostaining of nerve bodies (synaptophysin), macrophages (F4/80), and NGF was conducted. Mesenteric vessels were imaged using confocal microscopy where Z-stack images were collected through the vessel. Ang II-infusion significantly increased sympathetic hyperinnervation of mesenteric arteries as shown by a 2-fold increase in synaptophysin staining (38±3% vs 19±2% positive staining; P<0.001, n=6). Macrophage infiltration into mesenteric arteries was also elevated in Ang II-infused mouse vessels compared to vehicle (F4/80+ cells; 26±8 vs 12±3; P<0.01, n=7), as were NGF-producing macrophages (5±2 vs 2±2 NGF+cells; P<0.05, n=6). In clodronate-liposome-treated mice, Ang II-induced pressor responses (135±8 vs 161±3 mmHg; P<0.01, n=8), vascular hyperinnervation (% synaptophysin area: 34±1 vs 40±2%; P<0.01, n=7) and macrophage infiltration (13±5 vs 20±7 cells; P<0.05, n=8) was significantly blunted compared to PBS-liposome-treated mice. Interestingly, despite reducing blood pressure to a similar degree as clodronate-liposome treatment, hydralazine had no effect on vascular hyperinnervation or macrophage infiltration. Ang II-induced hypertension is associated with elevated macrophage infiltration into resistance arteries, which induce local sympathetic hyperinnervation and represents a potential pathophysiological mechanism of immune-mediated hypertension.

10.14
IMPAIRED RIGHT CORONARY VASODILATIVE FUNCTION IN PULMONARY HYPERTENSIVE RAT ASSESSED BY SYNCHRONTRON MICROANGIOGRAPHY

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Pulmonary hypertension (PH) causes cardiac hypertrophy in right ventricles (RV), and eventually leads to RV failure due to persistently elevated ventricular afterload. We hypothesized that the mechanical stress of RV associated with increased afterload impairs vasodilative function of the right coronary artery (RCA) in PH. Using microangiography, we compared the vascular function of RCA in the two different models of PH rats. All experiments were conducted in accordance with guidelines for experimental procedures as set forth in the Declaration of Helsinki and the APS “Guiding Principals in the care and Use of Animals.” Rats were divided into 4 groups: (1) MCT group (5 wk after subcutaneous injection of monocrotaline, 60 mg/kg), (2) age-matched control group, (3) SuHx group (subcutaneous injection of Su5416, 20 mg/kg, with subsequent exposure to hypoxia (10%) for 3 wk followed by reexposure to normoxia for 5 wk), (4) age-matched control group. Coronary endothelial function was assessed using microangiography with synchrotron radiation in anesthetized rats. Imaging was performed at baseline and during acetylcholine (ACh, 5 μg/kg/min), sodium nitroprusside (SNP, 5 μg/kg/min), blockade of both NO synthases (NOS, L-NAME, 50 mg/kg) and cyclooxygenases (COX, meclofenamate, 3 mg/kg), and post blockade ACh. Since all rat of the SuHx group died immediately after NOS/COX blockade, we examined the effects of EDHF inhibition by charybdotoxin (0.15 μM/hr) and apamin (1.5 μM/hr) instead of NOS/COX blockade. In both PH rats, there was no difference in RCA vessel calibers at baseline compared to each control group. Coronary endothelial function was assessed using microangiography with synchrotron radiation in anesthetized rats. Imaging was performed at baseline and during acetylcholine (ACh, 5 μg/kg/min), sodium nitroprusside (SNP, 5 μg/kg/min), blockade of both NO synthases (NOS, L-NAME, 50 mg/kg) and cyclooxygenases (COX, meclofenamate, 3 mg/kg), and post blockade ACh. Since all rat of the SuHx group died immediately after NOS/COX blockade, we examined the effects of EDHF inhibition by charybdotoxin (0.15 μM/hr) and apamin (1.5 μM/hr) instead of NOS/COX blockade. In both PH rats, there was no difference in RCA vessel calibers at baseline compared to each control group. ACh and SNP mediated dilation was reduced in second and third order resistance arteries in both PH rats. MCT group displayed focal stenoses and segmental constrictions during NOS/COX blockade. On the other hand, SuHx group displayed focal stenoses and segmental constrictions during EDHF blockade. In conclusion, endothelium-dependent and independent vasodilative responses were significantly attenuated in the middle and small arteries in both severe PH rats and the presence of abnormal constriction mechanism was revealed in the right coronary circulation of severe PH rat. The observed impaired vasodilative function of RCA in these two PH models suggests that impaired RCA function might have causal relationship with RV failure in the patients with severe PH.
10.15
CARD9 KNOCKOUT AMELIORATES FIBROSIS AND HYPERTROPHY IN A TAC PRESSURE-OVERLOAD MODEL
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Nearly 1 in 3 adults aged over 20 in the US presents with hypertension, which can lead to left-ventricular hypertrophy, hypertensive heart disease, and heart failure. The progressions of each are tied closely to elevated systemic inflammation. CARD9, a cytosolic protein expressed in macrophages and neutrophils, mediates secretion of inflammatory cytokines by these cells. We recently reported rescued myocardial function by knockout of CARD9 (CARD9-/-) in high-fat diet overfeeding [1]. Macrophage deletion ameliorates the progression of TAC-induced left ventricular hypertrophy and fibrosis [2]. Therefore, the objective of this study is to investigate if CARD9 knockout rescues progression of TAC pressure-overload induced hypertrophy. Investigators adhered to standards of ethical animal treatment as stipulated by the IACUC. C57BL/6 wild-type (WT) and CARD9-/- mice were assigned to thoracic aortic constriction (TAC) or to sham (CONT). To assess heart function, fractional shortening (FS), was measured by echocardiogram 1, 2, or to sham (CONT). Mean arterial pressure (MAP) was measured acutely in anesthetized rats after 10 months (mo) by indwelling vascular catheter. Splenic T-cells were assessed by flow cytometry at 10 mo. Results: As expected, at 10 mo DS rats developed HT while DR rats did not. [MAP (mmHg): DS-Ovx, 190±12*; DS-Sham, 177±6*; DR-Ovx, 114±1; DR-Sham, 102±3; *p<0.005 vs DR, same surgical status; n=5-8/group]. Similarly, the frequency of CD8+ interleukin (IL)-17a+ T-cells were higher in DS compared to DR rats [Frequency at 10 mo (%): DS-Ovx, 5.2±1; *p<0.0001 vs DR, same surgical status; n=5-8/group]. Histological results of Masson’s trichrome stain suggests concentric hypertrophy in both TAC groups, but revealed less fibrosis in CARD9-/-TAC than in WT-TAC. In conclusion, CARD9 is a novel target for TAC-induced dysfunction, possibly through reductions in fibrosis and hypertrophy. Further study is needed to investigate signaling transduction involved, as well as the extent to which CARD9-/- may be protective in order to determine if it is a potential target for intervention in hypertensive heart disease. This study is funded by INBRE. References: [1] L. Cao, X. Qin, M. R. Peterson, S. E. Haller, K. A. Wilson, N. Hu, X. Lin, S. Nair, J. Ren, and G. He, “CARD9 knockout ameliorates myocardial dysfunction associated with high fat diet-induced obesity,” J. Mol. Cell. Cardiol., vol. 92, pp. 185–195, 2016. [2] D. Kain, U. Amit, C. Yagil, N. Landa, N. Naftali-Shani, N. Molotski, V. Aviv, M. S. Feinberg, O. Goitein, T. Kushnir, E. Kohn, F. H. Epstein, Y. Yagil, and J. Leor, “Macrophages dictate the progression and manifestation of hypertensive heart disease,” Int. J. Cardiol., vol. 203, pp. 381–395, 2016.

10.16
CYTOTOXIC CD8+ T-CELLS PLAY A ROLE IN HYPERTENSION-ASSOCIATED INFLAMMATORY RESPONSES IN FEMALE DAHL RATS
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Introduction: Oophorectomy is associated with increased body weight (BW) gain and incidence of hypertension (HT) compared with age-matched women. Increased BW gain and HT coincide with increased inflammatory biomarkers. To separate immune responses due to HT from those due to BW gain, we compared T-cell subpopulations in normotensive Dahl (DR) and hypertensive Dahl (DS) rats after ovariectomy. Methods: Six week old DS and DR rats were ovariectomized (Ovx) or sham operated (Sham). Mean arterial pressure (MAP) was measured acutely in anesthetized rats after 10 months (mo) by indwelling vascular catheter. Splenic T-cells were assessed by flow cytometry at 10 mo. Results: As expected, at 10 mo DS rats developed HT while DR rats did not. [MAP (mmHg): DS-Ovx, 190±12*; DS-Sham, 177±6*; DR-Ovx, 114±1; DR-Sham, 102±3; *p<0.005 vs DR, same surgical status; n=5-8/group]. Ovariectomy increased BW to a similar extent in both DS and DR rats [Ovx BW-Sham BW (g): DS, 179±13 vs DR, 202±14; *ns]. This model allows us to study immune mechanism as involved in HT that are disassociated from the magnitude of BW gain. The hypertensive DS rats showed signs of ongoing kidney disease as compared to the normotensive DR rats. The frequency of CD8+ interleukin (IL)-17a+ T-cells were higher in DS compared to DR rats [Frequency at 10 mo (%): DS-Ovx, 9.6±2*; DS-Sham, 7.6±2*; DR-Ovx, 1.3±0.4; DR-Sham, 3.1±1.6; *p<0.0001 vs DR, same surgical status; n=5-8/group]. Similarly, the frequency of CD8+ Foxp3+ T-cells were higher in DS compared to DR rats [Frequency at 10 mo (%): DS-Ovx, 26±6*; DS-Sham, 15±2*; DR-Ovx, 5±1.5; DR-Sham, 5.2±1; *p<0.0001 vs DR, same surgical status; n=5-8/group]. Conclusion: Imbalance in the “pro” and “anti” inflammatory cytotoxic CD8+ T-cell population may contribute to the hypertension-associated inflammatory response that is independent of body weight gain in female rats.
10.17
ARTERIAL STIFFNESS DUE TO CAROTID CALCIFICATION DISRUPTS NEUROVASCULAR COUPLING AND LEADS TO COGNITIVE DEFICITS

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Arterial stiffness is a strong risk factor for cognitive decline and dementia. It refers to the diminished capacity of an artery to buffer pulsatile blood flow from ventricular ejection. Arterial stiffness increases with age and is associated to high systolic blood pressure; however its effects on the brain remain poorly understood. Therefore, this study aimed to examine the effects of arterial stiffness on learning and memory, neurovascular coupling, blood-brain barrier (BBB) permeability and features of Alzheimer’s disease pathology, in a novel murine model based on carotid calcification. Arterial stiffness was induced by the application of a 0.3M CaCl₂-soaked pad on the right carotid artery. Arterial stiffness led to a shift in the Aβ40/Aβ42 ratio in the frontal cortex, without affecting tau protein phosphorylation. Analysis of cerebral autoregulation, vascular amyloidosis and BBB permeability are underway. These initial results show that arterial stiffness has a negative impact on cognitive and cerebrovascular functions, and should therefore be considered as a target to protect the brain in the elderly and in hypertensive individuals. This research was supported by funding from the Heart and Stroke Foundation of Canada (HG), the Fonds de recherche du Québec-Santé (HG), the Canadian Foundation for Innovation (HG), the Natural Sciences and Engineering Research Council of Canada (EP) and the Canadian Institutes of Health Research (GF, EP, HG). MFI was the recipient of a postdoctoral fellowship from the Groupe de Recherche sur le Système Nerveux Central (GRSNC, and MG and FRP were recipients of a Biomedical Doctoral Award from the Alzheimer Society of Canada.

10.18
DYNAMIC VASCULAR T CELL-ANTIGEN PRESENTING CELL INTERACTIONS DURING HYPERTENSION

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T cells contribute to the development of experimental hypertension, which is associated with significant accumulation of T cells into the perivascular fat surrounding the aorta and renal vasculature. While a hypertension-specific neoantigen has been implicated in T cell activation, whether vascular-infiltrating T cells recognize and are locally activated by an antigen within the vessel wall remains unclear. We have developed an explanted aorta model to examine live dynamic interactions between vascular T cells and antigen presenting cells (APCs) within healthy and hypertensive mouse vessels. Indicators of antigen presentation are slower T cell velocities, greater interaction time and a greater proportion of T cells interacting with APCs. The aim of this study was to identify whether hypertension-specific cognate antigens are presented to and recognised by T cells within the vessel wall interacting with APCs. The aim of this study was to identify whether hypertension-specific cognate antigens are presented to and recognised by T cells within the vessel wall interacting with APCs.

A C57BL6/J male mice aged 10-12 weeks. Control mice received 0.9% NaCl, in identical conditions. Animals were sacrificed between 2 and 3 weeks after surgery. The application of CaCl₂ led to increased collagen deposition, elastin fragmentation and macrophage infiltration in the carotid artery. In the brain, carotid calcification attenuated the cerebral blood flow response to whisker stimulation and to the topical application of the endothelium-dependent vasodilator acetylcholine, monitored by laser-Doppler flowmetry in vivo. Mice with arterial stiffness also exhibited a slower spatial learning acquisition in the Morris water maze test and significant impairments in spatial reference memory. Although modest, arterial stiffness led to a shift in the Aβ40/Aβ42 ratio in the frontal cortex, without affecting tau protein phosphorylation. Analysis of cerebral autoregulation, vascular amyloidosis and BBB permeability are underway. These initial results show that arterial stiffness has a negative impact on cognitive and cerebrovascular functions, and should therefore be considered as a target to protect the brain in the elderly and in hypertensive individuals. This research was supported by funding from the Heart and Stroke Foundation of Canada (HG), the Fonds de recherche du Québec-Santé (HG), the Canadian Foundation for Innovation (HG), the Natural Sciences and Engineering Research Council of Canada (EP) and the Canadian Institutes of Health Research (GF, EP, HG). MFI was the recipient of a postdoctoral fellowship from the Groupe de Recherche sur le Système Nerveux Central (GRSNC, and MG and FRP were recipients of a Biomedical Doctoral Award from the Alzheimer Society of Canada.
detected a ~2-fold increase in CCR5 ligand (CCL3, CCL4 and CCL5) secretion from hypertensive mouse aorta compared to vehicle-treated mouse aorta (*P<0.05; n = 4). Using 2-photon microscopy, we observed a greater number (~2-fold) of hT cells compared to nT cells within Ang II-infused mouse aorta (390±113 Vs 198±49). Importantly, time-lapse recordings of hypertensive mouse aorta revealed hT cells exhibited significantly slower velocity (hT cells 2.6 μm/min Vs nT cells 4.4 μm/min; P<0.01, n=8-11), longer duration of interaction (hT cell 36.0±5.2 min Vs nT cells 25.7±5.1; P<0.01, n=8-11) and a greater proportion of interactions with APCs (hT cells 10.7±2.3 Vs nT cells 1.5±0.7%; P<0.01, n=8-11). Consistent with current evidence, chronic treatment of Ang II-infused CD11c-YFP mice with an isoketal scavenger, 2-hydroxybenzylamine (2-HOBA), blunted pressor responses to Ang II, but interestingly, also abolished antigen presentation to hT cells within the aorta. Collectively, these data are the first evidence that vascular-infiltrating T cells recognize cognate antigens that are presented by APCs within the diseased vessel wall during hypertension.

10.19 LOSS OF ETB RECEPTOR FUNCTION ACTIVATES NOD-LIKE RECEPTOR AND INFLAMMASOME SIGNALING PATHWAYS IN RENAL OUT MEDULLA DURING TYPE 1 DIABETES THROUGH AN ER STRESS-INDEPENDENT MECHANISM

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Renal infiltration of immunocompetent cells and increased production of inflammatory markers are key in the pathogenesis of diabetic kidney disease. Endothelin-1 (ET-1), a potent vasoactive peptide that acts through two receptors (ETA and ETB), has been implicated in diabetes and is upregulated in patients with diabetic nephropathy (DN) and in animal models of diabetes-induced kidney injury. ETB receptors are highly expressed in renal outer medulla (OM). ET-1 exerts pro-inflammatory actions in the kidney; however, the mechanism(s) by which ET-1 mediates these effects are unclear. The present studies were designed to determine the role of the ETB receptor in the activation of inflammasome and NOD-like receptor signaling pathways in the renal OM during type 1 diabetes (T1D). ETB deficient (ETB def) and transgenic (TG) control rats were made diabetic by i.v. injection of streptozotocin (STZ). 10 wk later, OM were isolated and expression of inflammasome genes was assessed by RT-PCR array. Diabetes led to upregulation of NLRP5 (~4-fold increase vs. TG controls; n=3/group; p<0.05) and IL-1β (~3-fold increase vs. TG controls; n=3/group; p<0.05) in OM of ETB def rats. In addition, PSTPIP1, a negative regulator of the inflammasome, was decreased in OM of diabetic ETB def rats compared to TG controls. Together, these results demonstrate an overactivation of the downstream signaling of NOD-like receptor and inflammasomes in the absence of functional ETB receptor. Recently, endoplasmic reticulum (ER) stress has been identified as an inducer of inflammasome activation; thus, we tested if diabetic ETB def rats had an exaggerated ER stress response in the OM. RNA expression of GRP78, ATF-4, ATF-6, s-XBP-1, CHOP and caspase-12 was not different in OM of diabetic TG control and ETB def rats. Our data suggest that the activation of NOD-like receptor and inflammasome signaling pathways in this diabetic model is not mediated by ER stress. Funded by NIH T32 DK007545 to CDM and P01 HL95499 to DMP and JSP.

A HIGH FAT DIET INCREASES BLOOD PRESSURE AND LEADS TO A PROINFLAMMATORY IMMUNE CELL AND CYTOKINE PROFILE IN THE AORTAE OF FEMALE DAHL SALT-SENSITIVE RATS (DSS)

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A high fat (HF) diet has been linked to hypertension, which is often associated with an accumulation of macrophages and other leukocytes in the artery wall. Indeed, T lymphocytes were recently found to mediate HF-diet induced increases in blood pressure (BP) in male DSS. Women represent ~50% of hypertensive cases and are more likely than men to be obese; yet there is limited data on the impact of HF-diet on BP and immune cell activation in females. We hypothesized that a HF diet increases BP and proinflammatory immune cells and cytokines in the aortae of female DSS. Five-week old females were maintained on normal fat (NF; 7.2% fat n=4) or HF diet (35% fat, n=3) for 10 weeks. Systolic BP was measured by tail-cuff and aortic T cells, macrophages, and cytokines were measured by flow cytometric analysis. Total fat intake was significantly higher in the HF group after 10 wks (kcal/24hr: 127 ± 11 HF vs 80 ± 10 NF, P=0.03), and this likely contributed to the greater increase in body weight in HF-fed females after 10 wks (% increase: 196 ± 4 HF vs 160 ± 9 NF, P=0.01). A 10 wk HF diet increased BP (systolic BP in mmHg: 178 ± 7 HF vs 150 ± 8 NF, P=0.05). HF diet also led to an increase in total T cells (expressed as % total aortic cells, P=0.05) and
T cell activation (expressed as % total T cells, P<0.005). A 10 wk HF diet also resulted in greater numbers of pro-inflammatory Th17 cells (expressed as % CD3+CD4+ T cells: P=0.004), M1 macrophages (expressed as % total aortic cells, P=0.01), and TNFα+ cells (expressed as % total aortic cells, P=0.06). Although there was no change in anti-inflammatory T regulatory cells (expressed as % total aortic cells, P=0.67), M2 macrophages (expressed as % total aortic cells: P <0.0001), and IL-10+ cells (expressed as % total aortic cells, P=0.01), and TNFα+ cells (CD3+CD4+ FoxP3+), Effector Th17 cells (CD3+ CD4+ IL-17), M2 macrophages (CD11b/c+ CD206+ IL-10+), T regulatory cells (CD3+CD4+ IL-10+), TNFα+ cells, and IL-10+ cells were lower following a HF-diet. We conclude that a HF diet increases BP and the pro-inflammatory T cell, macrophage, and cytokine profile in the aorta of female DSS. Future studies will address the role of female sex hormones on BP and immune cell status in response to HF-diet, as well as the contribution of T cells and macrophages in HF-diet induced increases in BP.

<table>
<thead>
<tr>
<th>Type of Immune Cell</th>
<th>Normal Fat</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T cells (CD3+)</td>
<td>25 ± 0.3</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Activated T cell (CD3+CD44+)</td>
<td>19 ± 3</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>M1 macrophages (CD11b/c+CD206+TNFα+)</td>
<td>1 ± 0.01</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>M2 macrophages (CD11b/c+CD206+IL-10-)</td>
<td>1 ± 0.01</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>Effector Th17 cells (CD3+CD4+IL-17)</td>
<td>5.3 ± 0.9</td>
<td>12.8 ± 1.1</td>
</tr>
<tr>
<td>T regulatory cells (CD3+CD4+FoxP3+)</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>TNFα+ cells</td>
<td>4 ± 0.9</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>IL-10+ cells</td>
<td>3 ± 0.5</td>
<td>6 ± 0.3</td>
</tr>
</tbody>
</table>

10.21

**INDUCTION OF AP-1, NF-kB, miR-21 EXPRESSION, AND ANGIOTENSIN BY TUNGSTEN CARBIDE-COLBALT NANOPARTICLES INVOLVES ROS-MEDIATED MAPK PATHWAYS AND RESULTS IN TRANSFORMATION OF JB6 AND BEAS-2B CELLS**

Tabatha Barber1, Joni Aldinger1, Linda Bowman1, Terence Meighan1, and Min Ding1

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Tungsten carbide-cobalt (WC-Co) nanoparticle composites have wide applications because of their hardness and toughness. WC-Co has been classified as Group 2A (probably carcinogenic to humans) by the IARC. The present study examined the alterations of miR-21-PDCD4 signaling in JB6 cells after exposure to WC-Co nanoparticles. The results showed that (1) WC-Co induced AP-1 and NF-kB activity as well as ERK 1/2, p38, AKT, and JNK signaling in JB6 and BEAS-2B cells. (2) WC-Co caused PDCD4 inhibition in JB6 cells; (3) exposure of cells to WC-Co caused a significant increase of miR-21 expression and decrease of PDCD4 expression; (4) inhibition of ERKs with U0126 reversed WC-Co-induced PDCD4 inhibition, but inhibition of p38 with SB203580 did not; and (5) ROS scavengers, N-acetyl-L-cysteine and catalase, reversed the inhibitory effect of WC-Co on PDCD4 expression, while superoxide dismutase promoted the inhibition. (6) Chronic exposure to WC-Co nanoparticles induces colony formation in JB6/AP-1 and BEAS-2B cells in the Soft Agar Transformation Assay. (7) Lastly, WC-Co nanoparticles also affected the cells to induce angiogenesis tested by chicken chorioallantoic membrane (CAM) assay. These findings demonstrate that WC-Co nanoparticles induce AP-1, NF-kB, miR-21 expression while inhibiting PDCD4, which may be mediated through ROS, especially by endogenous H2O2, and ERK pathways. Unraveling the complex mechanisms associated with these events may provide insights into the initiation and progression of WC-Co-induced carcinogenesis.

10.22

**SYMPATHETIC NERVES PROMOTE HYPERTENSION-SPECIFIC EFFECTOR MEMORY T CELL HOMING AND PROLIFERATION IN THE BONE MARROW OF MICE WITH ANGIOTENSIN II INFUSION**

Liang Xiao1, Hana Itani1, Jason Foss1, and David G. Harrison1


We have recently identified a critical role of hypertension-specific effector memory T lymphocytes (TEM cells) in response to repeated surges of blood pressure. Formed during an initial hypertensive challenge, a part of TEM cells reside in the bone marrow (BM) in a quiescent state for prolonged periods and can be reactivated upon re-exposure to the hypertensive stimulus. Hypertension is associated with increased sympathetic outflow, including the input to BM. We therefore hypothesized that sympathetic nerves regulate accumulation and reactivation of hypertension-specific TEM cells in BM. To test this, we performed unilateral superior cervical ganglionectomy (SCGx) in wild-type C57BL/6 mice, which selectively sympathectomizes the forelimb on the surgical side. After recovery from surgery, the mice receive angiotensin II infusion (490ng/kg/min) for two weeks. The number of TEM cells in the denervated BM following ang II was slightly but significantly less than observed in the innervated limb as measured by flow cytometry. To determine T cells in the BM that were specific to hypertension, 5×10⁶ BM
cells were isolated from either the SCGx or control limbs, loaded with proliferation marker CFSE, and co-cultured with 0.5×10⁶ splenic dendritic cells isolated from another ang II-infused mouse. We found 30% less CD8⁺ T cell proliferation in the SCGx BM compared to control side (1.8±0.1 vs. 2.6±0.3×10⁴), but no difference in CD4⁺ T cells. To further study the effect of sympathetic nerves on T cell homing in BM, 1×10⁷ pan T cells were isolated from wild-type mice (w/CD45.2 allele) after angiotensin infusion, and adoptively transferred into CD45.1 mice that had previously received SCGx. As determined by flow cytometry 7 days after transfer, 25% less CD8⁺ Tₐₐm cells from hypertensive donors homed to the SCGx BM than the innervated BM of recipients (20.8±2.5 vs. 27.8±2.6 per 10⁶ total BM cells). To study the response of these Tₐₐm cells to a repeated hypertensive challenge, a subset of these SJL mice was also subjected to angiotensin infusion after adoptive transfer. We found respectively 50% and 36% less CD4⁺ and CD8⁺ Tₐₐm cells from hypertensive donors were positive for the proliferation marker Ki-67. We conclude that sympathetic nerves promote Tₐₐm cell homing to the BM after an initial blood pressure elevation, and also promote proliferation of these cells upon a repeated hypertensive challenge.

10.23  
INDUCTION OF AP-1 SIGNALING AND DNA DAMAGE BY COPPER OXIDE NANOPARTICLES INVOLVE ROS-MEDIATED MAPK PATHWAYS

Tabatha Barber¹, Linda Bowman¹, Joni Aldinger¹, and Min Ding¹


Occupational exposures to copper dusts or fumes have been reported to be harmful to human health, with possible risk of cancer among copper smelter workers. Copper (II) oxide (CuO) nanoparticles have not, to our knowledge, been extensively examined for potential carcinogenic or genotoxic effects. To investigate the mechanisms of CuO-induced pathogenesis, the effect of CuO on AP-1-MAPKs and ROS generation were investigated. The results indicated that CuO caused a 2-fold increase in AP-1 activity in JB6 cells. The induction of AP-1 activity in cultured cell lines was time- and dose-dependent. The signal transduction pathways for AP-1 activation were also investigated. Western Blot analysis demonstrated that CuO stimulates phosphorylation of p38 MAPK and ERKs. CuO also generated ROS when incubated with the cells as measured by electron spin resonance (ESR). Span "inso-spacerun: yes;" Nano-sized CuO generated more ROS than the fine-sized particles when incubated with the cells. COMET assay suggests that exposure of the cells to CuO resulted in DNA damage. Soft agar transformation assays have concluded that there is a significant increase in colony formation in JB6/AP-1 cells treated with CuO particles as compared to the control. Unraveling the complex mechanisms associated with these events may provide insights into the initiation and progression of CuO-induced pathogenesis.

10.24  
A NEW ROLE OF SOX6 IN BLOOD PRESSURE CONTROL THROUGH RENIN REGULATION

Jose A. Gomez¹, Conrad P. Hodgkinson², Alan Payne³, David G. Harrison⁴, and Victor J. Dzau⁵


Hypertension afflicts 33% of the U.S. adult population. Despite current treatments, approximately 50% of people with hypertension have uncontrolled blood pressure. Thus, there is a critical need to develop new therapies to treat this disease and its complications. The Renin Angiotensin Aldosterone System (RAAS) plays a key role in regulating blood pressure in humans. Renin controls the rate-limiting step in the conversion of angiotensinogen to angiotensin I. In adults, renin is produced and stored by juxtaglomerular (JG) cells in the kidney. However, the transcriptional mechanisms that govern the specification of renin expressing cells under normal or pathophysiological conditions and the contribution of the immune system remain poorly understood. During blood pressure changes the number of adult renal cells expressing renin increases through a process known as JG cell expansion. Mesenchymal stromal-like cells (MSCs) are pluripotent cells that can have immunomodulatory actions. We found that MSCs can differentiate to renin expressing cells and sought to determine regulators of renin expression and blood pressure control. Renin expression was induced in adult renal MSCs by treatment with 3-isobutyl-1-methylxanthine (IBMX) and Forskolin in vitro. Gene array experiments between renal MSC and JG cells identified a number of potential candidates that control MSC differentiation, including Sox 6. In vitro silencing of Sox6 by lentivirus-mediated shRNA decreased the differentiation of renal MSCs to renin producing cells (3.5 fold, n=4, P=
new approaches for understanding the physiological regulation of renin by HH in both high and low passage cells. However, DNMT1 and DNMT3b expression were significantly downregulated by HH in low passage Cfibs, correlating with DNA hypomethylation in these cells. In low passage cells, RNA-seq identified 2115 genes significantly changed in HH including 193 transcriptional regulators, 21 genes involved in DNA methylation, and 105 inflammatory genes. We verified enrichment of inflammatory signaling by qRT-PCR and found HH significantly upregulated Interleukin-1β and Interleukin-6 only in low passage cells. Experiments aimed at identifying differentially regulated pathways in high passage RV Cfibs by RNA-seq are ongoing. Together, these data suggest the combination of pressure overload and hypoxia is correlated with epigenetic reprogramming of Cfibs and increased inflammatory signaling. This unique pro-inflammatory Cfib phenotype is maintained for multiple passages but is eventually attenuated over time with removal of the PH insult. Future investigations will identify specific genes regulated by PH-induced fibroblast reprogramming. We propose that therapies which target this acute fibroblast-mediated inflammatory process have the potential to prevent RV dysfunction.

10.25 PULMONARY HYPERTENSION-INDUCED RIGHT VENTRICULAR PRESSURE OVERLOAD TRIGGERS ACUTE EPIGENETIC REPROGRAMMING OF PRO-INFLAMMATORY CARDiac FIBROBLASTS

Danielle Bruns1, Stephen Thoemmes1, Kurt Stenmark2, Peter Buttrick1, and Lori Walker1

1Med., Div. of Cardiology, Univ. of Colorado, Denver, 12700 E. 19th Ave., Aurora, CO, 80045, 2Pediatrics, Univ. of Colorado, Denver, 12700 E. 19th Ave., Aurora, CO, 80045.

Right ventricular (RV) function is a strong predictor of survival in a variety of clinical contexts including pulmonary hypertension (PH), highlighting the importance of delineating signaling pathways that contribute to RV dysfunction. We have previously demonstrated increased inflammation in PH-induced RV dysfunction and hypothesized that PH stimulates pro-inflammatory changes in RV cardiac fibroblasts (Cfibs). Further, we hypothesized that this process may be mediated by epigenetic changes in the fibroblast such as DNA methylation, which allows rapid and dynamic regulation of gene expression. We explored this hypothesis using a large animal model with significant resonance with human disease-the neonatal calf exposed to hypobaric hypoxia (HH). We assessed DNA methylation, gene expression and inflammation in low (2, 3) and high (6, 7) passage control (CO) and HH RV Cfibs. Exposure to HH resulted in global DNA hypomethylation in low passage RV Cfibs while global DNA methylation was not different between CO and HH high passage Cfibs. Further, we quantified expression of regulators of global DNA methylation, DNA-methyltransferases (DNMT), DNMT3a expression was unchanged by HH in both high and low passage cells. However, DNMT1 and DNMT3b expression were significantly downregulated by HH in low passage Cfibs, correlating with DNA hypomethylation in these cells. In low passage cells, RNA-seq identified 2115 genes significantly changed in HH including 193 transcriptional regulators, 21 genes involved in DNA methylation, and 105 inflammatory genes. We verified enrichment of inflammatory signaling by qRT-PCR and found HH significantly upregulated Interleukin-1β and Interleukin-6 only in low passage cells. Experiments aimed at identifying differentially regulated pathways in high passage RV Cfibs by RNA-seq are ongoing. Together, these data suggest the combination of pressure overload and hypoxia is correlated with epigenetic reprogramming of Cfibs and increased inflammatory signaling. This unique pro-inflammatory Cfib phenotype is maintained for multiple passages but is eventually attenuated over time with removal of the PH insult. Future investigations will identify specific genes regulated by PH-induced fibroblast reprogramming. We propose that therapies which target this acute fibroblast-mediated inflammatory process have the potential to prevent RV dysfunction.

10.26 MIR-762 INHIBITION PREVENTS AND REVERSES ANGIOTENSIN II INDUCED AORTIC FIBROSIS AND STIFFENING

Kim Ramil C. Montaniel1, Jing Wu1, Matthew R. Bersi2, Liang Xiao1, Hana A. Itani1, Kasey C. Vickers3, Jay D. Humphrey2, and David G. Harrison1


We and others have shown that hypertension (HTN) is associated with a striking deposition of collagen in the aortic adventitia. This leads to aortic stiffening and eventually end-organ damage. Through a screen of microRNAs (miRNAs) in the aorta, we found that miR-762 is the most upregulated miRNA in mice with angiotensin II (Ang II)-induced HTN. qRT-PCR confirmed that miR-762 is upregulated 6.35±1.22 (p=0.03) fold in Ang II-infused mice compared to controls. To study the role of miR-762 in HTN, we administered a locked nucleic acid inhibitor of miR-762. MiR-762 inhibition did not influence the hypertensive response to Ang II, yet it normalized stress-strain relationships and aortic systolic energy storage (ASE) (Table1). Moreover, miR-762 inhibition in the last 2 weeks of Ang II infusions reversed aortic stiffness in mice treated with Ang II for 4 weeks (ASE, 4 wk Ang II [51±5.18 kPa] vs 4wk Ang II + LNA-762 (last...
two weeks) [20±1.76 kPa], p<0.0001). Further studies showed that miR-762 inhibition reduced mRNA for several collagens and fibronectin and dramatically upregulated collagens MMP1a, 8 and 13 (Table 1). We also found that miR-762 inhibition during Ang II infusion led to a 9.11±1.92 (p=0.007) fold upregulation of Sprouty1 mRNA, suggesting that miR-762 targets the degradation of Sprouty1 mRNA. Sprouty1 inhibits activation of p38-MAPK which is central in the pathogenesis of aortic stiffening. Hence, miR-762 modulates aortic stiffening and fibrosis through a Sprouty1-p38-MAPK dependent mechanism. Thus, miR-762 has a major role in modulating aortic stiffening and its inhibition dramatically inhibits pathological fibrosis, enhances matrix degradation, prevents and reverses aortic stiffness. miR-762 inhibition might represent a new approach to prevent aortic stiffening and its consequent end-organ damage. Table 1: The effect of miR-762 inhibition on aortic fibrosis, stiffening and matrix gene expression. All values are presented as mean±SE.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ang II</th>
<th>Ang II + LNA-762</th>
<th>P value (One way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitial Collagen</td>
<td>75.8±10.0</td>
<td>283.9±15.6</td>
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<tr>
<td>Systolic energy storage (W sys, kPa)</td>
<td>66±2.49</td>
<td>17±1.33</td>
<td>61±4.73</td>
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<td>Collagen 1α*</td>
<td>5.05±0.55</td>
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<td>Collagen 3α*</td>
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<td>6.93±1.37</td>
<td>0.00025±0.00001</td>
<td>0.0004</td>
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<tr>
<td>Collagen 5α</td>
<td>3.34±0.61</td>
<td>5.09±0.96</td>
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<td>Fibronectin</td>
<td>1.0±0.36</td>
<td>2.10±0.18</td>
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<tr>
<td>MMP1α</td>
<td>1.0±0.21</td>
<td>1.84±1.22</td>
<td>0.010±0.0056</td>
<td>&lt;0.0001</td>
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<td>MMP1β</td>
<td>1.0±0.14</td>
<td>5.27±2.83</td>
<td>12.74±2.09</td>
<td>&lt;0.0001</td>
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<tr>
<td>MMP1γ</td>
<td>1.0±0.21</td>
<td>3.76±1.55</td>
<td>39.35±2.22</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

*Note: All fold change values were normalized to the sham group.

10.27
THE R213G POLYMORPHISM IN EC-SOD PROTECTS EARLY BLEOMYCIN-INDUCED PULMONARY INFLAMMATION AND ATTENUATES INDUCTION OF GENES INVOLVED IN LEUKOCYTE EXTRAVASATION
Kalin Swain1, Gary Mouradian1, Rohit Gaurav2, Steve Pugliese3, Ana-Laura Hernandez-Lagunas1, Karim El-Kasmi1,4, Anis Karimpour-Fard1, Russell Bowler3, Carmen Sucharov3, and Eva Nozik-Grayck1,4

Extracellular superoxide dismutase (EC-SOD) is a key lung antioxidant enzyme, protecting against oxidative stress and inflammation in acute lung injury (ALI). A single nucleotide polymorphism (SNP) substituting arginine to glycine at 213 (R213G) in SOD3 decreases tissue binding, thereby increasing EC-SOD in the extracellular fluids such as epithelial lining fluid and plasma. Higher EC-SOD in extracellular fluids confer protection against ALI in humans, and the R213G polymorphism protects against lipopolysaccharide induced ALI in mice. In this study, we analyzed how the R213G SNP modulates lung proinflammatory signaling pathways in an intratracheal bleomycin mouse model of ALI, characterized by early alveolar inflammation progressing to lung fibrosis. In wild type (WT) mice, bleomycin increased proinflammatory cytokines, measured with V-plex (mesoscale discovery), over 21 days. In contrast, R213G mice developed a more robust induction of inflammation 3 days post-bleomycin, including proinflammatory cytokines IL-1β, TNFα, IL-6, IFNγ, and anti-inflammatory cytokine, IL-10. However, the inflammation resolved quickly in R213G mice by day 7 (n=6-8). Unlike the WT mice, R213G mice did not progress to lung fibrosis, shown by pulmonary mechanics and collagen deposition. To identify molecular mechanisms by which the R213G SNP protects against ALI, we performed high throughput RNAseq in lung RNA and bioinformatics to evaluate gene pathways that were altered 7 days post-bleomycin in WT and R213G mice. The top differentially activated pathway, determined by ingenuity pathway analysis, was leukocyte extravasation (WT = 2.121, 9.55E-05; R213G = 0.59, 9.77E-08; z-score and q-value, respectively). This innate immune response has an important role in the recruitment, engagement/adhesive interactions and trans-endothelial migration of leukocytes. Genes involved in leukocyte diapedesis (ITGAM, ITGA4, ITGA1, ICAM1, and PECAM1), superoxide generation through NADPH oxidase (CYBs and NCFs) and hydrolyzation of extracellular matrix (MMP8, MMP12, MMP13), were differentially expressed between WT and R213G mice. Studies are ongoing to validate these findings. RNA sequencing and bioinformatic analysis identifies potential pathways responsible for the protective effects of the R213G SNP and provides a foundation to better understand the role of redox regulation in inflammatory lung diseases.

10.28
INTERLEUKIN 21 PROMOTES HYPERTENSION AND END-ORGAN DYSFUNCTION

52
Bethany L. Dale¹, Fanny Laroumanie², Mohamed A. Saleh³, Holly M. Scott Algood⁴, and Meena S. Madhur¹²


We have previously shown that T cell derived pro-inflammatory cytokines such as interleukin 17A (IL17A) and interferon gamma (IFNγ) are upregulated by and can promote angiotensin II-induced hypertension. IL21 is a pleiotropic cytokine produced primarily by T follicular helper (Tfh) cells and Th helper 17 (Th17) cells. It was recently demonstrated that IL21 deficient mice have reduced gastric inflammation following Helicobacter pylori infection accompanied by a decrease in local production of IL17A and IFNγ. The role of IL21 in hypertension is unknown. We hypothesized that IL21 deficient mice would be protected from hypertension and hypertensive end-organ damage in response to angiotensin II (Ang II) infusion, in part through reduced production of IL17A and IFNγ. We found that indeed IL21−/− mice exhibited a 28 mmHg reduction in blood pressure in response to 4 weeks of Ang II infusion compared to age-matched wild type (WT) mice (p=0.0155). Preliminary data demonstrates that IL21 mRNA expression increases 1.5 fold in splenic T cells in response to Ang II (p=0.015). IL17A production from splenic CD4+ T cells and IFNγ production from splenic CD8+ T cells was reduced in IL21−/− mice compared to WT mice after 4 weeks of angiotensin II infusion. Finally, renal function was assessed by measuring albumin to creatinine ratio in spot urine following Ang II infusion. Wild type mice developed 3.6 times more albuminuria compared to IL21−/− mice (p=0.02). Taken together, these studies suggest that IL21 may play a key role in hypertension, in part through modulation of IL17A and IFNγ production. Immunobiology of Blood and Vascular Systems Training Program, NIH 2 T32 HL069765-11A1. 1. Carbo A, Olivares-Villagomez D, Honetcillas R, Bassaganya-Riera J, Chaturvedi R, Piazuvo MB, Delgado A, Washington MK, Wilson KT, Algood HM. Systems modeling of the role of interleukin-21 in the maintenance of effector CD4+ T cell responses during chronic Helicobacter pylori infection. MBio. 2014; 5 (4).

10.30
INVESTIGATING THE MECHANISMS BY WHICH IgG ANTIBODIES CONTRIBUTE TO ANGIOTENSIN II-DEPENDENT HYPERTENSION

Maggie Lieu¹, Christopher T. Chan¹, Christopher G. Sobe⁵, Antony Vinh¹, and Grant R. Drummond¹


Recent studies by our laboratory have shown that angiotensin (Ang) II-induced hypertension in mice is associated with elevated serum and aortic IgG levels. Moreover, inhibition of B cell activation and IgG production protected mice against Ang II-induced increases in blood pressure.

Aerobic exercise induces a stress response, including an increase in epinephrine and inflammatory cytokines. Based on results from cell culture models, monocyte inflammatory cytokine response is dependent on β1 adrenergic receptors (AR). However, data from human in vivo studies is limited. The purpose of this study was to determine post-exercise cytokine changes when participants exercised after taking a non-selective or selective (β1 AR) beta blocker. Methods: This is a preliminary report of an on-going study. To date, 12 healthy, young adults (30.9±6.0 yrs; body mass index 26.9±1.1) have completed the double-blind randomized cross-over design protocol. Three hours after ingestion of placebo, nadolol (non-selective beta blocker), or bisoprolol (selective β1 AR beta blocker) a resting blood sample was collected. The subject exercised 30 minutes on a leg cycle ergometer at a workload 10% above lactate threshold. Immediately and one-hour post-exercise blood samples were collected. Serum cytokine analysis is underway (current analysis n=3). Results: Compared to pre-exercise, immediately post exercise serum TNF percent change was: placebo -6.7%, nadolol 44.0%, and bisoprolol -32.0%; and one-hour post exercise serum TNF percent change was: placebo -31.2%, nadolol 12.2%, and bisoprolol -68.5%. Discussion: Under the selective β1 AR beta blocker (bisoprolol) condition TNF percent change from resting was lower immediately and one-hour post exercise, compared to placebo and non-selective beta blocker (nadolol). Similarly as to what is reported in cell culture models, the human in vivo post-exercise cytokine response may be dependent on β1 AR. Although our subjects were healthy, there may be immune system considerations for those who exercise and are prescribed a beta blocker medication.

10.29
EFFECT OF BETa BLOCKERS ON POST-EXERCISE CYTOKINE RESPONSE

Melissa Markofoški¹, Chad Dolan¹, Hawley Kunz¹, Rachel Graft², Nadia Agha¹, Rod Azadan¹, Forrest Baker¹, Preetesh Mylabathula¹, and Richard Simpson¹

¹Dep.T. of Hlth. and Human Performance, Univ. of Houston, 3875 Holman St., Houston, TX, 77204.

Aerobic exercise induces a stress response, including an increase in epinephrine and inflammatory cytokines. Based on results from cell culture models, monocyte inflammatory cytokine response is dependent on β1 adrenergic receptors (AR). However, data from human in vivo studies is limited. The purpose of this study was to determine post-exercise cytokine changes when participants exercised after taking a non-selective or selective (β1 AR) beta blocker. Methods: This is a preliminary report of an on-going study. To date, 12 healthy, young adults (30.9±6.0 yrs; body mass index 26.9±1.1) have completed the double-blind randomized cross-over design protocol. Three hours after ingestion of placebo, nadolol (non-selective beta blocker), or bisoprolol (selective β1 AR beta blocker) a resting blood sample was collected. The subject exercised 30 minutes on a leg cycle ergometer at a workload 10% above lactate threshold. Immediately and one-hour post-exercise blood samples were collected. Serum cytokine analysis is underway (current analysis n=3). Results: Compared to pre-exercise, immediately post exercise serum TNF percent change was: placebo -6.7%, nadolol 44.0%, and bisoprolol -32.0%; and one-hour post exercise serum TNF percent change was: placebo -31.2%, nadolol 12.2%, and bisoprolol -68.5%. Discussion: Under the selective β1 AR beta blocker (bisoprolol) condition TNF percent change from resting was lower immediately and one-hour post exercise, compared to placebo and non-selective beta blocker (nadolol). Similarly as to what is reported in cell culture models, the human in vivo post-exercise cytokine response may be dependent on β1 AR. Although our subjects were healthy, there may be immune system considerations for those who exercise and are prescribed a beta blocker medication.

10.30
INVESTIGATING THE MECHANISMS BY WHICH IgG ANTIBODIES CONTRIBUTE TO ANGIOTENSIN II-DEPENDENT HYPERTENSION

Maggie Lieu¹, Christopher T. Chan¹, Christopher G. So-
and vessel stiffening. However, the mechanism(s) by which IgG mediates these processes remains to be determined. Therefore, we investigated the potential role of two known effector mechanisms of IgG-mediated immunity in Ang II-induced hypertension, namely macrophage activation and upregulation of the complement system. Treatment of male C57BL6/J mice with Ang II (0.7 mg/kg/d, s.c.) for 28 days increased tail cuff blood pressure by ~37 mmHg compared to saline-treated mice. Immunohistochemistry and flow cytometry revealed that Ang II-infusion was also associated with IgG deposition in the aortic adventitia as well as an increase in the number of CD206+ M2-like macrophages. Importantly, IgG was found to co-localise with these cells. As evidence that hypertension-specific antibodies can activate macrophages, stimulation of cultured RAW246.7 macrophages with purified IgG from Ang II but not saline-treated mice promoted the production of TGF-β. Finally, Ang II-dependent increases in aortic IgG were also associated with upregulation of C1q, C1r, C1s, C2, C3, C4 and C6, highlighting a possible role for the complement cascade. Taken together, these observations provide insights into the likely mechanisms by which B cell activation and IgG antibodies may promote hypertension and vascular remodeling, and suggest that targeting IgG-dependent macrophage and complement activation may represent novel therapeutic approaches to treating the condition.

10.31 MACROPHAGE-DERIVED IGF-1 CONTRIBUTES TO AORTIC FIBROSIS AND STIFFENING IN HYPERTENSION MICE

Grant R. Drummond1, Christopher T. Chan1, Alexei Ilinykh2, Antony Vinh1, Shalini M. Krishnan1, Maggie Lieu1, Caitlin V. Lewis1, Henry Diep1, Alex Pinto2, and Christopher G. Sobey1


M2 macrophages accumulate in the vessel wall during hypertension and are important mediators of vascular remodeling, fibrosis and stiffening. However, the mechanisms involved are yet to be defined. M2 macrophages are an important source of insulin-like growth factor-1 (IGF-1). In other disease settings, IGF-1 is known to contribute to fibrosis and tissue growth but its role in vascular remodeling during hypertension is unknown. Therefore, we examined whether macrophage-derived IGF-1 contributes to vascular fibrosis, aortic stiffening and elevated blood pressure (BP) in hypertensive mice. In male C57BL6/J mice, infusion of angiotensin II (Ang II, 0.7 mg/kg/d for 14 days, s.c.) elevated systolic BP by >50 mmHg (P<0.05) and increased collagen content and stiffness of the aorta, each by ~2-fold (P<0.05). These changes were accompanied by a 6.8-fold increase in M2 macrophage numbers (CD45<sup>-</sup>CD11b<sup>+</sup>Ly6G<sup>-</sup>F4/80<sup>-</sup>CD206<sup>+</sup> cells) in the aortic wall (P<0.05), and importantly expression of the M2 marker, CD206, was strongly positively correlated with that of IGF-1 (r²=0.57; P<0.001). Depletion of monocytes (macrophage precursors) by treatment with clodronate-containing liposomes (50 mg/kg, every 3 days, i.v.) reduced the Ang II-dependent influx of M2 macrophages into the aorta by 75% (P<0.05) and simultaneously inhibited aortic IGF-1 expression by 32%, collagen deposition by 34%, aortic stiffness by 43% (P<0.05), and systolic BP by 25 mmHg (all P<0.05). Macrophage-specific IGF-1 deficient mice (LysM<sup>Cre<sup>+</sup> x IGF-1<sup>fl/fl</sup>) treated with Ang II also displayed reduced IGF-1 expression in the aorta (by 33%) compared to similarly treated control mice (LysM<sup>Cre<sup>+</sup> x IGF-1<sup>±</sup>); P<0.05). Moreover, IGF-1 deficient animals were protected from Ang II-induced increases in aortic collagen deposition, stiffening and systolic BP (all P<0.05). Finally, confirming that the effects of clodronate and macrophage-specific IGF-1 deficiency on vascular remodeling occurred upstream of their BP-lowering actions, an equivalent anti-hypertensive dose of the non-specific vasodilator, hydralazine (25 mg/L, p.o.), had no effect on Ang II-induced increases in aortic macrophages, IGF-1 expression or collagen deposition. In conclusion, M2 macrophage-derived IGF-1 plays a crucial role in the aortic fibrosis that contributes to vascular stiffening and elevated systolic BP during Ang II-induced hypertension in mice. Future studies aimed at unraveling the cellular targets and second messengers activated by IGF-1 in the aortic wall have the potential to reveal new targets for novel anti-hypertensive therapies.

10.32 OPPOSING EFFECTS OF C3A AND C5A ON KIDNEY INJURY IN ANGIOTENSIN II INDUCED HYPERTENSION

Ulrich Wenzel1, Sebastian Weiss1, Alva Rosendahl1, Catherine Meyer-Schwesinger1, Jörg Koehl2, and Heimo Ehmke3

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Adaptive and innate immune responses participate in the pathogenesis of hypertension and hypertensive end-organ
AngII treatment with high salt intake reduces TNF-α. The role in hypertension is unknown. Using anaphylatoxin receptor reporter and knockout mice, we investigated the pathogenic role of the C5a and C3a in hypertensive renal injury. Out of the CD45+ cells isolated from the kidney of GFP-C5aR1 reporter knock-in mice 28% expressed C5aR1. The majority of CD45+ cells carrying C5aR1 were dendritic cells (89%) followed by neutrophils and macrophages. Using confocal microscopy, the C5aR1 was detected mainly on infiltrating cells, but a minor population of resident podocytes and parietal cells also expressed C5aR1. Since most mouse strains are resistant to hypertensive end-organ damage induced by Ang II, we used an aggravated model of hypertension by combining Ang II infusion with unilateral nephrectomy and salt in the drinking water. Deletion of C5aR1 ameliorated albuminuria compared to wildtype mice and reduced the expression of renal injury (NGAL) and the inflammation marker gene CCL2 in hypertensive mice. Additional deletion of C3aR eliminated the protective effects of C5aR1 deficiency, while deletion of C3aR alone aggravated albuminuria and renal injury (wildtype 18.7±4.1, C3aR-/- 35.7±8.3, C3aR/C5aR1-/- 6.0±1.6 mg albumin/mg creatinine). These findings suggest that the complement system regulates hypertensive kidney damage via opposing signaling pathways. While the C5a/C5aR1 axis promotes renal inflammation and albuminuria, the C3a/C3aR axis exerts protective effects.

10.33 INTRARENAL ANGIOTENSINOGEN FORMULATION IN RESPONSE TO CHRONIC HIGH SALT INTAKE AND ANGIOTENSIN ADMINISTRATION IS AUGMENTED IN TNF-α RECEPTOR KNOCKOUT MICE
Eamonn Mahaffey¹, Alexander Castillo¹, L. Gabriel Na-⁴, and Dewan Majid¹
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Background: Chronic angiotensin II (AngII) treatment enhances both angiotensinogen (AGT) and tumor necrosis factor- alpha (TNF-α) formation in the kidney. However, the link between such TNF-α increase and AGT formation remains unclear. We examined the hypothesis that AngII treatment with high salt (HS) intake reduces TNF-α receptor type 1 (TNFR1) activity and increases intrarenal AGT formation. Methods: Responses to AngII infusion (25 ng/min; implanted minipump) with HS (4% NaCl) diets for 4 weeks were evaluated in wild-type (WT; n=6) as well as knockout mice (KO) for TNFR1 (n=7) and TNFR2 (receptor type 2; n=6). Systemic blood pressure (SBP) was measured by tail-cuff plethysmography and 24-hour urine collections were done using metabolic cages. The urinary excretion rate of AGT (uAGT) was measured using ELISA. Results: AngII + HS induced increases in mean SBP was significantly greater in TNFR1KO (77±2 to 115±3 mmHg), but similar in TNFR2KO (78±2 to 99±5 mmHg) compared to WT (76±1 to 102±2 mmHg). Interestingly, the increase in uAGT was also significantly greater in TNFR1KO (6±2 to 167±75 ng/24hr) but not different in TNFR2KO (8±7 to 65±44 ng/24hr) compared to WT (6±3 to 46±16 ng/24hr). Conclusion: The results suggest that TNFR1 activity mitigates the hypertensive response to chronic AngII infusion with HS intake, likely by attenuating the increase in intrarenal AGT formation.

10.34 NLRP3 Inflammasome-mediated Immunity in Dahl Salt-Sensitive Hypertension
Justine Abais-Battad¹, Hayley Lund¹, and David Mattson¹
¹Physiology, Med. Coll. of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI, 53226.

Previous studies demonstrated the importance of infiltrating renal T lymphocytes in the pathogenesis of hypertension and renal damage in Dahl salt-sensitive (SS) rats. It remains unclear how this adaptive immune response becomes activated during the course of this sterile disease. The NLRP3 inflammasome identifies exogenous and endogenous danger signals and initiates innate immunity and host defenses. It is comprised of the sensory protein NLRP3, the adaptor protein ASC, and the effector pro-caspase-1, and when stimulated, allows for the processing of pro-inflammatory cytokines like IL-1β and IL-18. The following preliminary studies sought to characterize the expression of the NLRP3 inflammasome in SS kidneys during a low 0.4% NaCl and high 4.0% NaCl diet, and test whether pharmacological inhibition of NLRP3 inflammasome activation attenuates salt-induced hypertension and renal damage in SS rats. RNA sequencing data revealed a significant upregulation in RNA expression of NLRP3 (33.8%) and caspase-1 (26.4%), and a similar trend for IL-1β (25.5%), in the renal medulla of SS rats fed the high salt diet compared to low salt controls (n=4/group). Western blotting also showed increased protein expression of NLRP3 (1.5-fold) and ASC (3.2-fold) in the renal medulla of SS rats after 3 weeks of high salt, together demonstrating increased NLRP3 inflammasome
mRNA and protein expression in the renal outer medulla of SS rats during high salt. To assess the role of IL-1β, SS rats implanted with telemeters were administered IL-1 receptor antagonist anakinra (IL-1RA; 600 μg/day i.p., n=4-6) daily during high salt challenge. Rats receiving IL-1RA tended to have lower mean arterial pressure (MAP) than those receiving saline, reaching significance on day 17 of treatment (159±6 mmHg saline vs. 141±5 mmHg IL-1RA) of high salt. An additional preliminary study using NLRP3 inflammasome inhibitor glyburide (Gly, 100mg/kg/day p.o., n=4-5) also demonstrated a trend for reduced MAP (156±8 mmHg vehicle vs. 145±9 mmHg Gly) and albuminuria (69±17 mg/day vehicle vs. 41±7 mg/day Gly). Since pharmacological NLRP3 and IL-1β inhibition appeared to mitigate the SS phenotypes, the NLRP3 inflammasome in SS kidneys may mediate the initiation of adaptive immunity and the subsequent infiltration of T lymphocytes that amplifies salt-induced hypertension and renal damage. (DK96859, HL116264, 5T32HL007792, 15SFRN2391002)

11.0: CAREER WORKSHOP

11.1 GET A JOB: BUILD THE SKILLS EMPLOYERS WANT!
Jennifer Sasser
Dept. of Pharmacology and Toxicology, Univ. of Mississippi Med. Ctr., Jackson, MS.
Data show that less than 8% of doctoral students will become tenure track faculty, and more and more graduates are in a wide array of non-academic positions. Therefore, it is crucial for trainees to develop additional “employability skills” during their PhD and postdoctoral training in order to be well qualified for the diverse job opportunities available to PhD’s in the workforce. The overall objectives of the symposium are to provide information for trainees on the following: 1) PhD job market trends; 2) Employability Skills; 3) the APS/ACDP Professional Skills Document; and 4) the importance of an Individual Development Plan.

12.0: INFLAMMATION, IMMUNITY, INTESTINAL FLORA AND THE METABOLIC SYNDROME

12.2 SYSTEMIC OLFACTORY RECEPTORS AND THE GUT MICROBIOTA

Jennifer Pluznick1, Niranjana Natarajan1, Daishiro Hori2, Sheila Flavahan2, Jochen Steppan2, Nicholas Flavahan2, and Dan Berkowitz2
SCFA metabolites (acetate, propionate and butyrate) are byproducts of gut microbial metabolism which have been shown to dilate blood vessels ex vivo. Recently, we reported that two SCFA receptors, Gpr41 and Olfr78, both play roles in blood pressure regulation. Here, we will highlight novel data showing the localization and function of Gpr41 – we find by RT-PCR that Gpr41 localizes to the vascular endothelium, where it plays a hypotensive role (Gpr41 KO mice have isolated systolic hypertension, as measured by telemetry). In agreement with a phenotype of systolic hypertension, KO mice also exhibited elevated pulse wave velocity in vivo, but surprisingly, no increase in ex-Â-vivo aorta stiffness (measured by tensile testing experiments). In sum, these studies demonstrate that endothelial Gpr41 lowers baseline BP, likely by decreasing vascular tone. Finally, we will explore the implications of these findings, and will integrate these new data with previously published work by ourselves and others regarding the role of the gut microbiota in the control of blood pressure.

12.3 METABOLIC SYNDROME
Kamal Rahmouni1
1Pharmacology, Univ. of Iowa, 51 Newton Rd., Iowa City, IA, 52242.
The worldwide prevalence of obesity is associated with an escalating incidence of morbidity and mortality. This because the epidemic of obesity has been paralleled by an increase in the incidence of the metabolic syndrome, a cluster of risk factors including hypertension and that raises the risk for heart disease and other health problems. This has triggered a great interest in understanding the mechanisms underlying the adverse cardiovascular effects of obesity and metabolic syndrome which has led to significant progress in recent years. Defects in various biological processes ranging from genetic and humoral factors to basic cellular signaling pathways in different tissues have been involved in the pathogenesis of the obesity and metabolic syndrome. The diversity in the processes implicated is consistent with the polygenic and multifactorial nature of these conditions and their comorbidities. The new knowledge gained in recent years should be taken into account when seeking novel diagnostic and therapeutic approaches for the cardiovascular disorders caused by excess adiposity. Given the evidence pointing
to the significance of the neurogenic mechanisms in metabolic syndrome new strategies that disrupt these processes should be favored.

12.4 ALTERING THE MICROBIOTA FOR WEIGHT CONTROL

Sean Davies1, Zhongyi Chen1, Linda Zhang1, Lilu Guo1, Yonglin Zhang1, Youmin Zhang2, Lei Ding2, Patricia Yancey2, Arion Kennedy3, Alyssa Hasty3, MacRae Linton4, and Kevin Niswender5


Alterations in the gut microbiota have been implicated in the development of obesity-related diseases, establishing the gut microbiota as a therapeutic target. To alter the microbiota, we have focused on incorporating bacteria that sustainably biosynthesize metabolites with therapeutic effects. To assess the potential of this approach, we engineered E. coli Nissle 1917 (EcN) to produce N-acetylphosphatidylethanolamine (NAPE), by transformation of these bacteria with A. thaliana NAPE acyltransferase (pNAPE-EcN). When pNAPE-EcN are administered in drinking water concurrently with high fat diet (60% kcal), this pNAPE-EcN administration markedly inhibits gain of weight concurrently with high fat diet (60% kcal), this pNAPE-EcN administration markedly inhibits gain of body weight and body fat compared to mice administered standard drinking water, vehicle (0.125% gelatin) or control bacteria (pEcN). Treatment of LDLR-/- mice receiving standard drinking water, vehicle (0.125% gelatin) or control bacteria (pEcN) failed to reduce weight gain or food intake in Napepld+/- mice, but the effects were restored if the administered bacteria heterologously co-expressed NAPE-PLD along with NAPE acyltransferase (pNAE-EcN). Together, these results demonstrate that incorporation of therapeutic bacteria into the gut microbiota can be used to treat obesity and that this treatment can potentially be tailored according to the specific phenotype of the individual. NIH R01 AT007830 Reference: Incorporation of Therapeutically Modified Bacteria into Gut Microbiota Inhibits Obesity. J. Clin Invest. 124:3391-3406 PMC4109548.

13.0: INFLAMMATION, HYPERTENSION, AND END-ORGAN DAMAGE

13.1 ALTERING THE MICROBIOTA FOR WEIGHT CONTROL

David Harrison1

1Internal Med., Vanderbilt Univ., 2220 Pierce Ave., Nashville, TN, 37232.

Hypertension remains an enormous health care burden that affects 30% of Western populations. Despite its prevalence the cause of most cases of hypertension remain unknown. Our laboratory has defined a novel mechanism for hypertension involving adaptive immunity. We found that mice lacking lymphocytes (RAG-1-/- mice) develop blunted hypertensive responses to a variety of stimuli including chronic angiotensin II infusion, DOCA-salt challenge and norepinephrine infusion. Adoptive transfer of T cells, but not B cells, restores the hypertensive responses to these stimuli. Hypertension is associated with the infiltration of T cells into the kidney and vasculature, where they release cytokines, including IFN-γ, IL-17A, and TNFα, which promote sodium retention, vasoconstriction and oxidative injury. Recently, we have found that angiotensin II has striking effects on dendritic cells (DCs), promoting their propensity to activate T cells. Our data indicate that angiotensin II infusion increases DC superoxide production by 5-fold and causes a striking accumulation isoketals, oxidized products of arachidonic acid in these cells. These form covalent bonds to lysines of proteins and these modified proteins become immunogenic. Several isoketal scavengers, including 2-hydroxybenzylamine (2-HOBA) prevent DC activation, the ability of these cells. These form covalent bonds to lysines of proteins and these modified proteins become immunogenic. Several isoketal scavengers, including 2-hydroxybenzylamine (2-HOBA) prevent DC activation, the ability of DCs to stimulate T cell proliferation and prevent hypertension. A major impetus for immune cell activation seems to be increased sympathetic outflow, stimulated by the central actions of angiotensin II. By lesioning the AV3V region of the forebrain of mice or inactivating the NADPH oxidase in the subfornical organ using Cre Lox technology, we have prevented the central actions of angiotensin II and found that this inhibits both T cell activation and hypertension. Renal denervation likewise pre-
vents activation of DCs in the kidney and the accumulation of activated DCs in the spleen. Thus, the kidney seems to be a major site of DC activation in hypertension. In summary, we have identified a new mechanism underlying hypertension and a potential new therapy for this common and yet difficult to manage disease.

13.2
T REGULATORY LYMPHOCYTES IN HYPERTENSION

Ernesto Schiffrin1


In angiotensin II and aldosterone-infused mice, adoptive transfer of Treg blunted BP changes, vascular inflammatory cell infiltration, cytokine changes and oxidative stress increases induced by angiotensin II and by aldosterone. Endothelial dysfunction was also prevented when Treg were administered to angiotensin II or aldosterone-infused mice. Scurfy mice are deficient in Treg due to a mutation in the transcription factor forkhead box P3 gene. Angiotensin II induced endothelial dysfunction and oxidative stress in perivascular adipose tissue (PVAT) in mesenteric artery of wild-type T cell-injected \( \text{Rag}^1\)), whereas these were exaggerated in Scurfy T cell-injected \( \text{Rag}^1\). Angiotensin II enhanced microvascular remodeling and stiffness in vehicle- and Scurfy T cell-injected \( \text{Rag}^1\). Angiotensin II increased monocyte chemotactic protein-1 expression in the vascular wall and PVAT, monocyte/macrophage infiltration and pro-inflammatory polarization in PVAT and the renal cortex, and T cell infiltration in the renal cortex only in Scurfy T cell-injected \( \text{Rag}^1\). Wild-type Treg co-injection with vehicle or Scurfy T cells prevented or reduced these effects of angiotensin II. In DOCA-salt rats, mineralocorticoid receptor blockade was associated with enhanced Treg action and reduced Th17. In conclusion, Treg counteract proinflammatory effects and microvascular injury by either angiotensin II or mineralocorticoids by modulating innate and adaptive immune responses. (CIHR Grants grants 82790 and 102606 and First Pilot CIHR Foundation Grant). Didiion SP et al. Endogenous Interleukin-10 Inhibits Angiotensin II–Induced Vascular Dysfunction. Hypertension. 2009;54:619-624. Viel EC et al. Immune regulation and vascular inflammation in genetic hypertension. Amer J Physiol Heart Circ Physiol. 2010;298:H938-H944. Barhoumi T et al. T regulatory lymphocytes prevent angiotensin II-induced hypertension and vascular injury. Hypertension. 2011;57: 469-476. Kasal DAB et al. T regulatory lymphocytes prevent aldosterone-induced vascular injury. Hypertension 2012;59:324-330. Leibowitz A et al.


13.3
RENAL IMMUNE CELLS AND HYPERTENSION

David Mattson1


Experiments in our laboratory have demonstrated that feeding a high NaCl diet to Dahl Salt-Sensitive (SS) rats results in a significant infiltration of T-lymphocytes into the kidney that is accompanied by the development of hypertension and renal disease. Since this disease phenotype closely resembles observations in patients, we performed studies to investigate the pathological role of immune cells in the kidney in hypertension and renal disease. Using zinc finger nuclease technology, we generated three mutant rat strains in the Dahl SS genetic background: one with a null mutation in recombination activating gene 1 (Rag1); a second with a null mutation in CD247 which encodes the CD3 zeta chain; and the third with an in-frame, 6 bp deletion in the Src Homology 2 domain of Sh2b3. As a result, we have rat strains with a full complement of T- and B-cells (the wild type Dahl SS), rats deficient in T cells with the Dahl SS genetic background (the CD247 mutant strain), rats deficient in T- and B-cells on the Dahl SS background (the Rag1 mutant rats), and rats with mutant Sh2b3 (a putative T-cell signaling molecule). Adding clinical significance to this work, mutations in CD247 and Sh2b3 are associated with hypertension and/or renal disease in patients. For example, studies on SS and Rag1 null rats fed a 4.0% NaCl diet for three weeks (n=5/group) demonstrated that infiltration of T-cells in the kidney following high salt was significantly blunted in Rag1 null rats (1.7±0.6 x 10^5 cells/kidney) compared to the Dahl SS (5.6±0.9 x 10^5 cells/kidney). Accompanying the reduction in T cells in the kidney, mean arterial blood pressure and urinary albumin excretion rat were significantly lower in Rag1 null mutants (158±3 mmHg and 60±16 mg/day, respectively) than in SS rats (180±11 mmHg and 251±37 mg/day). Finally, a histological analysis revealed that the glomerular and tubular damage in the kidneys of the SS rats fed high salt was also attenuated in the Rag1 mutants. Subsequent experiments demonstrated that mutation of CD247 and Sh2b3 also attenuates the infiltration of T-cells into

13.4
INFLAMMATION AND RENAL FIBROSIS
Steven Crowley¹

Interstitial fibrosis predicts organ failure in most tissues including the kidney. Macrophages have been implicated in the pathogenesis of kidney fibrosis, and type 1 angiotensin (AT₁) receptors are expressed on the macrophages that infiltrate the renal interstitium during the pathogenesis of hypertensive and normotensive kidney fibrosis. Recently, we examined the actions of AT₁ receptors on macrophages in progressive renal fibrosis. We found that deficiency of AT₁ receptors on macrophages exacerbates kidney fibrosis during angiotensin II-dependent hypertension or following unilateral ureteral obstruction (UUO). Macrophages isolated from obstructed kidneys of mice lacking AT₁ receptors solely on macrophages had heightened expression of pro-inflammatory M1 cytokines, including interleukin (IL-1), and kidney cross-transplant studies revealed that stimulation of IL-1 receptors in the kidney mediates the augmentation of renal fibrosis instigated by AT₁ receptor-deficient macrophages. In this session, we will report these findings and discuss signaling pathways through which the macrophage AT₁ receptor may regulate macrophage differentiation during renal fibrogenesis. (VA Medical Research Service BX-000893). Reference: Zhang, J. D., Patel, M. B., Griffiths, R., Dolber, P. C., Ruiz, P., Sparks, M. A., Stegbauer, J., Jin, H., Gomez, J. A., Buckley, A.F., Lefer, W. S., Chen, D., and Crowley, S. D. (2014). Type 1 angiotensin receptors on macrophages ameliorate interleukin-1 receptor-mediated kidney fibrosis. Journal of Clinical Investigation 124,2198-2203.

13.6
HIGH SALT, GUT MICROBIOME AND BLOOD PRESSURE
Dominik Muller¹
¹Cardiovascular, Experimental and Clinical Res. Ctr. & Max-Delbrück Ctr., Lindenberger Weg 80, Berlin, 13125, Germany.

The presentation will cover the role of high salt on the gut microbiome, the gut immune system and its consequences on salt-sensitive hypertension.

13.7
T CELL SERUM AND GLUCOCORTICOID-REGULATED KINASE 1 (SGK1) AND HYPERTENSION
Meena Madhur¹, and Allison Norlander²

T lymphocytes play a central role in the pathophysiology of hypertension by secreting cytokines that have a direct effect on the kidneys and vessel wall to both promote further increases in blood pressure and contribute to hypertensive end-organ damage. We previously showed that angiotensin II (Ang II) increases T cell production of the pro-inflammatory cytokine, interleukin 17A (IL-17A), and that mice deficient in IL-17A have blunted hypertension and reduced renal and vascular dysfunction. It was recently shown that salt enhances IL-17A production from CD4+ T helper cells via an SGK1 dependent pathway. SGK1 can be activated by multiple environmental stimuli including certain cytokines and Ang II. We hypothesized that SGK1 signaling in T cells is critical for T cells to promote hypertension and end-organ dysfunction. To test this hypothesis, we generated mice with T cell specific deletion of SGK1 (SGK1fl/fl x TgCD4cre). Loss of T cell SGK1 deletion compared to SGK1 floxed control mice. Moreover, renal and vascular inflammation was abrogated in these mice compared to SGK1 floxed control mice. Importantly, renal injury, assessed by albuminuria, was blunted in mice with T cell SGK1 deletion compared to control mice. Finally, vascular reactivity studies using isolated mesenteric vessels demonstrated that vessels from mice with T cell SGK1 deletion were protected from Ang II-induced endothelial dysfunction. Taken together, these studies demonstrate that T cell SGK1 is necessary for the full development of hypertension and hypertensive end-organ damage, and thus, this pathway may be a novel therapeutic target for hypertension. (NIH K08 HL121671, AHA Prevention SFRN, NIH F31 HL127986). References: Madhur et al. Hypertension 55: 500-507, 2010. Wu et al. Nature 496: 513-517, 2013. Kleinewietfeld et al. Nature 496: 518-522, 2013.
15.0: INFLAMMATION AND ATHEROSCLEROSIS

15.2
B CELL SUBSETS IN ATHEROSCLEROSIS

Coleen McNamara

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B cells have emerged as important immune cells in murine atherosclerosis, regulating lesion development in a subset-dependent manner. B-2 B cells promote atherosclerosis through poorly defined mechanisms, and B-1 B cells exert atheroprotective effects largely through production of natural IgM antibodies (NAb). Natural IgM to oxidation-specific epitopes (OSE) that accumulate in atherosclerosis such as malondialdehyde (MDA) and phosphorylcholine (PC) present on oxidized low-density lipoprotein can antagonize oxLDL stimulation of macrophages limiting inflammation. B-1 cells are the major source of circulating IgM in mice. A human equivalent to the murine B-1 cell was recently identified through its ability to spontaneously produce IgM and data implicates this cell in producing IgM to OSE on LDL. Plasma levels of IgM to MDA-LDL are associated with less CAD and fewer CV events in humans. As such, unraveling the pathways that lead to B-1 cell production of IgM to OSE may enable targeted immune strategies to bolster production of IgM to OSE on LDL and protect from atherosclerosis in humans. Our work has identified the helix-loop-helix factor, Id3 as a key regulator of B-1 cells in mice and humans and implicated chemokine receptors as functionally important proteins on B-1 cells that are regulated by Id3. Using loss and gain of function studies in murine atherosclerosis models, we identified a key role for chemokine receptors on B-1 cells in promoting their atheroprotective function. Moreover, analyzing a human cohort with indices of coronary artery plaque volume and stability measured by intravascular ultrasound-virtual histology (IVUS-VH), we implicate chemokine receptors in B-1 cell atheroprotection in humans. Support: NIH R01 HL107490 and P01 HL055798. Reference: Rosenfeld SM, Perry HM, Gonen A, Prohaska TA, Srikakulapu P, Grewal S, Das D, McSkimming C, Taylor AM, Tsimikas S, Bender TP, Witztum JL, McNamara CA. B-1b Cells Secrete Atheroprotective IgM and Attenuate Atherosclerosis Circ Res. 2015 Jul 17;117(3):c28-39.
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**Inflamation, Immunity, and Cardiovascular Disease**

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* Indicates Invited Speaker