Hosted by the UAB School of Medicine
Immunology, Autoimmunity and Transplantation Committee

Harry W. Schroeder, Jr, MD PhD
Hubert Tse, PhD

Co-Organizers of Symposium
Welcome to UAB!

On behalf of the Immunology, Autoimmunity and Transplantation Committee and the Program in Immunology of the University of Alabama at Birmingham School of Medicine, we would like to welcome the attendees of the first Spring Immunology Symposium: Immunologic Diseases and Basic Immunology to UAB.

There is a strong tradition in the United States of creating regional conferences in immunology with national and international implications. The Midwinter Conference of Immunologists, which has been held yearly in Asilomar, California, is now in its 52nd year; and the Autumn Immunology Conference, which is held yearly in Chicago, Illinois, is now in its 41st year. Our schools in the Southeastern Region of the United States have a strong tradition of basic, translational and clinical research in the fields of immunology, autoimmunity, vaccination and transplantation. It is our hope that we can build on the success of this first inaugural meeting to create our own symposium to meet the needs of our faculty, students and trainees for a forum where they can present and share their latest findings, find new opportunities for collaborations, and have the opportunity to hear and speak with outstanding immunologists from the nation and the world.

Harry W Schroeder Jr, MD, PhD  
Director, UAB Program in Immunology  
Co-Organizer, Spring Immunology Symposium

Hubert Tse, PhD  
Assistant Professor of Microbiology  
Co-Organizer, Spring Immunology Symposium

Robert Kimberly, MD  
Director, Comprehensive Arthritis, Musculoskeletal and Autoimmunity Center  
Co-Chair; Immunology, Autoimmunity and Transplantation Committee

Casey Weaver, MD  
Professor of Pathology  
Co-Chair; Immunology, Autoimmunity and Transplantation Committee

Ray L. Watts, MD  
Dean, School of Medicine  
Senior Vice President for Medicine  
UAB
**Acknowledgments**

*Supported by the UAB School of Medicine*

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<th>Thanks to the members of the <strong>UAB Immunology, Autoimmunity and Transplantation Committee</strong>!</th>
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<td>Allan Zajac, PhD</td>
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|* Co-Chair, UAB Immunology, Autoimmunity and Transplantation Committee  
** Co-director, Spring Immunology Symposium  
+, Member, Spring Immunology Symposium Planning and Hosting Committee |

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<th>Thanks for reviewing the abstracts submitted for presentation at the Spring Immunology Symposium!</th>
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<th>Thanks to Ada Elgavish, PhD, for developing the website for the symposium and for coordinating all the logistics of the symposium, from interaction with speakers, submission of abstracts by the trainees, venues for the various events, to developing a budget.</th>
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<th>Thanks to Paula Robinson for her efforts with the marketing of the event and for her assistance at the symposium.</th>
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| Thanks to Marsha Brand, RN, for her assistance at the symposium. |
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Agenda

Saturday, June 9, 2012

Place: Heritage Hall, room 102

7:30 am Pick up badges

Welcome
Harry W. Schroeder, Jr, MD, PhD
Professor of Medicine

8:00 – 8:15 a.m.
Division of Clinical Immunology and Rheumatology
Director, UAB Program in Immunology
Director, Immunologic Diseases & Basic Immunology Training Program
UAB

Basic and Human Immunology Session 1

Chairs: Dr. Jeremy M. Boss, Emory & Dr. Frances Lund, UAB

Jeremy (Jerry) Boss, PhD
Professor & Chair, Department of Microbiology and Immunology
Emory School of Medicine

8:15 – 8:45 a.m.
Title: Genetic and Epigenetic Regulation of Programmed Cell Death-1

Frances Lund, PhD
Professor & Chair, Department of Microbiology
UAB

8:45 – 9:15 a.m.
Title: Modulating allergic disease by controlling positioning of dendritic cells within the lymph

Keynote Speaker: Max D. Cooper, MD
Georgia Research Alliance Eminent Scholar
Professor of Pathology and Laboratory Medicine
Emory University School of Medicine

9:15 – 10:00 a.m.
Title: How did our adaptive immune system evolve?
10:00 – 10:15 a.m.  **Coffee Break**

**Hans Martin Jäck, PhD**
Head, Division of Molecular Immunology
Department of Internal Medicine III
University Hospital of the Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Title: Micro RNA controls plasma cell differentiation

10:15 – 10:45 a.m.

Xiaoqian Wang, Trainee, Emory University, Travel Award Recipient

Title: A division-linked mechanism for the generation of IL-10 producing human B cells

10:45 – 11:00 a.m.

Hao Li, Trainee, University of Alabama at Birmingham, Travel Award Recipient

Title: IL-23 controls autoimmunity by facilitating clearance of apoptotic bodies in the marginal zone

11:00 – 11:15 a.m.

Rachel A. Henry, PhD, Trainee, Vanderbilt University, Travel Award Recipient

Title: Autoantigen-Specific B Lymphocyte Depletion Overcomes Failed Immune Tolerance in Type 1 Diabetes

11:15 – 11:30 a.m.

Sarah Mollo, Trainee, University of Alabama at Birmingham, Travel Award Recipient

Title: B Cells Have Multiple Roles for the Generation of CD4 T Cell Memory and Recall Responses

11:45 – 12:00 p.m.  **Trainee Awards Ceremony**

12:00 - 1:00 p.m.  **Lunch**

**Basic and Human Immunology Session 2**

**Chairs:** Dr. Casey Weaver, UAB & Dr. James E. Crowe, Vanderbilt

**George Georgiou, PhD**
Department of Chemical Engineering
Department of Biomedical Engineering
Institute for Cell and Molecular Biology
The University of Texas at Austin

Title: Serum Immunoglobulins and V Gene Repertoires

1:00 - 1:30 p.m.
James Crowe, MD
Ingram Professor of Cancer Research
Professor of Pediatrics, Microbiology and Immunology
Director, Vanderbilt Alliance for Nanomedicine
Director, Vanderbilt Program in Vaccine Sciences
Vanderbilt School of Medicine

Title: Genetic and structural basis for development of human neutralizing antibodies against viruses

Harry W Schroeder Jr, MD PhD
Professor of Medicine
Division of Clinical Immunology and Rheumatology
Director, UAB Program in Immunology

Title: Diseases of Immune Function and the Structured Antibody Repertoire: Arginine delenda est!

Thomas (Tom) M. Aune, PhD
Professor of Medicine
Vanderbilt University Medical Center

Title: Diverse functions of distal regulatory elements at the IFNγ locus

Keynote Speaker: Lawrence Steinman, MD
Professor of Neurology & Neurological Sciences
Stanford University, CA

Title: Mechanistic Biomarkers in Autoimmune Disease

3:00 – 3:20 p.m.  Coffee Break

Brian Evavold, PhD
Associate Professor of Microbiology and Immunology
Emory University

Title: Frequency and two dimensional affinity of CD4+ T cells

Troy Randall PhD
Professor of Medicine
Division of Rheumatology and Clinical Immunology
UAB

Title: The control of Tfh and germinal centers by IL-2
John F. Kearney, PhD
Professor of Microbiology
UAB

Title: Antibodies and the Hygiene Hypothesis

5:15 - 5:45 p.m.  Business Meeting - Heritage Hall, room 422

Change of Place:  Alumni Hall

6:00 – 8:00 p.m.  Reception and Poster Session

Sunday, June 10, 2012

Place:  Heritage Hall, room 102

Immunologic Diseases Session

Chairs:  Dr. Ignacio (Iñaki) E. Sanz, MD, Emory & Dr. James (Tom) W Thomas, II, Vanderbilt

Welcome
Robert P. Kimberly, MD
8:15 – 8:30 a.m.
Howard L. Holley Professor of Medicine
UAB
Director, Comprehensive Arthritis, Musculoskeletal and Autoimmunity Center

S. Louis Bridges, Jr., MD, PhD
Marguerite Jones Harbert - Gene V. Ball, MD Professor of Medicine
Director, Division of Clinical Immunology & Rheumatology
UAB

Title: The Role of B Lymphocytes in Rheumatoid Arthritis

Ignacio (Iñaki) E. Sanz, MD
Professor of Medicine
Director, Division of Rheumatology
Georgia Research Alliance Eminent Scholar
Director, Kathleen B. and Mason I. Lowance Center for Human Immunology
Emory University

Title: Using B cells to understand and treat SLE

Peggy Kendall, MD
Assistant Professor of Medicine (Allergy/Immunology)
Vanderbilt University Medical Center

Title: The Role of B Lymphocytes in Type 1 Diabetes
Casey Weaver MD  
10:00 – 10:30 a.m.  
Professor of Pathology  
UAB

10:30 – 11:00 a.m.  
Coffee Break

Jian Han, MD PhD  
Faculty Investigator  
11:00 – 11:20 a.m.  
HudsonAlpha Institute for Biotechnology  
Title: High throughput sequencing of immune repertoire: Technology and applications

Sung Sam Lim, MD MPH  
Associate Professor of Medicine  
11:20 – 11:40 a.m.  
Emory University School of Medicine  
Title: The Disparities of Systemic Lupus Erythematosus

Charles O. Elson, III, M.D.  
Professor of Medicine  
11:40 – 12:00 a.m.  
University of Alabama at Birmingham  
Title: Innate and Adaptive Immunity in IBD

Keynote Speaker: Noel Rose, MD PhD  
Professor,  
Director, Center for Autoimmune Disease Research  
12:00 – 1:00 p.m.  
Department of Molecular Microbiology and Immunology  
Johns Hopkins Bloomberg School of Public Health  
Title: Immunologic Diseases: Views of the Past, Visions of the Future
1. **Israr Ahmad**, Purushotham Guroji, Hugo Jimenez, Eva Simanyi, Anusuiya Nagar, Priyamvada Nagar, Nabiha Yusuf. **Toll-Like Receptor-4 mediated cutaneous immune responses augment ultraviolet radiation induced DNA damage and tumor development.** Department of Dermatology and Skin Diseases Research Center, University of Alabama at Birmingham.

2. **Rakieb Andargachew**¹, Cheng Zhu², Brian Evavold¹. **2D Micropipette Detection of Mycobacterium Antigen 85B Specific Low Affinity CD4+ T Cells in the Response to Peptide Immunization and Mycobacterium marinum Infection.** ¹Department of Microbiology and Immunology, Emory University, Atlanta, GA. ²Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA.

3. **Jeremy Auerbach**¹ and Vitaly V. Ganusov¹⁻². **Mathematical modeling reveals kinetics of lymphocyte recirculation between major murine organs.** ¹Department of Mathematics, ²Department of Microbiology, University of Tennessee, Knoxville.

4. **André Ballesteros-Tato**¹, Beatriz León¹, Beth A. Graf¹, Amy Moquin², Pamela Scott Adams², Frances E. Lund¹ and Troy D. Randall¹. **Interleukin-2 inhibits germinal center formation by limiting T follicular helper differentiation.** ¹Department of Medicine, Division of Clinical Immunology and Rheumatology. University of Alabama, Birmingham, AL ²Trudeau Institute, Saranac Lake, NY.

5. **J. Lori Blanchfield**¹, Maria Bettini², Cheng Zhu³, and Brian Evavold¹. **The contributions of high affinity, MOG-specific T cell clones to the course of murine demyelinating autoimmune disease.** ¹Department of Microbiology and Immunology, Emory University, Atlanta, GA. ²Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN. ³Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA.

6. **Davide Botta**¹, André Ballesteros-Tato¹, Kyle Martin², Louise Hartson², Tirumalai Rangasamy², Thomas J. Mariani², Troy D. Randall¹, Debra A. Cockayne³, Christopher S. Stevenson³, Frances E. Lund¹. **Deficiency Of The Transient Receptor Potential Melastatin 2 (TRPM2) Cation Channel Provides Protection Against Pulmonary Inflammation In A Murine Model Of Chronic Obstructive Pulmonary Disease (COPD).** ¹Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA. ²Department of Medicine, University of Rochester Medical Center, Rochester, NY, USA. ³Hoffmann-La Roche Inc., Pharma Research & Early Development (pRED), DTA Inflammation, Nutley, NJ, USA.

8. Juan J. Calix¹,², Allison M. Brady², Robert L. Burton³, Thomas R. Larson², Janet Yother², Moon H. Nahm³. **Discovery of subtypes in serotype 20 of Streptococcus pneumoniae.** ¹University of Alabama at Birmingham, Medical Science Training Program, ²University of Alabama at Birmingham, Department of Microbiology, ³University of Alabama at Birmingham, Department of Pathology.

9. Amber L. Buel², Rodrigo Naves⁴, Tethia Mbana³, Chander Raman²,³ and Patrizia De Sarno¹. **Therapeutic effectiveness of lithium in EAE is dependent on IFN-γ signaling.** Departments of ¹Psychiatry and Behavioral Neurobiology, ²Microbiology, ³Medicine, University of Alabama at Birmingham, Birmingham, AL, and ⁴Catholic University, Santiago, Chile.

10. Ashley R. Burg¹,²,³, and Hubert M. Tse, PhD²,³. **Lack of Phagocytic NADPH Oxidase Activity Decreases Anti-Viral Innate Immune Responses In Type 1 Diabetes.** Immunology Theme- UAB Graduate Biomedical Sciences¹, UAB Department of Microbiology² and the UAB Comprehensive Diabetes Center³.

11. Kevin S. Cashman³, Christine Sestero¹, Patrizia De Sarno⁴, and Chander Raman¹,². **CD5 through CK2 activation regulates B1-a B cell survival, proliferation, and both artificial and physiologic T-independent type II responses.** Departments of Medicine¹, Clinical Immunology and Rheumatology², Microbiology³, and Psychiatry and Behavioral Neurobiology⁴, University of Alabama at Birmingham, Birmingham, AL, 35294.

12. Benjamin Christmann¹, Zdenek Hel¹, Charles Bernstein², Bengt Björkstén³, Maria Jenmalm⁴, Charles O. Elson¹. **The Human Adaptive Immune Response to Intestinal Microbiota Antigens.** ¹University of Alabama at Birmingham, Birmingham AL, USA ²University of Manitoba, Winnipeg, Manitoba, Canada ³ Karolinska Institutet, Stockholm, Sweden, ⁴Linköping University, Linköping, Sweden.

13. Sarah P. Collier*, Patrick L. Collins†*, Christopher L. Williams*, Mark R. Boothby† and Thomas M. Aune†*. **Tmevpg1, a long intergenic non-coding RNA, is required for Ifng expression by Th-1 cells.** Department of Pathology, Microbiology and Immunology and †Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232.

14. Lindsay J. Edwards and Brian D. Evavold. **T cell specific deletion of the phosphatase SHP-1 decreases the incidence and severity of experimental autoimmune encephalomyelitis.** Emory University Department of Microbiology and Immunology.

15. Michael W. Edwards¹, Ping Zhang², Suzanne M. Michalek¹ and Jannet Katz¹,². **Regulation of Akt phosphorylation by mTOR and MAP kinases during Francisella tularensis LVS infection of murine macrophages.** Departments of Microbiology¹ and Pediatric Dentistry², University of Alabama at Birmingham, Birmingham, Alabama 35294, USA.

16. Daniel R. Frederick, James B. McLachlan. **Tissue-specific Antigen Presentation during persistent Salmonella typhimurium infection.** Department of Microbiology and Immunology, School of Medicine, Tulane University.
17. Hao Li\textsuperscript{1,2}, Hui-Chen Hsu\textsuperscript{2}, Jun Li\textsuperscript{2}, PingAr Yang\textsuperscript{2}, Qi Wu\textsuperscript{2}, Daniel J Cua\textsuperscript{3}, Mohamed Oukka\textsuperscript{4}, John D Mountz\textsuperscript{1,2,5}. \textbf{IL-23 controls autoimmunity by facilitating clearance of apoptotic bodies in the marginal zone.} \textsuperscript{1}Microbiology, University of Alabama at Birmingham, Birmingham, AL, \textsuperscript{2}Division of Clinical Immunology & Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, \textsuperscript{3}Merck Research Laboratory, Palo Alto, CA, \textsuperscript{4}Seattle Children's Research Institute, University of Washington, Seattle, WA, \textsuperscript{5}Birmingham VA Medical Center, Birmingham, AL, United States.

18. Ashley S. Harms\textsuperscript{1,2}, Aaron D. Thome\textsuperscript{1,2}, David G. Standaert\textsuperscript{1,2}. \textbf{The role of fractalkine signaling in an AAV-alpha-synuclein model of PD.} \textsuperscript{1}Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham, \textsuperscript{2}Department of Neurology, University of Alabama at Birmingham.

19. Rachel A. Henry\textsuperscript{1}, Jonathan M. Williams\textsuperscript{2}, Amita Rachakonda\textsuperscript{1}, Peggy L. Kendall\textsuperscript{3}, and James W. Thomas\textsuperscript{1,2}. \textbf{Autoantigen-Specific B Lymphocyte Depletion Overcomes Failed Immune Tolerance in Type 1 Diabetes.} \textsuperscript{1}Vanderbilt University, Division of Rheumatology and Immunology, Department of Medicine, \textsuperscript{2}Vanderbilt University, Department of Pathology, Microbiology, and Immunology, \textsuperscript{3}Vanderbilt University, Division of Allergy, Pulmonary, and Critical Care, Department of Medicine.

20. Kristen L. Hoek\textsuperscript{1}; Tara Allos\textsuperscript{1}; Leigh M. Howard\textsuperscript{2}; Parimal Samir\textsuperscript{1}; Xinnan Niu\textsuperscript{1}, Kathryn M. Edwards\textsuperscript{2}, Andrew J. Link\textsuperscript{1}. \textbf{Optimized Human Immune Cell Isolation for Application in Systems Vaccinology.} \textsuperscript{1}Department of Pathology, Microbiology, and Immunology; \textsuperscript{2}Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee.

21. Amelia R. Hofstetter\textsuperscript{*}, Aron E. Lukacher\textsuperscript{†}. \textbf{Virus-specific class Iib-restricted CD8 T cells are protective across MHC class Ia haplotype barriers.} \textsuperscript{*}Department of Microbiology & Immunology, Emory University, Atlanta, Georgia 30322, USA. \textsuperscript{†}Department of Microbiology & Immunology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033, USA.

22. Jennifer D. Hood\textsuperscript{1}, Cheng Zhu PhD\textsuperscript{2}, Brian D. Evavold PhD\textsuperscript{1}. \textbf{Determining the antigen specificity of diabetogenic BDC2.5 cells.} \textsuperscript{1}Emory University, Atlanta, GA, \textsuperscript{2}Georgia Institute of Technology, Atlanta, GA.

23. Dennis J. Horvath, Jr.\textsuperscript{1,2}, Jennifer A. Gaddy\textsuperscript{1,2}, Vicki A. Cope\textsuperscript{1,2}, and Holly M. Scott Algood\textsuperscript{1,2}. \textbf{Presence of functional type IV secretion system impacts ability of mouse neutrophils to engulf Helicobacter pylori.} VA Tennessee Valley Healthcare System\textsuperscript{1}, Vanderbilt University Department of Medicine\textsuperscript{2}, Nashville, TN, USA.

24. Ji Young Hwang, Javier Rangel-Moreno, Maria de la Luz Garcia-Hernandez, and Troy D. Randall. \textbf{Role of Inducible Bronchus-Associated Lymphoid Tissue (iBALT) in Allergic Airway Disease.} Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA, 14624.

25. Jun Li\textsuperscript{1}, Hui-Chen Hsu\textsuperscript{1,2}, PingAr Yang\textsuperscript{1}, Qi Wu\textsuperscript{1}, Hao Li\textsuperscript{1}, David M Spalding\textsuperscript{1}, W. Winn Chatham\textsuperscript{1}, Robert P Kimberly\textsuperscript{1}, S. Louis Bridges\textsuperscript{1}, John D Mountz\textsuperscript{1,2}. \textbf{Death Receptor 5 (DR5) marks the highly pathogenic interacting GM-CSF\textsuperscript{+} T helper cells and IL-23\textsuperscript{+} macrophages rendering it as an attractive therapeutic target of autoimmunity.} \textsuperscript{1}Division of Clinical Immunology and Rheumatology,
26. Tetyana V. Pedchenko, Allison M. Sullivan, James B. Case, Peggy L. Kendall. **S100A4 is expressed in myeloid cells invading inflamed pancreatic islets and supports development of Type 1 diabetes.** Vanderbilt University Medical Center

27. Mohamed Khass, Yingxin Zhuang and Harry Schroeder. **Surrogate light chain component of the Pre-BCR selects for immunoglobulin heavy chains based on their CDR-H3 contents.** Departments of Medicine and Microbiology, University of Alabama at Birmingham

28. Zachary A.-F. Kistka, Dan Moore, Peggy Kendall, Tom Thomas. **Antigen specificity in oral tolerance: the role of the anti-insulin B lymphocyte.** Vanderbilt University Medical Center.

29. Kate Kosmac¹,², Glenn Bantug², Stipan Jonjic³ and William J. Britt²,⁴. **Inflammation, following MCMV infection, results in altered cerebellar development.** Neuroscience Program¹, Department of Microbiology², and Pediatric Infectious Diseases⁴, University of Alabama at Birmingham, AL, Department of Anatomy and Histology, Faculty of Medicine: University of Rijeka, Croatia³.

30. Kurtz, Jonathan R and McLachlan, James B. **Liver induced immunotolerance by Salmonella-specific CD4+ T-cells during chronic infection.** Department of Microbiology & Immunology, Tulane University School of Medicine, New Orleans, Louisiana.

31. Srilalitha Kuruganti¹, Shane Miersch², Ashlesha Deshpande, Bethany D. Harris, Kumar Putcha¹, Sachdev Sidhu², Winn Chatham³, Mark R. Walter¹. **Quantitative detection of Interferon-α subtypes in lupus patients.** ¹Department of Microbiology, ³Division of Clinical Immunology and Rheumatology, University of Alabama, Birmingham, ²University of Toronto-Canada.

32. Vy L. Le and Richard W. Comans. **Influenza A virus pathogenesis: Identification of host factors that contribute to severe respiratory distress.** Department of Microbiology and Immunology, Emory Vaccine Center, Emory University, School of Medicine.

33. Beatriz León¹, André Ballesteros-Tato¹, Jeffrey L. Browning², Robert Dunn³, Troy D. Randall¹ and Frances E. Lund¹. **B cell-dependent positioning of CXCR5-expressing dendritic cells drives Th2 development.** ¹University of Alabama at Birmingham, AL, ²Biogen Idec, Cambridge MA USA and San Diego CA, ³Pfizer-Centers for Therapeutic Innovation, San Diego CA.

34. M.S. Levinson, A. Silva-Sanchez, Y. Zhuang and H.W. Schroeder, Jr. **Role of Dβ germline sequence on constraining TCR CDRβ3 diversity.** Department of Medicine, University of Alabama at Birmingham.

35. Seth Levy, Anandi Sawant, and Selvarangan Ponnazhagan. **Myeloid-derived suppressor cells as key immune regulators of non-union fractures.** Department of Pathology, University of Alabama at Birmingham.

36. Yudong Liu¹, Andrew T. Holdbrooks¹, Patrizia De Sarno ², Lora L. Yanagisawa¹, Braden McFarland¹, Laurie E. Harrington¹, Chander Raman³, Etty N. Benveniste ¹ and Hongwei Qin¹. **Therapeutic**
efficacy of the JAK1/JAK2 inhibitor AZD1480 in experimental autoimmune encephalomyelitis.
Departments of Cell, Developmental and Integrative Biology\textsuperscript{1}, Psychiatry and Behavioral Neurobiology\textsuperscript{2} and Medicine\textsuperscript{3}, University of Alabama at Birmingham.

37. Jian Han\textsuperscript{1,2}, Chunlin Wang\textsuperscript{1}, Quanying Yang\textsuperscript{1,2}, Catherine M. Sanders\textsuperscript{1,2}, Jessica McClellan\textsuperscript{1}, Miranda Byrne-Steele\textsuperscript{1,2}, C. Lu\textsuperscript{1}. Identification and temporal monitoring of breast cancer-associated T cell receptors with high throughput sequencing. \textsuperscript{1}iRepertoire, 601 Genome Way, Huntsville, AL, United States. \textsuperscript{2}HudsonAlpha Institute for Biotechnology, 601 Genome Way, Huntsville, AL, United States.

38. Lisa M. McEwen\textsuperscript{1}, Cac T. Bui\textsuperscript{1}, Yvonne Paterson\textsuperscript{2} and Donald A. Harn\textsuperscript{1}. Driving HIV-specific vaccine responses in immune suppressed recipients. \textsuperscript{1}Department of Infectious Diseases, University of Georgia, Athens, GA, USA, \textsuperscript{2}Department of Microbiology, University of Pennsylvania, Philadelphia, PA.

39. Donald McGuire\textsuperscript{1}, Christine Sestero\textsuperscript{2}, Chander Raman\textsuperscript{2}. CD5 Enhances T cell cytokine signaling. Department of Microbiology\textsuperscript{1}, Department of Medicine\textsuperscript{2} University of Alabama at Birmingham.

40. Ian L. McWilliams and Laurie E. Harrington. A critical role for STAT4 in the accumulation of CD4 T cells in the CNS during EAE. University of Alabama at Birmingham.

41. Gordon P. Meares, Hongwei Qin, and Etty N. Benveniste. Regulation of STAT1 by AMPK Signaling in Astrocytes. Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham.

42. Mollo, S. and Harrington, L.E. B Cells Have Multiple Roles for the Generation of CD4 T Cell Memory and Recall Responses. University of Alabama at Birmingham

43. Tahseen H Nasti\textsuperscript{1}, Kyle Rudemiller\textsuperscript{1}, George Twitty\textsuperscript{1}, Hee Kyung Kim\textsuperscript{1,2}, Yuko Tsuruta\textsuperscript{1,2}, Mohammad Athar\textsuperscript{1,2}, Craig Elmets\textsuperscript{1,2} and Laura Timares\textsuperscript{1,2,3}. Effective immunoprevention against chemical carcinogenesis is induced by genetic immunization vectors that selectively expand mutant H-ras-specific CD8 T cells. \textsuperscript{1}Department of Dermatology, \textsuperscript{2}UAB Skin Diseases Research Center, \& \textsuperscript{3}Division of Human Gene Therapy, The University of Alabama at Birmingham School of Medicine.

44. Michelle H Nelson\textsuperscript{1}, Logan W Huff\textsuperscript{1}, Carolyn E Rogers\textsuperscript{1}, Sreenath Kundimi\textsuperscript{1}, and Chrystal M Paulos\textsuperscript{1,2}. Inducible costimulator augments antitumor Tc17 cell activity. \textsuperscript{1}Department of Microbiology and Immunology; and \textsuperscript{2}Department of Surgery, Hollings Cancer Center, Medical University of South Carolina (MUSC), Charleston, SC.


46. Catherine H. Poholek\textsuperscript{1,2}, Laurie E. Harrington\textsuperscript{3}. Interleukin-21 is required for the pathogenesis of inflammatory bowel disease. \textsuperscript{1}Medical Scientist Training Program, \textsuperscript{2}Graduate Biomedical Sciences, Immunology Theme, \& \textsuperscript{3}Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham.
47. Travis Ptacek, Chuanyi Ji, Xinrui Li, Jeffrey C Edberg, Robert P Kimberly. **Association of a novel fc-gamma receptor 2B (FCGR2B) variant with systemic lupus erythematosus (SLE).** Department of Medicine, Division of Clinical Immunology &n Rheumatology, University of Alabama at Birmingham.

48. Whitney Rabacal, Delphine Cendron, Kristen Hoek, Sudheer Pabisetty, Eric Sebzda. **Küppel like factor 2 in innate like lymphocyte development and function.** Department of Pathology Microbiology and Immunology, Vanderbilt University, Nashville.

49. T.D. Rohrbach, J.S. Deshane, C. Steele, C.D. Willey. **The Affect Macrophage Conditioned Media has on Lung Cancer Cell Line Migration, Proliferation and Radiosensitivity.** 1Department of Radiation Oncology and 2Department of Medicine, University of Alabama at Birmingham.

50. Anandi Sawant, Jonathan Hensel, Jessy Deshane, Brittney Harris, Diptiman Chanda, Carnella Lee, and Selvarangan Ponnazhagan. **Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis of the breast cancer cells.** Departments of Pathology and Medicine, University of Alabama at Birmingham.

51. Cara C. Schaefer, Anandi Sawant, Tong Huan Jin, Jaroslav Zmijewski, Hubert Tse, Justin Roth, Zhihuan Sun, Gene P. Siegal, Victor J. Thannickal, Stefan C. Grant, Selvarangan Ponnazhagan, and Jessy S. Deshane. **Targeting regulatory myeloid cell pathways to enhance anti-tumor immunity and long-term survival against lung cancer.** 1Departments of Medicine, 2Pathology, 3Microbiology & 4Pediatrics, University of Alabama at Birmingham.

52. Christine M. Sestero, Rodrigo Naves, Simer Preet Singh, Patrizia De Sarno, and Chander Raman. **Negative feedback regulation of encephalitogenic Th1 Cells by IFNγ in experimental autoimmune encephalomyelitis.** 1University of Alabama at Birmingham, Birmingham, AL, 2Universidad Pedro de Valdivia, Santiago, Chile, 3University of Rochester Medical Center, Rochester, NY.

53. Charles F. Spurlock, III, John T. Tossberg, Brittany K. Matlock, Lily Wang, Nancy J. Olsen, Subramaniam Sriram, Thomas M. Aune, PhD, Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, 2John T. Tossberg, MS, Research Department, ArthroChip, Franklin, Tennessee, 3Brittany K. Matlock, BS, Vanderbilt Vaccine Center, Vanderbilt University School of Medicine, Nashville, TN, 4Lily Wang, Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, 5Nancy J. Olsen, MD, Department of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, PA, 6Subramaniam Sriram, MD, Department of Neurology, Vanderbilt University School of Medicine, Nashville, TN, Thomas M. Aune, PhD, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.

54. Emily K. Stefanov, Nicholas W. Kin, Brian L.P. Dizon and John F. Kearney. **Antibodies against conserved antigens suppress allergic airway disease.** Department of Microbiology, University of Alabama at Birmingham.

Identical, HLA*B44 Female Twins Discordant for Common Variable Immunodeficiency and Recurrent Sino-Pulmonary Infection. Departments of Microbiology¹, Medicine², Biochemistry ³, Epidemiology ⁴ University of Alabama at Birmingham, Department of Chemical and Molecular Engineering⁵ University of Texas at Austin, and Department of Pediatrics ⁶ University Marburg, Germany.

56. Scott M. Tanner and Robin G. Lorenz. Decreased regulatory T cell numbers in the intestine precede the development colitis in the FVB.mdr1a⁻/- mouse. Department of Pathology, University of Alabama at Birmingham.

57. Xiaoqian Wang¹, James Roger², Ichikawa Travis Ichikawa³, Chungwen wei⁴, Ignacio Sanz⁵. A division-linked mechanism for the generation of IL-10 producing human B cells. ¹Division of Rheumatology and Immunology, Department of Medicine, Emory University ²Department of Medicine, University of Rochester.

58. Leticia Watkins, Zina Moldoveanu, Wen-Qiang Huang and Harry Schroeder. Enriching the BCR repertoire with charged CDR-H3s impairs protection against heterosubtypic influenza virus infection. Department of Medicine and Microbiology, University of Alabama at Birmingham.


60. LaTonya D. Williams¹, Steffanie Sabbaj², Anju Bansal², Tiffanie Mann², Victor Du¹, Nilesh Amatya², and Paul A. Goepfert¹². CD4-like Immunologic Function by IL-21-producing CD8 T cells in HIV-1-infected Individuals. Department of Microbiology¹, Department of Medicine², University of Alabama at Birmingham.

61. Colleen J Winstead, Sing Sing Way, James J Moon, and Casey T Weaver. Using cytokine reporter mouse models to track CD4 T cell memory in vivo. ¹University of Alabama at Birmingham, Department of Pathology, Birmingham, AL, ²University of Minnesota, Department of Pediatric Medicine, Minneapolis, MN, ³Massachusetts General Hospital, Department of Medicine, Boston, MA.
Abstracts
Toll-Like Receptor-4 mediated cutaneous immune responses augment ultraviolet radiation induced DNA damage and tumor development.

Israr Ahmad, Purushotham Guroji, Hugo Jimenez, Eva Simanyi, Anusuiya Nagar, Priyamvada Nagar, Nabiha Yusuf

Department of Dermatology, and Skin Diseases Research Center, University of Alabama at Birmingham, Birmingham, AL.

UV (ultraviolet) B induced DNA damage plays a critical role in development of skin cancer. The molecular basis for this biologic activity resides at least in part from the ability of UVB to damage DNA, predominantly in the form of cyclobutane pyrimidine dimers (CPD). When UVB induces DNA damage in cells, there is a meticulous attempt to repair it through the activation of DNA repair genes. Toll-like receptors (TLR), one component of innate immunity, are intricately associated with host immunity, and their involvement now extends to host responses to cancer. We have observed that UVB-induced DNA damage is greatly reduced in TLR4 deficient mice indicated by fewer CPD in their skin. We hypothesize that deficiency in TLR4 will prevent suppression of antigen presenting cells to produce IL-12, following UVB exposure. This will cause efficient DNA repair of CPD and fewer tumors in TLR4 deficient mice. The purpose of this study was to determine the mechanisms through which TLR4 contributed to UVB-induced cutaneous DNA damage responses and tumor development. To evaluate UVB induced DNA damage, TLR4 deficient and wild type (WT) mice were subjected to a single dose of UVB radiation (90mJ/cm$^2$) on their dorsal skin. There was significant ($p<0.05$) DNA damage in the form of CPD in the skin of wild type (WT) mice than the skin of TLR4 deficient mice. There were fewer CPD in bone marrow dendritic cells (BMDC) from TLR4 deficient mice than WT mice. The expression of DNA repair gene, Xeroderma pigmentosum complementation group A (XPA) was significantly less ($p<0.05$) in skin and BMDC from WT mice than TLR4 deficient mice after UVB exposure. When cytokine levels were compared in these two strains after UVB exposure, UVB-irradiated BMDC from TLR4 deficient mice produced significantly more IL-12 and IL-23 cytokines ($p<0.05$) than BMDC from WT mice. To evaluate UVB induced tumor development, TLR4 deficient and WT mice were subjected to a multiple doses of UVB radiation (200mJ/cm$^2$) for 40 weeks. We observed that UVB induced skin carcinogenesis was retarded in terms of tumor incidence, and tumor latency, in TLR4 deficient mice than WT mice, whereas significantly greater ($p<0.05$) numbers of tumors occurred in WT mice. CD4$^+$CD25$^+$ regulatory T-cells from WT mice produced more IL-10 ($p<0.05$) than regulatory T-cells from TLR4 deficient mice. There was significant increase ($p<0.05$) in Foxp3 expression in skin of WT mice than TLR4 deficient mice. Thus, strategies to inhibit TLR4 may allow us to develop immunopreventive and immunotherapeutic approaches for management of UVB induced cutaneous DNA damage and skin cancer.

This work was supported by Research Career Development Award from Dermatology Foundation, and New Investigator Award from Department of Defense (W81XWH-10-1-0763) to Dr. Nabiha Yusuf.
2D Micropipette Detection of *Mycobacterium* Antigen 85B Specific Low Affinity CD4+ T Cells in the Response to Peptide Immunization and *Mycobacterium marinum* Infection

Rakieb Andargachew¹, Cheng Zhu², Brian Evavold¹

¹Department of Microbiology and Immunology, Emory University, Atlanta, GA  
²Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

The widely used method for tracking antigen specific T cells is major histocompatibility (MHC) class I and II tetramers. The efficiency of these reagents relies on the monomeric T cell receptor (TCR) affinity for peptide MHC (pMHC) and as a result only high affinity T cells are detected with this technique. A novel method used for measuring affinity and frequency of antigen specific cells is a two dimensional (2D) micropipette adhesion frequency assay. Using this method, we have previously shown that low affinity MHC II tetramer negative I$\alpha$$\beta$ GP$_{66-77}$ specific CD4+ T cells participate in the immune response to lymphocytic choriomeningitis virus (LCMV) infection and are more prevalent than high affinity tetramer positive responders. To determine if low affinity cells also participate in the response to other pathogens, we examined *Mycobacterium Antigen 85B$_{280-294}$* (Ag85B) specific CD4+ T cells in the response to peptide immunization and *Mycobacterium marinum* infection in the C57Bl/6 strain. As compared to tetramer, 2D micropipette identified more I$\alpha$ Ag85B$_{280-294}$ specific CD4+ T cells from lymph nodes of peptide immunized mice. Of interest, continued in vitro restimulation maintained a wide range in overall affinity including the population of low affinity tetramer negative T cells. Analysis of the T cell response after infection with *M. marinum* showed low affinity cells were also prevalent with an overall 100 fold range of affinities being represented.
Mathematical modeling reveals kinetics of lymphocyte recirculation between major murine organs

Jeremy Auerbach\textsuperscript{1} and Vitaly V. Ganusov\textsuperscript{1,2}

\textsuperscript{1}Department of Mathematics, Knoxville, TN 37996, USA
\textsuperscript{2}Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA

Email: vitaly.ganusov@gmail.com

Migration patterns of naive, effector, and memory lymphocytes have important implications for activation of primary and secondary immune responses and for efficient control of pathogens in peripheral tissues. Yet, quantitative details of how long it takes for lymphocytes to migrate from one tissue to another has been poorly quantified. Using novel mathematical models we estimate the rates of migration of thoracic duct lymphocytes (TDLs) from blood to multiple lymphoid and nonlymphoid tissues of a rat and migration rates of TDLs back to the blood. Our analysis suggests extremely rapid recirculation of TDLs between blood, lung, and liver with an average residence time of cells in these tissues being around 1 minute. The model also predicts longer residence times of TDLs in the spleen (2 hours) and major lymph nodes and Peyer’s patches (9 hours). The same model provides a good match for independently measured output of TDLs via the thoracic duct in cannulated animals. By comparing accumulation of transferred lymphocytes in resting and antigen-stimulated lymph nodes we predict that enlargement of lymph nodes occurs because of increased entrance of lymphocytes into the inflamed LN and not because of decreased output as has been previously argued. Thus, in this work we provide for the first time the kinetics of entrance and exit of lymphocytes into multiple organs of rats. We also illustrate how within one modeling framework we can accurately describe multiple phenomena on lymphocyte recirculation in the whole organism including steady state distribution of lymphocytes in unmanipulated animals. This framework also allows to predict changes in lymphocyte distribution in the whole organism following local inflammation or treatments affecting lymphocyte entrance into or exit from lung or lymph nodes.
Interleukin-2 inhibits germinal center formation by limiting T follicular helper differentiation

André Ballesteros-Tato¹, Beatriz León¹, Beth A. Graf¹, Amy Moquin², Pamela Scott Adams², Frances E. Lund¹ and Troy D. Randall¹*

¹Department of Medicine, Division of Clinical Immunology and Rheumatology. University of Alabama, Birmingham, AL 35294-2182 ²Trudeau Institute, Saranac Lake, NY 12983

T follicular helper (Tfh) cells promote T-dependent humoral immune responses by providing T cell help to B cells and by promoting germinal center (GC) formation and long-lived antibody responses. However, the cellular and molecular mechanisms that control Tfh differentiation in vivo are incompletely understood. Here we show that interleukin-2 (IL-2) administration impaired influenza-specific GCs, long-lived IgG responses and Tfh cells. IL-2 did not directly inhibit GC formation, but instead suppressed the differentiation of Tfh cells, thereby hindering the maintenance of influenza-specific GC B cells. Our data demonstrate that IL-2 is a critical factor that regulates successful Tfh and B cell responses in vivo and regulates Tfh cell development.

This work was supported by the University of Rochester and by NIH grants HL069409, AI072689 and AI061511 to T.D.R. and NIH grants AI078907 and AI068056 to F.E.L.
The contributions of high affinity, MOG-specific T cell clones to the course of murine demyelinating autoimmune disease.

J. Lori Blanchfield¹, Maria Bettini², Cheng Zhu³, and Brian Evavold¹
1. Department of Microbiology and Immunology, Emory University, Atlanta, GA. 2. Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN. 3. Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA.

The design of antigen specific therapies for patients with demyelinating autoimmune disease, such as multiple sclerosis (MS), is dependent on a clear understanding of how pathogenic T cell populations recognize self-antigen. In the experimental autoimmune encephalomyelitis (EAE) model of MS, CD4⁺ T cell infiltrates from the central nervous system (CNS) exhibited a range of affinities (low to high) for MOG peptide:MHC class II during peak disease. Preliminary data have indicated that high and low affinity T cell populations fluctuate over time and that this dynamic can influence disease progression. Our hypothesis, based on these data, is that T cell affinity dictates the severity and chronic nature of autoimmune disease in the CNS. We propose to study this dynamic with high and low affinity T cells clones, particularly because of the rarity of isolating sufficient numbers of high affinity T cells ex vivo. We isolated a high affinity T cell clone, expressing the Vα4β1 T cell receptor (TCR), from mice primed with the myelin oligodendrocyte glycoprotein (MOG) self antigen. This clone has a high affinity TCR as measured by tetramer analysis and by the two-dimensional (2D) micropipette adhesion frequency assay. This clone exhibited a strong proliferative response, and was encephalitogenic upon adoptive transfer into to T cell deficient (Cα−/−) mice. We generated retrogenic mice in a lymphocyte deficient mouse strain (Rag−/−) by reconstituting the bone marrow with T cells expressing this high affinity Vα4β1 TCR as an alternative to maintaining T cell clones in culture. The T cells in this EAE model survived thymic selection, maintained a measurable 2D affinity, and exhibited spontaneous disease. The retrogenic model can provide an important platform for studying the contributions of TCR affinity to the course of demyelinating autoimmune disease.

Source of funding: NIH grant 5R01NS071518.
Deficiency Of The Transient Receptor Potential Melastatin 2 (TRPM2) Cation Channel Provides Protection Against Pulmonary Inflammation In A Murine Model Of Chronic Obstructive Pulmonary Disease (COPD)

Davide Botta1, André Ballesteros-Tato1, Kyle Martin2, Louise Hartson2, Tirumalai Rangasamy2, Thomas J. Mariani2, Troy D. Randall1, Debra A. Cockayne3, Christopher S. Stevenson3, Frances E. Lund1

1 Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA.
2 Department of Medicine, University of Rochester Medical Center, Rochester, NY, USA.
3 Hoffmann-La Roche Inc., Pharma Research & Early Development (pRED), DTA Inflammation, Nutley, NJ, USA.

Transient Receptor Potential Melastatin 2 (TRPM2) is a non-selective cation-permeable channel that is gated in response to oxidative stress via the intracellular binding of ADP-ribose. We hypothesized that TRPM2 plays an important role in the pathogenesis of Chronic Obstructive Pulmonary Disease (COPD) because of the close link between oxidative stress and inflammation in this pulmonary disease. The goal of this study was to determine whether the loss of TRPM2 altered the response to cigarette smoke (CS)-induced lung injury.

Wild-type B6 (WT) and Trpm2-deficient (Trpm2-/-) mice were exposed to sidestream CS (5 hours/day, 5 days/week) for up to 24 weeks (COPD model). As an alternative model of lung injury, a single dose of 5 μg lipopolysaccharide (LPS) was administered intranasally (Acute Respiratory Distress Syndrome model, ARDS). To assess inflammatory changes, bronchoalveolar lavage (BAL) and lung tissue digestions were performed and cellular infiltrates in the airways and lung tissue were determined by FACS. Expression of pro-inflammatory cytokines/chemokines and COPD-related genes in lung tissue and airways was measured by quantitative real-time PCR and cytokine multiplex assays, respectively. Histological changes in the lung were monitored on H&E-stained formalin-fixed tissue sections.

In the COPD model, Trpm2-/- mice had reduced cellular infiltrate in the lung tissue compared to WT mice due in part to a reduction in monocytes and monocyte-derived cells. Conversely, Trpm2-/- mice had a significantly higher number of cells recovered in the BAL fluid due to an increase in macrophages, which comprised >95% of BAL cells. Trpm2-/- mice had decreased expression of pro-inflammatory cytokines/chemokines in the lung, most notably CXCL1/KC, CXCL3/MIP-2β, CXCL5/LIX and IL-6. Furthermore, the expression of MMP12, a key protease implicated in the development of emphysema, was significantly lower in cells from lungs of Trpm2-/- mice. A similar attenuated inflammatory response was observed in Trpm2-/- mice exposed to LPS, making ARDS an ideal experimental model for mechanistic studies on TRPM2. Bone marrow (BM) chimera experiments in the ARDS model revealed that Trpm2-/- recipient mice had a suppressed cellular infiltration into the lung tissue regardless of the BM cell genotype, and identified functional defects in Trpm2-deficient macrophages. Indeed, purified Trpm2-deficient macrophages showed an impaired expression of pro-inflammatory mediators and proteolytic genes.

Lack of TRPM2 provides protection against pulmonary inflammation following exposure to CS or LPS, predominantly due to defects in non-hematopoietic cells. Interestingly, TRPM2 deficiency altered the activation state of lung macrophages resulting in reduced expression of cytokines/chemokines and proteases, including MMP12.

This work was funded by the Roche Postdoc Fellowship (RPF) Program, grant 09-534.
Neutrophil Activation Linked to T-Cell Suppression in HIV-1 Immune Dysfunction.


Human immunodeficiency virus-1 (HIV-1) infection leads to a state of chronic inflammation and immune dysfunction. T cells from HIV-1-infected individuals exhibit an exhaustion phenotype, limited functional capacity and reduced responsiveness to antigenic stimuli that is only partially restored following administration of highly active antiretroviral therapy (HAART). The mechanisms underlying T cell dysfunction in HIV-1 infection are not fully understood. Recently, interest has grown in the role of suppressive myeloid cell population, also known as myeloid-derived suppressor cells (MDSCs), that expands in cancer, infection, and trauma. This population constitutes a fundamental immune regulatory feedback mechanism via its capability to suppress T cell-mediated immune responses by multiple means. Despite the fact that the field addressing the role of MDSCs in immune regulation is rapidly expanding, little information is available on the role of MDSCs in HIV-1 infection. Using a detailed analysis of alteration of frequencies and activation status of subpopulations of myeloid cells, we have identified a population of activated neutrophils in the blood of HIV-1 infected individuals that is absent in healthy donors. This subset of activated neutrophils harbors the ability to suppress T cell function through the production of arginase-1 and reactive oxygen species (ROS). Increased ROS production by neutrophils as well as increased plasma levels of arginase-1 in HIV-1 patients was observed. Furthermore, neutrophils from HIV-1-infected patients express high levels of surface inhibitory molecule PD-L1. Interaction between PD-L1 on myeloid cells and PD-1 on T cells has been recently identified as a key mechanism inducing T cell exhaustion in HIV-1 and other chronic infections. Currently, we are investigating potential mechanism behind the development of this immunosuppressive neutrophil population in HIV-1 infection. Initial observations point towards both direct and indirect roles of HIV-1, mediated by bacterial translocation as well as production of IFN-α, a potent anti-viral cytokine, in the expansion of a suppressive neutrophil subpopulation. To our knowledge, this study is the first of its kind to suggest that chronic stimulation of neutrophils in HIV-1 infection results in an immunosuppressive phenotype characterized by a potent inhibitory effect on T cell function.

This study was supported by NIH Grant AI087178.
**Discovery of subtypes in serotype 20 of Streptococcus pneumoniae**

Juan J. Calix\(^1,2\), **Allison M. Brady**\(^2\), Robert L. Burton\(^3\), Thomas R. Larson\(^2\), Janet Yother\(^2\), Moon H. Nahm\(^3\)

\(^1\)University of Alabama at Birmingham, Medical Science Training Program  
\(^2\)University of Alabama at Birmingham, Department of Microbiology  
\(^3\)University of Alabama at Birmingham, Department of Pathology

*Streptococcus pneumoniae* (pneumococcus) synthesizes a polysaccharide (PS) capsule using proteins encoded by the capsular PS (*cps*) locus. While *S. pneumoniae* has diverse *cps* loci and produces various capsular PSs, each serotype of pneumococci produces a specific capsule type that is genetically, biochemically, and serologically distinct. Although 93 different pneumococcal serotypes have been defined, it is important to recognize additional diversity in serotypes because pneumococcal vaccines are designed to induce serotype-specific immune protection. We obtained evidence for heterogeneity within serotype 20 when serotype 20 capsular PS from a standard strain (ATCC6320) was unable to inhibit opsonization of a separate serotype 20 clinical isolate by immune sera. To investigate potential heterogeneity, we chose two serotype 20 strains (ATCC6320 from ATCC and 5931-06 from CDC) and purified their capsular PS, which was reduced to repeating units by alkali hydrolysis. Mass spectroscopy showed that the size of the ATCC6320 repeat unit was consistent with the published serotype 20 PS structure, but the repeat unit of 5931-06 PS was ~162 Daltons larger. Analysis with gas chromatography revealed that the molar ratio of Glc:Gal:GlcNAc was 3:2:1 for ATCC6320 and 4:2:1 for 5931-06. Further, the \(^1\)H NMR spectrum of 5931-06 PS revealed an additional anomeric chemical shift compared to ATCC6320 PS. The *cps* loci of the two strains were sequenced and were highly homologous. However, ATCC6320 contained a slip-strand mutation resulting in truncation of the putative glycosyl transferase *whaF*, while in 5931-06 *whaF* was intact. The *whaF* sequences of 13 other serotype 20 clinical isolates are identical to that of 5931-06. Despite genetic and biochemical differences, the two strains are indistinguishable by using available serotyping sera. We conclude that ATCC6320, which produces PS described in the literature, has an inactivated *whaF* and that *whaF* in 5931-06 mediates the addition of an additional glucose in its repeat unit. In view of this heterogeneity, we propose that serotype 20 should be renamed serogroup 20 with serotype “20A” used for ATCC6320-like strains and serotype “20B” for 5931-06-like. Serotype 20B strains appear to be more prevalent than serotype 20A.

This study was supported by AI-007051 (A.B.), AI-093103 (J.C.), AI-28457 (J.Y.), and AI-31473 (M.N.) from the NIH.
Therapeutic effectiveness of lithium in EAE is dependent on IFN-γ signaling

Amber L. Buel\textsuperscript{1}, Rodrigo Naves\textsuperscript{4}, Tethia Mbana\textsuperscript{2}, Chander Raman\textsuperscript{2,3} and Patrizia De Sarno\textsuperscript{1}

Departments of \textsuperscript{1}Psychiatry and Behavioral Neurobiology, \textsuperscript{2}Microbiology, \textsuperscript{3}Medicine, University of Alabama at Birmingham, Birmingham, AL, and \textsuperscript{4}Catholic University, Santiago, Chile

We previously reported that lithium, a GSK3 inhibitor, ameliorates experimental autoimmune encephalomyelitis (EAE). In active EAE induced by immunization with myelin oligodendrocyte peptide\textsubscript{35-55} (MOG\textsubscript{35-55}), pathogenic CD4 T cell populations include both Th1 cells and Th17 cells. However, in humans, relapsing remitting MS, the most frequent disease form, segregates predominantly into a Th1 or Th17 disease. In order to determine if lithium is beneficial for treatment of both Th1- or Th17-mediated disease, we performed passive transfer EAE experiments. We polarized encephalitogenic T cells towards a Th1 or Th17 phenotype, and then transferred the cells into naïve untreated or lithium-treated recipient mice. We found that lithium ameliorated Th1 EAE, but surprisingly exacerbated Th17 disease. Encephalitogenic Th17 cells cultured in the presence of lithium hypersecreted IL-17A, a mechanism that might contribute to the exacerbated disease in lithium-treated mice with Th17 disease. Our findings suggest that the cytokine milieu contributed by Th1 or Th17 disease impacts lithium’s therapeutic effectiveness in EAE. To determine what role IFN-γ plays in the ability of lithium to be therapeutically effective in Th1 disease, we induced EAE in untreated and lithium-treated IFN\textgamma R1\textsuperscript{−/−} and WT mice. Consistent with our previous findings, we found that EAE in lithium-treated WT mice was less severe than that in untreated WT mice. In IFN\textgamma R1\textsuperscript{−/−} mice, treatment with lithium slightly delayed progression of disease; however, the overall severity of EAE reached that of untreated IFN\textgamma R1\textsuperscript{−/−} mice. These findings indicate that the therapeutic activity of lithium is partially dependent on IFN-γ signaling. To begin exploring the underlying mechanism for this phenomenon, we characterized the infiltrating cell populations and cytokine profiles of the cerebellum and spinal cords from immunized untreated and lithium-treated WT and IFN\textgamma R1\textsuperscript{−/−} mice. We observed significant infiltration of T-cells within the cerebellum of untreated IFN\textgamma R1\textsuperscript{−/−} mice which was unhindered by lithium treatment. Additionally both the spinal cord and cerebellum of lithium-treated IFN\textgamma R1\textsuperscript{−/−} mice with EAE contained higher levels of IL-6 than that in untreated IFN\textgamma R1\textsuperscript{−/−} or WT mice and lithium-treated WT mice. We next assessed the effects of lithium cells in innate immune cells that also contribute to disease pathology in EAE and MS. Using bone marrow-derived dendritic cells and T-cells from TCR-transgenic OTII (OVA\textsubscript{323-339}) or 2D2 (MOG\textsubscript{35-55}) mice, we found that lithium inhibited antigen processing but not antigen presentation. Our results reveal that the GSK3 inhibitor, lithium, is therapeutically effective in EAE by attenuating the pathogenesis of innate and adaptive effector populations. We also report that the therapeutic activity of lithium requires functional IFN-γ signaling perhaps contributing to its effectiveness in attenuating Th1 but not Th17 disease. \textit{Supported by grants from NIH/NINDS-5RO1NS064261 to PD, NIH/Al-5RO1AI1076562 to CR. AB was a trainee of NMSS Collaborative Research Center, UAB.}
Lack of Phagocytic NADPH Oxidase Activity Decreases Anti-Viral Innate Immune Responses In Type 1 Diabetes

Ashley R. Burg1,2,3, and Hubert M. Tse, PhD2,3

Immunology Theme- UAB Graduate Biomedical Sciences1, UAB Department of Microbiology2 and the UAB Comprehensive Diabetes Center3

The autoreactive destruction of pancreatic beta-cells in Type 1 diabetes (T1D) can occur anytime between the first months of life to young adulthood. The impressive variance in age of onset for this autoimmune disease drives the notion that T1D involves more than just genetic susceptibility, and that an environmental trigger such as a viral infection may be required as the initiating event. Upon detecting a viral infection, the innate immune system will rapidly synthesize reactive oxygen species (ROS) and pro-inflammatory cytokines, not only as an anti-viral defense mechanism, but also as a “third signal” necessary for efficient synergy with the adaptive immune arm. We have previously shown that absence of phagocytic NADPH Oxidase (NOX) activity from Non-Obese Diabetic (NOD) mice, through a loss-of-function mutation (Ncf1m1J) in the p47phox subunit of NOX, confers significant T1D-resistance. The ability of NOD.Ncf1m1J mice to prevent spontaneous autoimmune T1D was partially due to dysfunctional TLR3-dependent acute anti-viral responses. In this study, we intend to define the role of superoxide on the initiation of T1D triggered by diabetogenic viral infections. We have found superoxide promotes the proinflammatory and anti-viral responses of both macrophages and dendritic cells in the NOD mouse model. Here we show that bone marrow-derived NOD.Ncf1m1J macrophages and dendritic cells stimulated with poly(I:C), a viral dsRNA mimic, exhibited significantly dampened TNF-alpha and IFN-beta production at the transcriptional and translational level, compared to the NOD wild-type response. By providing an exogenous source of superoxide with xanthine oxidase treatment, we were able to rescue the TNF-alpha response in poly(I:C)-stimulated NOD.Ncf1m1J macrophages back to wild-type levels. Surprisingly, however, exogenous superoxide did not rescue IFN-beta production, and in fact nearly abolished the IFN-beta response. Therefore, the production of superoxide during innate immune activation will promote the synthesis of NF-κB-dependent proinflammatory cytokines without affecting Type I interferons. Subsequently, p47phox functions in a NOX-independent manner to facilitate efficient IFN-beta synthesis. We are currently examining this differential effect of superoxide on the signaling pathways downstream dsRNA recognition. Furthermore, we are investigating dysfunctional responses of other dsRNA pattern-recognition receptors, aside from TLR3, such as MDA-5 and RIG-1. Finally, we are beginning a comprehensive in vitro and in vivo evaluation of NOX-dependent innate immune responses after infection with encephalomyocarditis virus and the onset of T1D.
CD5 through CK2 activation regulates B1-a B cell survival, proliferation, and both artificial and physiologic T-independent type II responses

Kevin S. Cashman³, Christine Sestero¹, Patrizia De Sarno⁴, and Chander Raman¹,²

Departments of Medicine¹, Clinical Immunology and Rheumatology², Microbiology³, and Psychiatry and Behavioral Neurobiology⁴,
University of Alabama at Birmingham, Birmingham, AL, 35294

B1-a B cells are a unique subset of innate-like B lymphocytes that have been heavily cited as potential contributors towards the pathogenesis of many disease states, especially autoimmune disorders such as systemic lupus erythematosus (SLE), Sjögrens syndrome, and rheumatoid arthritis. An interesting characteristic that sets B1-a B cells apart from other B cell subsets is the expression of the population defining surface marker, CD5. However, the physiological role of this receptor on the development, persistence, and/or function of this B cell population remains undefined. To begin addressing this question we generated a CD5 mutant knock-in mouse (CD5ΔCK2BD/ΔCK2BD) in which the serine threonine kinase binding domain, Casein kinase-2 binding domain (CK2BD), in the CD5-cytoplasmic tail was deleted. CD5 plays a critical role in regulating T cell survival following antigen receptor engagement and this activity is dependent on CK2 activation. In the CD5ΔCK2BD/ΔCK2BD mouse, the ability to activate CK2 is abrogated. B1-a B cells in the CD5ΔCK2BD/ΔCK2BD mouse turnover at a rate two-fold greater than that in WT mice. This increased turnover rate compensated for the decreased B cell survival to maintain a near normal B1-a B cell number in the peritoneal cavity. However, total serum IgM and IgA was significantly lower than that in WT mice. CD5ΔCK2BD/ΔCK2BD B1-a B cells proliferated poorly in response to anti-IgM stimulation but not LPS stimulation. Immune responses to T-independent type 1 antigen were relatively unaltered in the CD5ΔCK2BD/ΔCK2BD mouse. However the CD5ΔCK2BD/ΔCK2BD mouse exhibited an impaired anti-phosphorylcholine (PC) response following immunization with heat killed S. pneumoniae, a prototypic B1-a B cell response, as well as a decreased survivability following S. pneumoniae infection. In addition, B1 B cells from the CD5Δ/Δ and CD5ΔCK2BD/ΔCK2BD mice exhibited a decreased ability to clear apoptotic thymocytes. These data provide the first evidence that CD5 has a direct role in regulating B1-a B cell physiology and peritoneal homeostasis. Supported by NIH AI 1076562.
The human adaptive immune response to intestinal microbiota antigens

Benjamin Christmann 1, Zdenek Hel 1, Charles Bernstein 2, Bengt Björkstén 3, Maria Jenmalm 4, Charles O. Elson 1.

1 University of Alabama at Birmingham, Birmingham AL, USA 2 University of Manitoba, Winnipeg, Manitoba, Canada 3 Karolinska Institutet, Stockholm, Sweden, 4 Linköping University, Linköping, Sweden.

The intestinal microbiota has a major impact on the immune system which may play a role in immune mediated diseases, but the normal human adaptive immune response to gut microbiota is poorly defined. In this study we measured the human IgG response to a panel of gut microbiota antigens. We developed a microbiota antigen microarray containing 40 recombinant antigens from the murine microbiota. We then examined IgG seroreactivity in 224 adult individuals, both male and female, from Europe and North America, and in 28 Swedish children from 6 to 24 months of age. Most of the antigens selected for the microarray had matches of identity with genome sequences of the Human Microbiome Project. The antigens were from various classes of proteins and from different species of bacteria. Despite differences in geographic location, nearly every individual recognized a common set of antigens, although reactivity to any given antigen varied substantially. One antigen was differentially recognized in European vs North American samples. Some antigens in the panel had little or no serologic reactivity, despite being represented in the human microbiome. Children expressed the highest level of seroreactivity to antigens from the gut microbiome, and developed this response between the ages of 6 and 12 months. The human response to the gut microbiome begins between 6 and 12 months of age, and peaks near this time, but is maintained in adulthood. This study establishes a baseline for future studies of serologic reactivity of individuals with immune mediated diseases.
Tmevpg1, a long intergenic non-coding RNA, is required for Ifng expression by Th-1 cells

Sarah P. Collier*, Patrick L. Collins†*, Christopher L. Williams*, Mark R. Boothby†*
and Thomas M. Aune†,*

*Department of Pathology, Microbiology and Immunology and †Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232

The majority of the genome is noncoding and was believed to be nonfunctional. However, it is now appreciated that transcriptional control of protein coding genes resides within these noncoding regions. Thousands of genes encoding long intergenic noncoding RNAs, lincRNAs, have been recently identified throughout the genome, and these lincRNAs positively or negatively regulate transcription of neighboring genes. Both TMEVPG1 and its mouse orthologue encode lincRNAs and are positioned near IFNG. Here we show that transcription of both mouse and human TMEVPG1 genes is Th-1 selective and dependent upon Stat4 and T-bet, key transcription factors that drive the Th-1 differentiation program. Further, we demonstrate that Tmevpg1 expression is necessary but not sufficient for Th-1 dependent expression of Ifng. As with other developmental programs, our results demonstrate that a lincRNA plays an essential role initiating Th-1 differentiation.

This work was supported by US NIH Grants AI044924 (TMA) and AI077528 (MRB). The Vanderbilt University Medical Center Flow Cytometry Shared Resource is supported by the Vanderbilt Ingram Cancer Center (P30 CA68485) and the Vanderbilt Digestive Disease Research Center (DK058404).
T cell specific deletion of the phosphatase SHP-1 decreases the incidence and severity of experimental autoimmune encephalomyelitis

Lindsay J. Edwards and Brian D. Evavold

Emory University Department of Microbiology and Immunology

T cell activation is regulated by a dynamic balance between kinase and phosphatase activity. The tyrosine phosphatase SHP-1 is a critical negative regulator of the immune system, and a proximal regulator of T cell receptor signaling. Here, we have utilized a novel conditional deletion model to study the deletion of SHP-1 specifically in T cells. We have bred mice bearing a floxed SHP-1 allele that deletes the majority of the gene with mice bearing Cre under the control of the CD4 promoter, resulting in SHP-1 deletion during thymocyte development. We have determined that selective loss of this phosphatase in mature T cells dramatically attenuates experimental autoimmune encephalomyelitis (EAE). Both severity and incidence were decreased significantly in the conditional knockout mice relative to controls (mean max score 1.98, and 3.41, respectively, p=0.0022). Despite the observed differences in incidence and severity of disease, both effector and regulatory T cell infiltration into the CNS was similar in knockout and control groups. These data differ from results in mice heterozygous for the mothereaten allele, a naturally occurring SHP-1 mutant, which exhibit EAE severity that is similar to or slightly worse than controls. Collectively, these data suggest that lack of SHP-1 activity in T cells may protect from disease, while deletion in other cell types may exacerbate susceptibility or symptoms.

Funding provided by NIH grant R01 NS062358.
Regulation of Akt phosphorylation by mTOR and MAP kinases during *Francisella tularensis* LVS infection of murine macrophages

Michael W. Edwards¹, Ping Zhang², Suzanne M. Michalek¹ and Jannet Katz¹,²
Departments of Microbiology¹ and Pediatric Dentistry², University of Alabama at Birmingham, Birmingham, Alabama 35294, USA

*Francisella tularensis* is a gram-negative intracellular pathogen and the etiologic agent of tularemia. The attenuated *F. tularensis* live vaccine strain (LVS) is non-pathogenic in humans, but in mice it causes a pathology that resembles that caused by virulent *Francisella* strains in humans. Hence, *F. tularensis* LVS is a good model to study the immune host/pathogen interactions in an experimental mouse model. Central to the host immune response during an infectious process, is the signaling molecule Akt (protein kinase B), a serine/threonine kinase vital in pro-survival events, such as cell proliferation and metabolism, as well as protein translation and apoptosis. Full activation of Akt involves phosphorylation at Thr³⁰⁸ by phosphoinositide dependent kinase 1 (PDK1) and at Ser⁴⁷³ by the mammalian target of rapamycin complex 2 (mTORC2) via the PI3K pathway. Although mTORC2 has been shown to be the long sought “PDK2”, other kinases, i.e., integrin-linked kinase (ILK) and the p38 MAP kinase have been reported, albeit not without controversy, to also have PDK2 activity, thus capable of AktSer⁴⁷³ phosphorylation. Since Akt is such a central kinase in cellular immune events, understanding Akt regulation is critical and can lead to the future development of therapies by which the host response can be manipulated to the benefit of the host. The goal of the present study was to determine the kinases involved in the regulation of AktSer⁴⁷³ following *F. tularensis* LVS infection of murine-derived peritoneal macrophages. Three days after i.p injection of thioglycolate, macrophages were harvested, washed and cultured. Cells were incubated with the exquisite inhibitor of mTOR, rapamycin and/or the inhibitor of ILK, QLT0267 and/or the inhibitor of p38, SB203580, and then stimulated with LVS. At various times, cells were harvested, lysed and assessed by Western analysis. A severe downregulation in AktSer⁴⁷³ phosphorylation was seen in cultures treated with rapamycin, suggesting that mTORC2 played a role in this event. To determine if mTORC2 plays a role, we immunoprecipitated rictor, the critical binding partner of mTORC2, and assessed its interaction with mTOR by Western analysis. Although only a slight downregulation in AktSer⁴⁷³ phosphorylation was seen in cultures incubated with the ILK inhibitor, a more notable downregulation was seen in cultures incubated with QLT0267 and rapamycin, than with rapamycin alone. Since ILK is a positive regulator of the MEK/ERK pathway, we reasoned that if the synergistic effect between ILK and rapamycin was due to MEK/ERK involvement, we should observe similar results with the MEK/ERK inhibitor UO126. Indeed, similar results were seen. Inhibition of p38 MAP kinase rendered a notable downregulation in the levels of phosphorylated AktSer⁴⁷³ and a synergistic effect was seen in cultures incubated with SB203580 and rapamycin. However, when the MEK/ERK inhibitor UO126 was co-incubated with the p38 inhibitor SB203580 in cell cultures prior to LVS stimulation, the suppressive effect of SB203580 exerted on phosphorylated AktSer⁴⁷³ was dampened. Overall, our results show that after *F. tularensis* LVS infection of primary peritoneal macrophages AktSer⁴⁷³ phosphorylation is modulated by the mTORC2 and p38 pathways. Furthermore, regulation of AktSer⁴⁷³ by mTORC2 involves ERK phosphorylation, whereas, this is not the case for the regulation exerted by p38 MAP kinase. This work was supported by NIH-T32-AI-07051
Tissue-specific Antigen Presentation during persistent *Salmonella typhimurium* infection

Daniel R. Frederick, James B. McLachlan

Department of Microbiology and Immunology, School of Medicine, Tulane University

Antigen presentation is a cellular process involving the loading of exogenous and endogenous peptides for display on Major Histocompatibility Complexes (MHC) I and II. This is especially important in the initiation of a CD4 T cell immune response where dendritic cells present pathogenic antigen to CD4 T cells in the context of MHCII. While this interaction is well characterized in antigen-injection models, it remains unclear what antigen presenting cells (APCs) are displaying antigen, and what the impact this has on CD4+ T cells during persistent bacterial infection. In this study, we used a mouse model that mimics human typhoid fever by orally infecting mice with a virulent strain of *Salmonella typhimurium* tagged with a known immunogenic epitope called Eα52-68 peptide (pEα). This allows for the visualization of antigen presentation directly *ex vivo* at various times after infection through the use of an antibody that specifically recognizes only the pEα:MHCII complex. We have found that antigen-presentation can be detected in the spleens and livers in mice injected i.v. with purified pEα. More importantly, we could detect antigen presentation the spleens of mice infected with pEα-tagged *S. typhimurium* at 14 and 40 days post infection. Also, initial experiments show presentation by a diverse group of cells including dendritic cells and monocytes. These results demonstrate that antigen presentation can be detected during chronic infection and paves the way to understanding what role this might have in regulating CD4 T cell responses during persistent infection.
IL-23 controls autoimmunity by facilitating clearance of apoptotic bodies in the marginal zone

Hao Li1, 2, Hui-Chen Hsu2, Jun Li2, PingAr Yang2, Qi Wu2, Daniel J Cua3, Mohamed Oukka4, John D Mountz1, 2, 5

1Microbiology, University of Alabama at Birmingham, Birmingham, AL,
2Division of Clinical Immunology & Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL,
3Merck Research Laboratory, Palo Alto, CA,
4Seattle Children’s Research Institute, University of Washington, Seattle, WA, 5Birmingham VA Medical Center, Birmingham, AL, United States.

IL-23 helps expansion of IL-17 producing CD4 T (Th17) cells which are important in the pathogenesis of spontaneous autoimmune disease in BXD2 mice. In this study, we surprisingly found that IL-23 p19 subunit-deficient BXD2 mice (BXD2-Il23−/−) exhibit accelerated development of spontaneous germinal centers (GCs) and production of pathogenic autoantibodies, compared with wild-type BXD2 mice. Expression of IL-23 by adenovirus in BXD2-Il23−/− mice at early age can efficiently prevent the development of pathogenic autoantibodies and immune complex glomerulonephritis, suggesting that development of autoimmune disease is directly associated with the absence of IL-23. In both B6 and BXD2 mice, IL-23 was mainly produced by red pulp macrophages (RM) in the spleen. In GFP-IL-23R reporter or BXD2 mice, IL-23R was mainly expressed by marginal zone macrophages (MZMs) in the spleen of mice. In BXD2-Il23−/−, although there was a dramatic decline in the frequency of Th17 cells, there was a dramatic and age-related accelerated loss of both MZMs and CD21hiIgMhi marginal zone (MZ) B cells. MZMs are a small subset of specialized splenic macrophages essential for clearance of apoptotic cells entering to the spleen from circulation to prevent generation of immunogenic autoantigens, and there was a severe impairment in clearance of apoptotic bodies in the spleens of BXD2-Il23−/− mice. Absence of IL-23 correlates with enhanced expression of type I interferon (IFN) genes including Ifna1, Ifna4, Ifna7, Ifna11 and Ifnb, and type I IFNs have been identified to be produced by plasmacytoid dendritic cells (pDCs) localized in the marginal sinus in the spleen of BXD2 mice. Selective depletion of MZM via repeated injection of IL-23 intact BXD2 mice with clodronate liposome mimicked the effects of Il23−/−, leading to impaired clearance of apoptotic bodies and severely accelerated autoimmune disease progression by promoting type I IFN release from pDCs. Our results suggest a novel concept that IL-23 can act as a double edge sword to control the development and severity of autoimmunity in that over-expression of IL-23 may provoke autoimmunity through the induction/maintenance of Th17 yet complete deficiency of IL-23 also induced autoimmunity through the loss of marginal zone barrier that can prevent the evasion of apoptotic autoantigens. Our results further warrant an urgent need to carefully analyze the effects of IL-23 blocking biological reagents to treat autoimmune disease.

This work is supported by grants from NIH/NIAID (1AI 071110-01A1, ARRA 3RO1AI71110-02S11 and R01AI083705-01A2), ACR-Within-Our-Reach, Alliance for Lupus Research, VA Merit Review (1I01BX000600-01) and Lupus Research Institute
The role of fractalkine signaling in an AAV-alpha-synuclein model of PD

Ashley S. Harms (1,2), Aaron D. Thome (1,2), David G. Standaert (1,2)
(1) Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham
(2) Department of Neurology, University of Alabama at Birmingham

Parkinson’s disease (PD), the most common neurodegenerative movement disorder, is characterized by a progressive loss of dopamine producing neurons in the substantia nigra pars compacta (SNpc) and widespread intracellular aggregates of the protein alpha-synuclein (a-syn). Increasing evidence points to inflammation and innate immune system activators as a chief mediator in the progression and neurodegenerative activity involved with the disease. Reactive microgliosis in post mortem brain tissue, increased inflammatory cytokine expression in patient cerebrospinal fluid, and a genetic polymorphism in the HLA region associated with later onset PD all support immune involvement in dopaminergic neuron degeneration. Currently, the Standaert lab utilizes an adeno-associated virus vector (AAV) to introduce a-syn into the SNpc. At six months post-transduction, a 30% loss of dopaminergic neurons has been characterized along with microgliosis, elevated cytokine expression, leukocyte infiltration and IgG deposition. Most of these processes are mediated via the neuron-microglia signaling cascades which have been well documented in similar neurodegenerative disease models such as Alzheimer’s disease and multiple sclerosis. CX3CL1 (fractalkine) and its receptor CX3CR1 are expressed on neurons and microglia respectively and have strong neuroprotective implications in PD. Previous studies have implicated fractalkine involvement in different diseases, little is known about how the ligand-receptor signaling contributes to the pathogenesis of neurodegenerative diseases. In these studies we utilized the AAV vector to overexpress the full-length human a-syn protein into the SNpc of both WT and transgenic CX3CR1-/- mice. We assessed microgliosis using immunohistochemistry following 4 weeks post-AAV transduction in WT and CX3CR1-/- Fractalkine protein concentrations were analyzed by ELISA assays. Human aggregated a-syn protein was also used in vivo via direct injection into the SNpc to analyze internalization and localization of activated, phagocytic microglia. In vitro studies were performed utilizing cultured microglia from p1 WT and CX3CR1-/- pups into chamber slides. Time course treatments of a-syn and fractalkine ligand were performed and analyzed using ICC with subsequent confocal microscopy. Phagocytosis was also analyzed in vitro using fluorescent microspheres and analogous treatments performed earlier. Results from these studies implicate fractalkine signaling as a potential therapeutic target for regulating the microglial inflammatory response.

Funding: 5T32AR007450
Autoantigen-Specific B Lymphocyte Depletion Overcomes Failed Immune Tolerance in Type 1 Diabetes

Rachel A. Henry¹, Jonathan M. Williams², Amita Rachakonda¹, Peggy L. Kendall³, and James W. Thomas¹,²

¹ Vanderbilt University, Division of Rheumatology and Immunology, Department of Medicine
² Vanderbilt University, Department of Pathology, Microbiology, and Immunology
³ Vanderbilt University, Division of Allergy, Pulmonary, and Critical Care, Department of Medicine

Type 1 diabetes (T1D) results from immune tolerance loss in both T and B lymphocyte compartments. Genetically engineered T1D-prone NOD mice that lack anti-insulin B cells are protected from disease, as are mice in which the MHC class II molecule IAq is specifically deleted from B cells. A T1D clinical trial using anti-CD20 to deplete B cells has also identified B cells as candidate targets for therapy. To better understand how immune tolerance is lost, and how it might be restored, the VH125Tg/NOD T1D model was employed to track a small population of anti-insulin B cells (1-2%) in the repertoire. Examination of anti-insulin BCR shows that polymorphic alteration of CDR structure in NOD Vk genes enhances autoreactivity for insulin autoantigen. This is compounded by impaired receptor editing triggered by physiologic insulin in vivo. The increased frequency of insulin-binding B cells that forms in the developing repertoire fails to be censored throughout maturation, culminating in an increased frequency of anti-insulin B cells in the pancreas, the site of autoimmune attack. Insulin encounter increases costimulatory molecule expression on anti-insulin B cells, suggesting they may be competent to stimulate anti-insulin T cells. The presence of somatic hypermutations in some anti-insulin B cells, as well as interferon gamma production by T cells in the presence of anti-insulin B cells, suggests that productive T/B collaboration occurs during the disease process. To halt B cell stimulation of anti-insulin T cells, a novel strategy was employed to selectively deplete anti-insulin B cells using a monoclonal antibody that targets the insulin autoantigen bound to the BCR. This approach protects against disease in WT/NOD mice. These findings identify mechanisms that underlie autoreactivity in the B cell compartment of autoimmune mice, such as polymorphic enhancement of Vk autoreactivity and impaired receptor editing. These central tolerance problems permit escape of anti-insulin B cells into the periphery, where they accumulate with increased frequency at the site of autoimmune attack. Autoantigen targeting can specifically deplete anti-insulin B cells, and serves as a novel means through which immune tolerance balance can be restored to prevent disease. Successful clinical application of such an approach may prevent autoimmune islet destruction without compromising protective immunity generated by a broad B cell repertoire.

This work was funded by 5T32-HL-069765, 5T32-AR-059039, R01-AI-051448, and R01-DK-08246 NIH grants, as well as the Juvenile Diabetes Research Foundation grant 1-2008-108.
Optimized Human Immune Cell Isolation for Application in Systems Vaccinology
Kristen L. Hoek¹; Tara Allos¹; Leigh M. Howard²; Parimal Samir¹; Xinnan Niu¹; Kathryn M. Edwards²; Andrew J. Link¹

¹Department of Pathology, Microbiology, and Immunology; ²Department of Pediatrics
Vanderbilt University School of Medicine, Nashville, Tennessee

Systems vaccinology collects and integrates global biological information about immune responses after vaccination to better understand and predict immunogenicity. Our objective was to optimize the rapid processing of fresh human whole blood into immune cell subsets for functional genomic and proteomic analyses for future application in innovative clinical vaccine trials. Whole blood was collected from healthy adult donors with either a single 90 mL blood sample or 90 mL samples at four time points (Days 0, 1, 3, and 7) to optimize efficient and reproducible recovery of immune cell subsets. Blood was separated by Ficoll techniques to yield peripheral blood mononuclear cell (PBMC) and polymorphonuclear (PMN) cell fractions. The PBMC and PMN fractions were subsequently subjected to magnetic-activated cell sorting (MACS) to enrich for T-cells (CD3+), B-cells (CD19+), monocytes (CD14+), neutrophils (CD15+), natural killer cells (CD56+), and dendritic cells (CD11c+) prior to final purification by fluorescence-activated cell sorting (FACS). Procedures were adjusted based on data from each subject’s white blood count to improve purity, yield, and efficiency of collection. Quality control was performed on RNA and protein extracts from selected immune cell fractions. Using these procedures, targeted immune cells were collected with optimal yield, purity, and quality for functional genomic laboratory analysis by RNA-Seq and shotgun proteomics. Applications of these methods are being applied to subjects following an A/H5N1 vaccination with and without the AS03 adjuvant to construct a systems biology model of the human immune response.
Virus-specific class Ib-restricted CD8 T cells are protective across MHC class Ia haplotype barriers

Amelia R. Hofstetter*, Aron E. Lukacher†

*Department of Microbiology & Immunology, Emory University, Atlanta, Georgia 30322, USA.
†Department of Microbiology & Immunology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033, USA.

CD8 T cells recognize intracellular antigens primarily in the context of class I MHC molecules. The classical class Ia molecules are highly polymorphic. This presents a challenge for peptide-based vaccine strategies, as any single peptide will only bind a fraction of MHC molecules available in an outbred population. However, CD8 T cells also recognize class Ib molecules, which display little to no polymorphism. Our lab recently discovered a protective class Ib-restricted CD8 T cell response to Mouse Polyomavirus (MPyV) in H-2^b haplotype mice. Peptide VP2.139-147 from the VP2 capsid protein of MPyV binds to the class Ib molecule Q9 from the Qa-2 family of the MHC. H2-Q9 is non-polymorphic in mice. We hypothesized that Q9:VP2.139-restricted CD8 T cells can be expanded from non H-2^b haplotypes of mice, provided they express a Q9 gene product. Here we demonstrate that immunization of SJL (H-2^s) and NOD (H-2^g7) mouse strains expands Q9:VP2.139-specific CD8 T cells which are protective against MPyV. This work has important implications for peptide-based vaccine development.

Research supported by NIH grant RO1 CA139220
Determining the antigen specificity of diabetogenic BDC2.5 cells

Jennifer D. Hood¹, Cheng Zhu PhD², Brian D. Evavold PhD¹

1. Emory University, Atlanta, GA, 2. Georgia Institute of Technology, Atlanta, GA

Since low affinity CD4⁺ T cells dominate an autoimmune response like type I diabetes (T1D), standard techniques to identify the antigen may lack sufficient sensitivity to determine T cell frequency and affinity. Recently we made use of a micropipette adhesion frequency assay to define T cell frequency and affinities during experimental autoimmune encephalomyelitis. The micropipette revealed the presence of antigen specific T cells capable of producing cytokines but not detectable by pMHC tetramers and that these lower affinity T cells were the majority of the responding cells during an induced model of autoimmunity. To test whether these findings also applied to T1D, we turned to the BDC2.5 T cell receptor (TCR) transgenic, a widely used model of type I diabetes. It was generated using the TCR from a clone derived from a spontaneous non-obese diabetic (NOD) mouse that was capable of responding to pancreatic islets and inducing diabetes in adoptive transfer experiments. Synthetic peptide mimotopes that were capable of eliciting a strong response were generated due to the unknown self antigen from the pancreatic extracts. Recently, two studies have identified different epitopes within the chromogranin A (ChgA) protein as the antigen BDC2.5 cells recognize. Using the sensitivity of the micropipette adhesion assay, we determined the two dimensional effective affinity of BDC2.5 TCR cells to the ChgA and mimotope antigens. In addition, we were able to detect functional responses of BDC2.5 cells to ChgA by interferon γ production and proliferation but high doses of peptides were required to see the response. In accordance with our functional data, ChgA was lower affinity and elicited a weaker response from the BDC TCR cells compared to mimotope. Using the micropipette adhesion frequency assay we were able to assess the antigen specificity of the BDC2.5 TCR cells confirming its usefulness in identifying low affinity interactions with antigens and rare populations in a polyclonal response.

Funding:
NIH-NS071518
NIH-AI38282
NIH-F31DK089932
Presence of functional type IV secretion system impacts ability of mouse neutrophils to engulf *Helicobacter pylori*

Dennis J. Horvath, Jr.¹,², Jennifer A. Gaddy¹,², Vicki A. Cope¹,², and Holly M. Scott Algood¹,²

VA Tennessee Valley Healthcare System¹, Vanderbilt University Department of Medicine², Nashville, TN, USA

*Helicobacter pylori* is a highly successful Gram-negative pathogen that colonizes the stomach of 50% of the world’s population. *H. pylori* thrive within the inhospitable environment of the human stomach despite a vigorous immune response characterized by accumulation of T cells, macrophages, and neutrophils. Although neutrophils are required to control bacterial burden during infection models, the molecular mechanisms by which *H. pylori* are killed by neutrophils remains unknown. Toward this end, we developed a flow cytometric-based phagocytosis assay in order to quantitatively investigate the ability of mouse bone marrow neutrophils to bind, phagocytose, and undergo intracellular oxidative burst upon challenge with *H. pylori*. To validate the phagocytosis assay, we utilized yellow green microspheres as model particles and we were able to successfully distinguish between bound and internalized microspheres by Trypan blue quenching of extracellular fluorescence. These assays were performed with two *H. pylori* strains, which differ by their ability to translocate CagA (a toxin) into host cells. We observed that PM-SS1, an *H. pylori* strain with a functional type IV secretion system and SS1, which lacks a functional type IV secretion system, differed in their ability to modulate the phagocytosis and oxidative burst of polychromatic red microspheres. Furthermore, we found that neutrophils engulfed the SS1 strain much more effectively than PM-SS1 strain. Finally, we determined that phagocytic uptake of either strain was not enhanced by pre-treatment with recombinant IL-17A treatment. A better understanding of the methods used by phagocytes to successfully eradicate *H. pylori* and the molecular mechanisms by which this bacterium is able to actively modulate phagocyte function will help define how this organism is able to maintain chronic infection.

Funding for this project was provided by a VA Medical Center Merit Award (HMSA).
Role of Inducible Bronchus-Associated Lymphoid Tissue (iBALT) in Allergic Airway Disease

Ji Young Hwang, Javier Rangel-Moreno, Maria de la Luz Garcia-Hernandez, and Troy D. Randall. Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA, 14624

Inducible bronchus-associated lymphoid tissue (iBALT) is an ectopic lymphoid tissue formed in the lung after pulmonary infection or inflammation. This local lymphoid tissue is structurally similar to conventional secondary lymphoid organs (SLOs). Homeostatic chemokines, such as CXC chemokine ligand 13 (CXCL13) and CC chemokine ligand 19 (CCL19) regulate lymphocyte homing and are involved in compartmentalization of B and T cell areas in iBALT. Here, we induced iBALT in neonatal C57BL/6 mice by administering LPS prior to allergic sensitization, in order to understand how the presence of iBALT affects pulmonary immune responses to allergens. LPS administration in neonatal mice promoted iBALT formation, which correlated with increased mRNA expression of CXCL13 and CCL19. Although we observed the accumulation of more lymphocytes, both B and T cells, to the lungs of mice with iBALT, we observed fewer eosinophils in the lung and less Th2 cytokine production in bronchoalveolar fluid (BALF) after asthma induction in the presence of iBALT. We also transferred OVA-specific Th2 effector cells to mice with or without iBALT, and then challenged the mice with intranasal administration of OVA to address whether effector T cell homing is affected with iBALT. Whereas the presence of iBALT recruited higher number of effector T cells into iBALT area, the cells were dispersed throughout the whole lung in mice without iBALT. This may explain the discrepancy between the number of these cells and the actual Th2 cytokine levels detected in BALF.

These results suggest that iBALT induction reduces Th2-driven pathology and that the presence of iBALT is beneficial, rather than pathologic, in the context of allergic airway disease.

This research is supported by NIH grant HL69409 to T. D. R.
Death Receptor 5 (DR5) marks the highly pathogenic interacting GM-CSF* T helper cells and IL-23* macrophages rendering it as an attractive therapeutic target of autoimmunity

Jun Li1, Hui-Chen Hsu1,2, PingAr Yang1, Qi Wu1, Hao Li1, David M Spalding1, W. Winn Chatham1, Robert P Kimberly1, S. Louis Bridges1, John D Mountz1,2

1 Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, 35294; 2 Department of Medicine, Birmingham VA Medical Center, Birmingham, AL, 35233

The highly pathogenic granulocyte-macrophage colony-stimulating factor (GM-CSF)* IL-17+ CD4 T cells and IL-23*IRF5*M1 macrophages exhibit a bidirectional interaction in autoimmunity. Death receptor 5 (DR5) is a pro-apoptotic molecule and mediates apoptosis upon binding with its ligand, TRAIL, or an anti-DR5 agonistic antibody. DR5 can be upregulated by IRF5, the key transcription factor for the GM-CSF signal. TRAIL deficient mice develop increased inflammation. We therefore analyzed DR5 expression and apoptosis function of an anti-human DR5 antibody, TRA-8, in subpopulations of macrophages and T cells in humans and humanized DR5 transgenic mice with autoimmune diseases.

Expression of DR5 was highly correlated with that of GM-CSF in CD4 T cells and that of IL-23 and IRF5 in macrophages from synovia fluid or peripheral blood mononuclear cells (PBMC) of rheumatoid arthritis (RA) subjects (p<0.01). In vitro treatment of these samples with TRA-8 for 48 hrs resulted in 23.4% and 35.9% depletion of GM-CSF* CD4+ T cells and IL-23* macrophages, respectively. Similar DR5 expression pattern and TRA-8 depletion effects were observed in a 3kb mouse promoter/humanized DR5 transgenic mouse crossed with the Src-homology 2-domain phosphatase (SHP)-1 negative regulator deficient viable motheaten mice which exhibit a profound activation of inflammatory macrophages and Th17 cells with systematic autoimmune phenotypes. Three doses of TRA-8 (0.1 mg IP, weekly) lead to a 35.7% reduction of GM-CSF* CD4+ T cells and 45.1% reduction of IL-23*IRF5* macrophages in the draining LN. TRA-8 treatment also decreased expression levels of Csf-2 and p19, the genes encoding GM-CSF and IL-23p19 by 74.4% and 94.7% respectively, in joints. These findings were associated with amelioration of hemorrhagic pneumonitis and arthritis, reduction of autoantibodies, and increase of the lifespan after TRA-8 treatment.

Our study indicated that DR5 expression is highly correlated with GM-CSF in CD4+ T cells and IL-23 in macrophages, and thus can be used to recognize these two highly pathogenic cell populations. Importantly, we also demonstrated the high therapeutic efficacy of an anti-human DR5 antibody in autoimmunity.

Funding Support: This work was supported by an Arthritis Foundation fellowship award (to J.L.); and grants from Daiichi-Sankyo Co. Ltd (J.D.M.) and Lupus Research Institute (H.C.H.).
S100A4 is expressed in myeloid cells invading inflamed pancreatic islets and supports development of Type 1 diabetes

Tetyana V. Pedchenko, Allison M. Sullivan, James B. Case, Peggy L. Kendall

Vanderbilt University Medical Center

Type 1 diabetes (T1D) results from adaptive autoimmune destruction of insulin-producing beta cells in pancreatic islets. Dendritic cells (DCs) ferry antigen from islets to draining pancreatic lymph nodes for presentation to autoreactive T cells. S100A4 is a small calcium-activated molecule that contributes to cancer metastases and fibrosis, and has recently been linked to autoimmunity. We have discovered S100A4 among inflammatory infiltrates in pancreatic islets in a T1D model. Cells expressing S100A4 bear surface markers associated with antigen-presentation, such as MHCII and CD86. These cells include myeloid DCs and CD11b+ monocytes. Neither plasmacytoid DCs (PDCA1+/Ly6C-/B220+) nor lymphoid DCs (CD8+) express S100A4. S100A4-deficiency was engineered by GFP-targeting and introgressed onto the nonobese diabetic (NOD) mouse model of T1D for >10 generations. S100A4−/−/NOD mice are protected from the development of T1D, with a concomitant reduction in insulitis. However, GFP-expression in S100A4-deficient cells indicates that these cell populations remain in the inflamed islets. When compared to S100A4-sufficient counterparts, S100A4-deficient bone marrow-derived DCs have decreased ability to upregulate CD86, or to present antigen and activate T cells in vitro. Thus, S100A4 may contribute to the development of T1D by supporting myeloid cell antigen-presentation to autoreactive T cells.
Surrogate light chain component of the Pre BCR selects for immunoglobulin heavy chains based on their CDR-H3 contents

Mohamed Khass, Yingxin Zhuang and Harry Schroeder

Departments of Medicine and Microbiology, University of Alabama at Birmingham

Formation of the pre B cell receptor (Pre BCR) is a key step in B cell development. The Pre BCR is formed by the binding of the surrogate light chain proteins (SLC) and an in-frame re-arranged Mu heavy chain (Mu HC). We sought to test the hypothesis that the sequence of the heavy chain complementarity determining region 3 (CDR-H3) differentially affects the interaction between SLC and Mu HC and so formation pre BCR.

We previously showed that the sequence of the D₃H gene segment dictates the overall composition of CDR-H3. We used Bromo-deoxy Uridine (BrdU) incorporation and analysis of apoptotic markers to assess B cell turnover, cell cycle progression and cell loss as functions of CDR-H3 content and developmental checkpoint progression in the bone marrow. We measured the rate of formation of Pre BCR based on binding of the surrogate light chain to Mu HC in different mouse models.

B lineages enriched for hydrophobic CDR-H3 had difficulty forming Pre BCR, with increased cell loss and inefficient cell cycle progression at the stage of transition from early to the late pre-B cell. B cells limited to use of a single, normal D₃H with tyrosine enriched CDR-H3 followed the wild-type pattern.

Our findings suggest that immunoglobulin heavy chains with hydrophobic CDR-H3s have poor binding to the surrogate light chain, which results in a decreased cycling activity and increased apoptosis at the Pre B cell stage. These findings may explain how humans minimize the use of hydrophobic CDR-H3s in their developing B cells.

This work was supported by NIH A148115.
Antigen specificity in oral tolerance: the role of the anti-insulin B lymphocyte

Zachary A.-F. Kistka, MD; Dan Moore, MD; Peggy Kendall, MD; Tom Thomas, MD

Vanderbilt University Medical Center

Type 1 Diabetes (T1D) is an organ-specific autoimmune disease that results from pathologic loss of immune tolerance leading to the destruction of insulin-producing β-cells in the pancreatic islets. B lymphocytes play an integral role in T1D as requisite antigen presenting cells (APCs) for autoreactive T cells. Oral tolerance is a promising disease prevention mechanism by which immune non-reactivity is promoted by antigen exposure through gut lymphoid structures. Delivery of one β-cell autoantigen (β-CAA), insulin, to the gut prevents T1D in nonobese diabetic (NOD) mice by stimulating T regulatory (Treg) cell differentiation. In human studies, treatment with oral insulin reveals that individuals with the highest level of insulin autoantibodies (IAA) show the most benefit in disease prevention. The function of B lymphocytes in oral tolerance induction is not known. Although IAA are present in WT NOD mice, routine detection of anti-insulin B cells by flow cytometry is hindered by their low numbers. To circumvent this impasse, our laboratory developed lines of NOD mice expressing anti-insulin transgenes that have traceable numbers of insulin-binding B cells. Preliminary data validates the detection of anti-insulin B lymphocytes in mesenteric lymph nodes and Peyer’s patches. We hypothesize that exposure of anti-insulin B lymphocytes to insulin delivered in the gut facilitates oral tolerance through alteration of effector lymphocyte subpopulations and prevents T1D.

ELISPOT and flow cytometry will be used on NOD mice harboring anti-insulin transgenes to identify how anti-insulin B lymphocyte exposure to oral insulin a) skews differentiation from Th1 to Th2 cells, b) alters Th17 cell expression, and c) increases expression of FoxP3+ Tregs. Ig transgenic NOD mice which do not express anti-insulin B lymphocytes will receive anti-insulin B lymphocytes (treated or untreated) to test the hypothesis that exposure to oral insulin alters trafficking of anti-insulin B cells. Because multiple β-CAAs are attacked in T1D, it is important to determine if oral tolerance toward one autoantigens prevents autoaggression against other β-CAAs. We will use BDC2.5 T cell transfer to determine whether oral insulin-induced tolerance is restricted to insulin, or results in global Treg-mediated suppression of effector lymphocytes targeting other β-CAAs.

This work was supported by NIH grants R01 AI051448-09 and T32 DK 7061-37.
Inflammation, following MCMV infection, results in altered cerebellar development

Kate Kosmac¹,², Glenn Bantug², Stipan Jonjic³ and William J. Britt²,⁴

Neuroscience Program¹, Department of Microbiology², and Pediatric Infectious Diseases⁴: University of Alabama at Birmingham, AL; Department of Anatomy and Histology, Faculty of Medicine: University of Rijeka, Croatia³

Human cytomegalovirus (HCMV) is the most common viral infection transmitted from mother to unborn child and is one of the most significant infectious causes of developmental brain disorders. Several neurological deficits arise from infection with HCMV in utero, ranging from defects in perceptual senses to structural injury resulting in profound cognitive delays. Although neurological deficits resulting from CMV infection are well documented, the mechanisms leading to these abnormalities have yet to be elucidated.

We have developed a newborn mouse model of CMV infection that recapitulates many of the characteristics of human infection, including abnormal development of the cerebellum. Using this model, we have shown that infection of the developing CNS leads to decreased granule neuron precursor (GNPC) proliferation and morphological deficits within the cerebellum. Of note, these abnormalities coincide with a robust inflammatory response. Our preliminary data suggests that inflammation plays a significant role in the pathogenesis of the observed neurodevelopmental abnormalities; thus, we hypothesize that we can limit disease and long term consequences of early insults to the developmental program by modulating inflammation with the anti-inflammatory, prednisolone.

These experiments will provide insights into the role of host inflammatory responses in altered CNS development, within the context of CMV infection. Moreover, understanding how the immune system may alter the developmental program within the CNS may offer important insight into human CMV infection, as well as various other CNS diseases including autism, cerebral palsy and schizophrenia.

Supported by: T32 Training Grant in Immunological Diseases and Basic Immunology (5 T32 AI 7051-34)
LIVER INDUCED IMMUNOTOLERANCE BY SALMONELLA-SPECIFIC CD4+ T-CELLS DURING CHRONIC INFECTION

Kurtz, Jonathan R* & McLachlan, James B. *

*Department of Microbiology & Immunology, Tulane University School of Medicine, New Orleans, Louisiana, USA

Background: Salmonella spp., a genus of rod-shaped Gram-negative enterobacteriaceae, causes a range of disease in humans and animals, such as typhoid fever, paratyphoid fever, and foodborne illnesses. In mice, S. typhimurium causes a persistent bacterial infection analogous to typhoid fever in humans. Although a strong cellular immune response is initiated, the bacteria are never fully cleared. It is currently unknown what mechanisms govern this immunological “stalemate.” While it is known that CD4+ helper T cells are important effectors during Salmonella infection, it is not as clear how these cells respond to bacterial infection in infected tissues and what role these cells serve during infection. Our lab aims to illuminate the mechanisms of CD4+ T-cell immune responses generated toward Salmonella infection, especially during the chronic phase, and what host and microbial factors contribute to bacterial persistence.

Results: Using MHC class-II tetramers, we are able to visualize Salmonella-specific CD4+ T-cell responses during an ongoing infection in various organs. Following magnetic bead enrichment, we show that Salmonella-specific CD4+ T-cells adoptively transferred from lymphoid organs protect mice from subsequent Salmonella challenge. Conversely, we show Salmonella-specific CD4+ T-cells enriched and transferred from infected livers increases susceptibility to challenge, and that this phenomenon is dose dependent. Furthermore, we show that in comparison to liver CD4+ T-cells, lymphoid Salmonella-specific CD4+ T-cells produce higher levels of the potent inflammatory cytokine, interferon-γ, while Salmonella-specific CD4+ T-cells from the liver produce larger amounts of the immunosuppressive cytokine, interleukin-10. Lastly, we show that liver Salmonella-specific CD4+ T-cells have a decreased proliferative capacity in vivo compared to lymphoid T cells.

Conclusions: We have shown that during Salmonella infection different immunological responses occur at different sites of infection. Additionally, we have shown that the liver induces a more tolerogenic immune response during chronic infection. Therefore, we believe the liver may provide a privileged niche for Salmonella survival in vivo.
Quantitative detection of Interferon-α subtypes in lupus patients

Srilalitha Kuruganti¹, Shane Miersch², Ashlesha Deshpande, Bethany D. Harris, Kumar Putcha¹, Sachdev Sidhu², Winn Chatham³, Mark R. Walter¹

¹Department of Microbiology, ³Division of Clinical Immunology and Rheumatology, University of Alabama, Birmingham, ²University of Toronto-Canada.

Type-I interferons (IFNs) play a central role in the pathogenesis of systemic lupus erythmatosus (lupus). Type-I IFN family consists of 12 different IFN-α-subtypes, IFNβ,ω,κ,ε with 70-95% amino acid sequence identity. IFNs signal through IFNAR1/IFNAR2 heterodimeric receptor regulating gene expression leading to pleotropic biological activities. Increased levels of IFNs, consisting of an unknown mixture of IFN-I-subtypes have been detected in the serum of lupus patients. It is important to identify specific IFN-subtypes contributing to Lupus in order to develop protein-based-therapeutics. Therefore, our objective is to determine IFN-I-subtype protein levels in lupus patient serum using anti-IFN-I-subtype specific antibodies. To accomplish this, twelve IFNα subtypes were expressed, purified from E.coli and the biological activity was determined using luciferase assay. Anti-IFN-I-subtype specific neutralizing antibodies are being isolated by phage display technology. Serum samples of lupus patients were obtained from Kirklin clinic, for analysis.

Characterization of IFN-I subtypes suggests that IFNα1a exhibits the lowest bioactivity and IFNα14c is most active. An IFNAR1/2-FC protein has been engineered to form high affinity complexes with the IFN-I-s. It neutralizes all the IFN-I-s and provides an important reagent to determine total IFN-I in serum samples. The IFN-I subtype-specific Fabs are being developed using the purified IFN-subtype proteins. Initial studies using Fabs selected against IFNa2a, IFNa6 and IFNa8 will be presented to demonstrate the methods to quantify IFN-I-subtype bioactivity in lupus patient serum and normal donors.

These studies show promising approaches to identify and quantify IFN-subtype levels in the serum of lupus patients, which may be important to optimize anti-IFN lupus therapies.

This study is supported by a grant from Lupus Research Institute to M.R.W.
**Influenza A virus pathogenesis: Identification of host factors that contribute to severe respiratory distress**

*Vy L. Le and Richard W. Compans*

Department of Microbiology and Immunology, Emory Vaccine Center, Emory University, School of Medicine

Most influenza virus infections are cleared within a week, many infections cause severe respiratory damage resulting in hospitalization and death. According the Center for Disease Control, influenza virus infections contribute to 3,000-49,000 deaths in the United States annually. While several factors contribute to pathogenesis, the mechanisms that govern how some infections result in severe respiratory distress, while others are resolved inconsequentially, are only partially defined. To explore the host cellular components that contribute to influenza virus pathogenesis, we utilized two genetically related strains of the 2009 H1N1 pandemic influenza virus; A/Netherlands/602/2009 and A/California/07/2009. Although these viruses share nearly 100% sequence identity, mice that are infected with the viruses exhibit great disparity in the severity of symptoms as measured by body weight, mortality and viral replication in the respiratory tracts. Our study reveals several key components that are critical in the balance between an immune response against influenza virus in which the virus is rapidly cleared and damage to the host is moderate, versus a response resulting in severe pathogenic effects that is marked by destruction of the airway epithelium. This study is supported by NIH-A1074492
B cell-dependent positioning of CXCR5-expressing dendritic cells drives Th2 development

Beatriz León¹, André Ballesteros-Tato¹, Jeffrey L. Browning², Robert Dunn³, Troy D. Randall¹ and Frances E. Lund¹

¹University of Alabama at Birmingham, AL 35294, USA.
²Biogen Idec, Cambridge MA USA and San Diego CA USA
³Pfizer-Centers for Therapeutic Innovation, San Diego CA

T cells need to encounter antigen-bearing dendritic cells (DCs) in order to undergo clonal expansion and effector development. These interactions, which take place within lymphoid tissues, are orchestrated by finely-tuned expression of chemokines and chemokine receptors. Indeed, CCR7 expression by both T cells and DCs is thought to facilitate DC/T cell encounters within the T cell area and promote T cell activation and differentiation. However, it is unclear whether DC-dependent T cell priming must occur within the T cell area of the LN. Here, we show that, following infection with an intestinal nematode, antigen-bearing DCs and the responding CD4⁺ T cells upregulate expression of CXCR5 and migrate in a CXCR5/CXCL-13 dependent fashion to the B cell area of the mesenteric lymph node. We further demonstrate that CXCL13/CXCR5-mediated recruitment of T cells and DCs into the B cell areas of the mesenteric LN is required for the development of IL-4 producing Th2. Finally, we demonstrate that nematode-activated, lymphotoxin-producing B cell play an essential role in this process by inducing CXCL13 upregulation within the LN. This B cell and lymphotoxin-dependent process controls DC/T cell homing to the B cell area and is necessary for the subsequent differentiation of the T cells into IL-4 producing effectors. These results show that B cells orchestrate encounters between T cells and antigen-bearing DCs within a specialized microenvironment that facilitates Th2 differentiation.
ROLE OF Dβ GERMLINE SEQUENCE ON CONSTRAINING TCR CDRβ3 DIVERSITY

M.S. Levinson, A. Silva-Sanchez, Y. Zhuang and H.W. Schroeder, Jr.

University of Alabama at Birmingham Department of Medicine

A highly diverse T cell receptor (TCR) repertoire is necessary for the recognition of exogenous antigens. This diversity is developed through V(D)J rearrangement and N addition during TCR development. The product of V(D)J rearrangement in the beta chain of the TCR is the CDRβ3, a region of high variability that recognizes antigen and includes all of the D gene. Interestingly, the Dβ sequence, the D gene for the beta chain of the TCR, is highly conserved across various species, from trout to mouse to humans. This suggests that there are some natural constraints on the TCR; these constraints are thought to limit deleterious T cells from reaching the periphery. We hypothesize that altering the D region will have an effect on the development of thymocytes.

Thymocytes were sorted by flow cytometry based on their expression of CD44, CD25 and CD28. RNA from the thymocytes was extracted and the CDRβ3s were sequenced using primers to the Vβ13-1 and to the VβC1.

We have preliminary data of mice forced to use the DH, the D from the B cell receptor heavy chain, instead of Dβ. DH is used because it uses different amino acids than Dβ; DH is enriched with the hydrophobic amino acid tyrosine, while Dβ mainly uses neutral amino acids. When compared to mice using Dβ, the DH mice have an altered mature T cell repertoire in that they resemble the amino acid distribution seen in the B cell repertoire. However, we see a reduced proportion of hydrophobic sequences in the DH compared to the B cell repertoire, which is more in line with the natural T cell repertoire.

The germline sequence is clearly affecting the altered TCR repertoire, skewing it towards a more hydrophobic, BCR-like distribution. However, the altered TCR repertoire isn’t as hydrophobic as the BCR repertoire, suggesting mechanisms of somatic selection by preferring a less charged CDRβ3. Ongoing experiments using varied TCR DJβ locus mutants will elucidate the role of the germline sequence on the development of thymocytes.
Myeloid-derived suppressor cells as key immune regulators of non-union fractures

Seth Levy, Anandi Sawant, and Selvarangan Ponnazhagan

Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama

Every year in the United States there are more than 6 million fractures; approximately 10% of them result in delayed healing or develop into non-union fractures. The event of osteogenesis following fractures depends on and is influenced greatly by associated vasculogenesis and stimulatory signals in the bone microenvironment, including an influx of immune cells in the fracture site. The hypoxic microenvironment aids in the survival of many immune cell types, necessitating understanding of the role of infiltrating immune cells in non-union fractures and possibly targeting them if found involved in the cascade of signaling leading to non-union fractures. To this end, we have evaluated the immune cell populations that play a key role during stages of fracture healing in an immunocompetent mouse model of femoral fracture. Results of these studies so far indicate that during the initial phase of fracture healing there is a dramatic increase in the number and percentage of myeloid derived suppressor cells (MDSC) in the fracture area followed by a decrease in the number and percentage of MDSC as the fracture heals. MDSC are a heterogeneous subpopulation of immune cells that are known to survive in hypoxic environment, are known to promote angiogenesis, and suppress many lymphocyte populations. Using gemcitabine to specifically decrease the levels of MDSC, we observed that fractures in the absence of MDSC demonstrate delayed healing further signifying the role of MDSC in fracture healing. Based on this data we predict that MDSC may play a significant role in non-union fractures and targeting MDSCs during the earlier phase of fracture would minimize the chances of eventual non-union pathology.

NIH T32 AR047512-10
Therapeutic Efficacy of the JAK1/JAK2 Inhibitor AZD1480 in Experimental Autoimmune Encephalomyelitis

Yudong Liu¹, Andrew T. Holdbrooks¹, Patrizia De Sarno², Lora L. Yanagisawa¹, Braden C. McFarland¹, Laurie E. Harrington¹, Chander Raman³, Etty N. Benveniste¹* and Hongwei Qin¹*

Departments of Cell, Developmental and Integrative Biology¹, Psychiatry and Behavioral Neurobiology² and Medicine³, University of Alabama at Birmingham, Birmingham, AL

Increasing evidence indicates that pathogenic T helper cells as well as myeloid cells, including dendritic cells (DCs), macrophages (MΦ), and microglia, are involved in the pathogenesis of Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE), an animal model of MS. It is well known that the Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway is the major signaling pathway regulating inflammatory responses in both Th1/Th17 cells and myeloid cells. Inhibitors of the JAK/STAT pathway have demonstrated clinical efficacy in rheumatoid arthritis and other inflammatory disorders; however, few studies have focused on MS/EAE. In this study, we use a novel JAK1/2 inhibitor, AZD1480, which has been shown to potently inhibit the JAK/STAT pathway in solid and hematologic malignancies, to investigate its therapeutic potential in EAE.

We found that AZD1480 effectively controls encephalitogenic T cell differentiation, and ameliorates the development of classical and atypical EAE. In vivo, decreased STAT1/3 signaling and expression of inflammatory cytokines/chemokines, including IL-1β, IL-6, IL-12, IL-23, IFN-γ, IL-17A, iNOS, TNF-α, CCL2 and CXCL10, is observed in both cerebellum (CB) and spinal cord (SC) in AZD1480 (50 mg/kg) treated mice. In addition, the number of CNS infiltrating cells in the CB and SC is much less in AZD1480 treated mice, especially for myeloid cells and CD8⁺ T cells. Histology results also confirmed less infiltration of inflammatory cells and demyelination in both the CB and SC in AZD1480 treated mice. In vitro, AZD1480 inhibits the JAK/STAT pathway in Th1/Th17 cells at 0.25 μM and bone marrow-derived MΦ at 0.5 μM, resulting in less production of pro-inflammatory cytokines/chemokines. AZD1480 does not promote T cell apoptosis. Interestingly, no significant effect of AZD1480 was observed in inhibiting T cell proliferation in vitro, but a significant inhibition of proliferation was observed in CD4⁺ T cells and CD11b⁺ cells in AZD1480 treated mice during the peak phase of EAE. In addition, AZD1480 influences the priming phase of EAE, as demonstrated by decreased MOG-induced Th1 and Th17 cell differentiation in spleen and lymph nodes in AZD1480 treated mice.

Our previous study showed that mice with conditional knockout of SOCS3 in cells of the myeloid lineage developed early onset of a severe and non-resolving disease with features of atypical EAE. We further evaluated the effect of AZD1480 in these LysMCreSOCS3⁻/⁻ mice, and a most striking beneficial effect of AZD1480 was observed. Our data highlight the potential therapeutic role of JAK inhibitors in EAE, and possibly MS, and provides new insight into novel mechanisms for treatment of autoimmune diseases.

Acknowledgements: This work was funded by National Institutes of Health Grants NS45290 (to E.N.B.), NS64261 (to P.D.), DK84082 (to L.E.H.), and AI76562 (to C.R.); National Multiple Sclerosis Society Grants RG 3892-A-12 (to E.N.B.) and RG 3891 (to C.R.); and Collaborative Research Center Grant CA 1059-A-13 (to E.N.B.).
Identification and temporal monitoring of breast cancer-associated T cell receptors with high throughput sequencing

Jian Han¹,², Chunlin Wang¹, Qunying Yang¹,², Catherine M. Sanders¹,², Jessica McClellan¹, Miranda Byrne-Steele¹,², C. Lu¹

¹iRepertoire, 601 Genome Way, Huntsville, AL, United States. ²HudsonAlpha Institute for Biotechnology, 601 Genome Way, Huntsville, AL, United States

Tumor-specific antigens may trigger an immune response that leads to T lymphocytes infiltrating the tumor tissue. We have developed a method to study the immune repertoire of a sample by utilizing a patented multiplex PCR amplification strategy, arm-PCR (Patent No. 7,999,092), coupled with high throughput sequencing (Wang et al., PNAS 2010). Using this method, we studied a patient’s surgically removed breast cancer tissue, the nearby normal tissue, and sorted peripheral blood. The patient’s sorted peripheral blood was also examined three months, six months, and one year post-surgery. Dominant T cell clones with specific CDR3 sequences were identified from the breast cancer tissue. Some of these same clones were found expressed at high levels in the nearby normal tissue and peripheral CD8+ cells. After treatment, dynamic changes in these cancer-associated clones were apparent, demonstrating the capability of the current technology to identify specific T cells associated with a patient’s cancer tissue. These specific T cells can serve as personalized biomarkers for prognosis, treatment evaluations, and early detection of recurrence. They can also be used to develop personalized treatment strategies. Currently, this study has been extended to examine 10 additional breast cancer patients.
Driving HIV-specific vaccine responses in immune suppressed recipients

Lisa M. McEwen¹, Cac T. Bui¹, Yvonne Paterson² and Donald A. Harn¹

¹Department of Infectious Diseases, University of Georgia, Athens, GA, USA, 30602-7387
²Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA, 19104-6076

Malaria, TB and HIV-1 remain tremendous disease burdens in much of the world’s population. Vaccines for these diseases are desperately needed. However, vaccine efficacy is likely to be compromised due to the systemic Th2 biasing and immune suppression caused by infection with parasitic helminthes. Our lab and others have shown that helminth infection suppresses Th1-type vaccine-specific responses. Although Sub-Saharan populations will benefit most from these vaccines, a large percentage of these populations live in helminth endemic regions. A goal of our research is to find vaccine vectors that drive significant HIV-specific immune responses in helminth infected recipients, despite the immune status of the individual. In the current study, we demonstrate that administration of an HIV vaccine (Listeria monocytogenes expressing HIV-1 IIIB gag protein) drives significant HIV-specific CTL and Th1 responses in mice chronically infected with the helminth parasite Schistosoma mansoni. These results suggest that Listeria vector vaccines likely will drive HIV-specific responses in helminth-infected human populations. Kinetic studies show that the HIV-vaccine-specific responses generated in schistosome infected mice are durable. Further, Listeria vectors should be considered in the development of new generation HIV-1, malaria or TB vaccines to be administered to populations in sub-Saharan Africa where helminth infection is endemic.

Supported by grants NIH-AI071883 and NIH-AI-078787 awarded to DAH.
CD5 Enhances T cell cytokine signaling

Donald McGuire¹, Christine Sestero², Chander Raman²

Department of Microbiology¹, Departement of Medicine² University of Alabama at Birmingham

CD5 plays an important role as an inhibitor of the T cell receptor though an ITAM like domain and enhancer of cell survival through activation of CK2. We have previously showed that loss of CK2 dependant pro survival signals in CD5/-/- mice result in less severe experimental autoimmune encephalomyelitis (EAE). Interestingly, changes in T helper cell polarization in CD5 mutant mice suggest a role for CD5 beyond its classical role as regulator of cell activation and survival. We have found that CD5 also plays an important role as an enhancer of cytokine signaling. External engagement of CD5 enhances STAT 1 phosphorylation following stimulation with IFN gamma. The increased STAT1 phosphorylation correlates with a decrease in Th1 polarization and is dependent on the CK2 binding domain of CD5. We also found that CD5 enhances IL-6 and IL-10 activation of STAT3. The increase in STAT3 phosphorylation was greatest in Treg cells. Surprisingly this enhancement of STAT3 phosphorylation is through an unknown mechanism that is independent of CK2 and PI3K. These results clearly show that CD5 plays substantial role in regulating signal strength of key cytokines and may represent a means to alter T cell responses in context of autoimmune diseases.

NIH/NIAID T32 AI007051-34
NIH RO1 and NMSS
A critical role for STAT4 in the accumulation of CD4 T cells in the CNS during EAE

Ian L. McWilliams and Laurie E. Harrington

University of Alabama at Birmingham.

Multiple Sclerosis (MS) is an autoimmune disease characterized by demyelination of neurons in the central nervous system (CNS). In order to study the mechanisms underlying MS, we use the well-described mouse model, experimental autoimmune encephalomyelitis (EAE). During EAE, the CD4 T cell subsets, TH1 and TH17, are associated with immunopathology. STAT4 is an important TH1 transcription factor that, when activated by IL-12, results in the production of IFNγ. Deletion of STAT4 protects mice from EAE; in contrast, deletion of IL-12 and IFNγ does not ameliorate disease. This disconnect has prompted the study of the association between STAT4 and pathogenesis. We utilize bone marrow (BM) chimeric mice to provide a physiological environment in which to study the intrinsic role of STAT4 in CD4 T cells during EAE. Previous reports have shown that STAT4 regulates expression of IL-18Rα, however, using this BM chimera system we did not observe differences in IL-18Rα expression on WT and STAT4−/− CD4 T cells during EAE. Interestingly, we find that during EAE, the in vivo cell frequency of STAT4−/− CD4 T cells is greatly reduced in the CNS of BM chimeric mice compared to the equivalent allelically marked WT CD4 T cells in the control group. Further experimentation has shown there is no defect in the proliferative capacity of these cells by Ki67 staining in the CNS of these BM chimeric mice. Taken together, these data suggest an important role for STAT4 in maximal migration or maintenance of STAT4−/− CD4 T cells in the CNS during EAE.
Regulation of STAT1 by AMPK Signaling in Astrocytes

Gordon P. Meares, Hongwei Qin, and Etty N. Benveniste

Department of Cell, Developmental and Integrative Biology
The University of Alabama at Birmingham

Inflammation in the central nervous system (CNS) contributes to most neurological disorders. Neuroinflammation involves the release of inflammatory molecules from glial cells such as astrocytes and microglia and can lead to neuronal damage. In multiple sclerosis (MS), peripheral immune cells, including interferon-γ (IFN-γ)-producing Th1 cells, infiltrate the CNS and have a key role in shaping the inflammatory micro-environment in the CNS, in part through cytokine-mediated interactions with glial cells. Recent evidence suggests that AMP-activated protein kinase (AMPK) can regulate inflammatory gene expression. AMPK is a cellular fuel sensor, becoming activated in response to energetic stress to restore energetic homeostasis. In this study, we have found that AMPK signaling during experimental autoimmune encephalomyelitis (EAE), an animal model of MS, is down-regulated in the brain, confirming previous reports. Diminution of AMPK signaling correlates with increased expression of IFN-γ in the CNS. Using primary astrocyte cultures, we have identified that IFN-γ induces biphasic AMPK signaling, with early inhibition followed by later activation, suggestive of negative feedback mechanisms. We have used genetic and pharmacological approaches to study how the AMPK pathway regulates astrocyte-mediated inflammation. Forced activation of AMPK suppresses several cytokines and chemokines induced by IFN-γ in primary astrocytes and microglia. IFN-γ regulates gene expression through activation of STAT1, suggesting that AMPK may influence STAT1 function. Consistent with this hypothesis, deletion of AMPK or the upstream kinase LKB1 results in a marked increase in basal expression of STAT1 and, conversely, activation of AMPK blocks IFN-γ-induced STAT1 expression. Deletion of AMPK leads to increased basal and IFN-γ-induced expression of inflammatory molecules including TNF-α, iNOS, CXCL10 and CCL2. AMPK does not regulate IFN-γ-induced activating phosphorylation of STAT1, but instead attenuates nuclear translocation of STAT1 and subsequent DNA binding. Overall, these findings provide the first link between AMPK and STAT1, and may provide important clues about how bioenergetics and inflammation are linked.

B Cells Have Multiple Roles for the Generation of CD4 T Cell Memory and Recall Responses

Mollo, S. and Harrington, L.E.

University of Alabama at Birmingham (UAB)

Patients with B cell lymphomas and autoimmune diseases are often treated with Rituximab, a monoclonal antibody that depletes CD20 expressing B cells; however, the effect of this depletion on newly forming and pre-existing CD4 memory T cells is unclear. While experiments in B cell-deficient mice suggest these cells play a critical role in the development of CD4 memory T cells, it has been difficult to ascertain when during infection B cells influence the CD4 T cell response or if the differences are due to the disrupted splenic architecture. In order to determine if B cells are necessary during priming, we utilized the anti-CD20 antibody to deplete B cells prior to infection with Listeria monocytogenes (LM). We found a reduced frequency and number of antigen specific CD4 T cells capable of producing IFNγ and IL-2 at effector and memory time-points. Importantly, the CD4 T cell response was further diminished in B cell-deficient mice suggesting that in addition to their role during priming, B cells’ effect on splenic architecture can impact CD4 T cells. Using bone marrow chimeras, we found that antigen presentation by B cells is dispensable for the CD4 effector response, however, it is necessary for the generation of an optimal memory response. Due to the differential activation requirements between naïve and memory cells, we analyzed the recall response of CD4 memory T cells in the absence of B cells. Similar to naïve CD4 T cells, CD4 memory cells require B cells in order to generate an optimal secondary response.

NIH/NIAID T32 AI007051-32
Effective immunoprevention against chemical carcinogenogenesis is induced by genetic immunization vectors that selectively expand mutant H-ras-specific CD8 T cells.

Tahseen H Nasti¹, Kyle Rudemiller¹, George Twitty¹, Hee Kyung Kim¹, ², Yuko Tsuruta¹, ², Mohammad Athar¹, ², Craig Elmets¹, ² and Laura Timares¹, ², ³

¹Department of Dermatology, ² UAB Skin Diseases Research Center, & ³ Division of Human Gene Therapy, The University of Alabama at Birmingham School of Medicine.

Polyaromatic hydrocarbons (PAHs) possess mutagenic and carcinogenic properties that are associated with cancers of skin, lung, pharynx, mouth, breast gastrointestinal tract and many other cancers. The carcinogenic PAH 7,12-dimethylbenzanthracene (DMBA) generates a characteristic point mutation, Q61L, in the H-ras oncogene (H*ras). The mice that are subjected to DMBA carcinogenesis develop tumors with 80-100% having this type of mutation. Our previous studies suggest that mice that develop a Delayed hypersensitivity response (DTH) by polyaromatic hydrocarbons (PAHs) are more resistant to tumor development than mice that lack this response. So we tested the hypothesis that a vaccine designed to induce T cell specific immunity to H*ras should eliminate cells with DMBA-induced mutations and protect against tumor development. We observed that mutant, but not wild type (WT) H-ras peptide induced DTH responses when challenged with mutant but not WT peptide in A/J and C3H/HeN mice. Hapten-specific DTH responses are mediated by CD8⁺ T cells but suppressed by CD4⁺ T cells. Therefore vectors were designed to focus epitope loading into MHC class I molecules by generating a chimeric gene encoding a ubiquitin (Ub) tagged/H*ras or WT H-ras epitope/EGFP fusion protein. H*ras-specific DTH and CTL responses were detected in mice vaccinated with plasmid DNA or engineered DC encoding Ub/H*ras/EGFP, but not control Ub/WT H-ras/EGFP. In contrast, WT H-ras peptide was unable to elicit DTH responses, indicating that tolerance to endogenous H-ras was maintained. Engineered DC lines generated increased numbers of IFN producing CD8⁺ T cells. Detectable DTH responses were elicited by DMBA in Ub/H*ras/EGFP vaccinated mice, indicating that DMBA-induced H*ras expressing cells were recognized. Mice that were Ub/H*ras/EGFP vaccinated and then subjected to a two-step DMBA/TPA chemical carcinogenesis protocol developed fewer (p=0.004) and smaller (p=0.02) tumors compared to tumors in control cohorts. H*ras specific mRNA expression was low in H*ras vaccinated mice than untreated or Ub/WT-ras/EGFP treated groups indicating immunoeediting by H*ras-specific T cells. These “proof of concept” studies provide a foundation for further development of focused vaccination strategies for successful immunoprevention of chemically induced tumors in skin and potentially other tissues.
Inducible costimulator augments antitumor Tc17 cell activity


1Department of Microbiology and Immunology; and 2Department of Surgery, Hollings Cancer Center, Medical University of South Carolina (MUSC), Charleston, SC

IL-17-producing CD8+ T cells, called Tc17 cells, have been identified in both mice and humans. Tc17 cells exhibit potent antitumor immunity in melanoma-bearing mice and enhanced cell memory properties. The programming cytokines have been identified for in vitro Tc17 generation, however the costimulatory molecules important for enhancing IL-17 production are unknown. We discovered that IL-17-producing CD8+ T cells’ superior antitumor immunity is regulated by the inducible costimulator ICOS. In this study, we examined the role of ICOS using a clinically relevant pmel-1 adoptive cell transfer therapy model for murine melanoma. We found that blocking ICOS signaling in mice dramatically impaired Tc17 cell-mediated tumor immunity. Conversely, activating Tc17 cells with an ICOS agonist augmented their functionality, thereby improving their capacity to eradicate large tumors. This enhanced antitumor immunity was associated with increased expression of ICOS, IL-2Rα, and IL-7Rα expression on Tc17 cells. To uncover the ideal signal(s) to generate human Tc17 cells for clinical use, antigen-specific human CD8+CD161+ Tc17 cells were expanded with K562 artificial APCs (aAPCs). We used aAPCs expressing ligands for the T cell receptor (CD3), ICOS, CD28, and/or 4-1BB to expand human Tc17 cells. Interestingly, combining ICOS with 41BB stimulation endowed Tc17 cells with superior multifunctionality, long-term expansion, and improved antigen-specific lytic ability compared to other costimulatory combinations. These findings have broad implications for the next generation of cellular therapies.

This work was supported by start up funds at MUSC, Charleston, SC (through the intramural program). Supported in part by the Flow Cytometry & Cell Sorting Shared Resource, Hollings Cancer Center, MUSC (P30 CA138313).
Modulation of Autoimmune Diabetes by Antibodies specific for N-Acetyl Glucosamine

J. Stewart New, Brian LP Dizon, and John F. Kearney

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

The hygiene hypothesis correlates the increasing incidence of autoimmune disease with decreased exposure to environmental microbes in developed societies, implying that microbial experience is necessary to develop an immune system that can efficaciously protect against autoimmunity. Vaccination with Group A Streptococci (GAS) produces a strong antibody response to the immunodominant epitope N-Acetyl-Glucosamine (GlcNAc), associated with the Group A cell wall carbohydrate (GAC). This GlcNAc moiety is conserved in mammals as a dynamic post-translational modification of proteins, which may serve regulatory roles on intracellular molecular function and is catalyzed by the enzymes O-GlcNAcase and O-GlcNAc transferase. O-GlcNAcylated proteins are highly enriched in the pancreas, where this modification has been implicated as a glucose-sensing mechanism governing insulin release in \( \beta \)-cells. We have shown that antibodies generated against GAS bind GlcNAc residues on \( \beta \)-cell granules in murine and human pancreatic islets. Developmental remodeling of the pancreas between 7-21 days of age in mice accompanied by significant cellular apoptosis has been suggested as an initial source of autoantigen priming in Type 1 Diabetes (T1D). We hypothesized that anti-GlcNAc Abs mediate non-inflammatory clearance of apoptotic \( \beta \)-cell antigens. Neonatal immunization of female NOD-mice with GAS but not Group C Streptococci reduced the incidence of diabetes. Furthermore, anti-GlcNAc immunotherapy on NOD mice primed with diabetogenic BDC2.5 T-cells protected from diabetes onset. These observations suggest the potential for T1D therapies involving immunization by GlcNAc-expressing microorganisms to prime antibody-producing cells capable of inhibiting T1D pathogenesis.

Funding. This work was supported by grants from the National Institutes of Health: AI4782 to JFK. BLPD was supported by 5T32GM008861 and DK082277.
Interleukin-21 is required for the pathogenesis of inflammatory bowel disease

Catherine H. Poholek^1,2, Laurie E. Harrington^3, University of Alabama at Birmingham, Birmingham, AL
^1 Medical Scientist Training Program; ^2 Graduate Biomedical Sciences, Immunology Theme; ^3Department of Cell Biology

Th17 CD4 T cells are necessary for protection against pathogens but have also been cast as pathogenic in the context of many autoimmune diseases, including Inflammatory Bowel Disease (IBD). Th17 cells produce several inflammatory cytokines, including IL-17, IL-21, IL-22, and IL-26. Although these cytokines may act in concert to induce inflammation in colitis, IL-21 is a strong candidate for further scrutiny. IL-21 expression is increased in biopsies from patients with ulcerative colitis compared to healthy controls, and recent Genome Wide Association Studies have shown an association between the locus containing il2/il21 and IBD. We have shown that IL-21 signaling is required for the full induction of IBD in a murine model of disease. While others have shown in vitro that exogenous IL-21 acts to induce IL-17 production by CD4 T cells, our in vivo data suggests that IL-21 deficient cells are capable of producing IL-17A and IL-17F to a greater degree than IL-21 competent cells during IBD. In addition, our data disputes the previous finding that IL-21 suppresses the transcription factor Foxp3 as we have shown that IL-21 deficient T-regulatory cells express equal or less Foxp3 during IBD. Taken together, these data indicate that IL-21 plays a role in the induction of IBD that is separate from modulating the balance between effector and regulatory T cell responses. This work highlights a previously unrecognized role for IL-21 in the pathogenesis of IBD.

NIH/NIDDK-1R01 DK084082-01
Association of a novel fc-gamma receptor 2B (FCGR2B) variant with systemic lupus erythematosus (SLE)

Travis Ptacek, Chuanyi Ji, Xinrui Li, Jeffrey C Edberg, Robert P Kimberly

University of Alabama at Birmingham

SLE is an autoimmune disease characterized by auto-antibodies and immune complex formation. Deposition of immune complexes can cause severe and potentially fatal complications like glomerulonephritis. Fc-gamma Receptors (FCGRs) on leukocytes that recognize the Fc domains of IgG antibodies are responsible for immune complex clearance and participate in antibody-mediated regulation of immune responses. SNPs in FCGRs have been strongly implicated in susceptibility to and severity of SLE. The low affinity FCGRs reside within a gene cluster on 1q23, and recently variation in the number of gene copies in the genome (copy number variation, CNV) of one the genes, FCGR3B, was also implicated in SLE. CNV has also been reported for two other genes in the cluster (FCGR2C and FCGR3A). Although FCGR CNV relatively is common, CNV of the only inhibitory receptor in the cluster, FCGR2B, has not been reported, despite the genomic structure of the 1q23 FCGR gene cluster suggesting its possibility. Prior studies using a FCGR2B knock out mouse and human studies examining functional SNPs of FCGR2B establish the gene’s importance in autoimmunity and SLE. We hypothesized that CNV of FCGR2B may exist, and that if it did, it would have significance in the SLE phenotype. We have used three pyrosequencing CNV assays, which quantify single nucleotide differences between homologous genes (FCGR3B relative to FCGR3A and of FCGR2A and FCGR2B relative to FCGR2C), to determine relative CNV across the FCGR cluster in a population of 2172 SLE cases and 2682 healthy controls. In addition to previously reported CNVs involving FCGR2C, FCGR3B, and FCGR3A, we identified individuals that appeared to have duplications and deletions of FCGR2B. Further CNV analysis by other pyrosequencing assays and qPCR assays indicated that these variants did not include the cytoplasmic tail-encoding exons. Analysis by logistic regression showed that the FCGR2B duplication variant (variant 2B) is statistically associated with SLE (p<0.01, OR = 1.8), and that this effect is independent of FCGR2B functional SNPs and functional alleles and CNVs of other genes. Preliminary analysis of mRNA and protein from EBV transformed cell lines derived from variant 2B and no CNV control donors indicate the presence of a novel transcript and protein product in variant 2B lines, but not control lines. We are currently working on recalling donors (variant 2B donors vs controls) to characterize variant 2B mRNA transcripts, protein size and antibody reactivity and protein expression on primary cells. We also plan on future studies to characterize the function of variant 2B.

NIAMS P01 AR49084
HHMI Med to Grad Fellowship Program
KÜPPEL LIKE FACTOR 2 IN INNATE LIKE LYMPHOCYTE DEVELOPMENT AND FUNCTION

Whitney Rabacal, Delphine Cendron, Kristen Hoek, Sudheer Pabisetty, Eric Sebzda

Innate immunity underpins key aspects of pathogen clearance and tissue homeostasis, including tumor surveillance. We have previously demonstrated that the transcription factor Krüppel-like factor 2 (Klf2) maintains homeostasis within the adaptive arm of the immune system. We now report that Klf2-deficient mice display significant functional and differentiation defects in an innate-like lymphocyte lineage that may contribute to tumor surveillance and viral clearance. These results reveal a novel, cell-intrinsic role for Klf2 in an innate-like lymphocyte lineage.

Funding: This work was funded by NIH-HL-094773.
The Affect Macrophage Conditioned Media has on Lung Cancer Cell Line Migration, Proliferation and Radiosensitivity

T.D. Rohrbach¹, J.S. Deshane² C. Steele², C.D. Willey¹

¹Department of Radiation Oncology; University of Alabama at Birmingham; Birmingham, Al.
²Department of Medicine; University of Alabama at Birmingham; Birmingham, Al.

Background: Each year lung cancer takes the lives of over one million people worldwide with about a quarter of advanced patients forming metastatic tumors. Increased infiltration of macrophage populations in lung cancer patients have correlated with high microvessel density and poor prognosis. Tumor associated macrophages associated with the tumor microenvironment are believed to predominantly possess an alternatively activated (M2) phenotype. Hallmarks of this phenotype are production and secretion of signaling molecules aiding in wound repair, angiogenesis, and extracellular matrix remodeling. We wanted to investigate the migratory, proliferative and radiosensitizing effects that M2 macrophage have on lung cancer cell lines in in vitro models.

Methods: Human lung cancer cell lines A549, H1299, H1792, and H1975 were used in this study along with the murine lung cancer cell line Lewis Lung Carcinoma (LLC). Macrophages were differentiated in vitro from murine bone marrow and stimulated with IFN-γ or IL-4 to achieve classically activated (M1) and alternatively activated (M2) populations. Conditioned media (24 h) was collected from the various macrophage stimulations and used in migration and proliferation assays. Migration was assessed using BD 24 well FluorBlok cell culture inserts. Proliferation was measured using Perkin Elmer’s ATPlite assay with or without ionizing radiation.

Results: A549 cells experienced a 20% increase (p<0.0001) in cell migration when cultured with M2 conditioned macrophage media compared to non-stimulated conditioned media. When A549 cells were cultured in M1 conditioned macrophage media, they experienced a decrease in cell migration. ATP levels increased 55% in LLC, 69% in H1792, 36% in H1299, and 50% in H1975 (p<0.05) when cultured in M2 conditioned macrophage media. A549 cells were trending in this direction. The LLC cell line cultured in M2 conditioned macrophage media demonstrated significantly higher (p<0.05) ATP levels when exposed to 0 Gy, 3 Gy and 9 Gy radiation compared to M1 and non-stimulated conditioned media. H1299, H1792, H1975, and A549 cell lines demonstrated trends of higher ATP levels in cultures with M2 macrophage conditioned media, relative to M1 and non-stimulated conditioned macrophage media, when exposed to 3 Gy, 6 Gy, and 9 Gy radiation.

Conclusion: Our studies suggest that M2 conditioned macrophage media promote lung cancer migration and proliferation, particularly, for the human lung adenocarcinoma cell line, A549. Furthermore, treatment of murine lung cancer cells with M2 conditioned macrophage media increased radiation resistance. We predict that pro-survival signaling molecules present in M2 conditioned macrophage media contribute to proliferation and radioresistance in lung cancer cells.

Future Directions: We will attempt to block the release of cytokines from both M1 and M2 macrophage population with different therapeutics and use the Milliplex ELISA Kit from Millipore to identify different cytokine populations between M1 and M2 macrophage.

Funding: Financial support was provided by Dr. Willey's Lab Development Funds
Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis of the breast cancer cells

Anandi Sawant, Jonathan Hensel, Jessy Deshane, Brittney Harris, Diptiman Chanda, Carnella Lee, and Selvarangan Ponnazhagan

Departments of Pathology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35226

Osteolytic bone metastasis is associated with increased morbidity and mortality in breast cancer patients with median survival rate of less than 2 years. Current therapies for treating bone metastasis are limited and focus mainly on its symptomatic management. Therefore, a better understanding of the molecular mechanisms involved in the formation and development of bone metastases will offer new strategies for therapeutic intervention and extending survival. As the osteolytic bone metastasis is triggered by interplay of bone homing cancer cells, osteoclasts and the immune cells in the bone microenvironment, the main purpose of the current study was to understand the influence and interaction of metastasizing cancer cells with cells of the skeletal system and the immune system. Towards this goal, female BALB/c mice were injected with $10^5$ osteolytic mouse breast cancer cell line 4T1, expressing firefly luciferase, via intra-cardiac route. Mice were sacrificed at different times as bone metastasis increased. Immunophenotyping was performed on cells obtained from bone marrow and spleen to enumerate various immune cells such as macrophages, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), myeloid-derived suppressor cells (MDSC), Th1, Th2, Treg and CD8$^+$ T cells by flow cytometry. Micro-CT and histological analysis was performed on femur and tibia to assess the extent of bone destruction. Results of this study indicated that increased bone metastasis and osteolytic damage correlated with significant increase in pDC numbers in the bone which in-turn led to elevated Th2 response. Levels of Treg and MDSC were drastically elevated with bone metastasis of the breast cancer. The significance of elevated pDC numbers with increased growth and metastasis was further confirmed by depleting pDC population in vivo which led to a significant decrease in the tumor burden and absence of bone metastasis. Depletion of pDC population increased Th1 response and decreased the levels of osteolytic cytokines. Tumor-specific cytotoxic CD8$^+$ T cell population was enhanced after pDC depletion and led to killing of disseminated breast cancer cells both in the bone and other organs. Depletion of pDC also led to decreased levels of MDSC.

Although, elevated MDSC levels are reported when cancer metastasizes to the bone, the significance of this observation remains undefined. As MDSC are the progenitors of macrophages, which differentiate into osteoclasts, we hypothesized that MDSC may undergo osteoclast differentiation and contribute to enhanced bone destruction and tumor growth. Indeed, multi-nucleated, tartrate resistant acid phosphatase positive (TRAP$^+$) osteoclasts were observed when MDSC isolated from mice with breast cancer bone metastasis were cultured in the presence of RANKL and M-CSF. The osteoclasts differentiated from MDSC were also found to be functional in degrading bone in vitro and in vivo. Collectively these data suggest that pDC play a pivotal role in bone metastasis of the breast cancer cells with skewing of Th1 response to Th2. Thus, depletion of pDC during cancer progression may provide adjuvant effects on therapeutic approaches. Further, the discovery that MDSC have osteoclastogenic potential and play a vital role in increased bone destruction and growth of tumors in the bone microenvironment, suggests the potential of MDSC-targeted therapies in reducing bone damage while promoting tumor immunity.
Targeting regulatory myeloid cell pathways to enhance anti-tumor immunity and long-term survival against lung cancer

Cara C. Schafer1, Anandi Sawant2, Tong Huan Jin1, Jaroslaw Zmijewski1, Hubert Tse3, Justin Roth4, Zhihan Sun1, Gene P. Siegal2, Victor J. Thannickal1, Stefan C. Grant1, Selvarangan Ponnazhagan5 & Jessy S. Deshane2

1Departments of Medicine, 2Pathology, 3Microbiology & 4Pediatrics, University of Alabama at Birmingham

In the United States and worldwide, lung cancer is the leading cause of cancer related death. Current front line therapies, including chemotherapeutic combination strategies, have produced limited success in prolonging patient survival prompting the necessity to improve multi-modal therapies to manage the disease.

Oxidative stress, with elevated levels of Reactive Oxygen Species (ROS), is implicated in the initiation and progression of cancer. Myeloid Derived Suppressor Cells (MDSCs) are immunosuppressive immature myeloid cells which promote tumor progression and metastasis by producing high levels of ROS and inhibiting the host protective anti-tumor T cell responses. Memory CD8+ T cells are a critical component of protective immunity, and inducing effective memory T-cell responses is a major goal of vaccines against tumors. We hypothesized that a combination therapeutic strategy using gemcitabine, a current front line therapy for lung cancer and a known inhibitor of MDSC expansion, along with Superoxide Dismutase (SOD) mimetic, which targets ROS in the tumor microenvironment will enhance the CD8+ T cell response, inhibit tumor progression, and prolong survival. We developed an immunocompetent mouse model of lung cancer with Lewis Lung Carcinoma (LLC) cells. Tumor challenged mice treated with gemcitabine and SOD mimetic showed significant reduction in ROS levels, reduced tumor burden, and prolonged survival in tumor challenged mice compared to controls treated with gemcitabine or the SOD mimetic alone. Most importantly, this novel strategy to target MDSC infiltration and function reduced apoptosis of CD8+ T Cells and enriched the quantity and quality of the CD8+ memory T cell response. Adoptive transfer of tumor specific CD8+ memory cell subsets derived from treated mice into recipient mice with established lung cancer resulted in prolonged survival of the treated mice, with persistent memory CD8+ T cells which responded vigorously to tumor re-challenge. A possible mechanism for improved memory response of CD8+ T cells may involve the activation of transcription factor STAT-3 which was increased in memory T cell subsets from the mice treated with the combination therapy. This study clearly delineates a role of MDSC in lung cancer progression and offers an efficient therapeutic strategy targeting MDSC, their effector molecules and mechanistic pathways, for treatment of early lung cancer and prevention of lung cancer relapse. This study was supported by NCI CA 13148-39 UAB CCC CDGP awarded to JD and SP.
Negative feedback regulation of encephalitogenic Th1 Cells by IFNγ in experimental autoimmune encephalomyelitis

Christine M. Sestero¹, Rodrigo Naves², Simer Preet Singh³, Patrizia De Sarno¹ and Chander Raman¹

¹ University of Alabama at Birmingham, Birmingham AL 35294
² Universidad Pedro de Valdivia, Santiago, Chile
³ University of Rochester Medical Center, Rochester, NY 14642

*Ifngr1*⁻/⁻ mice develop more severe experimental autoimmune encephalomyelitis (EAE) with delayed onset compared to WT mice. Enhanced disease severity in *Ifngr1*⁻/⁻ mice was associated with elevated numbers of both Th1 and Th17 cells in the central nervous system. Elevated Th17 populations were expected, but the expanded numbers of Th1 cells in *Ifngr1*⁻/⁻ mice contradicts the paradigm that IFNγ signaling is a positive feedback loop for Th1 expansion. Furthermore, cultures of encephalitogenic T cells isolated from these mice restimulated under Th1 skewing conditions contained several fold greater numbers of IFNγ-expressing Th cells than WT T cell cultures. These observations led us to investigate the possibility that IFNγ participates in a negative feedback loop associated with Th1 differentiation. Addition of suboptimal levels of neutralizing anti-IFNγ mAb during *in vitro* polarization of encephalitogenic WT T cells to Th1 cells led to at least a two-fold greater number of CD4⁺IFNγ⁺ T cells. Suboptimal neutralization of IFNγ didn’t alter the generation and/or expansion of Th1 cells when encephalitogenic T cells were obtained from *Ifngr1*⁻/⁻ or *Stat1*⁻/⁻ mice, confirming the requirement for intact IFNγ signaling. Partial neutralization of IFNγ also leads to decreased NO production and iNOS expression in Th1 cells, suggesting a mechanism for negative regulation in these populations that is mediated by IFNγ-induced NO production. Contrary to the traditional dogma, these data suggest that IFNγ can negatively regulate Th1 differentiation, which may partly explain the opposing biology of IFNγ in EAE and multiple sclerosis. Supported by grants from the NMSS (RG3891) and the NIH (AI1076562); support to CS from NIH IRACDA and support to RN from Beca Chile.
Overexpression of the long intergenic non-coding RNA (lincRNA)-p21 in multiple sclerosis

Charles F. Spurlock, III¹, John T. Tossberg², Brittany K. Matlock³, Lily Wang⁴, Nancy J. Olsen⁵, Subramaniam Sriram ¹,⁶, and Thomas M. Aune¹,⁷

¹ Charles F. Spurlock, III, BS, Subramaniam Sriram, MD, Thomas M. Aune, PhD, Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA
² John T. Tossberg, MS, Research Department, ArthroChip, Franklin, Tennessee 37069, USA
³ Brittany K. Matlock, BS, Vanderbilt Vaccine Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA
⁴ Lily Wang, Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232
⁵ Nancy J. Olsen, MD, Department of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033, USA
⁶ Subramaniam Sriram, MD, Department of Neurology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA
⁷ Thomas M. Aune, PhD, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA

Multiple sclerosis (MS) is a multifactorial disorder of unknown etiology leading to CNS demyelination. Despite decades of research, there are still significant gaps in our approach to treating MS patients because we lack a clear understanding of how the disease is sustained. Our work has sought to uncover defects in the cellular programs that initiate cell proliferation and cell death. In two prototypic autoimmune diseases, MS and rheumatoid arthritis, defects in DNA repair mechanisms and, in particular, expression and function of p53 are widely present in the peripheral blood mononuclear cells. The long intergenic non-coding RNA (lincRNA)-p21 mediates p53-induced apoptosis by activating or repressing transcription of p53 target protein-coding genes and thus plays a key role in determining a cell’s transcriptional program. Our results demonstrate that lincRNA-p21 is weakly expressed in subjects with clinically isolated syndrome, a precursor of MS, and increased 3-4 fold in subjects once they develop MS. Increased levels of lincRNA-p21 in MS are not regulated by p53, but rather, our results demonstrate that JNK enzymes negatively regulate and ERK enzymes positively regulate lincRNA-p21 expression. A MAP kinase imbalance, low JNK, high ERK, exists in MS, which we propose is responsible for increased lincRNA-p21 expression. Further, comparison of gene expression profiles between subjects with MS to lists of lincRNA-p21 target genes suggests that a major portion of the MS-specific transcriptome is a direct result of over-expression of lincRNA-p21.

Grant Support
Supported by grants from the National Institutes of Health (R42AI53948, R01AI044924), the American College of Rheumatology ‘Within Our Reach’ grant program (ACR124405), National Multiple Sclerosis Society (RG4576A2/1), and the National Science Foundation Graduate Research Fellowship Program. The Vanderbilt Medical Center Flow Cytometry Shared Resource is supported by the Vanderbilt Ingram Cancer Center (P30 CA68485) and the Vanderbilt Digestive Research Center (DK058404).
Antibodies against conserved antigens suppress allergic airway disease

Emily K. Stefanov, Nicholas W. Kin, Brian L.P. Dizon and John F. Kearney

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

There has been a sharp rise in allergic asthma and asthma-related deaths in the developed world. The hygiene hypothesis proposes that excessively sanitary conditions early in life result in autoimmune and allergic phenomena because of a failure of the immune system to receive proper microbial stimulation during development. Chitin, a biopolymer of N-acetyl glucosamine (GlcNAc), is produced by many allergen-bearing organisms including: fungi, the exoskeleton of insects, crabs, shrimp and parasitic nematodes. We and others have found that instillation of chitin into the lungs induces the influx of innate cells and the release of pro-inflammatory cytokines. We propose that allergens are cargo in association with conserved molecules such as chitin and glucans and antibodies against these molecules block interactions between the molecules and their innate receptors. We demonstrate that antibodies against GlcNAc and other conserved bacterial polysaccharides, induced by neonatal vaccination or passive transfer, are reactive with and dampen the immune response against chitin and Aspergillus fumigatus. In the presence of anti-polysaccharide antibodies, we observed a marked reduction in antigen uptake, cell influx, cellular activation, and cytokine production, resulting in a striking decrease in the severity of allergic airway disease in mice. Our results suggest that antigen exposure, during the neonatal period, from environmental sources, self-antigens, or vaccination, have dramatic effects on the adult B cell repertoire and subsequent antibody responses, modulating the immune response to allergens later in life and thus dampening development of allergic airway disease. We propose an adjunct hypothesis that antibodies may contribute to the mechanism of protection proposed by the hygiene hypothesis.

This work was supported by NIH R01 AI14782-32 and AAF Senior Investigator Award to JFK.
Phenotypic Analysis of B cells in a Pair of Identical, HLA*B44 Female Twins Discordant for Common Variable Immunodeficiency and Recurrent Sino-Pulmonary Infection

E. Szymanska 1, G.C. Ippolito 5, M. Zemlin 6, T.A. Hwangpo 2, M.G. Brand 2, Y.Zhuang 2, D. Schneider 3, G. Georgiou 5, E.E. Brown 4, and H.W. Schroeder, Jr 1,2

Departments of Microbiology 1, Medicine 2, Biochemistry 3, Epidemiology 4 University of Alabama at Birmingham, Department of Chemical and Molecular Engineering 5 University of Texas at Austin, and Department of Pediatrics 6 University Marburg, Germany

In our immune deficiency clinic, HLA*B44 is present in almost half of patients with Common Variable Immunodeficiency (CVID) or recurrent sino-pulmonary infections (RESPI) with normal, or near normal, IgG levels. In our clinic, one of pair of HLA*B44 positive identical female twins suffers with severe infections and an IgG of 477 mg/dl (CVID), whereas the other has milder infections and an IgG of 733 mg/dl (RESPI). Whole genome sequencing and analysis of the twin is in progress. Phenotypic characterization of peripheral blood B cell subpopulations by FACS revealed higher numbers of immature B cells in the CVID twin, but progressively lowers numbers of transitional, mature, memory IgD+ (p=0.012), memory IgD− (p=0.017), and plasmacytes when compared to the RESPI twin. Deep sequencing of immunoglobulin transcripts from the transitional, memory IgD+, memory IgD− and plasmacyte fractions revealed a consistently lower prevalence of tyrosine in the CDR-H3 loop (p<0.0001). The relative paucity of tyrosine was most pronounced in the plasmacyte fraction (12.4% CVID vs. 16.1% control). These findings suggest that in addition to a progressive block in mature B cell differentiation starting from transitional B cells, CVID in the discordant twin may be associated with an altered development of the antibody repertoire, which may help explain why, in spite of the presence of IgG, the CVID patient suffers more severe infections than her RESPI twin. A comprehensive analysis of the BCR in CVID may help extend the disease definition and help identify the mechanisms that underlie the immune deficiency in of CVID. Work supported by UO1AI90902-2 and T32AI007051-35.
Decreased regulatory T cell numbers in the intestine precede the development of colitis in the FVB.\textit{mdr1a}^{-/-} mouse

Scott M. Tanner and Robin G. Lorenz

University of Alabama at Birmingham, Department of Pathology

The FVB.\textit{mdr1a}^{-/-} mouse, lacking the small molecule pump P-glycoprotein (P-gp), is a commonly used model for the study of spontaneous T cell mediated colitis. In addition, \textit{MDR1} polymorphisms and P-glycoprotein deficiency in humans has been linked to the development of ulcerative colitis. Currently, the mechanism by which intestinal inflammation develops in FVB.\textit{mdr1a}^{-/-} mice is unknown. Recently, our lab has shown FVB.\textit{mdr1a}^{-/-} mice, prior to the development of histological inflammation, have a significant decrease in Foxp3\textsuperscript{+} regulatory T cells (Tregs) in Peyer's patches (PP) and intestinal lamina propria (LP), but not in spleen and mesenteric lymph nodes (MLN). Because Tregs are vital to maintain intestinal homeostasis, a decrease in Tregs could be a potential mechanism for the intestinal inflammation, leading us to further investigate the reduction in Foxp3\textsuperscript{+} cells. We hypothesize 3 mechanisms could be responsible for the decrease in Tregs: an increase in Foxp3\textsuperscript{+} cell apoptosis, decreased Foxp3\textsuperscript{+} cell trafficking to the intestines, or a shift from Foxp3\textsuperscript{+} cells to an effector T cell phenotype. Immunohistochemical caspase 3 studies indicate FVB.\textit{mdr1a}^{-/-} splenic Foxp3\textsuperscript{+} cells do not display increased apoptosis compared to wild type controls. Similarly, no change in the intestinal homing molecules \( \alpha_4\beta_7 \), CCR9 or CD103 was observed in FVB.\textit{mdr1a}^{-/-} spleen, MLN, PP, and intestinal (LP) by FACS and immunohistochemistry. Lastly, it is possible that FVB.\textit{mdr1a}^{-/-} Tregs are converting to an effector T cell phenotype, such as the pro-inflammatory Th17 lineage. We show that, while FVB.\textit{mdr1a}^{-/-} mice do not have increased levels of Foxp3\textsuperscript{+}IL-17\textsuperscript{+} cells, naïve CD4\textsuperscript{+} cells treated with TGF-\( \beta \) fail to develop into induced Treg cells and instead favor an IL-17 secreting Th17 phenotype. This failure of iTregs to develop explains the decrease in Foxp3\textsuperscript{+} Tregs in the FVB.\textit{mdr1a}^{-/-} intestine, representing a need to investigate Treg frequencies and function in human IBD patients with \textit{MDR1} polymorphisms.

Funding Acknowledgements: HHMI Med-to-Grad Fellowship, UAB Carmichael Scholarship, Crohn's and Colitis Foundation of America Student Research Fellowship Award, NIH P01 DK071176, UAB Digestive Diseases Research Development Center #P30 DK064400
A division-linked mechanism for the generation of IL-10 producing human B cells

Xiaogian Wang1, James Roger2, Ichikawa Travis Ichikawa1, Chungwen wei1, Ignacio Sanz1

1Division of Rheumatology and Immunology, Department of Medicine, Emory University
2Department of Medicine, University of Rochester

Regulatory B cells are active participants in down-regulating inflammation and autoimmunity, through the production of IL-10. Failure to produce IL-10 from regulatory B cells leads to the development of diseases such as Lupus and Rheumatoid Arthritis. In this study, we explored the development of IL-10 producing B cells in different in vitro and in vivo systems and determined the kinetics of IL-10 production. By analyzing the kinetics of IL-10 production from healthy individuals, we has identified that IL-10 production is linked to cell division. After several cell divisions, cells expressing the highest level of IL-10 were observed and also become antibody secreting cells. This represents the first evidence that antibody secreting plasma cell or plasma blasts are capable of producing IL-10 in human. Moreover, Ex vivo IL-10 producing B cells were predominantly found within the IgD(+)CD27(+) non-switched memory B-cell subpopulation following CpG IL2 stimulation and that their strong production of IL-10 correlates with high expression of TLR9. This point was further addressed in patients with autoimmune diseases SLE. Correlating with reduction of non-switched memory population, IL-10 levels were significantly lower in SLE patient than in healthy donors after culture with CpG IL2. Moreover, within the pool of SLE patients were subgroup with normal secretion of IL-10, and this subgroup is characterized by its normal distribution of B cell subsets. Taken together, our data identified the B cell subset for IL-10 secretion and clarified the development of regulatory B cells.
ENRICHING THE BCR REPERTOIRE WITH CHARGED CDR-H3s IMPAIRS PROTECTION AGAINST HETEROSUBTYPIC INFLUENZA VIRUS INFECTION

Leticia Watkins, Zina Moldoveanu, Wen-Qiang Huang and Harry Schroeder

Department of Medicine and Microbiology, University of Alabama at Birmingham, Birmingham, AL USA

While individuals previously infected with or vaccinated against influenza virus typically express protective levels of antibody toward homologous virus, heterosubtypic immunity (HI) requires production of a broadly neutralizing antibody repertoire. Intriguingly, the ability to generate HI varies with the age of the individual, with the elderly at particularly high risk. We have previously shown that both human and mouse produce a primary antibody repertoire that is enriched for tyrosine containing, neutral CDR-H3s while, conversely, preventing expression of other categories of sequences, including highly charged sequences enriched for cationic amino acids. Therefore, we believe that the breakdown in protection against influenza virus results, in part, from an accumulation of highly charged amino acids in the immunoglobulin (Ig) CDR-H3 repertoire. In this study, we sought to determine how, and to what extent, controlling composition of the antibody repertoire permits or prevents HI to influenza viruses.

Here, we immunized mice with an H3N2 strain (A/Udorn) of influenza, waited 4 weeks, and then challenged with an H1N1 strain (A/PR/8/34). We variably challenged five types of mice: wild-type (WT), mice homozygous for a single, normal D₃ gene segment (ΔD-DFL), mice homozygous for a single, frameshifted D₃ that express hydrophobic antigen binding sites (ΔD-D₃FS), mice that express highly charged antigen binding sites (ΔD-iD), and mice heterozygous for a normal D₃ allele and a charged allele (WT/ΔD-iD). To measure protection from infection, we calculated body weight loss and measured influenza specific antibody (Ab) titers from collected sera.

Heterozygous WT/ΔD-iD mice experienced increased morbidity relative to WT, as if they had not been immunized; and displayed lower Ab titers against different strains of H3N2. These data show impaired immunity in mice heterozygous for the charged D₃ allele and suggest that inclusion of disfavored CDR-H3s can have a dominant negative effect on protection against T-dependent viral infection. That is, the mere presence of disfavored antigen binding sites, despite the normal D₃ allele, impairs heterosubtypic immunity.

Funding provided by: HWS NIH R21AI088498
Tbet Up-regulation in Effector CD4 T-cells Independent of Conventional Th1 Cytokines During Chronic Autoimmune Disease

Wen-I Yeh and Laurie E. Harrington

Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294

CD4 T-cells play a central role in both eradication of infectious agents and promotion of autoimmunity. Classically, effector CD4 T-cells have been divided into distinct subsets based on their functional properties: Th1 cells produce IFN-\(\gamma\), Th2 cells secrete IL-4, IL-5, and IL-13, Th17 cells make IL-17A, IL-17F, IL-21, and IL-22, and regulatory T cells are Foxp3+ and produce IL-10. Tbet (Tbx21), a member of the T box transcription factor family, is a master regulator of Th1 cell differentiation and critical for IFN-\(\gamma\) production. Using a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), it has been reported that Tbet is essential for disease development, in contrast, IFN-\(\gamma\)-deficient mice remain susceptible to EAE. Therefore, we hypothesized that Tbet is critical for disease development, even in the absence of the cardinal Th1 cytokine IFN-\(\gamma\). We discovered that CNS-infiltrating IFN-\(\gamma\)-deficient CD4 T-cells express comparable levels of Tbet (50-80% Tbet+ cells) to WT CD4 T-cells during EAE suggesting that, against the conventional Th1 differentiation, IFN-\(\gamma\) signaling is not required for Tbet expression. Therefore, we investigated other Th1 factors in Tbet regulation as well as the correlation between disease progression. First, we examined the role of STAT1 in Tbet expression and observed ~60% Tbet-positive STAT1-deficient CD4 T-cells in CNS indicating STAT1 is dispensable for Tbet up-regulation in vivo. Since IL-12 is commonly used for in vitro Th1 polarization, we sought to determine Tbet expression in mice deficient of IL-12 signaling. IL-12p35-deficient mice develop EAE and, interestingly, the level of Tbet in those CNS-infiltrating CD4 T-cells is still similar to WT CD4 T-cells. Moreover, to assess if there is redundancy between IL-12 and IFN-\(\gamma\) for Tbet expression, we abrogated IFN-\(\gamma\) signaling in IL-12p35-deficient mice and determined the level of Tbet in CNS CD4 T-cells. Surprisingly, blockade of both signaling pathways did not terminate Tbet expression in CD4 T-cells during EAE implying IFN-\(\gamma\) and IL-12 do not compensate for Tbet regulation. In order to explore which factors regulate Tbet, either positively or negatively during disease, we examined Tbet levels in CD4 T-cells after stimulation with various cytokines in vitro. These data may provide further insights into Th1 development and, more importantly, potential therapeutic targets for multiple sclerosis.

This work was supported by NIH-DK-084082 and National Multiple Sclerosis Society Award CA-1059-A-13.
A hallmark of HIV-1 pathogenesis is the rapid destruction of CD4 T cells, resulting in impaired cognate help to CD8 T cells and B cells. CD4 T cells are principal producers of interleukin-21 (IL-21), a vital helper factor required for the generation and maintenance of CD8 T cell functional competence and viral containment. We recently demonstrated that IL-21, produced primarily by CD4 T cells, is also produced by CD8 T cells in individuals who spontaneously control HIV-1 replication. However, virus-specific IL-21-producing CD8 T cells were not identified outside the context of HIV-1 infection. As such, we hypothesized that under conditions of reduced CD4 T cell help, as seen in HIV-1 infection, CD8 T cells producing IL-21 may partially compensate for this loss. To test this hypothesis, we analyzed the phenotype of IL-21-producing CD8 T cells for functions generally attributed to CD4 T cells and compared them to those producing IFN-γ in 20 HIV-1-infected individuals. Upon polyclonal stimulation with PDBu/ionomycin, IL-21-producing CD8 T cells upregulated CD40 ligand at levels equivalent to that found in CD4 T cells, and a fraction of these cells were capable of expressing IL-2 and TNF-α. Interestingly, these CD8 T cells did not co-express CD4, and there was no evidence that these ever expressed this molecule, unlike what has been shown in African green monkeys naturally infected with SIV. Furthermore, compared to their IFN-γ+ counterparts, IL-21-producing CD8 T cells produced significantly lower amounts of perforin and granzyme B, functions generally attributed to cytotoxic CD8 T cells. These findings suggest that IL-21+ CD8 T cells, uniquely seen in HIV-1 infection, may be compensating for certain functions that are normally performed by CD4 T cells and serve a helper role in the control of HIV-1 infection.

This work was supported by NIH grants R21 AI 73103 and R01 AI 084772 and Bill & Melinda Gates Foundation grant 37874 (all to P.A.G.) and NIH grants T32 AI 007051 and F31 AI 085970 (to L.D.W.).
Using cytokine reporter mouse models to track CD4 T cell memory in vivo

Colleen J Winstead, Sing Sing Way, James J Moon, and Casey T Weaver

1 University of Alabama at Birmingham, Department of Pathology, Birmingham, AL
2 University of Minnesota, Department of Pediatric Medicine, Minneapolis, MN
3 Massachusetts General Hospital, Department of Medicine, Boston, MA

CD4 T cells respond to in vivo activation stimuli, in part, by producing the homeostatic cytokine IL-2. Because the chromatin in naïve T cells is primed to make this cytokine, the kinetics of expression post-activation with a specific antigen are very rapid (<24 hours). It is thought that this acute production of IL-2 is necessary for full activation and fate determination of both the conventional CD4 T cells themselves, as well as other components of the adaptive (Tregs, CD8 T cells, B cells) and innate immune responses (e.g. NK, NKT, DCs) to infection. Published basic and translational research in the area of cytokines and T cell memory focused specifically on IL-2 are concentrated on components requiring IL-2 signaling for survival, differentiation, and function. Therapeutics with demonstrated efficacy for treatment of viral infection (e.g. HIV), autoimmunity (e.g. diabetes), and cancer (e.g. lymphoma) based on modulation of IL-2 almost exclusively target signaling through the tri-molecular receptor complex (IL-2Ra <CD25>, IL-2Rb <CD122>, common g chain <CD132>) on cells responding to the cytokine. Within a population of antigen-specific CD4 T cells responding to an acute stimulus, whether in the context of in vivo infection or non-antigen-specific chemical cross-linking of T cell receptors in a culture dish, only a percentage of cells will secrete IL-2. Though much is known regarding regulation of IL-2 production by CD4 T cells and the downstream consequences of both autocrine and paracrine signaling, there is virtually nothing known about the quantity, quality, or fate of CD4 T cells that produce IL-2 upon acute stimulation. Using strains of Listeria monocytogenes engineered to express foreign MHCII peptides and peptide-loaded MHCII tetramers, we are able to track and assess the fate of endogenous, antigen-specific CD4 T cells in infected mice. Combining this methodology with novel transgenic IL-2 reporter mouse models generated in the Weaver lab, we are able to address questions regarding fate by isolating, depleting, and/or adoptively transferring live, antigen-specific CD4 T cells to secondary hosts based on acute expression of IL-2. Preliminary data suggests IL-2-‘competent’ and –‘incompetent’, antigen-specific CD4 T cells differ in their ability to traffic to peripheral tissues following an acute, systemic infection, resulting in a potential functional, as well as proliferative and survival advantage for competent cells over those acutely activated, but incompetent for IL-2 expression. This work is being supported, in part, by NIH-AI-035783-13 and NIAID R01-A1-035783.