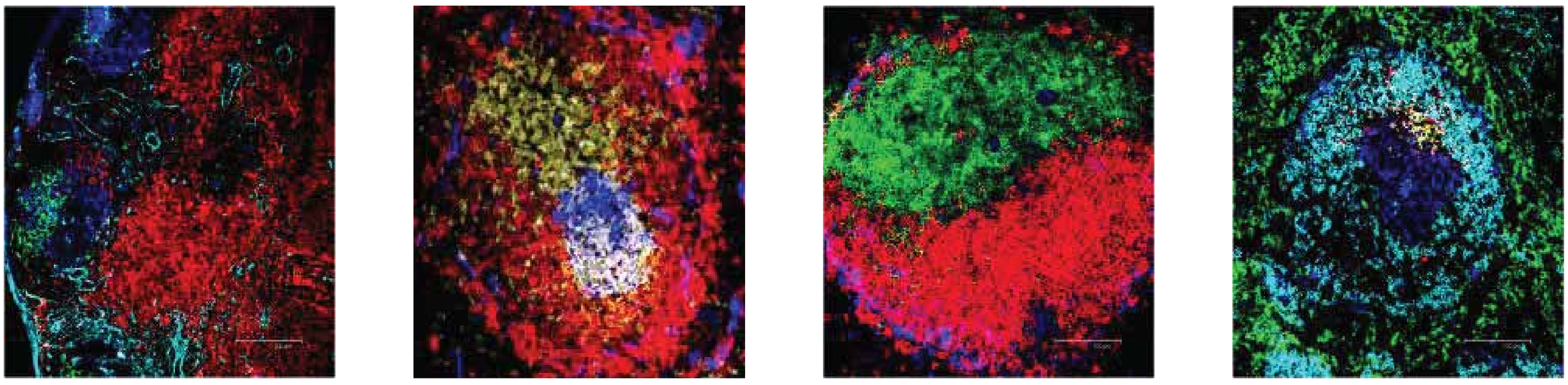
UAB MEDICINE

2nd Spring Immunology Symposium

Immunologic Diseases and Basic Immunology



June 22, 8 a.m. – 8 p.m. & June 23, 8 a.m. – 1 p.m.

Alys Robinson Stephens Performing Arts Center
University of Alabama at Birmingham

Keynote Speakers

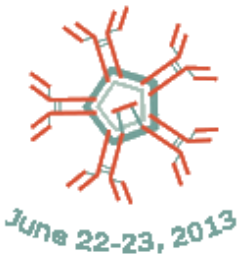
Emil R. Unanue, MD
Paul & Ellen Lacy Professor
Pathology and Immunology
Washington University School of Medicine
Albert Lasker Award for Basic
Medical Research

Prof. em. Rolf Zinkernagel
Professor of Experimental Immunology
University of Zurich, Switzerland
1996 Nobel Prize for Medicine

John Cambier, PhD
Ida and Cecil Green Distinguished Professor
Chair, Tntegrated Department of Immunology
University of Colorado Denver
and National Jewish Health

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

Harry W. Schroeder, Jr. MD, PhD
Hubert Tse, PhD
Co-Organizers of the Symposium



Spring Immunology Symposium

Immunologic Diseases & Basic Immunology

University of Alabama at Birmingham

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 the 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.

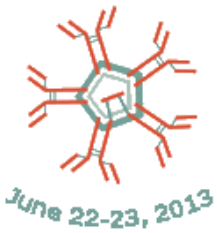
Harry W Schroeder Jr, MD, PhD
Professor of Medicine, Microbiology and Genetics
Director, UAB Program in Immunology
Co-Organizer, Spring Immunology Symposium

Robert Kimberly, MD
Howard L. Holley Professor of Medicine
Director, UAB Center for Clinical and Translational Science

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

Casey Weaver, MD
Wyatt and *Susan Haskell* Professor of Medical Excellence in Pathology
Co-Chair; Immunology, Autoimmunity and Transplantation Committee

Anupam Agarwal, MD
Professor of Medicine, Biochemistry, and Cell Biology
Director, Division of Nephrology
Interim Vice President and Dean, UAB School of Medicine



Spring Immunology Symposium

Immunologic Diseases & Basic Immunology

University of Alabama at Birmingham

Acknowledgments

Supported by the UAB School of Medicine

Thanks to the members of the **UAB Immunology, Autoimmunity and Transplantation Committee**!

Tika Benveniste, PhD
S. Louis Bridges, Jr, MD, PhD
Donald Buchsbaum, PhD
Randy Cron, MD, PhD
Devin Eckhoff, MD
Charles O. Elson, III, MD
Robert Gaston, MD
Robert P. Kimberly, MD*
Frances E. Lund, PhD
Roslyn Mannon, MD
Brendan McGuire, MD
Jan Novak, PhD
David Randolph, MD
Harry W. Schroeder, Jr, MD, PhD**,+
Chad Steele, PhD+
Victor Thannickal, MD
Tim Townes, PhD
Hubert Tse, PhD**,+
Casey Weaver, MD*,+
Allan Zajac, PhD
Staff: Jennifer Croker, PhD

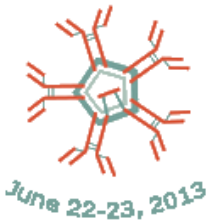
*, Co-Chair, UAB Immunology, Autoimmunity and Transplantation Committee
**, Co-director, Spring Immunology Symposium
+, Member, Spring Immunology Symposium Planning and Hosting Committee

Thanks for reviewing the abstracts submitted for presentation at the Spring Immunology Symposium!

Diane Bimczok, PhD
Harry W. Schroeder, Jr, MD, PhD
Chad Steele, PhD
Hubert Tse, PhD
Casey Weaver, MD

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.

Thanks to Marsha Brand, RN, for her assistance at the symposium.



Spring Immunology Symposium

Immunologic Diseases & Basic Immunology

University of Alabama at Birmingham

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Agenda

Saturday, June 22, 2013

Place: **Alys Robinson Stephens Performing Arts Center**, ***Sirote Theater***

7:30 - 8:10 am	Pick up badges
8:10 – 8:25 a.m.	Welcome **Harry W. Schroeder, Jr, MD, PhD** Professor of Medicine Division of Clinical Immunology and Rheumatology Director, UAB Program in Immunology UAB

Session 1

Chairs: Dr. Casey Weaver (UAB) and Dr. Linda Myers (University of Tennessee Memphis)

8:25 – 8:45 a.m	**Hubert Tse, PhD** Assistant Professor, Department of Microbiology, UAB **Title:** The Impact of Oxidative Stress on Autoimmune Responses in Type 1 Diabetes
8:45 – 9:15 a.m.	**Etty (Tika) Benveniste, PhD** Professor & Chair, Department of Cell, Developmental and Integrative Biology, UAB **Title:** Therapeutic Intervention of the JAK/STAT Pathway in Models of Neuroinflammation

9:15 – 10:00 a.m. ***Keynote Speaker:***

Emil R. Unanue, MD

Paul & Ellen Lacy Professor, Pathology and Immunology
Washington University School of Medicine
Member, National Academy of Sciences, the American Academy of Arts and Sciences and the Institute of Medicine

Title: Cellular and genetic events initiating diabetic autoimmunity

10:00 – 10:15 a.m. **Coffee Break**

10:15 – 10:45 a.m. **Andrew Mellor, PhD**

Professor, Medical College of Georgia

Director, Immunotherapy Center
Georgia Regents University

Title: A Tale with a STING: Inducing IDO to Control Autoimmunity

10:45 – 11:00 a.m. **Anupam Agarwal, MD**

Interim Senior Vicepresident for Medicine and Dean, School of Medicine
UAB

Title: Heme oxygenase-1 in the regulation of the immune response

11:00 – 11:15 a.m. *Immunology Trainee 1*

Han Dong and Timothy N.J. Bullock. Human Immune Therapy Center, Department of Pathology, University of Virginia, Charlottesville, VA 22908

Title: CD27 costimulation enhances IL-7 receptor re-expression on CD8+ T cells during viral infection and promotes CD8+ T cell memory

11:15 – 11:30 a.m. *Immunology Trainee 2*

Tahseen H. Nasti[1], Kyle Rudemiller[1], George Twitty[1], Hee Kyung Kim [1,2], Yuko Tsuruta [1,2], Mohammad Athar [1,2], Craig Elmets [1,2] and Laura Timares [1,2], [1] The Department of Dermatology, [2] The UAB Skin

Diseases Research Center, University of Alabama at Birmingham School of Medicine.

Title: An immune prevention strategy for protection against chemical carcinogenesis.

11:30 – 11:45 a.m *Immunology Trainee 3*

Ying Yi Zheng, Laurence Morel. Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL 32610.

Title: Contribution of marginal zone B cells to autoimmunity in the B6.Sle1.Sle2.Sle3 lupus prone mouse model.

11:45 – 12:00 p.m. *Immunology Trainee 4*

J Stewart New, Brian LP Dizon, M.D. Ph.D., John F Kearney Ph.D. Department of Microbiology, University of Alabama at Birmingham, Birmingham AL

Title: Modulation of autoimmune diabetes by antibodies specific for N-acetyl-D-glucosamine

12:00 - 1:30 p.m. **Lunch**

Session 2

Chairs: Dr. Frances Lund (UAB) and Sebastian Joyce (Vanderbilt University Medical Center)

1:30 – 2:00 p.m. **Laurence Morel, PhD**
Professor and Director, Experimental Pathology, Department of Pathology, University of Florida, College of Medicine

Title: CD4 T Cell Metabolism in Lupus

2:00 - 2:20 p.m. **Laurie E. Harrington, PhD**
Assistant Professor of Cell, Developmental and Integrative Biology
UAB

Title: Elucidating the factors that mediate protective and pathogenic CD4 T cell responses

2:20 - 2:40 p.m. **Jacob Kohlmeier, PhD**
Assistant Professor of Microbiology and Immunology
Emory University

Title: Maintenance and recall of lung airway resident memory CD8 T cells

2:40 - 3:00 p.m. **Phillip D. Smith, MD**
Mary J. Bradford Professor in Gastroenterology
Professor of Medicine and Microbiology
UAB

Title: Macrophages in Mucosal Homeostasis and Viral Infections

3:00 - 3:20 p.m. **Coffee Break**

3:20 - 3:40 p.m. **Chad Steele, PhD**
Professor of Medicine/Division of Pulmonary, Allergy & Critical Care Medicine
UAB

Title: Immunopathogenesis during fungal asthma

	Devin Absher, PhD
3:40 - 4:00 p.m.	Faculty Investigator HudsonAlpha Institute for Biotechnology **Title:** Epigenetics of Systemic Lupus Erythematosus
4:00 - 5:00 p.m.	***Keynote Speaker*** **Prof. Dr. med. Rolf M. Zinkernagel** Professor Emeritus, University of Zürich, University Hospital, Zürich 1996 Nobel Prize for Medicine **Title:** Immunology taught by viruses
5:15 - 5:45 p.m.	Immunology trainees meet with Nobel Prize for Medicine Laureate, Prof. Dr. med Rolf Zinkernagel
6:00 - 8:00 p.m.	**Reception and Poster Session** Place: Alys Robinson Stephens Performing Arts Center, *Daniel Lower Lobby and Abroms Patrons Lounge*

For fun and recreation ideas at the end of the day click here

Sunday, June 23, 2013

Place: Alys Robinson Stephens Performing Arts Center, *Sirote Theater*

Session 3

Chairs: Dr. James (Tom) W Thomas, II, Vanderbilt and Dr. Robin Lorenz (UAB)

8:15 – 8:30 a.m. **Welcome**

Robert P. Kimberly, MD
Howard L. Holley Professor of Medicine
UAB
Director, UAB Center for Clinical and Translational Science

8:30 - 9:00 a.m. **Sebastian Joyce, PhD**
Professor of Pathology, Microbiology and Immunology
Vanderbilt University Medical Center

Title: The limbic immune system according to natural killer T cells

9:00 - 9:30 a.m. **James (Tom) W. Thomas, MD**
Professor of Medicine
Professor of Pathology, Microbiology and Immunology
Vanderbilt University Medical Center

Title: Tracking B cells in Type 1 Diabetes

9:30 - 10:00 a.m. **Linda K. Myers, MD**
Professor of Pediatrics
University of Tennessee College of Medicine at Memphis

Title: Collagen peptides and autoimmune arthritis

10:00 - 10:30 A.M. **Arnold E. Postlethwaite, M.D.**
Goodman Professor of Medicine
University of Tennessee College of Medicine at Memphis

Title: Naturally Occurring Noncalcemic Analogs of Vitamin D
Possess Immunomodulatory and Anti-Fibrotic Properties

10:30 – 11:00 a.m. **Coffee Break**

11:00 – 11:20 a.m. **Allan J. Zajac, PhD**
Associate Professor of Microbiology
UAB

Title: Controlling effector and memory anti-viral CD8 T cell development

11:20 – 11:40 a.m. **Timothy Denning, PhD**
Assistant Professor of Pediatrics
Division of Neonatology
Emory University School of Medicine

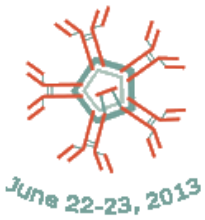
Title: Intestinal Macrophages: Regulators of Homeostasis and Inflammation

11:40 – 12:00 a.m. **Paul Goepfert, MD**
Professor of Medicine and Microbiology
Director, Alabama Vaccine Research Clinic
UAB

Title: Induction of CD8 T cell cryptic epitope responses by HIV infection and vaccines

12:00 – 1:00 p.m. ***Keynote Speaker***
John Cambier, PhD
Ida and Cecil Green Distinguished Professor and Chairman,
Integrated Department of Immunology
University of Colorado Denver and
National Jewish Health

Title: Subversion of BCR regulatory signaling circuitry by risk alleles promotes autoimmunity



Spring Immunology Symposium

Immunologic Diseases & Basic Immunology

University of Alabama at Birmingham

List of Abstracts

1. Toidi Adekambi[1], Chris C. Ibegbu[1], Ameeta S. Kalokhe[1], 2, Tianwei Yu[3], Susan M. Ray[2], and Jyothi Rengarajan[1,2*]. Depicting of Mycobacterium tuberculosis infection spectrum using antigen-specific CD27 and PD-1 expression. [1]Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, USA; [2]Division of Infectious Disease, Department of Medicine, Emory University, Atlanta, GA; [3]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Atlanta, GA, USA

2. Rakesh K. Bakshi[1], Rita Luther[2], Laurie E. Harrington[3], Casey T. Weaver[2] and Allan J. Zajac[1]. IL-2 production by CD8 T cells forecasts the formation of immunological memory. [1]Department of Microbiology, [2]Department of Pathology and [3]Department of Cell Biology, University of Alabama at Birmingham, Birmingham AL 35294

3. Anne Bet[1], Sarah Sterret[1], Alicia Sato[3], Anju Bansal[2], Paul A. Goepfert [1,2]. Cryptic epitopes enhance the breadth of HIV-1-specific T cell responses in recipients of a non-codon optimized vaccine. Department of Microbiology[1] and Department of Medicine[2], University of Alabama at Birmingham, Birmingham, AL USA 35294; Statistical Center for HIV/AIDS Research & Prevention (SCHARP)[3], Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024.

4. Siddheshvar Bhela, Sachin Mulik, and Barry T Rouse. MicroRNA-155: regulator of HSV-1 encephalitis but promoter of stromal keratitis. Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN

5. Rachel Henry Bonami[1], Amita B. Rachakonda[1], Chrys Hulbert[1], and James W. Thomas [1,2]. Stuck in a Rut: Impaired Receptor Editing in Type 1 Diabetes-Prone Mice. [1]Vanderbilt University, Division of Rheumatology and Immunology, Department of Medicine; [2]Vanderbilt University, Department of Pathology, Microbiology, and Immunology.

6. Allison M. Brady, Juan J. Calix, Jigui Yu, Moon H. Nahm. Ficolin-2 binds many pneumococcal serotypes with *wcjE*. Univ. of Alabama at Birmingham, Birmingham, AL.

7. Christian F. Bray[1]*, Patrice N. Mimche[1]*, Lauren M. Brady[1], Daniel Cox[1], David W. Fenne[2], Esmeralda Meyer[3], Mary Galinski[3] and Tracey J Lamb[1]. Exploring the role of EphA/EphrinA molecules in cerebral malaria. [1]Emory University, School of Medicine, Division of Pediatric Infectious Diseases; [2] Institute for Cardiovascular and Metabolic Research, University of Reading, UK; [3]Yerkes National Primate Centre, Emory University, Atlanta, Georgia. * Both of these authors contributed equally to this work.

8. Ashley R. Burg[1,2,3] and Hubert M. Tse, PhD [2,3] . Superoxide Production Mediates Anti-Viral Responses to Diabetogenic Viral Infections in NOD Bone Marrow-Derived Macrophages. Immunology Theme- UAB Graduate Biomedical Sciences[1], UAB Department of Microbiology[2] and the UAB Comprehensive Diabetes Center[3].

9. Kevin S. Cashman[3], Christine M. Sestero[1], Tamer Mahmoud[3], Patrizia De Sarno[4], John F. Kearney[3] and Chander Raman [1,2]. The CD5-CK2 signaling axis is critical for maintaining B-1a B cell responses to S. pneumoniae. Departments of Medicine[1], Clinical Immunology and Rheumatology[2], Microbiology[3], and Psychiatry and Behavioral Neurobiology[4], University of Alabama at Birmingham, Birmingham, AL, 35294.

10. Joseph G Daft [1,2], Charles O. Elson III[3], Robin Lorenz [1,2]. Antibodies to Commensal Microbiota are Present in Murine Models of Type 1 Diabetes. [1] Department of Pathology, UAB; [2] Comprehensive Diabetes Center, UAB; [3] Department of Medicine, UAB.

11. Han Dong and Timothy N.J. Bullock. CD27 costimulation enhances IL-7 receptor re-expression on CD8+ T cells during viral infection and promotes CD8+ T cell memory. Human Immune Therapy Center, Department of Pathology, University of Virginia, Charlottesville, VA 22908.

12. Ryan E. Doyle[2], Kirsten Neeck[2], Steven Palladino[2], and Tara M. DeSilva [1,2]. Regulation of Glial Glutamate Transporters in Multiple Sclerosis. [1]UAB Center for Glial Biology in Medicine, [2]UAB Department of Physical Medicine and Rehabilitation.

13. Victor Y. Du[1], Jonathan Carlson[2], Anju Bansal[1], Eric Hunter[3], Jesus F. Salazar-Gonzalez[4], Sonya Heath[1], and Paul A. Goepfert[1]. CD8 T cell responses are preferentially targeted to HIV-1 non-adapted epitopes during acute infection. [1]Department of Medicine, University of Alabama at Birmingham; [2]Microsoft Research, Redmond, Seattle, WA; [3]Emory Vaccine Center. Emory University; [4]Department of Genetics, University of Alabama at Birmingham.

14. Erdmann N[1], Du V[1], Bansal A[1], Carlson J[2], Hunter E, Tiffanie Mann[1], Sarah Sterrett[1], Anne Bet[1], Jonathan Carlson[2], and Paul A. Goepfert[1]. The use of HLA class II associated HIV polymorphisms in predicting novel CD4 T cell responses and viral escape. Departments of Medicine[1], University of Alabama at Birmingham, Birmingham, AL, Microsoft Research[2], Redmond, Seattle, WA, Emory Vaccine Center Emory University, Atlanta, GA.

15. Kirsten S. Evonuk [1,2,3], Brandi J. Baker[3], Christine M. Sestero[4], Ryan E. Doyle[5], Matthew A. Timberlake II[5], Chander Raman[4], and Tara M. DeSilva [2,3]. System xc- inhibition improves histopathological and clinical outcomes in experimental autoimmune encephalomyelitis. [1]Neuroscience Graduate Theme, [2]Center for Glial Biology in Medicine, [3]Department of Physical Medicine and Rehabilitation, [4]Department of Medicine, [5]Undergraduate Neuroscience Program.

16. Forghani P [1,2], Waller EK[2]. MDSCs Modulation by silibinin in Murine Breast Tumor Model. [1] School of Public Health, Dept of Pathobiology, Tehran University of Medical Sciences, Tehran, IR; [2] Department of Hematology and Medical Oncology, WCI, Emory University, Atlanta, GA.

17. Cassandra Garbutt Shahid Mukhtar and Robert Kimberly. Understanding the Regulatory Mechanisms of Fragment Crystallizable GammaReceptor IIB in Rheumatoid Arthritis, University of Alabama at Birmingham, Birmingham, AL.

18. Kristen L Hoek[1], Leigh M Howard[2], Tara M Allos[1], Parimal Samir[3], Kirsten E Diggins[1], Qi Liu[4], Nripesh Prasad [5,6], Megan Shuey[1], Xinnan Niu[1], Shawn Levy[6], Sebastian Joyce[1], Kathryn M Edwards[2], and Andrew J Link[1]. Systems Biology Assessment of Human Immune Responses after Seasonal Trivalent Inactivated Influenza Vaccine. Departments of [1]Pathology, Microbiology and Immunology, [2]Pediatrics, [3]Biochemistry, [4]Biomedical Informatics, Vanderbilt University School of Medicine, Nashville, TN, USA; [5]Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL, USA; [6]HudsonAlpha Institute for Biotechnology, Huntsville, AL.

19. Tracy Hwangpo[1], Daniel Griffin[2], Ewa Szymanska[1], Thomas Rothstein[2] and Harry Schroeder[1], Characterizing Human B1 cells in the CVID and RESPI Cohort. [1]University of Alabama at Birmingham, Birmingham, AL, [2]The Feinstein Institute for Medical Research, Manhasset, NY

20. Sarah A. Ingersoll, Julie Laval, Marcela Preininger, Milton Brown and Rabindra Tirouvanziam. Arginase-1 and Programmed Death Ligand-1, Potent Inducers of Immune Tolerance, are Upregulated on Airway Neutrophils in Cystic Fibrosis. Department of Pediatrics, Emory University School of Medicine & Center for CF Research, Children's Healthcare of Atlanta, Atlanta, GA.

21. Shannon M. Kahan[1], Maureen A. Cox[1], Scott R. Barnum[1], Daniel C. Bullard[2], Allan J. Zajac[1]. The Regulation of CD8 T Cell Fate Decisions During Acute and Chronic Viral Infections by Intercellular Adhesion Molecule-1. [1] Department of Microbiology, [2] Department of Genetics, University of Alabama at Birmingham.

22. Zoltán Kellermayer, Martina Mihalj, Péter Balogh. MAdCAM-1 independent lymphocyte homing to GALT of Nkx2-3-/- mice. Department of Immunology and Biotechnology, University of Pécs, Hungary.

23. Srilalitha Kuruganti[1], Ashlesha Deshpande[1], Winn W. Chatham[2], Mark R. Walter[1]. Influence of cytokine auto antibodies on serum IFNα activity in SLE. [1]Department of Microbiology, [2]Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham.

24. Julie Laval [1,3], Sarah Ingersoll[1], Marcela Preininger[1], Milton Brown[1], Vin Tangpricha[2] and Rabindra Tirouvanziam[1]. Molecular components of the insulin/Insulin-like growth factor-1 signaling pathway are highly expressed by airway neutrophils in Cystic Fibrosis and Cystic Fibrosis-related diabetes. [1] Department of Pediatrics, Emory University School of Medicine, Center for CF Research, Children's Healthcare of Atlanta, Atlanta, GA, USA; [2] Department of Medicine, Division of Endocrinology Emory University School of Medicine, Atlanta, GA, USA; [3] IGMM, CNRS UMR 5535, Université Montpellier 2, Montpellier, FRANCE.

25. 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, UAB.

26. Hao Li [1,2], Qi Wu[1], Jun Li[1], PingAr Yang[1], Yangxin Fu[3], Hui-Chen Hsu[1] and John D Mountz [1, 2, 4]. Increased Type I Interferon Promotes Follicular Shift of Lymphotoxin-expressing B Cells Which Disrupts Marginal Zone Barrier Integrity and Promotes Follicular Network Activation. [1]Division of Clinical Immunology and Rheumatology, Department of Medicine, and [2]Department of Microbiology, University

of Alabama at Birmingham, Birmingham, AL 35294; [3]Department of Pathology, University of Chicago, Chicago, IL 60637, USA; and [4]Birmingham, VA Medical Center, Birmingham, AL 35233, UAB.

27. Donald McGuire[2], Amber Rowse[2], Binghao Peng, Hao Li[2], Christine Sestero[4], Robert Axtell[5], Patrizia De Sarno[3] and Chander Raman[1]. CD5 increases Th17 polarization through AKT and GSK3 signaling. Department of Medicine[1], Department of Microbiology[2], Psychiatry and Behavioral Neurobiology[3], University of Alabama at Birmingham. Department of Biology, Chemistry, and Mathematics, University of Montevallo[4]. Department of Neurology and Neurological Sciences, Stanford University[5].

28. Gordon P. Meares and Etty N. Benveniste. ER Stress and Neuroinflammation: Connecting the Unfolded Protein Response to JAK/STAT Signaling. Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham

29. Lauren Miller[1], Milo Fasken[2], Courtney McDermott[1], David Guiliano[3], Jan Mead[1], Anita Corbett[2], Tracey Lamb[1]. Development of Saccharomyces boulardii as a Mucosal Vaccine Delivery System. [1]Division of Pediatric Infectious Disease, Emory University School of Medicine; [2]Biochemistry Department, Emory University; [3]School of Health, Sport and Bioscience, University of East London, UK.

30. Patrice N. Mimche[1], Michael P. Schenk[1], 2, Chengjing Zhou[3], Hyojung Choo[3], Jonathan Gibbins[2] , Shawn Jobe[3] and Tracey J Lamb[1]. Does platelet activation mediate pathogenesis of malaria infection? [1]Emory University, School of Medicine, Division of Pediatric Infectious Diseases and Children's Healthcare of Atlanta at Egleston, Atlanta, USA; [2]Institute for Cardiovascular and Metabolic Research, University of Reading, UK; [3]Aflac Cancer Center and Blood Disorders Service, Emory University, Atlanta, USA.

31. Tahseen H. Nasti[1], Kyle Rudemiller[1], George Twitty[1], Hee Kyung Kim [1,2], Yuko Tsuruta [1,2], Mohammad Athar [1,2], Craig Elmets [1,2] and Laura Timares [1,2]. An immune prevention strategy for protection against chemical carcinogenesis. [1]The Department of Dermatology, [2]The UAB Skin Diseases Research Center, University of Alabama at Birmingham School of Medicine.

32. J Stewart New, Brian LP Dizon, M.D. Ph.D., John F Kearney Ph.D. Modulation of autoimmune diabetes by antibodies specific for N-acetyl-D-glucosamine. Department of Microbiology, University of Alabama at Birmingham, Birmingham AL.

33. Lindsey E. Padgett and Hubert M. Tse. Lack of reactive oxygen species exacerbates diabetogenic CD4 T cell effector responses. Department of Microbiology, Comprehensive Diabetes Center; University of Alabama-Birmingham, Birmingham, AL

34. Melissa A. Pegues, Mark A. McCrory, Abolfazl Zarjou, and Alexander J. Szalai. A C-reactive protein → FcγR → macrophage axis exacerbates acute kidney injury Department of Medicine, The University of Alabama at Birmingham, Birmingham, Alabama.

35. Catherine H. Poholek[1], Laurie E. Harrington[2]. Interleukin-21 drives intestinal inflammation by bridging the adaptive and innate immune compartments. [1]Medical Scientist Training Program, [2]Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL

36. Ptacek, TS, Muktar, S, Redden, DT, Li, X, Ji, C, Absher, D, Edberg, JC, Kimberly, RP. A novel variant of FCGR2B is a risk factor for systemic lupus erythematosus, University of Alabama at Birmingham.

37. Pradeep B. J Reddy[1], Sharvan Sehrawat[2], Amol Suryawanshi[3], Naveen K Rajasagi[1], Madhu Khatri[4] and Barry T Rouse[1]. Th17 mediated recurrence of virus-induced immunopathology following discontinued FTY720 treatment. [1]Biomedical & Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996.; [2]Whitehead Institute for Biomedical Research, Cambridge MA 02142.; [3]New England Eye Center, Tufts University School of Medicine, Boston, MA 02111; [4]Department of Work Environment, University of Massachusetts-Lowell, Lowell, MA, USA.

38. Raphael Richardson, Siddheshvar Bhela and Barry T Rouse. Gut bacteria modulates angiogenesis and corneal immunopathology after herpes simplex virus infection, University of Tennessee, Knoxville.

39. Tanya Robinson, Christina Ochsenbauer, John Kappes, Mingce Zhang, Anna Genin, Robert Lowe, Matthew Stoll, and Randall Q. Cron. The Mechanism by which Regulatory T Cells Inhibit HIV-1 Infection of Polarized Human Monocyte-Derived Macrophages. Immunology Theme, Graduate School of Biomedical Sciences, CFAR Virology Core and, Division of Pediatric Rheumatology. University of Alabama at Birmingham, Birmingham, AL 35294.

40. Juan R. Barrantes[1], Brian Dizon[2], Mark Lisanby[3], Nicholas W. Kin[2] and John F. Kearney[2]. C57BL/6 C5 Deficient as a new mouse model for Bacillus anthracis studies. [1]Department of Pathology, [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.

41. Amber L. Rowse[2], Rodrigo Naves[3], Kevin S. Cashman[2], Donald J. McGuire[2], Tethia Mbana[3], Chander Raman[2,3] and Patrizia De Sarno[1]. Lithium controls central nervous system autoimmunity through modulation of IFN-γ signaling. Departments of [1]Psychiatry and Behavioral Neurobiology, [2]Microbiology, and [3]Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

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Depicting of *Mycobacterium tuberculosis* infection spectrum using antigen-specific CD27 and PD-1 expression

Toidi Adekambi[1], Chris C. Ibegbu[1], Ameeta S. Kalokhe[1, 2], Tianwei Yu[3], Susan M. Ray[2], and Jyothi Rengarajan[1, 2*]

[1]Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, USA

[2]Division of Infectious Disease, Department of Medicine, Emory University, Atlanta, GA

[3]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Atlanta, GA, USA

Introduction
It has become apparent that host biomarkers are needed to help to diagnose tuberculosis (TB) due to the lack of suitable tests to detect *Mycobacterium tuberculosis* in host samples.

Methods
In this study, we phenotype IFN-γ^+CD4$^+$ T cells using PD-1 and CD27 cell surface markers using flow cytometry.

Results
We showed that IFN-γ^+CD27$^+$ T cells and IFN-γ^+PD-1$^+$ T cells in combination may potentially depicter the *M. tuberculosis* infection as continuous spectrum of individuals who have not been infected with *M. tuberculosis* (BCG group), follow by individuals who have been successfully treated from previous TB diseases (treated TB), to individuals incubating latent bacteria in absence of clinical symptoms (LTBI) to individuals with massive load of replicating organisms (active tuberculosis). The subpopulation overlapping between LTBI and active TB may be the one to benefit the most from intervention by preventive therapy.

Conclusion
We conclude that IFN-γ^+CD27$^+$ T cells and IFN-γ^+PD-1$^+$ T cells in combination may be surrogate markers for *M. tuberculosis* load and depicter LTBI that may benefit the isoniazid preventive therapy.

IL-2 production by CD8 T cells forecasts the formation of immunological memory

Rakesh K. Bakshi[1], Rita Luther[2], Laurie E. Harrington[3], Casey T. Weaver[2] and Allan J. Zajac[1]

Department of Microbiology[1], Department of Pathology[2] and Department of Cell Biology[3], University of Alabama at Birmingham, Birmingham AL 35294

The development of immunological memory following vaccination or natural infection is critical for conferring long-lived protection against tumor outgrowth and pathogens. Understanding how memory T cell responses are regulated and deciphering the signals which dictate their development and maintenance provides basic information for the rational design of immune-based strategies for preventing and treating infectious diseases and cancers. In this study we set out to test the hypothesis that CD8 T cells which produce IL-2 preferentially form the memory T cell pool, and confer superior immunological protection. To address this we harnessed double cytokine reporter mice which facilitate the analysis of CD8 T cells that do or do not produce IL-2. Comparative analysis of antigen-specific IL-2^+ and IL-2^- CD8 T cells show that the IL-2^+ CD8 T cells more rapidly attain a memory CD127^{high} KLRG1^{low} phenotype and are preferentially CD27^{high} CD43^{low}. Further experiments showed that the IL-2^+ CD8 T cell population preferentially survives the down-regulation phase of the CD8 T cell response and undergo more rapid homeostatic proliferation. Adoptive transfer of equalized numbers of IL-2^+ and IL-2^- CD8 T cells revealed that IL-2^+ effector cells give rise to memory populations that mount rapid secondary proliferative recall responses. Collectively, these studies highlight that the formation of IL-2^+ CD8 T cells following activation is a critical developmental step necessary for the differentiation of memory T cell populations that serve to protect the host.

This study was funded by NIH R01 AI049360 and U01 AI082966 grants awarded to Allan J Zajac and Rakesh K Bakshi is a postdoctoral fellow in Dr. Zajac's lab.

Cryptic epitopes enhance the breadth of HIV-1-specific T cell responses in recipients of a non-codon optimized vaccine

Anne Bet[1], Sarah Sterret[1], Alicia Sato[3], Anju Bansal[2], Paul A. Goepfert[1,2]

Department of Microbiology[1] and Department of Medicine[2], University of Alabama at Birmingham, Birmingham, AL USA 35294; Statistical Center for HIV/AIDS Research & Prevention (SCHARP)[3], Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024.

Cryptic epitopes (CE) are MHC-I-restricted peptides encoded by any of the 5 alternative reading frames (ARFs) of a gene. CE-specific T cells (Tc) have previously been detected in HIV-1 infected patients but whether responses to CE can be induced by vaccination is currently unknown. Our previous work demonstrated that vaccination with naturally-encoded, non-codon optimized vectors generate CE more frequently than codon-optimized vectors. We therefore analyzed samples from 126 individuals who received either a naturally-encoded MVA/HIV62 double prime-double boost regimen (vaccine) or saline (placebo) during the HVTN205 trial. Peripheral blood mononuclear cells collected at 2 weeks post final vaccination were stimulated in an IFN□ ELISpot assay with overlapping peptide pools (OLPs) for HIV-1 Gag, Pol, and all 5 ARFs of these regions. Vaccinees had significantly more positive responses toward Gag but not Pol than placebo recipients (p=0.020, Mann-Whitney U test). *Ex vivo* Tc responses to potential CE were low in magnitude and their frequency did not differ significantly between treatment groups. In a cultured assay however, the median magnitude of responses to ARF OLP subpools was significantly greater in vaccinees (p< 0.001, Mann-Whitney U test), indicating CE-specific Tc responses are present but below the IFN□ ELISpot assay's limit of detection. Thus, we recommend that future vaccines expand the breadth of HIV-1 Tc responses through designs that preserve naturally-encoded CE.

MicroRNA-155: regulator of HSV-1 encephalitis but promoter of stromal keratitis

Siddheshvar Bhela[1], Sachin Mulik[1], and Barry T Rouse[1]

[1]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN

Herpes simplex virus (HSV) infection of humans can lead to life threatening herpes simplex encephalitis (HSE) and sometimes blinding ocular lesion, stromal keratitis (SK). Here, we show that mice with a deficiency of miR-155 are highly susceptible to HSE with a majority of mice (75-80%) dying after ocular infection with HSV. Acyclovir treatment (a day after virus reaches brain) reduced brain viral levels and protected miR-155 KO mice from HSE thus supporting the role for virus replication in brain as the cause of encephalitis. Furthermore, HSV infected miR-155 deficient mice developed compromised virus specific CD8 T cell responses. Moreover, miR-155 KO survivors developed attenuated ocular lesions and revealed significant reduction in pathogenic Th1 cells in corneas and lymphoid organs. Local delivery of antagomir-155 did not increase the incidence of HSE but led to profound reduction in pro-inflammatory milieu and significantly diminished SK lesions. In conclusion, we have discovered dual role for miR-155, a regulator of brain damage while a promoter of ocular immunopathology after HSV infection.

Stuck in a Rut: Impaired Receptor Editing in Type 1 Diabetes-Prone Mice

Rachel Henry Bonami[1], Amita B. Rachakonda[1], Chrys Hulbert[1], and James W. Thomas[1,2]

[1] Vanderbilt University, Division of Rheumatology and Immunology, Department of Medicine

[2] Vanderbilt University, Department of Pathology, Microbiology, and Immunology

This work was supported by NIH T32 HL069765, T32 GM08554, R01 AI051448, and the JDRF.

A delicate balance exists between maintaining protective immunity and preventing autoimmunity. It is unknown whether the insulin autoantigen triggers receptor editing, or if this critical immune tolerance mechanism is altered in type 1 diabetes. An increased frequency of anti-insulin B cells enters the periphery of type 1 diabetes-prone VH125Tg/NOD mice, compared with non-autoimmune VH125Tg/C57BL/6 mice. A reduced frequency of Igλ+ B cells indirectly suggests that impaired receptor editing efficiency may enhance BCR repertoire autoreactivity in NOD mice. To directly assess receptor editing in response to insulin, a novel BCR transgenic model was developed in which anti-insulin Vκ125 was targeted to the kappa locus. These mice were intercrossed with anti-insulin VH125Tg mice to generate VH125Tg/Vκ125SD mice to track the fate of anti-insulin B cells. Edited (non-insulin-binding) B cells are observed, and RAG-2/GFP mice show an increased GFP MFI in VH125Tg/Vκ125SD mice compared to control VH281Tg/Vκ125SD mice that lack insulin-binding B cells. These data suggest that physiologic levels of insulin upregulate RAG in an antigen-specific way to induce receptor editing in anti-insulin B cells. Despite RAG-2 upregulation in insulin-binding B cells, the majority of insulin-binding B cells fail to replace the insulin-binding light chain. Surprisingly, the frequency of edited B cells in the transitional stage is reduced in mature B cell compartments, suggesting either positive selection of anti-insulin B cells or a survival disadvantage of B cells that have undergone receptor editing. PTPN22 polymorphisms in humans are associated with central tolerance defects and autoimmunity. Loss of the mouse correlate, PEP, did not alter receptor editing efficiency in the VH125Tg/Vκ125SD C57BL/6 model. Interestingly, a lower frequency of edited B cells was present in VH125Tg/Vκ125SD NOD mice, suggesting that this immune tolerance mechanism is flawed in type 1 diabetes. These data suggest that physiologic levels of insulin autoantigen can stimulate receptor editing, but with lower efficiency in NOD mice.

Ficolin-2 binds many pneumococcal serotypes with *wcjE*

Allison M. Brady, Juan J. Calix, Jigui Yu, Moon H. Nahm

Univ. of Alabama at Birmingham, Birmingham, AL.

Background: *Streptococcus pneumoniae* (pneumococcus) is a nasopharyngeal commensal that can spread to sterile sites and cause disease. The major virulence factor of pneumococcus is its capsular polysaccharide (PS), with each serotype producing a structurally distinct PS. It is not clear why some capsule types are more likely to cause disease, while others rarely progress beyond carriage state. One possible explanation may be the interaction of pneumococcus with innate opsonins. Ficolin-2 is one such innate opsonin that is known to bind to *S. pneumoniae* serotype 11A. Ficolin-2 is found in the serum, targets acetyl groups, and induces the lectin pathway of complement. We recently discovered serotype 11E, a variant of serotype 11A. The PS of serotype 11A and 11E differ by the O-acetylation (OAc) of one sugar residue. This difference is attributed to *wcjE* which encodes an O-acetyl transferase. The *wcjE* allele is intact in serotype 11A, but disrupted in 11E. Ficolin-2 binding to serotype 11E is unknown. **Methods:** We used flow cytometry to detect Ficolin-2 binding to pneumococcus or PS coated latex beads. **Results:** We found that Ficolin-2 binds to serotype 11A, but not serotype 11E, suggesting that Ficolin-2 binding is dependent on WcjE-mediated OAc. The *wcjE* allele is found in 14 other serotypes, thus we hypothesize that Ficolin-2 is able to bind many pneumococcal serotypes in a *wcjE*-dependent manner. We found that Ficolin-2 binds most *wcjE*-containing serotypes, but does not bind to serotypes that lack *wcjE*. Other serotypes containing OAc by similar O-acetyl transferases, like WciZ in serotype 10C and WciG in 15B and, are not bound by Ficolin-2. To show that *wcjE* is necessary for Ficolin-2 binding, we disrupted *wcjE* allele in serotype 11D and 20A strains. We found that loss of *wcjE* leads to loss of Ficolin-2 binding. Furthermore, Ficolin-2 binding appears to be PS-dependent, as Ficolin-2 binds to latex beads coated with serotype 11A and 20A PS. Moreover, we tested Ficolin-2 binding to capsule-deficient strains and found Ficolin-2 does not bind to strains lacking capsular PS. **Conclusions:** Our findings show that the innate opsonin Ficolin-2 can bind many pneumococcal serotypes. Binding to pneumococcus is dependent on WcjE-mediated OAc of the PS. Recognition by Ficolin-2 may be important for clearance during pneumococcal infection.

Funding acknowledgements: T32 AI-007051, 2R56AI031473

Abstract title: Exploring the role of EphA/EphrinA molecules in cerebral malaria

Authors: Christian F. Bray[1*], Patrice N. Mimche[1*], Lauren M. Brady[1], Daniel Cox[1], David W. Fenne[2], Esmeralda Meyer[3], Mary Galinski[3] and Tracey J Lamb[1].

*Both of these authors contributed equally to this work

1. Emory University, School of Medicine, Division of Pediatric Infectious Diseases.
2. Institute for Cardiovascular and Metabolic Research, University of Reading, UK.
3. Yerkes National Primate Centre, Emory University, Atlanta, Georgia.

Collectively, Eph receptors and their Ephrin ligands represent the largest family of receptor tyrosine kinases. This family of molecules is divided into EphA and EphB receptors, which bind respectively to EphrinA and EphrinB ligands. Beyond their well-defined role in developmental processes and cancer, nothing is known about how Eph receptor/Ephrin ligands modulate immune responses and associated immunopathology during infection. Red blood cells parasitized with malaria sequester in organs of the body causing a variety of pathological sequelae. In the case of cerebral malaria, published work in the *Plasmodium berghei* ANKA model of experimental cerebral malaria indicates that T cell trafficking to the brain is an integral feature to the breakdown of the blood brain barrier. T cell trafficking occurs via several different trafficking molecules, mainly integrins and cell adhesion molecules such as ICAM-1. T cells have previously been shown to express Ephrin ligands and this family of molecules has been implicated in T cell trafficking. We hypothesized that in malaria infection T cells upregulate the transcription of EphrinA ligands to facilitate cell trafficking to the brain.

Here we show that in *P. berghei* ANKA infected C57BL/6 mice, splenic T cells upregulate transcription of EphrinA ligands at day 3 post-infection suggesting that this may be a generalized feature of the T cell response to malaria infection. Additionally, peripheral blood mononuclear cells (PBMCs) from naïve volunteers stimulated with red blood cells parasitized by the human malaria parasite *P. falciparum* upregulate transcription for EphrinA ligands. Next, we explored whether brain endothelial cells upregulate reciprocal EphA receptors in response to sequestered malaria-infected red blood cells, as would be required to fulfill the hypothesis the EphrinA ligands are involved in T cell trafficking/adhesion. *In vivo*, we were able to show that EphA2 is the predominant Eph receptor transcript in the brain of mice at Day 6 post-infection with *P. berghei* ANKA. Lymphotoxin alpha (LTα), but not tumor necrosis factor- α (TNF-α), is required for the development of experimental cerebral malaria symptoms in C57BL/6 mice. *In vitro*, mouse primary brain endothelial cells also upregulate EphA2 transcription in response to lysate of *P. berghei* ANKA parasitized red blood cells, as well as stimulation with recombinant LTα but not recombinant TNF-α. This suggests that EphA2 may play a role in EphrinA ligand-expressing T cell adherence during cerebral malaria. The implications of this will be discussed.

Source of funding:

Emory University School of Medicine
Royal Society
Children's Healthcare of Atlanta
Emory Egleston Children's Research Committee

Superoxide Production Mediates Anti-Viral Responses to Diabetogenic Viral Infections in NOD Bone Marrow-Derived Macrophages

Ashley R. Burg[1,2,3] and Hubert M. Tse, PhD[2,3]

Immunology Theme- UAB Graduate Biomedical Sciences[1], UAB Department of Microbiology[2] and the UAB Comprehensive Diabetes Center[3]

Viral infections are proposed to trigger the onset of Type-1 diabetes (T1D). Reactive oxygen species, such as superoxide, are important during anti-viral responses and as signaling molecules. We recently demonstrated that superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are highly T1D-resistant, partly due to dampened TLR3 and RIG-I signaling in bone marrow-derived macrophages (BMMΦ) upon poly(I:C)-stimulation. Decreases in TLR3 and RIG-I expression, NF-κB activation, and concomitantly, dampened TNFα and IFNβ synthesis were observed in NOD.*Ncf1*^{m1J} BMMΦ as compared to NOD BMMΦ. In this study, we aim to define how superoxide mediates macrophage responses to Encephalomyocarditis viral (EMCV) infections, a putative viral trigger of T1D. To this end, NOD and NOD.*Ncf1*^{m1J} BMMΦ were stimulated with an MOI=1 of EMCV strain-B (non-diabetogenic) or strain-D (diabetogenic) for 3, 6, 12, 24 or 48h, then analyzed for production of anti-viral mediators. By 24h, NOD.*Ncf1*^{m1J} BMMΦ exhibited a 2.5- and 1.3-fold lower level of RIG-I protein than NOD in response to EMCV-B and EMCV-D, respectively. By 48h, NOD.*Ncf1*^{m1J} BMMΦ displayed a dramatic 5-fold reduction in IL-1β production (3.2±0.2 pg/ml vs.16.9±0.2 pg/ml; $p<0.0001$) in comparison to NOD BMMΦ after diabetogenic EMCV-D infection. However, IL-1β was barely detected by either NOD or NOD.*Ncf1*^{m1J} BMMΦ throughout EMCV-B infection. Interestingly, the production of nitric oxide, normally mediated by IL-1β, was not affected by the absence of superoxide. TNFα synthesis by NOD.*Ncf1*^{m1J} BMMΦ was 2-fold lower with EMCV-B (558.1±41.7 vs. 1141.6±13.9 pg/ml; $p=0.0002$), and 1.6-fold lower with EMCV-D (2567±172.2 vs. 4210.1±86.1 pg/ml; $p=0.001$) in contrast to NOD BMMΦ. Taken together, superoxide may act on redox-sensitive signaling pathways, such as NF-κB and IRF3, to potentiate viral triggers of T1D. Future studies will define the mechanism of superoxide in anti-viral responses and determine if redox modulation of innate immune responses can prevent viral triggers of T1D.

The CD5-CK2 signaling axis is critical for maintaining B-1a B cell responses to *S. pneumoniae*.

Kevin S. Cashman[3], Christine M. Sestero[1], Tamer Mahmoud[3], Patrizia De Sarno[4], John F. Kearney[3] and Chander Raman[1,2]

Departments of Medicine[1], Clinical Immunology and Rheumatology[2], Microbiology[3], and Psychiatry and Behavioral Neurobiology[4], University of Alabama at Birmingham, Birmingham, AL, 35294

B-1a B cells are a unique population of B lymphocytes that exhibit many characteristics attributable to an innate lineage (i.e. expression of surface molecules CD11b, MHC-II, CD80, CD86, and biology such as phagocytosis and antigen presentation). This population is primarily derived within the fetal liver during embryonic development and persists in a self-renewing capacity in adulthood, during which this population distributes throughout the body, primarily residing in serous cavities such as the peritoneal and pleural cavities, and to a lesser extent within the spleen. One of the more interesting features of this population is the expression of the lineage defining marker, CD5, which is classically considered a pan-T lymphocyte marker.

The importance of CD5 signaling on the biology of the B-1a B cell population is poorly understood. Using a knock-in mouse model which lacks the 4 amino acids of the cytoplasmic tail of CD5 which are necessary for the binding and activation of the prosurvival serine-threonine kinase CK2 (ΔCK2BD), we have found that the B-1a lymphocytes from these animals exhibit increased activation induced cell death, lower serum IgM and IgA levels, and a reduced capacity to respond to T-independent type II antigens. All of which are hallmark attributes of B-1a biology. Another hallmark of the B-1a population is the production of highly conserved germline antibodies directed to shared epitopes between bacteria and animals such as, phosphorylcholine (PC) and phosphatidylcholine (PtC).

Mice with a mutation of the CK2 binding domain of CD5 exhibit lowered anti-PC responses following heat-killed *Streptococcus pneumoniae* immunization as well as increased susceptibility to *Pneumococcal* infection. This susceptibility can be recovered following prophylactic injection of normal Wt serum into the $CD5^{\Delta CK2BD/\Delta CK2BD}$ animals. This suggested a difference in the antibody repertoire of the $CD5^{\Delta CK2BD/\Delta CK2BD}$ mice, and this difference can be attributed to a loss of idiotypic selection of immuno-dominate B-1a restricted clones. Interestingly the hallmark T15 idiotypic response showed little to no change between the Wt and $CD5^{\Delta CK2BD/\Delta CK2BD}$ strains in serum levels following heat-killed *S. pneumoniae* challenge suggesting the importance of other more rare B-1a restricted clones in the clearance of this ubiquitous pathogen. *Supported by NIH AI 1076562*

Antibodies to Commensal Microbiota are Present in Murine Models of Type 1 Diabetes

Joseph G Daft[1,2], Charles O. Elson III M.D[3], Robin Lorenz[1,2] M.D, PhD

Type 1 Diabetes (T1D) is defined as the selective immune destruction of insulin producing beta-cells within the islet. Alterations in the intestinal microbiota, changes in intestinal integrity, and an aberrant immune system have been postulated to play key roles in the development of diabetes. Auto-antibodies to insulin, islet associated antigens, and antigens found in the lumen of the intestine , such as cows' milk protein, have been linked to T1D, however, the role of these antibodies in disease progression is still unknown. We hypothesize that a break down in intestinal integrity results in the generation of antibodies to commensal microbial antigens and that these antibodies play a role in the pathogenesis of diabetes.

Non-obese diabetic (NOD) female mice were compared to age and sex matched H_2g7 control mice. Serum antibodies to CBir1 flagellin (from a cluster XIVa commensal *Clostridium*) were compared between strains. ELISAs showed that NOD mice have detectable levels of anti-CBir1 flagellin IgM and IgG at the pre-diabetic time point of 5 weeks, while H_2g7 mice do not. We postulated that anti-CBir1 antibodies could be involved in the pathogenesis of disease via two mechanisms: (1) cross-reactivity with pancreatic antigens, (2) modulation of the immune response against pancreatic antigens. Through western blotting and immunohistochemistry using an anti-CBir1 flagellin IgM antibody, we have shown that this antibody has the ability to cross react with islet antigens.

To directly test if antibodies to commensal antigens modulate disease progression, NOD mice were treated with 2.75% Dextran Sulfate Sodium (DSS) to alter intestinal integrity. DSS treatment increases anti-CBir1 flagellin antibodies, but does not alter diabetes incidence. However this treatment is non-specific for increased levels of anti-CBir1 flagellin antibodies in serum. Therefore to specifically test the effects of CBir1 flagellin antibodies, NOD mice have also been injected with anti-CBir1 flagellin IgM and IgG antibodies. These mice are being monitored to determine if this increase in anti-CBir1 flagellin antibodies will alter the incidence of diabetes.

1. Department of Pathology, UAB
2. Comprehensive Diabetes Center, UAB
3. Department of Medicine, UAB

CD27 costimulation enhances IL-7 receptor re-expression on CD8$^+$ T cells during viral infection and promotes CD8$^+$ T cell memory

Han Dong; Timothy N.J. Bullock

Human Immune Therapy Center, Department of Pathology, University of Virginia, Charlottesville, VA 22908

The development of long term memory is the major goal of vaccination, and therefore defining the factors that regulate the establishment and maintenance of memory cytotoxic CD8$^+$ T cells is of particular interest. In many cases of viral infection, the development of fully functional CD8$^+$ T cell memory is dependent upon helper CD4$^+$ T cell support. CD4$^+$ T cells play a critical role in inducing the expression of CD70, the ligand for CD27, on dendritic cells (DCs). In a vaccinia infection model, our previous data demonstrated that 1) the defects in CD8$^+$ T cell memory that occur in the absence of CD4$^+$ helper T cells were a consequence of inadequate CD27 costimulation; and 2) CD27 costimulation led to increased interleukin 7 receptor α (IL-7Rα) on CD8$^+$ T cells. IL-7 plays a critical role in the generation and long-term maintenance of memory CD8$^+$ T cells; and in fact increased IL-7Rα has been regarded as a defining marker to long-lived memory precursor effector cells. CD27 costimulation promotes CD8$^+$ T cell memory presumably via enhancing the abundance of IL-7Rα-expressing memory precursor pool. Here we explore the underlying molecular mechanism by which CD27 costimulation enhances IL-7Rα-expressing memory precursors and show that CD27 signal facilitates the re-expression of IL-7Rα on CD8$^+$ effector T cells. Naïve CD8$^+$ T cells uniformly express IL-7Rα. Stimulating CD8$^+$ T cells by antigen (Ag) peptide *in vitro* resulted in downregulated IL-7Rα within 48hr, which could not be alleviated by exogenous CD27 costimulation, indicating that CD27 costimulation during early priming phase does not modulate IL-7Rα downregulation upon TCR engagement. After adoptively transferring IL-7Rα-downregulated CD8$^+$ T cells from culture to cognate Ag-infected mice, however, treatment of CD27 agonistic antibody to hosts led to a dramatic increase in frequency and absolute number of IL-7Rα-expressing CD8$^+$ T cells, implying late CD27 costimulation supports IL-7Rα re-expression. Data will be presented determining whether this increase in IL-7Rα re-expression is via direct regulation or population expansion/survival. Importantly, CD27 costimulation promotes not only IL-7Rα, but also its downstream signaling mediated by pSTAT5. Our results provide insights into the mechanistic basis by which CD27 costimulation influences CD8$^+$ T cell memory differentiation, and highlight the potential of targeting CD27-CD70 axis to enhance IL-7 signaling for antiviral/antitumor immunotherapy.

(Robert Wagner Fellowship, the James and Rebecca Craig Research Scholars Award, Virginia Kincaid Cancer Research Fund, University of Virginia Cancer Center support grant, Department of Pathology)

Regulation of Glial Glutamate Transporters in Multiple Sclerosis

Ryan E. Doyle[2], Kirsten Neeck[2], Steven Palladino[2], and Tara M. DeSilva[1,2]

[1]UAB Center for Glial Biology in Medicine, [2]UAB Department of Physical Medicine and Rehabilitation

Multiple sclerosis (MS) is an autoimmune disease characterized by myelin loss and axonal damage due to infiltration of immune system cells into the central nervous system (CNS). The mechanisms that lead to loss of myelinating oligodendrocytes (OLs) are not fully understood. There are many lines of evidence which suggest that glutamate dysregulation plays a role in multiple sclerosis. Since glutamate transporters are efficient in regulating glutamate, we wanted to understand if this system is affected in multiple sclerosis. In the current study, we show that the GLT-1 glutamate transporter is down-regulated in an animal model of MS. Our in vitro studies demonstrate a possible underlying mechanism of how glutamate regulates the down-regulation of GLT-1. Furthermore, we demonstrate that blocking the system X_c^- transporter, a source of excess glutamate, prevents down-regulation of the GLT-1 glutamate transporter and decreases severity of motor symptoms in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. These data, taken together, suggest an important role for GLT-1 down-regulation contributing to glutamate dysregulation in MS.

This work was funded by NINDS P30-NS069324, The National Multiple Sclerosis Society, The Civitan International Research Foundation, and The University of Alabama The Health Services Foundation - General Endowment Fund

CD8 T cell responses are preferentially targeted to HIV-1 non-adapted epitopes during acute infection

Victor Y. Du[1], Jonathan Carlson[2], Anju Bansal[1], Eric Hunter[3], Jesus F. Salazar-Gonzalez[4], Sonya Heath[1], and Paul A. Goepfert[1]

[1]Department of Medicine, University of Alabama at Birmingham [2]Microsoft Research [3]Emory Vaccine Center, Emory University [4]Department of Genetics, University of Alabama at Birmingham

Background: HIV CD8 T cell escape mutations can be predicted based on the human leukocyte antigen class I (HLA I) alleles of the infected population, whereby an HIV-1 epitope adapts to HLA I-restricted pressure, leading to the development of an adapted epitope. Due to this immune pressure, the virus evolves inside a host and can then be transmitted into a new host. Therefore, infecting as well as circulating HIV-1 isolates contain some forms of adapted epitopes. By default, current HIV-1 vaccines encode adapted epitopes based on existing viral sequences; however, the extent to which these epitopes induce recognition by CD8 T cells has not been assessed during acute HIV-1 infection.

Methods: We determined the HLA I alleles of 11 acutely infected clade B HIV+ patients and also derived the HIV-1 sequences of the infecting virus for each individual, or the transmitted founder virus (TFV). This strategy allowed us to predict the number of HLA I-restricted non-adapted (NE) and adapted epitopes (AE) encoded by each person's TFV. We synthesized peptides matching the NE and AE that are present in the TFV of these 11 patients. Using IFN-γ ELISPOT and intracellular cytokine staining assays by stimulating patient PBMC with these peptides, we compared the immunogenicity and functionality of T cell responses elicited to the non-adapted and the adapted epitopes.

Results: CD8 T cell responses were induced more frequently to NE (frequency is 33.33% for NE, and 3.23% for AE, p=0.0077). Similarly, individuals with more NE encoded by their TFV (>50%) had a greater breadth (total number of epitopes targeted by CD8 T cells) of responses (r=0.7547, p=0.0149). Furthermore, in a cross-sectional study of a larger cohort of ART-naïve Zambian patients with acute clade C HIV infection, those with TFV encoding more NE than AE had lower set point viral loads (r=0.5539, p=0.0066). This suggests protective CD8+ T cell responses may be maintained in an individual who's TFV contains more NE. Although responses to AE were less frequent, they shared similar magnitude, functional avidity, and polyfunctionality compared to those elicited to the NE counterparts. Interestingly, the few AE that elicited a response (reactive adapted epitopes, or RA) exhibited higher HLA binding affinity compared to the ones that did not (non-reactive adapted epitopes, or NRA, p<0.001), as assessed by the HLA binding assay. The NRA were also not immunogenic when tested in a larger cohort of chronic patients.

Conclusions: While CD8 T cells did not recognize a majority of AE, few of them were recognized with similar immunogenic qualities as their NE counterparts. These AE warrant further studies and should be considered for inclusion in candidate vaccines to enhance the breadth of T cell responses. Therefore, these results have important implications for future T cell-based HIV vaccines.

Supported by NIH-AI-98551

The use of HLA class II associated HIV polymorphisms in predicting novel CD4 T cell responses and viral escape

Authors: Erdmann N[1], Du V[1], Bansal A[1], Carlson J[2], Hunter E, Goepfert P[1]

Anju Bansal[1], Tiffanie Mann[1], Sarah Sterrett[1], Anne Bet[1], Jonathan Carlson[2], and Paul A. Goepfert[1]

Departments of Medicine[1], University of Alabama at Birmingham, Birmingham, AL, Microsoft Research[2], Redmond, Seattle, WA, Emory Vaccine Center Emory University, Atlanta, GA.

CD4 T-cells are the primary targets of HIV infection; however, their role in controlling viral replication remains unclear. We hypothesized that HIV adapts in response to CD4 T-cell mediated immune pressure.

Using HIV sequence data and HLA-II alleles, we applied a novel computational approach to identify HIV-1 polymorphisms disproportionately associated with HLA-II alleles in a large African cohort of chronically HIV-infected individuals. Using this approach, we identified 17 HLA class-II associated adaptations. A panel of 34 predicted adapted and nonadapted CD4 T-cell epitopes (15-20mer) encompassing the identified polymorphisms were synthesized and evaluated for immunogenicity. Peripheral Blood Mononuclear Cells (PBMCs) from 10 uninfected controls, 20 chronically infected individuals on ART, 11 HIV-controllers (ART naive, viral load <2000) and 8 HIV non-controllers (ART naive, viral load >10,000) were depleted of CD8+ cells and CD4+ T-cell responses were quantified using interferon-γ ELISpot assay.

The median magnitude (SFC/10^6 cells) of HIV-specific CD4 T-cell responses seen in controllers (451; Range 70-2123) was higher compared to non-controllers (223; Range 60-997) p=0.004, and tended to be higher compared with ART treated individuals (343; Range 55-1303). Positive pool responses were mapped to the individual peptide. Some, but not all individuals encoded the HLA- II alleles associated with the predicted HIV polymorphism biologically confirming the CD4 T cell epitope predictions. The magnitude of responses for the adapted epitopes was lower compared to non-adapted epitopes (P=0.0003).

We have applied a novel computational approach to identify novel HLA class-II epitopes enabling us to correlate the magnitude and breadth of CD4 T-cell responses with HIV progression. The finding that adapted peptides had significantly lower responses also lend credence to the use of HLA-II associated HIV polymorphism analysis in predicting CD4-mediated viral evolution. Our findings will help identify novel CD4 T-cell targets relevant to future HIV vaccine design.

System xc- inhibition improves histopathological and clinical outcomes in experimental autoimmune encephalomyelitis

Kirsten S. Evonuk[1,2,3], Brandi J. Baker[3], Christine M. Sestero[4], Ryan E. Doyle[5], Matthew A. Timberlake II[5], Chander Raman[4], and Tara M. DeSilva[2,3]

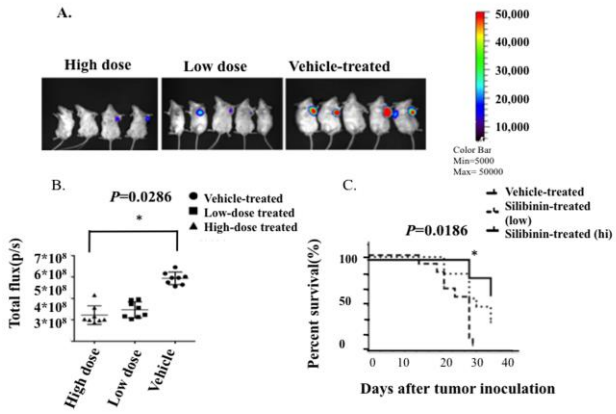
[1]Neuroscience Graduate Theme, [2]Center for Glial Biology in Medicine, [3]Department of Physical Medicine and Rehabilitation, [4]Department of Medicine, [5]Undergraduate Neuroscience Program

Multiple sclerosis (MS) is an autoimmune disease involving a number of pathophysiological mechanisms thought to contribute to myelin loss, axonal damage, and neurological dysfunction. One potential mechanism is excessive glutamate release and excitotoxic cell death. While excitotoxicity has been shown to be involved in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, the source of excess glutamate is not known. In this study, we characterize the role of the system xc- cystine/glutamate antiporter in causing excitotoxicity in EAE. Using primary cell cultures we show that microglia, in response to cytokines released by T_H1 cells, produce nitric oxide resulting in energy failure, glutamate release, and oligodendrocyte death. Inhibition of the system xc- antiporter alleviated cell death *in vitro* after stimulation of microglia with cytokines. Inhibition of the system xc- antiporter was also beneficial *in vivo*, improving histopathological and clinical outcomes of EAE in C57BL/6 and SJL/J mice. These data suggest a role for the system xc- antiporter in creating an excitotoxic cell environment in MS, and indicate that inhibition of the antiporter may be beneficial by preventing cell death in those with the disease. This work was funded by NINDS P30-NS069324, The National Multiple Sclerosis Society, The Civitan International Research Foundation, and The University of Alabama The Health Services Foundation - General Endowment Fund.

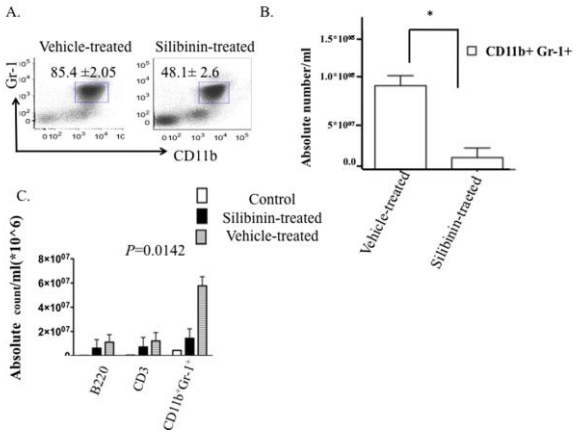
MDSCs Modulation by silibinin in Murine Breast Tumor Model

Forghani P [1,2], Waller EK[2] .1 School of Public Health, Dept of Pathobiology, Tehran University of Medical Sciences, Tehran, IR; 2 Department of Hematology and Medical Oncology, WCI, Emory University, Atlanta, GA

Introduction: Myeloid-derived suppressor cells (MDSC)s are now recognized as key mediators of immuno-suppression in cancer that in creased in the blood and in the tumor microenvironment (1-2). Silibinin, a natural flavonoid from the seeds of milk thistle, has been developed as an anti-inflammatory agent and supportive care therapy to reduce the toxicity of cancer chemotherapy (3). The goals of this study were to evaluate the effect of silibinin on MDSCs level and determine whether silibinin had anticancer activity in a breast tumor-bearing mice model. We hypothesized that silibinin could reduce cancer-associated inflammation and decrease accumulation of $CD11b^{+}Gr\text{-}1^{+}$ MDSCs in tumor bearing mice. Using female BALB/c mice harboring the 4T1 breast cancer cell line transfected with luciferase, we tested the biological effect of silibinin on breast cancer and MDSCs accumulation.



Results: Treatment with silibinin through gavages at dose (150 mg/kg) increased overall survival accompanied by a reduction in tumor volume (total flux). Also our data showed a significant selective effect of silibinin in decreasing the numbers of MDSCs in blood. Fig 1A&B: Silibinin treatments caused significant decreased in total flux parallel with increased survival ($p<0.05$). C: *In vivo* treatment with silibinin increases overall survival in tumor bearing mice. Fig 2. Silibinin treatment selectively reduces numbers of MDSCs in tumor-bearing mice. Fig 2. Silibinin treatment selectively reduces numbers of MDSCs in tumor-bearing mice.



Discussion and Conclusions: Here we showed a new mechanism for controlling tumor growth upon the effect of silibinin on MDSCs in mice bearing breast cancer cells. We have demonstrated the effectiveness of this natural herb on tumor modulation by inhibition of MDSCs expansion and delay in tumor growth. The results of our study lay the groundwork for designing clinical trials to explore the use of this natural compound in oncology patients. Further experiments need to identify inflammatory mediators that influenced by silibinin and affect on MDSCs accumulation.

Acknowledgment: Experiments involved flow cytometry in this research project was supported in part by the Flow Cytometry Core Facility of the Emory University School of Medicine.

Understanding the Regulatory Mechanisms of Fragment Crystallizable Gamma Receptor IIB in Rheumatoid Arthritis

Cassandra Garbutt Shahid Mukhtar and Robert Kimberly
University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA

Human immune systems have the abilities to discriminate self from non-self. Failure of an organism to recognize its own constituent parts as self would allow the production of autoantibodies that attack its own cells, tissues, and/or organs. This causes inflammation and damage that leads to autoimmune disorders such as rheumatoid arthritis (RA). RA is a chronic systemic inflammatory condition that may affect many tissues and organs, primarily joints. About one percent of the world's population and 1.6 million patients in the USA are afflicted by RA—women three times more often than men. In recent years, systems biology approaches—specifically network analyses that integrate experimentally derived information with computational modeling—have emerged as powerful tools for studying complex diseases.

In humans, the fragment crystallizable receptors (FcRs) bind to the most common immunoglobulin and are thought to play crucial role(s) in immunity, as well as in the pathogenesis of RA. Most of the FcRs are activating receptors but intriguingly, FcγRIIb is the only inhibitory Fcγ receptor. FcRs maintains cellular immune homeostasis by simultaneous triggering of activating and/or inhibitory receptors.The goal of our ongoing research is to understand the regulatory mechanisms of FcγRIIb in autoimmune diseases, for which genomics and computational modeling are essential. Two approaches were taken, namely the candidate and unbiased approaches. The candidate approach includes finding: known and statistically overrepresented cis-regulatory elements; evolutionary conserved regions (ECRs) across diverse animal species; and FcγRIIb overrepresented motifs using MEME and POBO. The unbiased approach includes: systematic mapping of FcγRIIb promoter and identify transcription factors; copy-number variations in FcγRIIb regulation; and microRNA mediated regulation of FcγRIIb.

Systems Biology Assessment of Human Immune Responses after Seasonal Trivalent Inactivated Influenza Vaccine

Kristen L Hoek[1], Leigh M Howard[2], Tara M Allos[1], Parimal Samir[3], Kirsten E Diggins[1], Qi Liu[4], Nripesh Prasad[5,6], Megan Shuey[1], Xinnan Niu[1], Shawn Levy[6], Sebastian Joyce[1], Kathryn M Edwards[2], and Andrew J Link[1]

Departments of [1]Pathology, Microbiology and Immunology, [2]Pediatrics, [3]Biochemistry, [4]Biomedical Informatics, Vanderbilt University School of Medicine, Nashville, TN, USA; [5]Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL, USA; [6]HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA

Systems biology represents a novel approach to comprehensively study the human immune response to vaccines at the global transcriptional and proteomic level. However, most systems vaccinology approaches utilize total PBMCs in their analyses. In this context, responses of under-represented immune cell types in the blood are potentially obscured by the predominant cells in the PBMC fraction, and the contribution of PMNs is completely ignored. To investigate the contribution of individual cell types in the immune response following vaccination, we developed a rapid and efficient method for purifying large numbers of T cells, B cells, monocytes, NK cells, myeloid DCs and neutrophils from fresh venous human blood for systems vaccinology studies. This optimized protocol was applied to adult volunteers vaccinated with 2011-12 seasonal TIV. 100mL blood was obtained prior to (day 0) and on days 1, 3, and 7 following vaccination. Whole blood, PBMC and PMN fractions were subjected to phenotypic analysis by flow cytometry. Immune cells were fractionated and processed for RNA and protein extraction in a single day. RNA-Seq and quantitative proteomics were performed on purified cells in order to determine individual expression profiles. Our results demonstrate that expression profiles generated from purified immune cells differ significantly from PBMC, and that cell-specific information is likely lost when using only PBMC for analysis. This innovative systems approach is currently being utilized to evaluate vaccine safety and efficacy in an adjuvanted H5 avian influenza clinical trial.

This project was funded in part with Federal funds from the National Institutes of Allergy and Infectious Disease, National Institutes of Health, Department of Health and Human Services, under Contract No. 272200800007C, the Vanderbilt Clinical and Translational Science Award grant NIH RR024975, and the Childhood Infections Research Program grant IT21AI095202-01.

Title: Characterizing Human B1 cells in the CVID and RESPI Cohort

Tracy Hwangpo, MD/PhD[1], Daniel Griffin, MD/PhD[2], Ewa Szymanska, Thomas Rothstein, MD/PhD[2] and Harry Schroeder, MD/PhD[1], (1)University of Alabama at Birmingham, Birmingham, AL, (2)The Feinstein Institute for Medical Research, Manhasset, NY

Rationale: A novel human B1 cell phenotype was described to be $CD20^{+}CD27^{+}CD43^{+}CD70^{-}$. It has been postulated that CVID patients are likely to have defects in B1 cells, contributing to their disease. We started a pilot study to examine B1 cells in our CVID and RESPI cohort.

Methods: Whole blood was collected from 51 consecutive subjects in 2011-2012. They were then processed by isolating PBMCS. Those cells were then sent to our collaborators who processed the samples without knowledge of the patient's diagnosis. Naive, memory, B1 cells, and Cd11bB1 specific cells were examined by flow cytometry. Those results were then sent to our lab to decode the diagnosis for analysis.

Results: We found that the percentage B1 cells were higher ($p<0.04$) in the CVID(4.2%) and RESPI (4.9%) population compared to the controls (2%). When Cd11bB1 cells were examined, CVID and RESPI patients also had more percentage of these cells than controls . However, when the absolute numbers of these cells were examined, the total B1 cells and Cd11bB1 cells were lower than controls. When precentage memory B cells and naïve B cells were examined among the groups, there was no significant difference. However, the absolute number of these cells were lower in the CVID population compared to the controls.

Conclusions: These results suggest that there is a preservation of B1 cells in the face of diminished total B cell numbers in CVID patients. This maybe important in the CVID population to help compensate for decrease generation of B cell subsets important in innate and humoral immunity.

T. Hwangpo is supported by T32 Immunology Training Grant; This research was also supported by NIH grant T0911170023.

Arginase-1 and Programmed Death Ligand-1, Potent Inducers of Immune Tolerance, are Upregulated on Airway Neutrophils in Cystic Fibrosis

Sarah A. Ingersoll[1], Julie Laval[1], Marcela Preininger[1], Milton Brown[1] and Rabindra Tirouvanziam[1]
[1]Department of Pediatrics, Emory University School of Medicine & Center for CF Research, Children's Healthcare of Atlanta, Atlanta, GA

Introduction: A long-standing paradox in cystic fibrosis (CF) is the chronic presence of viruses, bacteria and fungi in conjunction with high numbers of live neutrophils (PMNs) in the lung. We hypothesized that CF airway PMNs may express molecules that induce tolerance and dampen the immune response to airway pathogens. In the current study, we determined the expression of potential immunomodulating markers on CF airway PMNs in order to better understand the role of these cells in CF disease pathology.
Methods: Levels of Arginase-1 (Arg-1) and Programmed Death Ligand-1 (PD-L1) were quantified on live PMNs from blood and sputum of CF patients (n=19) and blood from healthy controls (HC) (n=11) using flow cytometry. Soluble PD-L1 was quantified in plasma and airway fluid from CF patients and healthy control plasma using an ELISA. Finally, an *in vitro* PMN activation assay was used to determine if Arg-1 and PD-L1 were present in the PMN granules.
Results: Arg-1 and PD-L1 levels were significantly increased on the surface of airway PMNs compared to blood PMNs. We observed a bimodal distribution of PD-L1 expressing PMNs, therefore we hypothesized that PD-L1 may be cleaved from the surface of airway PMNs by proteases present in the CF airway fluid. We compared levels of soluble PD-L1 in plasma and airway fluid by ELISA. Soluble PD-L1 levels in the plasma were not significantly different in CF patients compared to healthy controls; however, soluble PD-L1 was significantly increased in airway fluid compared to plasma in CF patients. When blood PMNs from CF patients (n=6) or HC (n=6) were stimulated with Latrunculin B and fMLF to initiate PMN granule release, we found that surface Arg-1 expression increased in conjunction with CD63 and CD66b, indicative of primary and secondary granule release. In agreement with our ELISA results, PD-L1 expression was significantly decreased after stimulation, suggesting this molecule is cleaved upon granule release.
Conclusions: Our results suggest that upon entering CF airways, PMNs upregulate molecules that may induce tolerance, either directly through cell-cell contact or remotely through mediators released in the extracellular fluid. Thus, the persistence of pathogens in CF airways may stem from active tolerance by live PMNs rather than PMN death as once believed, which is an important consideration for the development of targeted CF treatments.

The authors would like to acknowledge Dr. Tangpricha for access to patient samples. This work was supported by the Emory+Children's Center for CF Research Startup Fund (RT)

The Regulation of CD8 T Cell Fate Decisions During Acute and Chronic Viral Infections by Intercellular Adhesion Molecule-1

Shannon M. Kahan[1], Maureen A. Cox[1], Scott R. Barnum[1], Daniel C. Bullard[2], Allan J. Zajac[1]

[1] Department of Microbiology, [2] Department of Genetics,
University of Alabama at Birmingham

The clearance of acute viral infections and the control of chronic viral infections rely on the formation of an effective anti-viral CD8 T cell response. The development and properties of these responses is shaped early following activation by both the strength of the interaction between the T cell and the antigen-presenting cell, as well as by T cell-T cell interactions. Intercellular adhesion molecule-1 (ICAM-1) intensifies the interactions that influence CD8 T cell function and fate. The purpose of this study is to test the hypothesis that ICAM-1 configures the phenotypic, protective, and functional properties of CD8 T cell responses during infection. Mice lacking ICAM-1 (ICAM-1 $^{-/-}$) exhibit greater retention of effector-like CD127^{lo} KLRG-1^{hi} CD8 T cells after the clearance of acute lymphocytic choriomeningitis virus (LCMV) infection compared to wild type mice, demonstrating the role of ICAM-1 in shaping the late contraction of the short-lived effector cell subset. In addition, while ICAM-1$^{-/-}$ mice develop a pool of memory-like CD127^{hi} KLRG-1^{lo} CD8 T cells, these cells fail to accumulate upon secondary challenge. During chronic LCMV infection, ICAM-1$^{-/-}$ mice maintained higher numbers of virus-specific CD8 T cells compared to wild type mice in which this population usually succumbs to exhaustion and deletion. Surprisingly, this swollen effector pool did not accelerate viral control in ICAM-1$^{-/-}$ mice. Taken together these results demonstrate that ICAM-1 acts to modify the immune response both by directing the contraction of the effector population as well as enhancing the memory response. These studies also suggest that the strategic modulation of ICAM-1 may be an attractive therapy to bolster the levels of effector CD8 T cells during chronic infections, possibly preventing or reversing T cell exhaustion.

This study was supported by National Institutes of Health grants R21 AI099867, R01 AI049360 (A.J.Z) and T32 AI007051 (S.M.K.)

MAdCAM-1 independent lymphocyte homing to GALT of Nkx2-3-/- mice

Zoltán Kellermayer. Martina Mihalj, Péter Balogh
Department of Immunology and Biotechnology, University of Pécs, Hungary

PURPOSE: Nkx2-3 transcription factor regulates mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expression, and its sequence variants have been identified as susceptibility traits for inflammatory bowel diseases. Although mice lacking the Nkx2-3 transcription factor have no endothelial MAdCAM-1, Peyer's patches (PP) and mesenteric lymph nodes (mLN) still develop. As homing of lymphocytes to gut-associated lymphoid tissues (GALT) is dependent on high endothelial venules (HEV) displaying MAdCAM-1, we investigated molecular components of homing to GALT in Nkx2-3-/- mice.
MATERIALS AND METHODS: Adult and newborn Nkx2-3$^{-/-}$ and wild-type BALB/c mice were used. Phenotypic features of PP HEVs were studied by immunofluorescence. The kinetics of homing to GALT was tested by adoptive cell transfer using CFSE-labeled or GFP/MHC alloantigen-marked donor lymphocytes, followed by flow cytometry or tissue immunofluorescence. mRNA expression of addressins and posttranslational glycosylation enzymes was determined by qPCR. The involvement of MAdCAM-1 or PNAd adressins in GALT homing was studied using in vivo antibody-mediated blockade.
RESULTS: In PPs and mLNs of mutant mice there was enhanced staining for luminal MECA79 epitope against PNAd sulfoglycoepitope, and there was increased mRNA expression for several PNAd backbone proteins and modifying enzymes. Adoptively transferred lymphocytes homed efficiently to PNAd-positive GALT HEVs. Homing was blocked by MECA-79 anti-PNAd mAb injection, but not with anti-MAdCAM-1 mAb. The gut and GALT in Nkx2-3 deficient neonatal mice contain MAdCAM-1-positive vessels. Later in development in the organized lymphoid tissues of the gut mucosa HEVs gradually replace MAdCAM-1 with PNAd, whereas in the non-lymphoid segments of intestinal vasculature the loss of MAdCAM-1 is not coupled with the induction of PNAd.
CONCLUSIONS: Together these data indicate that in the absence of endothelial MAdCAM-1 in Nkx2-3-/- mice PNAd controls homing to GALT. Thus, HEV function is maintained, although with different adhesion molecule expression patterns.

Supported by:
Broad Foundation Proposal No. IBD-0341
This research was realized in the frames of TÁMOP 4.2.4. A/1-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system" The project was subsidized by the European Union and co-financed by the European Social Fund.

Influence of cytokine auto antibodies on serum IFNα activity in SLE

Srilalitha Kuruganti[1], Ashlesha Deshpande[1], Winn W. Chatham[2], Mark R. Walter[1]

[1]Department of Microbiology, [2]Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham AL.

The type-I interferon (IFN-I) family consists of 12 IFN-α subtypes as well as IFN-β and IFN-ω. IFN-α subtypes are thought to play a pivotal role in the pathogenesis of autoimmune diseases such as lupus by enhancing the production of anti-nuclear autoantibodies and driving end organ damage in patients with lupus nephritis. Despite the putative role of IFN-αs in disease, serum IFN-α activity levels are often low or undetectable in lupus patients. Auto antibodies (AAbs) against a single IFNα subtype (IFNα4) have been detected in lupus patients suggesting they may alter serum IFNα activity and disease severity. To better understand this relationship, we have measured total IFNα and IFNα subtype-specific AAbs levels in lupus patient serum. Our results thus far reveal an excellent correlation between IFNα activity and AAb levels, suggesting AAbs may provide an indirect marker of cytokine activity in some patients. Toward this end, we have determined the AAb profile for eleven cytokines implicated in lupus to understand their relationship to IFNα levels and overall disease severity. The resulting cytokine profile will be presented towards the goal of determining the best targets for anti- cytokine therapy in lupus.

This research was supported by Lupus research institute.

MOLECULAR COMPONENTS OF THE INSULIN/INSULIN-LIKE GROWTH FACTOR-1 SIGNALING PATHWAY ARE HIGHLY EXPRESSED BY AIRWAY NEUTROPHILS IN CYSTIC FIBROSIS AND CYSTIC FIBROSIS-RELATED DIABETES

Julie Laval[1,3], Sarah Ingersoll[1], Marcela Preininger[1], Milton Brown[1], Vin Tangpricha[2] and Rabindra Tirouvanziam[1]

1. Department of Pediatrics, Emory University School of Medicine, Center for CF Research, Children's Healthcare of Atlanta, Atlanta, GA, USA

2. Department of Medicine, Division of Endocrinology Emory University School of Medicine, Atlanta, GA, USA

3. IGMM, CNRS UMR 5535, Université Montpellier 2, Montpellier, FRANCE

Background: Insulin and insulin growth factor-1 (IGF-1) are potent hormones, which, by binding to surface receptors and activating a common signaling intermediate (insulin-receptor substrate-1, or IRS-1), trigger anabolic signaling and modulate inflammatory cell function. In CF and its complication, CF-related diabetes (CFRD), insulin and IGF-1 levels in the systemic compartment are low or very low, consistent with the poor metabolic status of these patients. Interestingly, airway epithelial cells can release IGF-1 and we recently found that CF airway neutrophils undergo anabolic reprogramming [Makam et al., PNAS 2009;106(14) and Laval et al., J Immunol, 2013:190(12)], suggesting a potential role for insulin/IGF-1 signaling in this compartment.

Methods: We used flow cytometry to characterize surface insulin and IGF-1 receptors (IR and IGF-1R, respectively) and intracellular IRS-1 levels in blood and airway neutrophils from CF and CFRD patients (N=12 and 10, respectively), and in blood neutrophils from healthy controls (HC, N=8). We also quantified insulin and IGF-1 levels in plasma (CF/CFRD and HC groups) and airway fluid (CF/CFRD group only), using ELISA. CF and CFRD patients were assessed at steady-state and in acute pulmonary exacerbations (APEs).

Results: Insulin and IGF-1 levels were lower in CF/CFRD than HC plasma, but there was no difference in surface IR and IGF-1R expression on blood neutrophils between the two groups, nor was there any modulation of these receptors between steady-state and APEs in the CF/CFRD group. However, intracellular IRS-1 level was increased in CF/CFRD blood neutrophils when compared to HC, and higher in APEs vs. steady-state.

Next, we compared blood and airway compartments within the CF/CFRD group. While insulin and IGF1 levels were lower in CF/CFRD airway fluid than plasma, downstream insulin/IGF-1 signaling components were upregulated in airway compared to blood neutrophils, including surface IR and IGF-1R expression and intracellular IRS-1 levels (at steady-state only, for the latter).

Interestingly, *in vitro* activation assays showed that surface IGF-1R levels were not regulated by exocytosis (a hallmark of CF airway neutrophils), suggesting another mechanism for the observed *in vivo* upregulation of insulin/IGF1 signaling components in CF airway neutrophils.

Conclusions: Our results suggest a potential role for insulin/IGF-1 signaling in live CF/CFRD airway neutrophils, with modulation during APEs. Further experiments will enable us to determine whether these observed changes are amenable to therapeutic modulation by drugs commonly used in patients with abnormal insulin/IGF-1 signaling, such as metabolic syndrome.

This work was supported by the Emory+Children's Center for CF Research Startup Fund (RT)

ROLE OF Dβ GERMLINE SEQUENCE ON CONSTRAINING TCR CDRβ3 DIVERSITY

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

Diversity of the T cell receptor is mainly developed through V(D)J rearrangement and N addition during TCR development. The product of V(D)J rearrangement is the CDRβ3, a region of high variability that recognizes antigen and includes all of the D gene. The Dβ sequence 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.

To do this we have created Db altered mice. These mutants are a Db2 KO (Db1); a replacement of the Db locus with a charged Db (DbDKRQ); and a replacement of the Db locus with a hydrophobic D_H (DbYTL). When compared to WT mice, the mutant Db mice have an altered T cell repertoire in both CDRb3 amino acid composition and length and these differences can be attributed to the changes in the germline sequence. Changing the Db also changes the total T cell number in both developing and mature T cells, with a charged Db being selected against. Ongoing experiments will elucidate the role of the Db sequence on the development of T cells and the role it plays in infection and inflammation.

Increased Type I Interferon Promotes Follicular Shift of Lymphotoxin-expressing B Cells Which Disrupts Marginal Zone Barrier Integrity and Promotes Follicular Network Activation

Hao Li[1, 2], Qi Wu[1], Jun Li[1], PingAr Yang[1], Yangxin Fu[3], Hui-Chen Hsu[1] and John D Mountz[1, 2, 4]

[1]Division of Clinical Immunology and Rheumatology, Department of Medicine, and [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294;
[3]Department of Pathology, University of Chicago, Chicago, IL 60637, USA; and
[4]Birmingham, VA Medical Center, Birmingham, AL 35233, UAB

Increased expression of type I interferon (IFN) signature genes and production of autoantibodies against apoptotic self antigens (Ags) are both hallmarks of systemic lupus erythematosus (SLE). How these two abnormalities are connected is, however, not completely understood. In this study, we provided evidence that these two abnormal phenotypes in lupus can be connected via deteriorations of marginal zone macrophages (MZMs) in the spleen. MZMs are a small subset of specialized splenic macrophages located in the marginal zone. They act as final follicular entry barrier to clear apoptotic cells entering to the spleen from circulation. We found that while there was a steady maintenance of MZM in normal B6 mice, there was a severe age-dependent decrease in the number of MZMs in the spleen of lupus prone BXD2 mice. A deficiency of type I IFN receptor in BXD2-*Ifnar*$^{-/-}$ mice preserved MZMs. To determine if type I IFNs affected MZ integrity through MZM or MZ B cells, B cells were transferred from BXD2 and BXD2-*Ifnar*$^{-/-}$ to BXD2-*Rag1*$^{-/-}$ mice. The results showed that in the absence of type I IFN signaling, B cells are retained B cells in the MZ and supported repopulation of MZMs. In contrast, a mixed bone marrow (BXD2-GFP^{+}*Ifnar*$^{+}$: BXD2-GFP^{-}*Ifnar*$^{-}$ =1:1) reconstitution in BXD2 *Rag1*$^{-/-}$ showed a 50:50 repopulation from the donor MZMs in recipient mice. These results suggest the role of type I IFNs on B cells and excluding their direct effects on maintenance of MZMs. Repeated injection of a low dose of recombinant LT receptor fusion protein (LTR:Fc, 10 μg/mouse per week, 4 weeks) to BXD2 mice selective depleted MZMs without affecting follicular dendritic cell (FDC) network and accelerated autoimmune phenotypes, suggesting that MZ local expression of LT by B cells is needed to support the integrity of MZMs. The location of LT^{+} cells were examined which further confirmed that MZ, not FO B cells, are the major population of B cells that express LT. Furthermore, confocal imaging analysis of LTβR expression suggests that the LT regulation direct target cells include not only MACAM1^{+} endothelial cells but also the MZMs. Blockade of LT by LTR:Fc induced apoptosis of MZMs in 20 hrs. Loss of MZMs in BXD2 is associated with increased uncleared apoptotic debris and production of type I IFNs which then further promoted follicular migration of apoptotic Ag delivery LT^{+} MZ B cells to promote FDC activation. Our present study suggests a novel mechanism associated with type I IFN-promoted immune reactions against apoptotic self-Ags as a result of follicular migration of LT expressing B cells. The shift of LT expressing B cells from the MZ to the follicles by type I IFNs not only affected maintenance of the number of MZM, but also promoted apoptotic Ag and LT stimulation of FDCs, thereby stimulated the autoimmune germinal center responses. The lack of MZM as a common lupus phenotype is supported by the consistent observation of an age-related loss of MZMs and follicular shift of LT^{+} B cells in another lupus prone B6-*Sle1.Sle2.Sle3* (TC) mice. In these mice, it was reported by Dr. Morel and colleagues that there was also extensive mislocation of CD1d^{+} MZ B cells in the follicles.

This work is supported by grants from VA Merit Review Grant (1I01BX000600-01), NIH/NIAID (1AI071110, ARRA 3RO1AI71110-02S1 and 1RO1 AI083705), Rheumatology Education Foundation, Arthritis Foundation, and Lupus Research Institute.

CD5 INCREASES Th17 POLARIZATION THROUGH AKT AND GSK3 SIGNALING

Donald McGuire[2], Amber Rowse[2], Binghao Peng, Hao Li[2], Christine Sestero[4], Robert Axtell[5], Patrizia De Sarno[3] and Chander Raman[1]

Department of Medicine[1], Department of Microbiology[2], Psychiatry and Behavioral Neurobiology[3], University of Alabama at Birmingham. Department of Biology, Chemistry, and Mathematics, University of Montevallo[4]. Department of Neurology and Neurological Sciences, Stanford University[5].

CD5 is an important enhancer of cell survival and regulator of antigen receptor signaling expressed on T and B1a cells. Recent work has shown that CD5 significantly increases Th17 differentiation. This effect is linked to activation of the serine/threonine kinase Casein Kinase 2(CK2). Loss of CD5 activation of CK2 ameliorates experimental autoimmune encephalomyelitis (EAE). Furthermore, recent studies have linked a CD5 polymorphism to risk of rheumatoid arthritis. However, the downstream mechanisms of CD5 signaling are not well understood. Here we report that CD5 activation of CK2 enhances generation of Th17 helper cells through increased AKT and GSK3β phosphorylation. Activation of AKT increased mTOR and S6K mediated RORγ nuclear localization. Furthermore, CD5 dampens IFNγ inhibition of Th17 differentiation. These results provide an important insight into how CD5 regulates T cell differentiation. They also show that CD5 is a novel regulator of cytokine responses. Consequently, inhibition of CD5 signaling could provide a valuable means to dampen Th17 responses in autoimmune diseases. Supported by NIH-AR-07450 NIH-NS-064261 NIH-AI-1076562 NMSS- RG3891

ER Stress and Neuroinflammation: Connecting the Unfolded Protein Response to JAK/STAT Signaling

Gordon P. Meares and Etty N. Benveniste

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

Neuroinflammation and endoplasmic reticulum (ER) stress are associated with many neurological diseases. ER stress is brought on by misfolded proteins. In turn, cells respond with activation of the unfolded protein response (UPR). The UPR is a highly conserved pathway that transmits both adaptive and apoptotic signals to restore homeostasis or eliminate the irreparably damaged cell. Recent evidence indicates that ER stress and inflammation are linked. In this study, we have examined the interaction between ER stress and JAK/STAT-dependent inflammation in astrocytes. The JAK/STAT pathway mediates the biological actions of many cytokines and growth factors. We have found that ER stress leads to the activation of STAT3 in a JAK1-dependent fashion. ER stress-induced activation of the JAK1/STAT3 axis leads to expression of IL-6 and several chemokines. The activation of STAT3 signaling is dependent on the protein kinase PERK, a central component of the UPR. Knockdown of PERK abrogates ER stress-induced activation of STAT3 and overexpression of PERK is sufficient to activate STAT3. Additionally, ER stressed astrocytes, via paracrine signaling, can stimulate activation of microglia leading to production of oncostatin M (OSM). OSM can then synergize with ER stress in astrocytes to drive inflammation. Together, this work describes a new PERK-JAK1-STAT3 signaling pathway that may elicit a feed-forward inflammatory loop involving astrocytes and microglia to drive neuroinflammation.

National Multiple Sclerosis Society: Career Transition Fellowship (TA 3050-A-1) (GPM)

Development of *Saccharomyces boulardii* as a Mucosal Vaccine Delivery System

Lauren Miller[1], Milo Fasken[2], Courtney McDermott[1], David Guiliano[3], Jan Mead[1], Anita Corbett[2], Tracey Lamb[1]

[1]Division of Pediatric Infectious Disease, Emory University School of Medicine

[2]Biochemistry Department, Emory University

[3]School of Health, Sport and Bioscience, University of East London, UK

The majority of human pathogens initially enter the body through the mucosa. Most current vaccination strategies rely upon needlestick injection and the development of systemic immune responses; however, this approach only poorly correlates with protection at the mucosa where most human pathogens first encounter the immune system. Optimal protection against many of these pathogens may instead be achieved by specifically targeting the gut-associated lymphoid tissue (GALT). We propose that development of the probiotic yeast, *Saccharomyces cerevisiae* subspecies *boulardii* (*S. boulardii*), as a mucosal vaccine delivery platform capable of efficiently inducing protective mucosal immune responses would significantly increase the ease and efficiency of vaccine development for a broad spectrum of mucosal diseases. *S. boulardii* is a generally recognized as safe (GRAS) organism already used clinically to treat diarrheal diseases and recurrences of inflammatory bowel disease. The tools for genetic manipulation of *S. boulardii* have already been well characterized for the closely related species, *S. cerevisiae*. Here we show that *S. boulardii* can be genetically manipulated to express proteins, and we report that this probiotic yeast has a better survival in the Peyer's patches of the small intestine than its non-probiotic counterpart *S. cerevisiae*. We are currently developing constructs using the alpha-mating factor to test the ability of *S. boulardii* to secrete proteins and testing different vector and promoter combinations to optimize protein expression in *S. boulardii.* (CCVI seed funding, CHOA)

Does platelet activation mediate pathogenesis of malaria infection?

Patrice N. Mimche[1], Michael P. Schenk[1, 2], Chengjing Zhou[3], Hyojung Choo[3], Jonathan Gibbins[2], Shawn Jobe[3] and Tracey J Lamb[1].

1. Emory University, School of Medicine, Division of Pediatric Infectious Diseases and Children's Healthcare of Atlanta at Egleston, Atlanta, USA
2. Institute for Cardiovascular and Metabolic Research, University of Reading, UK
3. Aflac Cancer Center and Blood Disorders Service, Emory University, Atlanta, USA

Platelet activation by malaria parasites is thought to cause pathology in malaria infection by acting as a bridge for infected red blood cells to adhere to the endothelium leading to blockage of the blood vessels in the brain, and in turn cerebral malaria. This event has most commonly been investigated using the *Plasmodium berghei* ANKA mouse model that causes experimental cerebral malaria (ECM) in C57BL/6 mice. Platelet depletion in mice using an anti-platelet monoclonal antibody can protect against death from ECM, but only when performed within the first few days of infection. This early effect of protection suggests that platelets mediate lethal inflammation in acute malaria infection, rather than act to enhance blood vessel blockage in chronic infection. Platelet depletion using monoclonal antibodies involves clearance of antibody-opsonized platelets. Fc triggering by the opsonized platelets and subsequent IL-10 production by macrophages may offer an alternative explanation for the protection offered by platelet depletion in these experiments. IL-10 can act to down-regulate the anti-malarial inflammatory response. Consistent with this hypothesis, following antibody removal of platelets the number of splenic T cells secreting protective IL-10 significantly was increased. Indeed, unlike antibody-mediated platelet removal, inactivation of platelets using aspirin or Plavix© neither protected against the manifestations of ECM nor increased the numbers of IL-10-producing T cells in the spleen.

To ascertain whether platelet presence truly alters the inflammatory landscape of mouse malaria infection we analyzed the effects of platelet depletion on malaria pathogenesis and immune response using a novel non-antibody-mediated method of platelet depletion. In this model, megakaryocyte and platelet-limited ectopic expression of the simian diphtheria toxin (DT) receptor allows for selective and nearly complete platelet depletion following DT administration. Upon DT administration platelets were ablated. However, unlike in antibody-mediated platelet depletion, no protective effect against the development of ECM was observed upon DT-mediated platelet ablation. Similarly, IL-10 production from splenic T cells was unaffected. These results suggest that studies showing a protective effect of platelet removal by mAb depletion may in fact reflect an artifact of the method of platelet depletion, and indicate that platelets do not play a critical role in the pathogenesis of ECM.

Source of funding:

Emory University School of Medicine
Royal Society
Children's Healthcare of Atlanta
Emory Egleston Children's Research Committee

An immune prevention strategy for protection against chemical carcinogenesis.

Tahseen H. Nasti[1], Kyle Rudemiller[1], George Twitty[1], Hee Kyung Kim[1,2], Yuko Tsuruta[1,2], Mohammad Athar[1,2], Craig Elmets[1,2] and Laura Timares[1,2]

[1] The Department of Dermatology, [2] The UAB Skin Diseases Research Center, University of Alabama at Birmingham School of Medicine

Polyaromatic hydrocarbons (PAHs) possess mutagenic properties that are associated with cancers of skin, lung, pharynx, mouth, breast gastrointestinal tract and others. The carcinogenic PAH 7,12-dimethylbenzanthracene (DMBA) generates a characteristic point mutation, Q61L, in the *H-ras* oncogene (*Mut-H-ras*), which is present in 80 – 100% of induced skin tumors in mice. Our previous studies showed that mice able to make a delayed-type hypersensitvity (DTH) response to polyaromatic hydrocarbons (PAHs) are more resistant to tumor development than mice lacking this response. Therefore, we tested the hypothesis that a vaccine designed to focus T cell specific immunity to Mut-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 peptides induced DTH in A/J and C3H/HeN mice in response to challenge with peptides of mutant, but not WT, H-ras epitopes. 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/ Mut-H-ras or WT-H-ras epitope/EGFP fusion protein. Mut-H-ras-specific DTH and CTL responses were detected in mice vaccinated with plasmid DNA or engineered DC encoding *Ub*/*Mut-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. Mice vaccinated with engineered DC lines encoding *Ub/H*ras/EGFP* generated increased numbers of IFN-γ producing $CD8^+$ T cells. This correlated with reduced levels of *Mut-H-ras* mRNA expression in DMBA-painted skin from immunized mice, in contrast to control unimmunized mice, measured by allele-specific blocker PCR. *Ub/Mut-H-ras /EGFP* vaccinated mice subjected to a two-step DMBA/TPA chemical carcinogenesis protocol developed up to 50% fewer ($p=0.004$) and 75% smaller ($p=0.02$) tumors per mouse compared to control cohorts. In contrast to the high *Mut-H-ras*-specific mRNA levels that were detected in the majority of tumors from control cohorts, the mutant allele was barely detectable in tumors from Mut-H-ras vaccinated mice, consistent with effective immunoediting by induced Mut-H-ras-specific T cells. These studies provide evidence that a significant portion of immunosurveillance control of carcinogen-initiated tumors is focused on tumor cell presentation of mutant epitopes derived from expressed carcinogen-induced oncogenes. These promising results lay a foundation for further development of epitope-focused vaccine strategies for successful immunoprevention of chemically induced tumors in skin, and potentially other tissues.

Acknowledgements. NIH R21 and P01 grants from **NIAMS** and **VA Merit Award**

Modulation of autoimmune diabetes by antibodies specific for N-acetyl-D-glucosamine

J Stewart New, Brian LP Dizon, M.D. Ph.D., John F Kearney Ph.D.

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

The increasing incidence of autoimmune disease in developed societies has been linked with decreased exposure to environmental antigens, implying that antigen exposure modifies immune system development. Vaccination with Group A Streptococci (GAS) produces a strong antibody response to N-acetyl-D-glucosamine (GlcNAc). This GlcNAc moiety is conserved in mammals, and GlcNAcylated proteins are enriched in pancreatic β-cells. Anti-GlcNAc antibodies generated against GAS bind GlcNAc epitopes in human and murine β-cells. Developmental remodeling of the pancreas is accompanied by significant β -cell apoptosis, which may serve as an initial source of autoantigen priming in Type 1 Diabetes (T1D). We therefore hypothesize that anti-GlcNAc IgM generated during GAS infection mediates non-inflammatory clearance of apoptotic β-cell antigens. Using the Min6 insulinoma cells, we demonstrate that β-cell apoptosis results in surface exposure of GlcNAc residues reactive with anti-GAS Abs. During DC priming with irradiated β-cells anti-GlcNAc Abs suppressed CD4 T cell activation and cytokine production. We show that neonatal immunization with GAS, but not Group C Streptococci, reduces the incidence of diabetes in female NOD-mice. Furthermore, passive transfer of anti-GlcNAc Abs protects NOD.Rag1ko mice from diabetes onset following adoptive transfer of diabetogenic BDC2.5 T-cells. Our data suggest that anti-GlcNAc Abs generated by vaccination with GAS can suppress development of T1D.

Our research is funded by AI4782-31 and DK082277-01, and JDRF.

Lack of reactive oxygen species exacerbates diabetogenic CD4 T cell effector responses

Lindsey E. Padgett and Hubert M. Tse, PhD.**

Department of Microbiology, Comprehensive Diabetes Center; University of Alabama-Birmingham, Birmingham, AL

In Type 1 diabetes (T1D), infiltrating leukocytes generate reactive oxygen species (ROS) and pro-inflammatory cytokines that collectively participate in β-cell destruction and enhance the effector response of autoreactive T cells. Our laboratory previously demonstrated that superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are T1D-resistant partly due to skewed T cell responses. To further dissect the role of ROS on autoreactive T cell effector responses, our laboratory characterized the NOD.BDC-2.5.*Ncf1*^{m1J} mouse, possessing diabetogenic CD4 T cells unable to synthesize superoxide. NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes stimulated with the BDC-2.5 mimotope or dispersed islets displayed a 2- and 5-fold increase in IFN-γ (210.0 ±7 vs. 96.7 ±5 ng/mL; $p<0.0001$); (31.9 ±0.6 vs. 7.4 ±0.2 ng/mL; $p<0.0001$), respectively, in comparison to NOD.BDC-2.5. Exogenous superoxide addition to NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes restored Th1 cytokine synthesis to NOD.BDC-2.5 levels, indicating that superoxide deficiency exacerbated IFN-γ synthesis. Moreover, synthesis of TNF-α, a pro-inflammatory cytokine implicated in T1D pathogenesis and responsible for β-cell destruction, was significantly upregulated by superoxide-deficient splenocytes upon stimulation with the BDC-2.5 mimotope (1039.2 ±20.9 vs. 614.0 ±42.9 pg/mL; $p<0.0001$) or dispersed islets (70.9 ±3.5 vs. 18.1 ±2.8 pg/mL; $p<0.0001$) compared to NOD.BDC-2.5. Interestingly, phosphorylation of lymphocyte-specific protein tyrosine kinase (P-Lck (Y505)) and linker for activation of T cells (P-LAT (Y191)), key redox-sensitive tyrosine kinases within the T cell receptor (TCR) signaling cascade, were increased 1.5- and 2-fold, respectively, in stimulated NOD.BDC-2.5.*Ncf1*^{m1J} CD4 T cells compared to NOD.BDC-2.5. Corroborating the enhanced Th1 response and TCR signaling, transfer of $1x10^6$ NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes induced T1D in all NOD.*scid* recipients by 14 days (n=7, $p=0.0778$), in contrast to only 50% of wild-type-transferred mice (n=8). Similarly, with a 20-fold reduction in cell dose, NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes remained more diabetogenic with T1D induction in 100% of NOD.*scid* recipients by 30 days (n=5), in contrast to only 40% of wild-type-transferred recipients (n=5). Future experiments will further analyze the effects of superoxide deficiency on the activation profile of TCR adaptor molecules within NOD.BDC-2.5.*Ncf1*^{m1J} T cells and determine the mechanism for enhanced diabetogenicity. Ultimately, unraveling the role of redox-dependent signals on autoreactive T cell responses may pinpoint novel targets for T1D prevention.

A C-reactive protein→ FcγR → macrophage axis exacerbates acute kidney injury

Melissa A. Pegues[1], Mark A. McCrory[1], Abolfazl Zarjou[1], and Alexander J. Szalai[1]
[1]Department of Medicine, The University of Alabama at Birmingham, Birmingham, Alabama

Renal ischemia-reperfusion injury (IRI) is a common cause of acute kidney injury (AKI), occurring in association with hypotension and cardiovascular surgery and inevitably during kidney transplantation. Mortality from AKI is high due to incomplete knowledge of the pathogenesis of IRI and the lack of an effective therapy. Inflammation accompanies IRI and increases the blood level of C-reactive protein (CRP), a biomarker of worsened outcomes in AKI. To test if CRP is causal in AKI we subjected wild type (WT) and human CRP transgenic (CRPtg) mice to bilateral renal IRI (both pedicles clamped for 30 min at 37°C then reperfused for 24 h). Human CRP blood level was increased ~six-fold after IRI in CRPtg (11.58 ± 1.40 µg/ml at baseline versus 65.75 ± 7.90 µg/ml at 24 h) but not after sham surgery (i.e. kidneys were manipulated but not clamped). The elevation of blood CRP was the result of increased production of CRP by the liver in response to renal injury, and not due to reduced filtration of blood or its production by the kidney. Nevertheless, higher levels of human CRP were recovered from the kidneys of CRPtg that had undergone IRI. Compared to WT, serum creatinine and urine albumin was increased after IRI in CRPtg. Histological evidence of kidney damage, including cast formation, brush border loss, and necrotic tubules, was greater in CRPtg than in WT. RT-PCR analysis of mRNA isolated from whole kidneys subjected to IRI revealed that expression of FcγRI increased and expression of markers of alternatively activated macrophages (M2) decreased in CRPtg compared to WT. Our findings show that CRP exacerbates IRI induced AKI, perhaps by shifting the balance of FcγRs and macrophages from beneficial to detrimental. CRP lowering promises to be an effective therapeutic modality for the treatment of AKI, particularly when AKI and CRP induction is inevitable.

Funding Support: This project was supported by a Pilot and Feasibility Award sponsored by P30DK079337 from the National Institute of Diabetes and Digestive and Kidney Diseases and by a predoctoral fellowship from AHA (13PRE14490057).

Interleukin-21 drives intestinal inflammation by bridging the adaptive and innate immune compartments

Catherine H. Poholek[1], Laurie E. Harrington[2]
[1]Medical Scientist Training Program, [2]Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL

Th17 CD4 T cells have been cast as pathogenic in the context of many autoimmune diseases, including Inflammatory Bowel Disease (IBD); however, the mechanism for this pathogenicity has yet to be elucidated. Based on the observation that IL-21 expression is increased in biopsies from patients with ulcerative colitis compared to healthy controls, we posit that IL-21 produced by Th17 cells mediates inflammation in the intestine. Using models of both spontaneous and CD4 T cell-dependent colitis, we show that a large number of IL-21-producing CD4 T cells are present in the intestines of mice with colitis and importantly, that IL-21 production is required for full disease progression. We find that although IL-21$^{-/-}$ CD4 T cells are unable to initiate disease, they produce elevated levels of IL-17A and IL-17F and are not selectively converted into Foxp3+ regulatory T cells, indicating that IL-21 is both crucial to the induction of colitis and surprisingly, is not acting via effects on the activation and differentiation of T cells. In direct support of this idea, IL-21R$^{-/-}$ CD4 T cells are capable of inducing disease unlike their IL-21$^{-/-}$ counterparts. In fact, our data demonstrate that IL-21 has an impact on the innate lymphoid cell compartment, which has previously been implicated in IBD pathogenesis. Taken together, our data show an important and previously unrecognized role for IL-21 that links CD4 T cells to innate lymphoid cells in the induction of chronic intestinal inflammation.

FUNDING SUPPORT: Supported by funding from the UAB Center for Clinical and Translational Science Grant Number TL1TR000167 from the National Center for Advancing Translational Sciences (NCATS) and National Center for Research Resources (NCRR).

A NOVEL VARIANT OF FCGR2B IS A RISK FACTOR FOR SYSTEMIC LUPUS ERYTHEMATOSUS

PTACEK, T.S., MUKTAR, S., REDDEN, D.T., LI, X., JI, C., ABSHER, D., EDBERG, J.C., KIMBERLY, R.P.

SLE is an autoimmune disease characterized by auto-antibodies and immune complex formation. 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. The low affinity FCGRS reside within a gene cluster on 1q23, and recently gene 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 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. Previously, we used three pyrosequencing CNV assays to determine relative CNV across the 1q23 FCGR gene cluster in a large SLE case control cohort. In addition to previously reported CNVs involving *FCGR2C*, *FCGR3B*, and *FCGR3A*, we identified individuals that appeared to have duplications and deletions of *FCGR2B*. Statistical analysis showed that the *FCGR2B* duplication variant is statistically associated with SLE, and that this effect is independent of *FCGR2B* functional SNPs and functional alleles and CNVs of other genes. Further CNV analysis using other assays suggested that these variants did not include the cytoplasmic tail. We recalled individuals with variant 2B, and genetically matched controls, and are currently characterizing variant 2B genomic sequence, mRNA transcripts, protein size and antibody reactivity and protein expression on primary cells. We were unable to find evidence for a truncated *FCGR2B* transcript or protein, although we did gather evidence that the variant may be a more complicated event that involves other gene loci. Future studies will begin with establishing the genomic sequence of the *FCGR2B* variant.

Th17 mediated recurrence of virus-induced immunopathology following discontinued FTY720 treatment

Pradeep B. J Reddy[1], Sharvan Sehrawat[2], Amol Suryawanshi[3], Naveen K Rajasagi[1], Madhu Khatri[4] and Barry T Rouse[1]

[1]Biomedical & Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 1414 Cumberland Ave, Knoxville, TN 37996.

[2]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge MA 02142.

[3]New England Eye Center, Tufts University School of Medicine, Boston, MA 02111.

[4]Department of Work Environment, University of Massachusetts-Lowell, Lowell, MA, USA.

In this report we show that the anti-inflammatory effect of FTY720 in Herpes simplex virus-1 induced ocular inflammation is lost upon the discontinuation of treatment. The lesions developed after FTY720 treatment withdrawals were characterized by the predominance of Th17 cells over Th1 cells. Th17 but not Th1 cells expressed higher levels of surface CD103, an integrin that permits migration to inflammatory sites. Furthermore, we demonstrate that $CD4^+$ T cells isolated from FTY720 treated naïve DO11.10 RAG-/- mice could be efficiently polarized to a Th17 and Treg but not to Th1 phenotype. The hyper acute emergence of inflammation in FTY720 treated animals could be protected upon administration of neutralizing antibody to IL-6, a proinflammatory cytokine involved in the generation of Th17 cells as well as genetic ablation of IL-17 signaling. These results suggest that approaches such as neutralization of proinflammatory cytokines might be considered along with FTY720 if interruption of the therapy is required to achieve the optimum anti-inflammatory effects of the drug.

This work is supported by NIAID grant AI063365.

Gut bacteria modulates angiogenesis and corneal immunopathology after herpes simplex virus infection

Raphael Richardson, Siddheshvar Bhela and Barry T Rouse

University of Tennessee, Knoxville

The ocular disease herpetic stromal keratitis results in blindness due to an immunopathological attack mainly orchestrated by T cells and neutrophils. Recently, it has become apparent that lesion severity at local sites may be influenced by the balance of microbes in distal locations such as the gut, respiratory tract and even the skin. Reasons are still being assembled but appear to involve an influence on both innate and adaptive immunity. Since HSK is an uncommon outcome of ocular HSV infections in the infected individual, we sought to determine if the nature of enteric flora would influence the expression of HSK. A murine model of HSK was used to evaluate the concept. Mice were ocularly infected with HSV and separated into two groups. The controls received only sucrolose while the experimental group received sucrolose and a combination of antibiotics (Ampicillin, Gentamicin, Metronidazole, Neomycin, and Gentamicin) 3 weeks before infection to deplete gut flora and treatment continued until termination of the experiment at day 15. Significant decreases in corneal angiogenesis and HSK was evident in the antibiotic treated recipients. Analysis of inflammation cell numbers and molecules involved in angiogenesis also showed significant differences between treated and control groups. Further mechanistic studies to explain for the differences observed will I be reported and discussed.

The Mechanism by which Regulatory T Cells Inhibit HIV-1 Infection of Polarized Human Monocyte-Derived Macrophages

Tanya Robinson, Christina Ochsenbauer, Ph.D., John Kappes, Ph.D., Mingce Zhang, M.D., Ph.D., Anna Genin, M.D., Ph.D., Robert Lowe, M.D., Matthew Stoll, M.D., and Randall Q. Cron, M.D., Ph.D.

Immunology Theme, Graduate School of Biomedical Sciences, CFAR Virology Core and, Division of Pediatric Rheumatology.
University of Alabama at Birmingham, Birmingham, AL 35294

Macrophages are a key target for HIV-1 infection and contribute to the establishment, pathogenesis and latency of HIV-1 disease. Unlike $CD4^+$ T cells, macrophages are relatively resistant to the cytotoxic effects of HIV-1, and because of their longevity and widespread distribution in tissues and organs they may serve as viral reservoirs throughout the course of HIV-1 disease. HIV-1 infected macrophages display an activated phenotype and altered cytokine/chemokine production profiles. Although the activation of macrophages is critical for the induction of an effective immune response their inappropriate or sustained polarization can lead to immune dysfunction. Macrophages are functionally classified into two types: M1 (classic) macrophages, which produce proinflammatory cytokines and contribute to tissue destruction and, M2 (alternative) macrophages which secrete anti-inflammatory cytokines and promote tissue repair and remodeling. Previous research has demonstrated a more profound suppression of CCR5 HIV-1 replication in M1 polarized macrophages. M1-dependent inhibition of HIV-1 replication has been shown to be associated with a sharp decrease in HIV-1 DNA synthesis and a decrease in the accumulation of HIV-1 proteins. However, M2 polarized macrophages have been shown to be preferentially targeted by HIV-1 and demonstrate a higher level of R5 HIV-1 replication as well as an increase in HIV-1 DNA synthesis compared to M1 polarized macrophages. Using an *in vitro* model of polarization, we generated M1 (GM-CSF+IFN-γ+LPS) and M2 (M-CSF+IL-4) macrophages with predicted phenotypes (e.g. increased CD163 on M2) and infected them with CCR5 HIV-1 reporter viruses. We consistently found higher levels of HIV-1 (GFP and luciferase reporter viruses) infection of the M2 cells. Our lab has previously demonstrated the repressive effects of Tregs on HIV-1 transcription in Tregs themselves, as well as in neighboring non-Treg CD4 T cells. We are currently exploring the potential inhibitory effect Tregs have on HIV-1 infection of polarized M1 and M2 macrophages. We are exploring the mechanism by which this Treg-mediated inhibition occurs (cytokine dependent and/or cell contact dependent) using a transwell system and inhibitory cytokine antibodies. There has been very limited research conducted in this area of HIV-1 disease. Therefore, the research we propose will allow for a better understanding of the role Tregs play in HIV-1, as well as a new understanding of how the M1 to M2 macrophage switch contributes to AIDS pathogenesis and possible latency during HIV-1 infection. **NIH Basic Mechanisms in AIDS Pathogenesis Training Grant: 5T32AI007493-17**

C57BL/6 C5 Deficient as a new mouse model for *Bacillus anthracis* studies

Juan R. Barrantes[1], Brian Dizon[2], Mark Lisanby[3], Nicholas W. Kin[2] and John F. Kearney[2]

[1]Department of Pathology, [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

Studies of *Bacillus anthracis* (B.a.) are problematic due to its inherent virulence and bio-safety. Many laboratories utilize the attenuated Sterne strain, which only requires a BSL II facility, but restricts researchers to the susceptible A/J or similar mouse strains. Since the majority of genetically manipulated mice are on the C57BL/6 background, we sought to determine if C5 deficiency renders C57BL/6 mice susceptible to the Sterne strain. In this study, we compared the LD50 of A/J, C57BL/6 and C57BL/6 C5 deficient mice (C5) to show that C5 mice are susceptible to i.t. and s.c. B.a. challenges. Higher bacterial loads in the spleen of C5 mice and evidence from histological analyses of other tissues confirm the pathology associated with Sterne B.a. infection. Use of C57BL/6 C5 deficient mice allows for the study of anthrax under moderately safe conditions with an increased variety of genetically manipulated mice. We have also developed highly specific monoclonal antibodies to BclA, a major component of the B.a. exosporium and noted a partial protective effect conferred by passive transfer of anti-BclA *in vivo* in mice infected with B.a. We have also noted increased uptake of spores *in vitro* and *ex vivo* in macrophages in the presence of anti-BlcA antibodies. This new mouse model will allow us to identify potential mechanisms involved in antibody-mediated inactivation of spores prior to establishment of infectious loci and vegetative cell outgrowth and resulting toxemia and septicemia. Therapeutic strategies of this nature would be a major supplement to the current PA-based vaccines as well as to the current recommended antibiotic regimens and in the case of multi-resistant *B. anthracis* strains engineered to produce additional toxins.

Funding sources: This work was funded by the National Institute of Health R01 AI083449-01 and 1F31AI094961-01.

Lithium controls central nervous system autoimmunity through modulation of IFN-γ signaling

Amber L. Rowse[2], Rodrigo Naves[3], Kevin S. Cashman[2], Donald J. McGuire[2], Tethia Mbana[3], Chander Raman[2,3] and Patrizia De Sarno[1]

Departments of [1]Psychiatry and Behavioral Neurobiology, [2]Microbiology, and [3]Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Inhibitors of glycogen synthase kinase 3 (GSK3) are being explored as therapy for chronic inflammatory diseases. We previously demonstrated that the GSK3 inhibitor, lithium, is beneficial in experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis (MS). The primary effector cells in active EAE, induced by immunization with myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}), are $CD4^+$ interferon-γ (IFN-γ)-producing T helper (Th1) cells, and $CD4^+$ interleukin (IL)-17-producing T helper (Th17) cells. Relapsing remitting MS, the most frequent disease form in humans, segregates into a Th1 or Th17 disease, and each form of disease is differentially responsive to IFN-β, the major current therapy for MS. To determine if lithium suppresses disease induced by Th1 or Th17 cells, or both, 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 suppressed Th1 EAE, but not Th17 disease. In accordance with lithium's suppression of Th1 disease, we found that inhibitors of GSK3 attenuated IFN-γ dependent activation of signal transducer and activator of transcription 1 (STAT1) in naïve T cells and in encephalitogenic Th1 cells. Additionally, we found the therapeutic activity of lithium in EAE required functional IFN-γ-signaling. Our study reveals a novel mechanism for the efficacy of GSK3 targeting in EAE, through the IFN-γ-STAT1 axis. *Supported by grants from NIH/NINDS-5RO1NS064261 (to P.D), NIH/AI-5RO1AI1076562 (to C.R.) and NMSS Collaborative Research Center Trainee Award (to A.L.R).*

The roles of CD36 in host defense and homeostasis

Emily K. Stefanov and John F. Kearney
Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, 35294.

The ability to recognize polysaccharide antigens is vital in both host defense against pathogens and general housekeeping functions in the body. CD36 is a Type-BI scavenger receptor expressed by multiple cell types including APCs and subsets of B cells. CD36 recognizes a variety of both endogenous and pathogen-derived ligands, including β-glucans, lipoteichoic acid, and oxidized phospholipids. Therefore, we hypothesized that CD36 would have important roles in the recognition of and host defense against pathogens bearing these ligands.

We observed that mice lacking CD36 show increased susceptibility to infection by *S. pyogenes* and *A. fumigatus,* which express these ligands and had a decreased ability to generate anti-N-acetyl-D-glucosamine (GlcNAc) antibodies in response to immunization with *S. pyogenes*. CD36-/- APCs also exhibit a reduced capacity to phagocytose these microorganisms *in vitro* and *in vivo*.

Additionally, we observed that mice lacking CD36 have an exacerbation of *A. fumagatus*-induced allergic airway disease and an accumulation of proinflammatory cells in the alveolar spaces of the lung. We propose that this is due to the reduced ability to clear *A. fumigatus* conidia and the diminished capacity of CD36-/- phagocytes to clear apoptotic cells. Our data suggest that CD36 on both B cells and APCs plays important roles in the development of anti-GlcNAc antibody responses and the clearance of allergens to modulate the development of allergic airway disease in mice.
This work was supported by NIH R01 AI14782-32 to JFK.

T-bet supports differentiation of B-effectors into antibody secreting cells

Sara Stone[1], Betty Mousseau[2], Frances Lund[2]

[1]Medical Scientist Training Program, University of Alabama at Birmingham; [2]Department of Microbiology, University of Alabama at Birmingham

Memory B cells (Bmem) and long-lived plasma cells (LLPC) arise from germinal center B cells (GCB). The transcription factor Bcl6 is required for GCB cell survival and the development of Bmem. By contrast, Bcl6 inhibits LLPC development by repressing the transcription factor Blimp1 which normally controls LLPC development. To date, it is not clear how these opposing transcription factors are regulated in GCB cells. Interestingly, in T lymphocytes, the transcription factor T-bet modulates the balance between Blimp1 and Bcl6 and controls their subsequent differentiation into memory and effector cells. We recently defined a B-cell subset, referred to as B-effector 1 cells (Be-1 cells), that express high levels of T-bet and secrete IFNγ. We showed that IFNγ or T-bet deficient Be-1 cells inefficiently seed the LLPC compartment, leading us to suspect that the Be-1 cells may be precursors to LLPCs and Bmem. We therefore hypothesized that T-bet expression by Be-1 cells promotes cytokine production and supports their differential development into antibody secreting cells (ASCs). To test this hypothesis, we co-cultured naïve splenic B cells from T-bet-/-, IFNγ-/-, or C57BL/6 mice with Th1 or Th2 cells. After 4 days, we sort purified total B-cells and the IFNγ producing CXCR3+ CCR6+ Be-1 cell subset. We evaluated the transcription profile of the cells and measured cytokine and antibody secretion. We found that the CXCR3+ CCR6+ Be1s expressed higher levels of Bcl6 and T-bet but lower levels of Blimp1, Ig J-chain, and XBP1 compared to the non-Be1 cells. T-bet-/- Be1s expressed lower Blimp1, XBP1, and J-chain expression, but higher Bcl6 compared to B6 Be1s. T-bet-/- and IFNγ-/- Be1s had attenuated IFNγ production and antibody secretion compared to B6 Be1s. These results support a role for T-bet in determining Blimp1 and Bcl6 levels in B-effectors (Beffs) and modulating their development into ASCs rather than Bmem cells.

Phenotypic Analysis of B cell Subsets in HLA*B44 Positive Identical Twins Discordant for Common Variable Immunodeficiency and Recurrent Sino-Pulmonary Infection

E. Szymanska Mroczek[1], G. C. Ippolito [6], M. Zemlin [7], T.A. Hwangpo [2], M. G. Brand[2], Y. Zhuang[2], L. Freeberg [1] D.K. Crossman[3], J.D Osborne[1], Schneider[4], C. Liu[2], E.J. Lefkowitz [1], M.R. Crowley[3],G. Georgiou [6], E.E. Brown [5], and Harry W. Schroeder, Jr[1,2*]

Departments of Microbiology[1], Medicine[2], Genetics Research Division[3,] Biochemistry [4], Epidemiology [5], University of Alabama at Birmingham , Department of Chemical and Molecular Engineering[6] University of Texas at Austin, and Department of Pediatrics [7]University Marburg, Germany

Depressed serum immunoglobulin levels (sIgs) and recurrent sinopulmonary infections mark Common Variable Immune Deficiency (CVID). Many family members of CVID patients also suffer recurrent sinopulmonary infection (RESPI) but have normal sIg. We identified HLAB44 positive identical female twins who suffer sinopulmonary infections and are discordant for CVID and RESPI. Flow cytometry subsets showed equivalent numbers of immature B cells (BC) in both twins, but lower numbers of transitional and mature BC in the CVID twin. Deep sequencing of the immunoglobulin (Ig) repertoires expressed by the transitional and mature BC showed a significant divergence in the utilization of VH1 and VH4 family gene segments, with CVID favoring VH4 and RESPI VH1. RESPI twin used JH6 more frequently, whereas CVID twin used JH3. The amino acid composition of CDR-H3 repertoire was compared with a control; the twin and control tyrosine usage in transitional BC was similar (~15%) but greatly diverged in mature BC (control 15%, RESPI 25%, CVID < 10%). Whole genome sequencing revealed homozygosity for a rare CD21 S639N polymorphism and heterozygosity for CD19 L174V. These findings suggest that in addition to an acquired block in BC development at the transitional stage, the CVID twin produces an Ig repertoire that is markedly depleted of tyrosine. This may explain why the function of the Ig repertoire in CVID is more impaired than what might be expected by sIgs levels.

The role of IL-21 in memory CD8 T cell differentiation and maintenance

Yuan Tian, Maureen A. Cox and Allan J. Zajac
Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

Following many infections populations of memory CD8 T cell develop that persist overtime and operate to confer protection against subsequent pathogen exposures. Memory CD8 T cells are heterogeneous and can be subdivided into subsets including central memory (T_{CM}), effector memory (T_{EM}) and tissue-resident memory (T_{RM}) cells based on their phenotypes, functions, and locations. Together, these memory CD8 T cell subsets cooperate to protect the host from reinfection. Thus, understanding how these different subsets of memory CD8 T cells are formed and maintained will assist the development of efficacious vaccines and treatments for infectious diseases, especially for chronic infections such as HIV. Here we utilized mixed bone marrow chimeras to test whether interleukin-21 (IL-21) regulates the differentiation and maintenance of memory CD8 T cells. By comparing the phenotypes of IL-21 receptor $(IL\text{-}21R)^{+/+}$ and $IL\text{-}21R^{-/-}$ CD8 T cells in the same host, we found that $IL\text{-}21R^{-/-}$ CD8 T cells had defective accumulation of $CD44^{hi}CD62L^{low}$ T_{EM} in the blood and spleen. In addition, expression of the IL-21R permitted the generation and/or maintenance of more effector-like populations of CD8 T cells, as identified by increased expression of KLRG-1, and decreased levels of CD122 (receptor for IL-2 and IL-15). Moreover, $IL\text{-}21R^{-/-}$ memory CD8 T cells showed defective accumulation in nonlymphoid tissues including liver, lung, kidney, small intestinal (SI) epithelium and lamina propria compared to their $IL\text{-}21R^{+/+}$ counterparts. In terms of phenotypic attributes, both $IL\text{-}21R^{+/+}$ and $IL\text{-}21R^{-/-}$ CD8 T cells detected in these nonlymphoid tissues were $CD44^{hi}CD62L^{low}$, and those present in the SI intraepithelial lymphocytes (IELs) had similar expression of T_{RM} markers including CD103 and CD69. Notably, memory CD8 T cells in SI IELs expressed low levels of CD122 and CD127 (receptor for IL-7) when compared to their splenic counterparts. Since IL-7 and IL-15 have been shown to promote the survival and proliferation of memory CD8 T cells, these data suggest that IL-21 may be required for the maintenance of memory CD8 T cells when IL-7 and IL-15 signals are inadequate. Taken together, these findings suggest that IL-21 is a key regulator of both systemic and local CD8 T cell responses and may provide useful information for the development of efficacious vaccines that can elicit and maintain optimal memory CD8 T cell responses for infectious diseases as well as cancers.

The Role of Igs CDR-H3 in Responses to HIV-1 Envelope Protein

Wang Y,[1] Sanchez-Silva A,[2] , Zhuang Y,[2] Devine S,[3] Levinson M,[1] Elgavish A,[2] Haynes BF[3] and Schroeder HW Jr, [1,2,*]

[1]Departments of Microbiology and [2]Medicine, The University of Alabama at Birmingham

[3]Duke Human Vaccine Institute, Duke University Medical Center

[*]Corresponding author. Present address: 1825 University Blvd, Shelby Building, 405, UAB, Birmingham, AL 35294. Email: hwsj@uab.edu

Background: HIV1-specific broadly neutralizing antibodies that arise during infection are rare and hard to elicit and frequency of B cells expressing immunoglobulins (Igs) with HIV-1 antigen binding site is kept low by both genetic and somatic mechanisms. HIV-1-specific broadly neutralizing antibodies, such as the membrane proximal external region (MPER) antibodies 2F5 and 4E10, contain a long H chain complementarity determining region 3 (CDR-H3) that lacks tyrosine and includes patches of hydrophobic and charged amino acids. However, B cells bearing Igs with charged or hydrophobic CDR-H3s are normally culled from mature B cell subsets. This has led us to the hypothesis that the difficulty in eliciting HIV1-specific broadly neutralizing antibodies reflects normal repertoire constraints.

Methods: *Mice*-We previously generated BALB/c mice limited to the use of single D_H gene segments. The ΔD-DFL allele provides access to only the germline DFL16.1 gene segment, increasing its contribution from 20% of the developing repertoire to 100%. The ΔD-DμFS allele contains a single DFL16.1 gene segment that has been doubly frameshifted to promote the use of valine-enriched RF2. In the ΔD-iD allele, the sequence of inverted DSP2.2 has been embedded within DFL16.1. The ΔD-iD allele lacks the DFL16.1 sequence altogether. *Immunization*-each strain of 10 mice were immunized with HIV-1 JR-FL gp140 protein. Immunization was carried out every two weeks for a total of 6 times. Blood samples were collected 10 days after immunization. *Epitope Identification*-Two mice (No. 4 & 5) of each strain were selected (prior to immunization, and after the 2nd and 4th immunizations) for PEPPERPRINT Chip to detect their epitopes on HIV1 JR-FL gp160. *Serum Assay*-Binding ability was examined by HIV1 envelope protein ELISA. Blocking activity was examined by competitive ELISA with soluble CD4 (sCD4) and 2F5, 2G12 antibodies.

Results: We obtained evidence of strong and clear polyclonal responses to immunization with JR-FL gp160. As a general rule, the epitopes identified were located in the α-helical structure of HIV-1 envelope, which includes HIV1 envelope variable regions 1 and 2 (V1V2) and gp41. The heterogeneity of the anti-JR-FL gp160 response varied by D_H genotype with □D-DFL>WT>>□□D-D□FS, □D-iD. Conversely, the intensity of the response was greatest in the □D-iD. However, anti-JR-FL gp160 antibodies present in the sera of the D_H altered did not block HIV-1 envelope CD4, 2F5, 2G12 binding site. This suggests reduced affinity, stability or on rates, or increased off rates for Igs obtained from D_H altered mice.

Conclusion: The pattern of epitope recognition and antigen binding in the response to HIV1 gp160 depends, in part, on D_H gene segment sequence. Restrictions imposed by natural selection of D_H sequence may underlie some of the difficulty that patients experience in creating broadly neutralizing antibodies.

This project is supported by NIH5R01AI0907

Using reporter mouse models to track CD4 T cell differentiation and fate based on IL-2 expression

[1]Colleen J Winstead, [2]Sing Sing Way, [3]James J Moon, and [1]Casey T Weaver
[1]University of Alabama at Birmingham, Department of Pathology, Birmingham, AL
[2]Cincinnati Children's Hospital Medical Center, Department of Pediatrics, Cincinnati, OH
[3]Massachusetts General Hospital, Department of Medicine, Boston, MA

CD4 T cells respond to in vivo activation stimuli, in part, by rapidly producing the homeostatic cytokine IL-2. 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/B cells) and innate immune responses (NK/NKT/DC) to infection. Within a population of responding CD4 T cells, 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 autocrine and paracrine signaling, little is known about the quantity, quality, or fate of CD4 T cells that produce IL-2. Using *L. 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, 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' 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 incompetent for IL-2 expression. This work is being supported, in part, by NIAID R01-A1-035783.

Inhibition of Neutrophil Extracellular Trap formation induced by the Cystic Fibrosis pathogen *Pseudomonas aeruginosa*

Dae-Goon Yoo, Lan Pang, Balázs Rada
Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia

Neutrophil extracellular traps (NETs) are an important innate immune response of neutrophil granulocytes contributing to elimination of invading pathogens by entrapping them[1]. NET formation requires superoxide generation, nuclear and/or mitochondrial DNA extrusion and secretion of several antimicrobial neutrophil proteins such as myeloperoxidase (MPO), neutrophil elastase, lactoferrin and bactericidal/permeability-increasing protein[2]. Our research focuses on understanding the mechanism of NET formation stimulated by the opportunistic bacterial pathogen, *Pseudomonas aeruginosa. P. aeruginosa* causes chronic infections in a variety of diseases such as cystic fibrosis (CF). CF is due to lack of cystic fibrosis transmembrane conductance regulator (CFTR) protein, a cyclic AMP-regulated anion channel expressed on the apical membrane of epithelial cells in various organs such as the airways, sweat glands and pancreas. CFTR deficiency causes irregular anion transport across the airway epithelium, limits mucociliary clearance resulting in bacterial settlement and infection. Presence of the main CF pathogen, *P. aeruginosa* promotes neutrophil recruitment to the airways. Neutrophils, however, are incapable of clearing the infection, are believed to undergo unnecessary activation and cause tissue damage and contribute to lung failure[3]. NETs have been detected in CF airways. Thus, understanding the mechanism of *Pseudomonas aeruginosa*-triggered NET formation is of clinical importance to CF pathophysiology. Because *Pseudomonas aeruginosa* is the most common infectious agents detected in CF patients, we aimed at investigating whether *Pseudomonas aeruginosa* triggers NETs in human neutrophils.

Our data show that *Pseudomonas aeruginosa* PA14 stimulates robust release of extracellular DNA from human neutrophils. MPO, a primary granule component and citrullinated histone H4 (citH4) co-localize with extracellular DNA in *Pseudomonas*-stimulated NETs. The superoxide generating NADPH oxidase complex and the c-Raf/Raf-1 – MEK1/2 – ERK1/2 signaling pathway have been implicated in NET formation. By using inhibitors of NADPH oxidase (DPI), c-Raf (GW5074), MEK1 (U0126) and cytoskeletal (Cytochalasin-D, cytoD), our data suggest their involvement in *Pseudomonas*-initiated DNA release since all inhibitors tested had significant reduction of NET formation. However, only DPI had a significant inhibitory effect on *Pseudomonas*-stimulated superoxide production. MPO and neutrophil elastase are both associated with poor lung function in CF patients. We detected their release from neutrophils by ELISA upon *Pseudomonas* exposure. MPO and elastase release was inhibited by DPI, U0126 and cytoD whereas GW5074 was without any effect.

This study was supported by startup fund of Dr. Rada provided by UGA (1026AR211001).

The role of microbiota in CT induction of intestinal homeostatic Th17 cells

Qing Zhao[1], Charles O. Elson[2]
[1]Department of Microbiology, [2]Department of Medicine, University of Alabama at Birmingham

Inflammatory bowel disease (IBD) results from an aberrant immune response to the intestinal microbiota in a genetically susceptible host. During recent years, multiple genes along the Th17 cell adaptive immune pathway have been found to relate with IBD susceptibility. However, $CD4^+$ Th17 cells are present in the normal intestine and do not cause inflammation in non-lymphopenic, specific pathogen-free mice, or in C57/BL6 IgA deficient (B6.$IgA^{-/-}$) mice whose colon lamina propria (LP) is enriched in $CD4^+$ Th17 and Th1 effector T cells. Besides systemic and mucosal humoral response, cholera toxin (CT) oral inoculation results in the expansion of non-inflammatory Th17 cells in the mucosal sites probably due to the induction of IL-6 production by dendritic cells (DCs). This CT-induced homeostatic Th17 population was amplified in B6.$IgA^{-/-}$ mice compared to wild type (WT) B6 mice. CT-pulsed DCs facilitated Th17 cell differentiation *in vitro* as well, but in a moderate level compared to *in vivo* induction. It has been shown that the differentiation of Th17 cells in the intestinal LP is regulated by the composition of intestinal microbiota, particularly with the presence of segmented filamentous bacteria (SFB). Using 16S rDNA Microbiome Sequencing, we found a difference between intestinal microbiota composition of B6.$IgA^{-/-}$ mice and WT B6 mice. We proposed that it is the combined effect of CT and the intestinal microbiota that leads to the amplified homeostatic Th17 population in B6.$IgA^{-/-}$ mice after CT immunization. In order to test this, B6.$IgA^{-/-}$ mice were orally administered vancomycin for a week, which resulted in the ablation of intestinal gram-positive bacteria including SFB. Both CT immunogenicity and adjuvanticity were greatly dampened compared to those in the control mice, indicating that commensal bacteria play a role in CT immunogenicity and adjuvanticity after oral immunization.

Because intestinal microbiota is established within weeks after birth and mainly from the mother, we crossed male B6 WT mice back to female $IgA^{-/-}$ mice, and used the F1s to generate homozygous B6.$IgA^{-/-}$ and B6 offspring with the same intestinal microbiota. When these mice were orally immunized with CT and ovalbumin (OVA), the previous observed differences between these two strains completely disappeared: lamina propria Th17 cells, and mucosal and systemic antibody responses to CT and OVA were the same. We conclude that intestinal microbiota play an important role in the CT induction of intestinal homeostatic Th17 cells and in the host immune response to CT.

This research was supported by DK 071176.

Contribution of marginal zone B cells to autoimmunity in the B6.Sle1.Sle2.Sle3 lupus prone mouse model.

Ying Yi Zheng, Laurence Morel.
Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL 32610.

Lupus is an autoimmune disease in which the affected individual generates antibodies (Abs) against a variety of autoantigens. The B6.Sle1.Sle2.Sle3 triple congenic (TC) murine model of lupus expresses lupus-like phenotype linked to three previously characterized lupus susceptibility loci derived from lupus-prone NZM2410 mice. Previous results indicated that when compared to non-autoimmune B6 mice, B cells in TC mice are released from the marginal zone (MZ) to enter the follicle (FO) where they can potentially activate autoreactive $CD4^+$ T cells. This breach of follicular exclusion by TC MZB cells corresponds to a breach of tolerance associated with lupus pathology. We predict that TC MZB cells contribute to autoimmunity in vivo by entering the FO where they can either receive $CD4^+$ T cell help to become anti-DNA IgM secreting plasma cells, or that TC MZB cells in the FO activate resident $CD4^+$ T cells which then proceed to activate auto-reactive FOB cells that contribute to the anti-DNA Ab production.
We found through characterization of splenic B cell subsets that there is a preferential expansion of TC MZB cells in TC mice overtime in contrast to age-matched B6 mice. This preferential expansion of TC MZB cells coincides with increase of anti-DNA IgM in TC mice with progression of disease. We then proceeded to identify the molecular mechanism employed by TC MZB cell precursors to differentiate into MZB cells. Early B cells entering the spleen are transitional B cells, and these cells will differentiate into MZB cells in the presence of Notch2 signaling. Our results indicate that TC MZB cells express a high level of Notch2 receptor (Notch2R) protein and an increased transcription of the Notch2R target gene Deltex 1 as compared to B6 MZB cells. Furthermore, we discovered that TC spleens have more myeloid cells expressing delta like 1 ligand ($DL1^+$), the ligand for Notch2R, than age-matched B6 spleens, although TC myeloid cells expressed a lower level of DL1 than B6 myeloid cells. Our studies suggested that TC MZB cells preferentially expand by upregulation of the Notch2 signaling pathway.
In order to explore how MZB cells contribute to autoimmunity in TC mice, we performed DL1 monoclonal Ab (mAb) mediated depletion of MZBs. Unlike B6 mice, TC mice are more resistant to DL1 mAb mediated MZB cell depletion. Old TC mice responded to DL1 mAb treatment with an increase of follicular type II (FOII) B cells not seen in similarly treated old B6 mice. Considering that FOII B cells can differentiate into marginal precursor B cells (MZP), old TC mice may resist DL1 mAb mediated MZB cell depletion by expanding FOII B cells to replenish the MZB cell population. Overall, we conclude that TC MZB cells preferentially expand in TC mice by enhanced Notch2 signaling provided by increased expression of Notch2R on MZB cells and a high proportion of DL1+ myeloid cells.
Additional research is underway to investigate whether TC MZB cells contribute to autoAb production in vivo. Expansion of autoreactive MZB cells in TC mice may present these cells as a specific target for depletion to thereby reduce manifestation of lupus disease.

Grants: 2R01 A058150, 5T32AR007603-12