6th Annual
Upstate New York
Immunology Conference

Sponsored by
National Institute of Allergy
And Infectious Diseases

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November 9-11, 2003

The Sagamore
Bolton Landing, NY
Cover Photo

Three color staining of normal mouse small intestine. aß-TCR+ appear green and CD8a+ cells are shown in red, yellow-orange cells represent double stained cells. Purple-blue staining recognizes cytokeratin expressed by epithelial cells.

Submitted by: Maria Lopez, Ph.D.  
Center for Immunology & Microbial Disease  
Albany Medical College
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome</td>
<td>4</td>
</tr>
<tr>
<td>Participating Institutions</td>
<td>5</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>6</td>
</tr>
<tr>
<td>Major Industry Supporters and Grants</td>
<td>7</td>
</tr>
<tr>
<td>Keynote Speaker - Ira Mellman, Ph.D.</td>
<td>10</td>
</tr>
<tr>
<td>Keynote Speaker – Laurie Glimcher, M.D.</td>
<td>12</td>
</tr>
<tr>
<td>Schedule of Events</td>
<td>14</td>
</tr>
<tr>
<td>Corporate Sponsors</td>
<td>18</td>
</tr>
<tr>
<td>Speaker Abstracts</td>
<td>23</td>
</tr>
<tr>
<td>Poster Abstracts</td>
<td>33</td>
</tr>
<tr>
<td>The Sagamore</td>
<td>59</td>
</tr>
</tbody>
</table>
MESSAGE FROM THE CO-CHAIRMEN ….

Welcome to the 6th Annual Upstate New York Immunology Conference and thank you for participating in another exciting 3-days filled with a wonderful exchange of science in these beautiful surroundings!

So much has happened since 1998 when the small group of 50 research immunologists, students and postdocs first attended this Conference at Garnet Hill. In five short years we have grown, but the most significant changes have occurred since we last met in 2002. With the assistance of Dennis Metzger, Dawn Bellville, and Lynn Arnold, we have:

- undertaken a mass mailing campaign which resulted in an increase in this year’s attendance by more than 36% over 2002 … and more than double the attendance in 1998!
- increased corporate sponsorship by 48%
- increased institutional donations by 22%
- obtained two educational grants from private industry totaling $10,000
- continued to receive support from NIAID to help cover registration fees for students and postdocs
- doubled the number of poster presentations
- developed a permanent NYIC web site at AMC (www.amc.edu/NYIC) which will be maintained for future Conference registrations, etc.

We are also excited to have this year’s keynote speakers Laurie Glimcher (Harvard) and Ira Mellman (Yale, Ludwig Institute) with us and they will join the prestigious list of speakers who have added so much to our Conferences in the past.

We anticipate an exciting, innovative and informative Conference and hope you enjoy all of the presentations and activities planned!

Jim Drake and Ed Gosselin

“All attendees, whether affiliated with AMC or not, are required to comply with the terms and conditions of AMC’s “Drug and Alcohol in the Workplace/Classroom” Policy. This policy is available for review.”
Participating Institutions

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Trudeau Institute
University of Rochester
University of Wuerzburg
Wadsworth Center
Acknowledgements

We would like to thank the following individuals and institutions:

National Institute of Allergy and Infectious Diseases for grant funding in support of Graduate Students and Postdoctoral Fellows participation.

Dr. Dennis W. Metzger for his dedicated efforts in acquiring major financial support for this meeting.

The Sagamore Hotel and Conference Center and all of their staff for hosting this event. Special thanks to Pat Jarett, Gary Dilmore, Don Vilmar and Michael Crawford for all of their efforts.

Dawn K. Bellville for coordinating the multi-faceted components needed to produce a well-organized meeting.

Albany Medical Center Foundation for their guidance and support.

Institutional Representatives:

- Jim Drake and Ed Gosselin  Albany Medical College
- Jerrie Gavalchin  Cornell University
- Yasmin Thanavala  Roswell Park Cancer Institute
- Sarah Gaffen  SUNY at Buffalo
- Allen Silverstone  SUNY Upstate
- Laura Haynes  Trudeau Institute
- Nick Cohen  University of Rochester
- Gary Winslow  Wadsworth Center

THANK YOU!!
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Best wishes for a successful meeting!
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Thank You
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continuing grant support for
Graduate Students and Postdoctoral Fellows
Ira Mellman, Ph.D. is the Sterling Professor and Chairman of Cell Biology; Professor of Immunobiology; Member, Ludwig Institute for Cancer Research; Editor in Chief, The Journal of Cell Biology.

Dr. Mellman received his A.B. from Oberlin College in 1973 and his Ph.D. from Yale University in 1978. His laboratory explores fundamental questions of membrane traffic as they relate to two specific problems. The first is the question of cell polarity and asymmetry. These efforts aim to solve the molecular mechanisms responsible for the sorting, intracellular targeting, and transport of membrane components to their appropriate membrane domains in epithelial cells, neurons, and lymphocytes. The second is the question of antigen processing by cells of the immune system. This work involves understanding how the endocytic and biosynthetic pathways are modified and regulated to facilitate the generation of immunogenic peptides that can be loaded onto MHC class I and class II molecules. Current interests involve analyzing dendritic cells, the most important of all antigen presenting cells and cells which carefully regulate their function concomitant with changes in endocytosis, class II transport, and gene expression. By exploring the interface between fundamental cell biology and the immune response, Mellman's group has identified and characterized important new principles that in both, and has also lead to innovations in ongoing clinical trials of novel cancer vaccine strategies.
Dendritic cells (DCs) play an essential role in initiating the immune response due to their ability to stimulate immunologically naïve T cells, an ability that reflects the DC’s exceptional capacity for antigen presentation. Given their importance, DC function is carefully regulated by a process of terminal differentiation termed maturation. Immature DCs populate peripheral tissues and are adapted to accumulate, but not process or present, foreign and self antigens. They then migrate to lymphoid organs, constitutively or in response to inflammatory signals, where they are converted into mature DCs having a diminished capacity for antigen uptake but a greatly enhanced capacity for antigen processing and presentation. DC maturation is accompanied by dramatic alterations in cellular organization, membrane traffic, and expression of proteins required for inducing T cell responses. We have systematically evaluated the cell biological events responsible for DC maturation. DCs exhibit specializations that allow them to enhance the abilities of otherwise conventional endocytic organelles to form immunogenic complexes of both MHC class I and class II. Notably, DCs control the degradative capacity of their lysosomal apparatus by markedly decreasing the expression of hydrolytic enzymes and by regulating their activity by carefully controlling lysosomal pH. Ironically, the attenuation of lysosomal function increases the efficiency of antigenic peptide formation by reducing the efficiency of complete degradation. This also allows protein antigens to escape endosomes, enabling their processing by proteasomes and presentation by MHC class I. DCs also express a numerous proteins, normally associated with epithelial cells and neurons, which play essential roles in DC maturation and in T cell stimulation. An understanding of these basic features of DC cell biology is emerging as being critical to the implementation of therapeutic strategies (e.g. vaccines) designed to target DC function.
Laurie H. Glimcher MD is the Irene Heinz Given Professor of Immunology at the Harvard School of Public Health, and Professor of Medicine at Harvard Medical School. She received her B.A. degree from Radcliffe College and her M.D. from Harvard Medical School. She received her postdoctoral training at Harvard and in the Laboratory of Immunology at the Institute of Allergy and Infectious Diseases in Bethesda. She is board certified in Internal Medicine and Rheumatology, and is an Associate Rheumatologist at the Brigham and Woman's Hospital. Dr. Glimcher received the Soma Weiss Award for Undergraduate Research, the Distinguished Young Investigator Award from the American College of Rheumatology, the Leukemia Society's Stohlman Memorial Scholar Award, the Arthritis Foundation's Lee S. Howley Award, the FASEB Excellence in Science Award and the American Society of Clinical Investigation Investigator Award. She is a Fellow of the American Academy of Arts and Sciences, a member of the Institute of Medicine of the National Academy of Sciences and a member of the National Academy of Sciences. She is the President of the American Association of Immunologists. Dr. Glimcher is a member of the Howard Hughes Medical Institute Scientific Advisory Board and serves on the Irvington Institute Fellowship Committee. She is on the Corporate Board of Directors of the Bristol-Myers Squibb Pharmaceutical Corporation and of the Waters Corporation.

Her laboratory uses biochemical and genetic approaches to elucidate the molecular pathways that regulate CD4 T helper cell development and activation. The complex regulatory pathways governing $T_{\text{helper}1}/T_{\text{helper}2}$ ($T_{H1}/T_{H2}$) responses are critical for both the development of protective immunity and for the pathophysiologic immune responses underlying autoimmune diseases. This developmental event is regulated by the IL-4 and IFN-$\gamma$ cytokines. During the last ten years, Dr. Glimcher’s laboratory has studied the transcriptional pathways that control this important immune checkpoint. They demonstrated that the B7-1/B7-2 co-stimulatory factors differentially regulate $T_{H1}/T_{H2}$ responses. The laboratory subsequently defined the genetic bases of both IL-4 and IFN-$\gamma$ expression in T cells. Her group identified the proto-oncogene $c$-$\text{maf}$ as the transcription factor responsible for TH2-specific IL-4 expression and, subsequently, isolated a second novel protein, NIP45, that together with $c$-$\text{maf}$ and the NFAT family of transcription factors controls IL-4 gene expression and $T_{H2}$ differentiation. Subsequently, her group discovered the first $T_{H1}$-specific transcription factor, T-Bet and demonstrated that this single factor is a master-regulator of both IFN-$\gamma$ gene expression and the $T_{H1}$ phenotype. Most recently her laboratory has expanded their interest in lineage commitment in lymphocytes to the B cell with the discovery of a transcription factor, XBP-1 that controls plasma cell differentiation. This body of work may provide a conceptual framework to therapeutically manipulate these responses in the settings of autoimmune disease and cancer.

Outside the lab, she is the mother of three children (and two dogs), ran the Boston Marathon last year and likes books, poetry, playing tennis and gardening.
T-bet, a Transcription Factor That Controls Type 1 Immunity

Laurie H. Glimcher, M.D.
Harvard Medical School, Boston, MA

T-bet is the first Th1 specific transcription factor described that plays a central role in Th1 development. T-bet expression correlates with IFNg expression in Th1, NK and B cells. Ectopic expression of T-bet both transactivates the IFNg gene and induces endogenous IFNg production. Remarkably, retroviral gene transduction of T-bet into polarized Th2 and Tc2 primary T cells redirects them into Th1 and Tc1 cells, respectively, as evidenced by the simultaneous induction of IFNg and repression of IL-4 and IL-5. Thus, T-bet initiates Th1 lineage development from naïve Thp cells both by activating Th1 genetic programs and by repressing the opposing Th2 programs.

We have recently published in vivo proof-of-principle studies that definitively demonstrate that T-bet controls the Th1/Th2 balance in vivo. Mice that overexpress T-bet (T-bet transgenic mice) have increased production of IFNg and decreased production of IL-4. Mice that are deficient in T-bet (T-bet ko mice), in contrast, have severely impaired production of IFNg and increased production of Th2 cytokines. T-bet controls IFNg production from CD4, NK, gd and in recent data CD8 and dendritic cells. T-bet ko mice are more susceptible to infection with Leishmania Major but are protected from the development of autoimmune diseases such as inflammatory bowel disease and systemic lupus erythematosus. T-bet deficient mice are, in contrast, highly susceptible to asthma as would be expected from their failure to mount Type I immunity which is protective in asthma and allergies. Small molecules and biologics that can effectively and selectively inhibit T-bet activity represent an attractive approach to controlling autoimmune, infectious and allergic disease.
Sunday, November 9th

3:00-5:00 p.m. Arrival and Registration

5:30-7:00 p.m. Dinner (Sagamore Dining Room)

7:00-8:00 p.m. **Keynote Speaker**
- Introduction by James Drake, Ph.D. (Albany Medical College)

**Ira Mellman, Ph.D.**
Sterling Professor and Chairman, Department of Cell Biology
Department of Immunobiology, Yale University
Affiliate Investigator, The Ludwig Institute for Cancer Research
Editor-in-Chief, The Journal of Cell Biology
“Cell Biology of Antigen Presentation by Dendritic Cells”

Monday, November 10th

7:00-8:00 a.m. Breakfast (Restaurant)

8:00-10:00 a.m. Session I: **Signaling Molecules and Immune Cell Function** (Dollar Island)
Session Chair: Michelle Lennartz, Ph.D. (Albany Medical College)

**Andrea Sant, Ph.D.** (University of Rochester)
“Regulation of Immuno-dominance in CD4 T-Cell Responses”

**Paul Massa, Ph.D.** (SUNY Upstate Medical University)
“Discordance of iNOS RNA and Protein Expression in Mice Lacking SHP-1 and Antiviral State”

**Lee Ann Sinha, Ph.D.** (SUNY at Buffalo)
“The Role of the Ets-1 Transcription Factor in Regulating B-Cell Activation Versus Quiescence”

**Michelle Lennartz, Ph.D.** (Albany Medical College)
“The Many Faces of a Macrophage”

10:00-10:30 a.m. Break
Monday, November 10th—Continued

10:30-12:30 p.m. Session II: **T-Cell Signaling and the Immunological Synapse**
(Dollar Island)

Session Chair: Nick Cohen, Ph.D. (University of Rochester)

*Jim Miller, Ph.D.* (University of Rochester)
“T-Cell Signaling Through the Immunological Synapse”

*James Clements, Ph.D.* (Roswell Park Cancer Institute)
“Modulating SLP-76 Dosage in the Thymus Compromises the Efficiency of Negative Selection”

*Xin Lin, Ph.D.* (SUNY at Buffalo)
“CARMA1, an Essential Component for T-Cell Receptor Signaling Pathway”

*Laura Haynes, Ph.D.* (Trudeau Institute)
“Impaired CD4 T-Cell Cognate Function is Responsible For Age-Related Reductions in Humoral Responses”

12:30-1:30 p.m. Lunch (The Trillium)

1:30-3:00 p.m. Session III: **Cutting-Edge Topics in Immunology**
(Dollar Island)

Session Chair: James Drake, Ph.D. (Albany Medical College)

*Timothy Powell, Ph.D.* (Trudeau Institute)
“Response of CD8+ Lymphocytes During Influenza Infection: Phenotype and Comparison with CD4+ Lymphocyte Expansion and Migrations”

*Hongmin Li, Ph.D.* (NYSDOH-Wadsworth)
“Crystal Structure of Superantigen *Mycoplasma-Arthritidis* Mitogen (MAM) Complexed With Peptide/Major Histocompatibility Complex”

*Daniel Beiting, B.S.* (Cornell University)
“IL-10 Controls Inflammation at the Surface of the Diaphragm and in the Pleural Cavity During Muscle Infection with *Trinclinella Spiralis*”

*Sonya Swing, Ph.D.* (Taconic)
“Transgenic Models in Immunology: Application and Exchange”
Monday, November 10th—Continued

3:00-5:30 p.m. Break and Poster Set-up (Dollar Island)

3:30-4:30 p.m. NYIC Executive Committee Meeting (Empire Room)

5:30-7:00 p.m. Dinner (Sagamore Dining Room)

7:00-8:00 p.m. **Keynote Speaker**
- Introduction by Susan Swain, Ph.D. (Trudeau Institute)

  **Laurie Glimcher, M.D.**
  Irene Heinz Given Professor of Immunology
  Department of Immunology and Infectious Diseases
  Harvard School of Public Health
  Member of the National Academy of Sciences
  President, American Association of Immunologists
  “T-bet, a Transcription Factor that Controls Type 1 Immunity”

8:00-10:00 p.m. **Cocktail Reception** and Poster Session (Shelving Rock)

Tuesday, November 11th

7:00-8:00 a.m. Breakfast (Restaurant)

8:00-9:30 a.m. Session IV: **Immunity and Viral/Parasitic Infectious Diseases**
(Dollar Island)

  Session Chair: Yasmin Thanavala, Ph.D. (Roswell Park)

  **Ed Barker, Ph.D.** (SUNY Upstate Medical University)
  “MHC Class I Molecules Decrease the Ability of Natural Killer Cell to Mediate Antibody Dependent Cell-mediated Cytotoxicity of HIV-infected Primary T-Cells”

  **Matthias Hesse, Ph.D.** (Cornell University)
  “Survival in Schistosomiasis Depends Upon a Cooperative Response by Suppressor Cells of Innate and Adaptive Origin”

  **Protul Shrikant, Ph.D.** (Roswell Park Cancer Institute)
  “*In Vitro* Studies to Evaluate Determinants of CD8+ T-Cell Developmental Pathways”
Tuesday, November 11th—continued

9:30-10:00 a.m. Break (Restaurant)

10:00-12:00 p.m. Session V: Bacterial Infections
(Dollar Island)

Session Chair: Gary Winslow, Ph.D. (Wadsworth)

Andrea Cooper, Ph.D. (Trudeau Institute)
“The Role of IL-27 Moderating T-Cell Responses to
Mycobacterial Infection”

Beth Rhoades, Ph.D. (Cornell University)
“A New Model of Granuloma Formation: Towards Dissection
of the Granulomatous Response to Mycobacterium spp.”

Timothy Sellati, Ph.D. (Albany Medical College)
“Evolution of Mycoplasma pneumoniae Infection in
T-bet-deficient Asthmatic Mice”

Constantine Bitsaktsis, Ph.D. (Wadsworth Center)
“Cellular Immunity During Ehrlichiosis”

12:00-1:00 p.m. Lunch (The Trillium)

Depart from Conference

NYIC 2003 Meeting Organizers would like to thank everyone
For participating in this year’s conference.
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Speaker Abstracts
Regulation of Immunodominance in CD4 T-cell Responses

Andrea J. Sant, Ph.D.
University of Rochester

CD4 T cells respond to a very limited subset of potential peptide epitopes within complex immunogens, a well-documented, but poorly understood phenomenon termed immunodominance. Our laboratory has explored the factors that regulate this selectivity in T cell responses and have found that a key parameter that controls T cell responses to antigen is a biochemical property of the peptide:MHC complex, the ligand recognized by antigen specific CD4 T cells. Using purified, soluble MHC class II molecules, we examined the kinetic stability of a number of CD4 epitopes from diverse antigens previously characterized with regard to immunodominance, including several model protein antigens and epitopes derived from pathogenic organisms. Remarkably, we found that immunodominant and cryptic peptides segregate at the extreme opposite ends of a broad kinetic stability spectrum. Immunodominant peptides, which dominate in immune responses, form highly stable complexes with the class II molecule ($t_{1/2}$ dissociation $>100$ hrs), whereas cryptic peptides, those that do not elicit T cell responses, interact very unstably with class II molecules ($t_{1/2}$ dissociation $<5$ hrs). Differential kinetic stability is also associated with different consequences of “editing” within antigen presenting cells (APC), promoted by the intracellular chaperone termed “DM”. Using genetically engineered forms of test antigens, we now have extended these studies to show that kinetic stability of peptide:class II complexes can be selectively manipulated and that such modified antigens display corresponding changes in DM editing within APC and in immunodominance in vivo. Taken together, our data suggest that the spontaneous kinetic stability of class II-peptide complexes is a critical parameter that dictates preferences for CD4 T cell priming and that this parameter can be selectively and rationally manipulated in vaccines to promote immune responses to pathogens or tumors.

Discordance of iNOS RNA and Protein Expression in Mice Lacking SHP-1 and Antiviral State

Dr. Paul T. Massa, and Kathryn L. Beuler
SUNY Upstate Medical University
Department of Neurology, Department of Microbiology/Immunology

We have observed a striking deficiency in the ability of mice lacking SHP1, a tyrosine phosphatase, to mount an effective anti-viral response to Theiler’s murine encephalomyelitis virus (TMEV). Using dsRNA to mimic infection, we have found an interesting discordance between transcription and expression of inducible nitric oxide synthase (iNOS), an interferon inducible gene, in SHP1 deficient “moth-eaten” mice. Glial cells of moth-eaten mice showed significantly higher transcripts of iNOS in response to dsRNA; however, the amount of protein was significantly less. This decreased expression was also evident in immunohistochemical stains. Furthermore, activity of iNOS in moth-eaten cultures was significantly less. The production of NO by synthases during a viral infection has been linked to reductions in replication and spread, thereby representing a critical step in the innate immune response to viral infection. We are currently analyzing whether dsRNA specifically affects translation or proteolysis of iNOS in an SHP1 dependent manner.
The Role of the Ets-1 Transcription Factor in Regulating B Cell Activation Versus Quiescence

*Duncheng Wang, §James Clements, *Brian Grabiner, and *Lee Ann Garrett-Sinha
*Department of Biochemistry, State University of New York at Buffalo, Buffalo, NY 14214
§Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263.

Tight regulation of B-cell activation is critical to prevent loss of tolerance to self-antigens. Therefore, B-cell activation is regulated by the coordinated action of specific transcription factors that control the expression of genes critical for maintaining B-cell quiescence and preventing spontaneous activation. The Ets-1 transcription factor is highly expressed in B and T lymphocytes where it acts downstream of various signaling pathways and regulates the expression of numerous genes. Mice deficient in the Ets-1 transcription factor (Ets-1−/−) have increased numbers of IgM-secreting plasma cells and increased levels of serum IgM. We have shown that Ets-1−/− mice lack marginal zone B cells and that the follicular B cells from these mice exhibit phenotypic characteristics of activated B cells. In addition, the serum from Ets-1−/− mice contains high titers of antibodies reactive against various self-antigens including double-stranded DNA, histones, immunoglobulins, myelin basic protein and cardiolipin. An examination of kidney sections demonstrates immune complex deposition in the glomeruli of Ets-1−/− mice, which leads to autoimmune glomerulonephritis as the mice age. Taken together, these observations indicate that Ets-1−/− animals develop autoimmunity likely as a result of hyper-activation of follicular B cells and their spontaneous differentiation to IgM-secreting plasma cells. Thus, Ets-1 is required for to inhibit B cell activation and to promote tolerance to self-antigens.

The Many Faces of the Macrophage

Michelle R. Lennartz, Ph.D1, Keylon Cheeseman1, Christopher Collins2, Lindsay A. Flax1, and 3Daniel J. Loegering, Ph.D.
1Cell Biology and Cancer Research, 2Immunology and Microbial Diseases, and 3Cardiovascular Sciences, Albany Medical College.

Macrophages are the sentinels of the body, strategically positioned to protect and defend against invading organisms. We are looking at several aspects of macrophage biology and will detail our studies concerning the macrophages’ role as defender of the realm, an innocent participant in chronic inflammatory disease, and as a target for terrorists. Macrophages recognize and destroy foreign particulates via antibody-mediated phagocytosis. We have shown that protein kinase C-epsilon (PKC-ε) is critical for phagocytosis. Catalytic activity is necessary as is localization of the enzyme to incoming targets. We are defining the domains required for localization and determining the downstream targets. The C1B DAG-binding domain is necessary, but not sufficient, for targeting. Our studies suggest that phospholipase D2 provides the DAG for PKC-ε translocation. Ongoing studies are identifying additional domains involved in PKC-ε movement in macrophages. In chronic inflammatory diseases, macrophages contribute to tissue damage in response to perceived invaders. We will summarize our results demonstrating that immune complexes stimulate matrix metalloproteinase (MMP) release in macrophages, an event which contributes to plaque rupture in atherosclerosis. Our results suggest that MMPs are stimulated by immune complexes in an oxidant-dependent manner. Finally, macrophages are the reservoir for Francisella tularensis, a Category A bioterror organism. We have developed a computerized method for quantitation of F. tularensis uptake by macrophages and will present our recent studies on the phagocytosis of this pathogen in macrophages from susceptible and resistant mouse strains. Supported by R01 AI050821 and an AMC Bridge Award.
T-cell Signaling Through the Immunological Synapse

Jim Miller, Ph.D.
University of Rochester

T cell activation requires a coordinated reorganization of cell surface receptors, cytoskeletal elements and cell signaling lipids and proteins into a spatially and temporally ordered structure termed the immunological synapse. The immunological synapse is thought to play to important roles in T cell function. First, polarization of the microtubule organizing center and associated secretory apparatus toward the antigen presenting cell allows for directional secretion of cytolytic granules and newly synthesized cytokines. Second, the assembly of cell signaling complexes and selective exclusion of phosphatases from the central domain of the immunological synapse provides for sustained signaling that is required for complete T cell activation. We have been examining to role of two cell surface proteins in regulating the formation of the immunological synapse, the specific targeting of signaling proteins to the central region of the synapse, and how these localization events regulate distinct outcomes in T cell activation. We have found that the integrin, LFA-1, which is a well known cell adhesion molecule can facilitate the recruitment of PKCtheta to the immunological synapse, while the costimulatory receptor, CD28, is required to target PKCtheta to the central region. This function of CD28 is mediated by its ability to activate PI-3kinase and results in the nuclear localization of NF-kB and upregulation of IL-2 transcription. In addition, CD28 can transduce PI-3kinase-independent signaling that upregulates IL-2 secretion through post-transcriptional mechanisms. The overall goal of these studies is to understand the specific molecular an cell biological events which contribute to establishing the threshold of T cell activation.

Modulating SLP-76 Dosage in the Thymus Compromises the Efficiency of Negative Selection


In T cells, several key adaptor proteins that function to link T cell receptor (TCR)-dependent activation of protein tyrosine kinases (PTKs) with more distal signaling events have been described. These include the SH2-domain leukocyte protein of 76 kDa (SLP-76), the Linker of Activated T cells (LAT), and the Grb2-related adaptor downstream of Shc (Gads). Mice made deficient for either SLP-76 or LAT manifest a severe block in T cell development at a stage that requires pre-TCR signaling for further differentiation. More recently, our laboratory has revealed a potential role for SLP-76 in propagating TCR-dependent signals that regulate thymic selection of the peripheral T cell repertoire. In transgenic mice expressing lower than normal levels of SLP-76 in the thymus, we find low levels of surface CD5 in the CD4⁺CD8⁺ subset and a skewing of the ratio of thymic CD4⁺ and CD8⁺ cells. The efficiency of negative selection is also impaired in thymocytes expressing low levels of SLP-76, consistent with the notion that TCR signaling is compromised. These observations suggest the hypothesis that SLP-76 expression must be maintained at some minimal level to ensure proper selection of the peripheral T cell repertoire. It will now be of interest to assess the influence of altering thymic SLP-76 expression levels on thymocyte selection using transgenic TCR mouse models where peptide ligands that promote either negative or positive selection have been defined.
CARMA1, an Essential Component for T-Cell Receptor Signaling Pathway

Xin Lin, Ph.D.
SUNY at Buffalo

T cell receptor (TCR) induces a series of signaling cascades and leads to activation of multiple transcription factors including NF-κB. Although the mechanism of TCR-induced NF-κB activation is not fully understood, recent studies indicate that Bcl10 and CARMA1, two adaptor/scaffold proteins, are critical signaling components and play essential roles in mediating TCR-induced NF-κB activation. CARMA1 (also known as CARD11) is a scaffold molecule and contains a caspase-recruitment domain (CARD) and a membrane-associated guanylate kinase-like (MAGUK) domain. It plays an essential role in mediating CD3-CD28 costimulation-induced NF-κB activation. However, the molecular mechanism by which CARMA1 mediates costimulatory signals remains to be determined. Here, we show that CARMA1 is constitutively associated with the cytoplasmic membrane. This membrane association is essential for the function of CARMA1, since a mutant of CARMA1, CARMA1(L808P) that is defective in the membrane association, cannot rescue CD3-CD28 costimulation-induced NF-κB activation in JPM50.6 CARMA1-deficient T cells. Although CD3/CD28 costimulation effectively induces the formation of the immunological synapse in CARMA1-deficient T cells, the recruitment of PKC-θ, Bcl10, and IKKβ into lipid rafts of the immunological synapse is defective. Moreover, expression of wildtype CARMA1, but not CARMA1(L808P), restores the recruitment of PKC-θ, Bcl10, and IKKβ into lipid rafts in CARMA1-deficient T cells. Consistently, expression of a mutant CARMA1, CARMA1(?CD) that cannot associate with Bcl10, failed to restore CD3/CD28 costimulation-induced NF-κB activation in JPM50.6 cells, whereas expression of Bcl10-CARMA(?CD) fusion protein effectively restored this NF-κB activation. Together, these results indicate that CARMA1 mediates CD3/CD28 costimulation-induced NF-κB activation by recruiting downstream signaling components into the immunological synapse.

Impaired CD4 T-cell Cognate Function is Responsible for Age-related Reductions in Humoral Responses

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In order to examine the effect of age on the helper function of CD4 T cells, we have developed an adoptive transfer model using TCR transgenic (Tg) CD4 T cells. This model allows us to compare the in vivo cognate helper function of identical numbers of antigen-specific naive T cells from young and aged donors. Upon transfer to young hosts, there is significantly reduced expansion and germinal center (GC) differentiation of the antigen-specific B cell population in the hosts which received aged CD4 T cells. This reduced cognate helper function was seen at all time points and over a wide range of transferred cell numbers. In hosts receiving aged CD4 cells, there were also dramatically lower levels of antigen-specific IgG. This age-related defect is most likely due to the inability of the aged CD4 T cells to migrate appropriately since few aged cells were found within germinal centers. In addition, we found that there was no difference in B cell expansion or differentiation or in IgG production when young CD4 T cells were transferred to young or aged hosts. Our results show that age-related reductions in humoral responses are due to defects in the cognate helper function of CD4 T cells from aged individuals.
Response of CD8+ T Lymphocytes During Influenza Infection: Phenotype and Comparison with CD4+ T Lymphocyte Expansion and Migration

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Naïve CFSE labeled TCR transgenic CD8+ T cells were adoptively transferred into mice subsequently infected with influenza. The kinetics of expansion, division, migration and effector function were followed for 30 days. Transferred CD8+ T cells were recovered from draining lymph nodes (DLN), non-draining lymph nodes (NDLN) and spleen at early time points (day 2-4 post infection (PI)) but are not found in lung and airways until day 5 PI. CFSE monitored division was seen first in the DLN, while cells that reach the lung had undergone more than six divisions. Transferred CD8+ T cells in DLN, NDLN, spleen, lung and airways had high surface levels of CD25, CD43, CD44 and Ly6C and had lost CD62L indicating activated cells. These activated CD8+ T cells produced IFNγ and TNFα and were cytotoxic ex vivo. The expansion and division of influenza specific CD4+ and CD8+ TCR transgenic T cells were compared by co-transfer into the same host, followed by influenza infection. CD8+ T cells expanded to a higher number than CD4+ T cells in lungs and airways, with comparable numbers in DLN, NDLN and spleen. CFSE monitored division of CD4+ and CD8+ T cells was similar in DLN and spleen. CD4+ T cells were detected in much smaller numbers than CD8+ T cells 30 days post infection. These data provide new insights into CD8+ T cell activation in response to a locally proliferating virus and, show, that CD4+ and CD8+ T cells are regulated differentially during influenza infection.
Funding: NIH PO1 HL639525 and T32AI49823 (DMB)

Crystal Structure of Superantigen Mycoplasma-arthritidis Mitogen (MAM) Complexed with Peptide/Major Histocompatibility Complex

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Mycoplasma arthritidis-derived mitogen (MAM) is a soluble bacterial protein that functions like a superantigen (SAG), which binds to major histocompatibility complex (MHC) class II molecules and stimulates the activation of a large fraction of T cells by recognizing specific T cell receptor (TCR) Vß subfamilies. Here we present the crystal structure, at 2.5 Å resolution, of MAM complexed with a human MHC class II molecule, HLA DR1, loaded with a haemagglutinin peptide (HA) of influenza virus. This complex structure reveals a novel fold for MAM composed of two a-helical domains, forming a L-shaped molecule. The N-terminal domain of MAM is a four-helical bundle with a long N-terminal loop of 25 residues wrapping around. In C-terminal domain, one central helix is surrounded by five others. This fold is entirely different from that of the pyrogenic superantigens, consisting of a ß-grasped motif and a ß-barrel. The DR1/HLA/MAM complex structure shows that the N-terminal of MAM mainly binds to the DR1 a1 domain and the bound HA peptide, and to a less extend, to the DR1 ß1 domain. Under crystallization condition, two MAM molecules form a homodimer through their C-terminal domains in an asymmetric fashion. The two MAM molecules in the dimer cross-link two MHC molecules to form a (DR1/HA/MAM)2 complex. Based on the structure, a hypothetical model of TCR2MAM2MHC2 is proposed.
IL-10 Controls Inflammation at the Surface of the Diaphragm and in the Pleural Cavity During Muscle Infection with Trichinella spiralis

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Adult Trichinella spiralis reside in the intestinal epithelium where they release first-stage, or newborn larvae (NBL). NBL enter circulation and, in rodents, a high concentration of the parasite establish in the diaphragm. Given the predilection of T. spiralis for this tissue and the established role of the diaphragm in lymphatic drainage, we sought to characterize the diaphragm-associated and body cavity responses to muscle infection, and to evaluate the influence of IL-10 on these responses. C57BL/6 (WT) and B6.129P2-IL10tmlCgn (IL-10 -/-) mice were synchronously infected with 15,000-25,000 NBL, administered intravenously. At 0, 5, 10, 20 or 100 days post-infection (dpi), diaphragms and body cavity exudates were recovered. Using standard histochemical, immunohistochemical, and flow cytometric techniques, we found that muscle infection induces an inflammatory response at the surface of the diaphragm as well as in the pleural cavity. In the absence of IL-10, cellular infiltration of the diaphragm was more pronounced, resulting in a 4-fold increase in cells recovered from diaphragms of IL-10 -/- mice compared to WT. Similarly, mice lacking IL-10 expanded the pleural B and T cell populations 6 fold and 9 fold, respectively. In addition, IL-10 controls the expansion of B-2 cells (11-fold) and B-1 cells (4-fold) in the pleural cavity following infection. In the peritoneal cavity, B-2 cells expand only 3-fold in the absence of IL-10, while the B-1 cell population is diminished following infection. Oral infection elicited both a pleural and a peritoneal response. Taken together, these results document a role for IL-10 in controlling a lymphocytic response at the surface of the diaphragm and in the body cavities during muscle infection with T. spiralis.

Transgenic Models in Immunology: Application and Exchange

Sonya Swing, DVM, Ph.D.
Taconic
Quality Laboratory Animals and Services for Research

MHC Class I Molecules Decrease the Ability of Natural Killer Cell to Mediate Antibody Dependent Cell-mediated Cytotoxicity of HIV-infected Primary T-cells

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Antibody controls HIV by neutralization, inducing complement mediated destruction of infected cells and triggering antibody dependent cell-mediated cytotoxicity (ADCC). We demonstrate that antibodies directed to HIV envelope protein gp120 triggers natural killer (NK) cells to kill autologous primary CD4+ cells infected with HIV. However, the level of killing of the antibody-coated HIV-infected cells by NK cells was significantly less than killing of an ADCC susceptible cell line. HIV selectively
decreases major histocompatibility complex (MHC) class I molecules HLA-A and HLA-B on the infected cells but leaves HLA-C and HLA-E on the surface. Since HLA-C and E may trigger inhibitory receptors on NK cells we wanted to determine whether HLA-C and -E specific inhibitory receptors on NK cells may prevent the NK cells from mediating ADCC of HIV-infected cells. When we block the interaction between NK cell inhibitory receptors (i.e., NKG2A, CD158a and CD158b) and HLA-C and HLA-E we observed a significant increase in killing of anti-gp120-coated HIV-infected cells by NK cells. Thus, HLA-C and E molecules on infected cells may help facilitate evasion of NK cell mediated destruction of antibody-coated HIV-infected cells.

Survival in Schistosomiasis Depends Upon a Cooperative Response by Suppressor Cells of Innate and Adaptive Origin

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IL-10 reduces immunopathology in persistent infections, yet the contribution of IL-10 from distinct cellular sources remains poorly defined. We generated IL-10/RAG2-deficient mice and dissected the role of T cell- and non-Tcell-derived IL-10 in schistosomiasis by performing adoptive transfers. Although IL-10 was generated by the innate and adaptive immune response, with both sources regulating the development of type-2 immunity, Tcell-derived IL-10 prolonged the survival of S. mansoni infected mice to a greater extent. Most of the CD4+ T cell-produced IL-10 was confined to a subset T cells expressing CD25. These cells were isolated from egg-induced granulomas and exhibited potent suppressive activity in vitro. Nevertheless, when naïve, natural-occurring CD4+CD25+ cells were depleted in adoptive transfers, recipient IL-10/RAG2 animals were more susceptible than RAG2 mice, revealing an additional host protective role for non-Tcell-derived IL-10. Thus, innate effectors and regulatory T cells producing IL-10 cooperate to reduce morbidity and prolong survival in schistosomiasis.

The Determinants for CD8+ T-cell Responses

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The signals emanating from TCR ligation by antigen/MHC (signal 1) integrate with signals generated by co-stimulatory molecules (signal 2) as well as cytokines (signal 3) to determine commitment of naïve CD8+ T cells for activation, proliferation, differentiation and survival. To assess the precise impact of these variable signals on T cell commitment, we have used adherent fibroblasts as surrogate APC’s that can provide signal 1 and signal 2 at defined dosages and duration. By adding cytokines that are produced by different immune cell types; IL-12 (mononuclear cells) and IL-21 (activated CD4+ T cells), we have evaluated the development of naïve CD8+ T cells response. Our results indicate that the duration and amount of antigen encountered affects early CD8+ T cell activation and but its cell division as determined by CFSE-DA dilution remains the same at 72h. However, the proliferation achieved by brief (1h) or limited antigen (16.7%) stimulation restricts the differentiation of naïve CD8+ T cells as demonstrated by reduced CTL activity and transient IFNg production. Provision of IL-12 can overcome the restricted antigen priming and augment differentiation in terms of both CTL activity and IFNg production without altering the number of cell divisions. Moreover, the ability of IL-12 to alter CD8+ T cell commitment as late as 18h post antigenic stimulation, suggests that T cell differentiation is plastic.
and cytokine signals may integrate with antigen mediated commitment at later time points. In contrast to IL-12, IL-21 is able to promote CD8+ T cell early activation, proliferation and differentiation into CTL’s, but has no impact on CD8+ T cell IFNγ production. The information to be presented will delineate determinants that govern different phases of an antigen driven CD8+ T cell response. This understanding will enable rational approaches for harnessing CD8+ T cells to control cancer and/or infectious diseases.

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**IL-23 is important in IL-17 induction in Tuberculosis**

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Tuberculosis caused by *Mycobacterium tuberculosis* is the most prevalent infectious disease worldwide which kills more than 3 million people per year. IL-12p70 a heterodimeric cytokine made up of two disulphide linked subunits p35 and p40 has an important role to play in the generation of a protective response to tuberculosis by promoting IFN-γ production and thereby inducing a cell-mediated immune response. Absence of p40 subunit is more detrimental to the generation of protective immune responses than just the absence of p35 subunit in *M.tuberculosis* murine infection. Recently, a four alpha helix moiety, p19 which is structurally related to p35, which has no biological activity by itself but forms a disulfide-bonded heterodimer with p40 subunit termed IL-23 has been identified. This suggested that the absence of IL-12 p70 was not the only critical factor resulting in severe infection in the p40 -/- mice. In the present study, mice lacking the p19 subunit of IL-23 were infected via the aerosol route with virulent *M.tuberculosis H37Rv* and the progression of disease was monitored by enumeration of bacteria, histological analysis and assessment of antigen-specific responses. We found that the IL-23 p19 deficient mice are capable of forming granulomas, successfully induce a Th1 response and control infection. However, in the IL-23 p19 deficient mice there were lower levels of IL-17, a proinflammatory cytokine in the lungs of infected mice. We hypothesize that IL-23 has a direct role in the induction of IL-17 in host response to *M.tuberculosis* infection.

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**A New Model of Granuloma Formation: Towards Dissection of the Granulomatous Response to *Mycobacterium* spp.**

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Infections with virulent mycobacteria are characterized by a chronic granulomatous response, which serves to contain the spread of the pathogen but may also destroy infected tissues when the response is dysregulated. It is not clear which bacterial and host factors are involved in protective versus pathogenic aspects of the granulomatous response. To this end, we have developed a new model of granuloma formation to examine the role of mycobacterial factors. Cell wall lipids from *Mycobacterium bovis BCG* were delivered via polystyrene beads along with bone marrow-derived macrophages in an ECM gel into mice intraperitoneally. Input macrophages and recruited peritoneal neutrophils and macrophages internalized the lipids in the matrix and secreted inflammatory cytokines including TNFa, IL-1, IL-6, IL-10 and various chemokines. Histological sections showed an early influx of granulocytes.
followed by waves of monocytes, epithelioid macrophages at 4 days which were followed by lymphocytes and sequelae of chronic mycobacterial lesions at 12 days. FACS immunophenotyping confirmed the early recruitment of neutrophils and eosinophils and ensuing arrival of macrophages, dendritic cells, and lymphocytes (TCRβ⁺, TCRδ⁺ or CD49b⁺ NK cells). Matrices containing BCG lipids tended to adhere to fatty tissue. Adherent matrices were rapidly vascularized, and neutrophils and lymphocytes arrived via the blood resulting in matrices that were more cellular and fibrotic than free-floating ones. BCG lipid-containing matrices recruited T cells and NK cells that were capable of producing IFNγ and TNFα or IL-4. There was a higher prevalence of these T cells in the granuloma matrices than in the surrounding peritoneal exudate or lymphoid organs. Mycobacterial proteins were also delivered in the matrices to evaluate the ability to form immunological granulomas in BCG-sensitized mice. Delivery of recombinant ESAT-6 in the matrix elicited a delayed-type hypersensitivity (DTH) response that was characterized by enhanced recruitment of IFNγ-producing CD4 and CD8 T cells as well as NK cells after 48 hours, a time the DTH response to mycobacterial protein antigens typically peaks. This model demonstrates several relevant features of tuberculosis: (1) the adjuvant and granulomagenic properties of BCG lipids and (2) the DTH response to mycobacterial protein antigens, features which could be carefully dissected using this approach.

Evolution of *Mycoplasma pneumoniae* Infection in T-bet-deficient Asthmatic Mice

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Asthma and community-acquired pneumonia represent two of today’s most significant respiratory disorders. Epidemiological evidence suggests that asthmatic patients are more likely than the general population to become infected with *Mycoplasma pneumoniae*, a significant cause of acute respiratory illness that accounts for 20% of all cases of community-acquired pneumonia. This finding raises the intriguing possibility that asthma may predispose individuals to contracting pneumonia. Conversely, several clinical studies have implicated mycoplasmal infection in the exacerbation of asthmatic symptoms through mechanisms that are not fully understood. We have used a murine model of asthma and mycoplasmosis to explore the cellular and molecular underpinnings of this complex association. The severity and clinical course of *M. pulmonis* infection was assessed in wild type and T-bet⁻/⁻ BALB/c mice that spontaneously develop asthma. Following intranasal challenge with *M. pulmonis*, IL-5 levels in the lungs of T-bet⁻/⁻ mice were significantly higher than in wild type control lungs. IL-5 plays a critical role in the development of allergic respiratory pathology owing to its profound effects on eosinophil proliferation, maturation, and recruitment. Thus, the observation that lungs from infected T-bet⁻/⁻ mice were populated with many more eosinophils than those from controls correlated nicely with the observed disparity in IL-5 expression. The lungs of infected T-bet⁻/⁻ mice also contained higher levels of TNF-a, MCP-1, and IL-6, potent pro-inflammatory cytokines. These differences may be reflective of the higher mycoplasmal burden observed in the lungs of T-bet⁻/⁻ mice compared with controls. Collectively, these data suggest that asthma may indeed modulate the host response to *M. pulmonis* and that mycoplasmal infection, for its part, may reciprocate by altering the influx and/or distribution of eosinophils within the lung.
An Enhanced Immune Response to Particulate Antigen Targeted to Fc Gamma Receptor Type I. Ramifications for *F. tularensis* Vaccine Development

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Attenuated *Francisella tularensis, F. tularensis* LVS, has been used to vaccinate against *F. tularensis* infection. However, attenuated vaccines represent substantial risk to the patient. Vaccines composed of inactivated organisms, or purified *F. tularensis* components, would be preferable, provided they can stimulate humoral and cellular immunity. By targeting soluble antigen (Ag) to Fc gamma receptor Type I (Fc?RI) on Ag presenting cells (APC), humoral and cellular immunity can be enhanced. The goal of these studies is to utilize this strategy to enhance the antigenicity of inactivated *F. tularensis* organisms. Therefore, we wanted to know whether the above Fc?RI targeting strategy could be applied to particulate Ag. A novel Fc?RI-specific, biotin-binding, targeting molecule was used to target biotin-labeled particulate Ag (biotinylated tetanus toxoid C-fragment adsorbed to microspheres) to Fc?RI. ELISA, Flow Cytometry, and Ag presentation assays were used to evaluate targeting molecule function and T cell activation in response to Ag. When targeted to Fc?RI, the T cell response to particulate Ag was 10-100 fold greater than the response to non-targeted Ag. Furthermore, this enhancement was 100-1000 fold greater than the response to soluble Ag.

These studies demonstrate that targeting particulate Ag to Fc?RI can significantly enhance the Ag-specific T cell response *in vitro*. In addition, they suggest that Fc?RI targeting of inactivated *F. tularensis* organisms, or purified *F. tularensis* Ag attached to microparticulates, may provide a means of generating a more potent cellular and humoral immune response to *F. tularensis*, without the use of attenuated organisms. Supported by NIH grant # A146968
IL-12 Enhances Local Humoral Immunity to Influenza Virus

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Previous work in our laboratory showed that intranasal coadministration of an influenza vaccine and IL-12 protected mice against influenza challenge and this protection was antibody-mediated (1). Using a similar influenza model, we have now further investigated the induction of mucosal immunity in the lungs of immunized mice.

BALB/c wild type and IFN-α KO mice were immunized i.n. with inactivated influenza virus Type A/Taiwan/85 alone, or together with IL-12. The mice were boosted on days 14 and 28. Induction of specific antibody producing cells in the lung was tested 35 days after the initial immunization using ELISPOT method. In a separate experiment, lung lymphocyte subsets were enumerated after immunization by flow cytometry. To determine if i.n. administration of IL-12 and influenza vaccine induced formation of bronchus-associated lymphoid tissue (BALT) in the lungs, histology was performed on lung sections. Levels of IgA and IgG - positive cells in the lungs were also visualized using immunofluorescence.

It was found that IL-12 treatment increased the numbers of total, IgA, and IgG2a antibody-secreting cells (ASC) in both BALB/c wild type and IFN-α KO mice. Interestingly, IgA ASC in the lungs of IFN-α KO mice were more numerous compared to wild type mice, demonstrating that IgA production induced by IL-12 is IFN-α independent. In contrast, the production of IgG2a was IFN-α dependent. The addition of IL-12 also increased the percentage of activated B cells in the lung compared with mice that received vaccine alone, and unvaccinated mice. Coadministration of the influenza vaccine and IL-12 also resulted in the appearance of mucosal inductive sites in the form of BALT in the lungs of treated mice. Increased numbers of both IgG and IgA – positive B cells were detected by immunofluorescence at these sites.

Taken together, the results show that IL-12 acts locally in the lung to enhance mucosal antibody production to influenza virus. (Supported by NIH grants AI41715 and HL62120)

Connexin43 Expression in Cultured Cells of the Monocyte/Macrophage Lineage

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The expression of connexin43 (Cx43) was studied in the J774A.1 murine macrophage-like cell line. Cx43 expression was increased after exposure to bacterial Lipopolysaccharide (LPS). Elevated Cx43 expression was detected at protein and RNA levels by immunoblot and ribonuclease protection assays respectively. The protein had two detectable immunoreactive bands, 43 and 19 kD. The smaller band was present at greater intensity under non-stimulated conditions, but the 43 kD immunoreactive band increased to a greater extent upon LPS stimulation. Significant induction was observed beginning at 0.1 ng/ml and maximal induction was observed with 1 µg/ml. The increase in RNA expression was detected at 2 hour after LPS (1 µg/ml) treatment, peaking at 10 hours. Cx43 protein expression was elevated at 6 hours after LPS treatment and peaked at 24 hours. In order to determine the signaling pathways involved in LPS-mediated induction of CX43, specific inhibitors were used. As NF-κB is pivotal in the LPS-induced transcription of many genes, J774A.1 cells were treated with TPCK or TLCK, agents
known to block activation of NF-κB. Both agents prevented the LPS-induced increase in CX43 expression. Pre-treatment of J774 cells with the p38 inhibitor SB202190 (10µM) for 1 hour prior to LPS-stimulation significantly decreased both Cx43 protein and mRNA expression. In contrast, pre-treatment with the MEK1 inhibitor, PD98059 (20 µM), had no effect on Cx43 expression, while the JNK inhibitor, SP600125 (10µM), increased Cx43 expression. These results imply that the MAPK p38 pathway, as well as the NF-κB pathway have a role in CX43 expression.

**IgA Increases the Severity of Inflammation in a Murine Model of Allergic Lung Inflammation**

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IgA is the most abundantly produced immunoglobulin at mucosal surfaces, where it plays an integral role in defense against pathogens. While considered an important mediator of immunity at these sites, its involvement in non-microbial initiated mucosal pathologies is unknown. The goal of this study was to determine the importance of IgA in the pathogenesis of allergic asthma, a prominent disease of the respiratory mucosa. To investigate this, we employed a murine model of allergic lung inflammation and mice with a targeted disruption of the ?-heavy chain gene that renders them unable to produce IgA. Mice were sensitized to ovalbumin (OVA) in alum followed fourteen days later by five consecutive daily intranasal (IN) doses of OVA in saline. Disease was evaluated by the presence of an eosinophil-rich inflammatory infiltrate in lung tissue and BAL fluids, allergen specific antibody levels in serum and BAL fluids, expression of Th2 cytokines, and the development of airway hyperresponsiveness (AHR). The inflammatory response was significantly reduced in IgA−/− mice as compared with IgA+/+ controls (p<0.05). There were significantly fewer inflammatory cells (p<0.05) in both lung tissue and BAL fluids from IgA−/− mice compared to IgA+/+ mice, and eosinophils and lymphocytes represented the most affected populations. In addition, there was a significant reduction (p<0.05) in total and IgG1 OVA specific antibody levels in BAL fluids from IgA−/− mice, as well as reduced serum IgE levels. IL-4 and IL-5 levels were significantly reduced (p<0.05) in BAL fluids from IgA−/− mice compared to IgA+/+ mice early during the IN challenge phase, but were similar by the completion of challenge. Airway hyperresponsiveness results have thus far proven inconclusive and further experiments are currently under way. The data clearly demonstrate a role for IgA in the development of a non-microbial mediated inflammatory response in the respiratory mucosa. Our cytokine data, together with previous results from our laboratory which demonstrated a defect in APC function in IgA−/− mice, suggest a delay in the kinetics of allergic lung inflammation due to the lack of IgA, possibly through diminished antigen uptake and presentation resulting in decreased T cell activation early in the response. We are currently conducting experiments to further examine this possibility (Supported by NIH grants AI41715, AI49822, HL62120, and Philip Morris, Inc.).

**BCL11B Repression Function in T Lymphocytes**


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35
BCL11 proteins are highly conserved zinc finger transcriptional regulators with crucial role in lymphocyte development. Ablation of BCL11A in mice results in impaired fetal B and T lymphopoiesis, while deletion of BCL11B is restricted to T cell defects with a block at the CD4^-CD8^- double negative stage. Homozygous deletion and point mutations of BCL11B in adult mice is associated with T cell lymphomagenesis and overexpression of BCL11B suppresses cell growth in cellular systems. In humans BCL11A was found associated with the translocation t(2;14)(p13;q32.3) in several cases of B cell chronic lymphocytic leukemias. All these results suggest that BCL11 proteins play complex functions in lymphocytes. However, the cellular roles of BCL11 proteins remain largely unknown. To gain insight into the function of BCL11B in T lymphocytes we employed affinity purification and mass spectrometric analysis and identified several complexes associated with BCL11B, among which a major transcriptional repression complex, as well as components of the nucleosome. Results presented here prove the functional association of BCL11B with the repression complex in T lymphocytes.

Lymphoid Chemokines in Central Nervous System (CNS) Autoimmunity

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the CNS that is mediated by CD4^+ T cells specific for myelin proteins. It is widely used as an animal model of multiple sclerosis (MS). In chronic stages of EAE the cellular infiltrate develops features of “organized” lymphoid tissue. We postulated that this process is driven by the expression of lymphoid chemokines, such as BLC, ELC and SLC, in the CNS. Indeed, we detected BLC in spinal cords of mice with acute EAE but not in naïve or mock-immunized controls. While ELC and SLC were present at low levels in cords from naïve mice, they were up-regulated during EAE. The expression of all lymphoid chemokines rose during clinical progression and relapse. Hematopoetic cells might be the source of ELC and BLC in the inflamed cords since CNS-infiltrating mononuclear cells showed expression of those lymphoid chemokines on the mRNA level. Our findings suggest that lymphoid chemokines might provide a novel target for the treatment of chronic autoimmune diseases such as MS.

MHC Class I Molecules Decrease the Ability of Natural Killer Cell to Mediate Antibody Dependent Cell-mediated Cytotoxicity of HIV-infected Primary T-cells

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Antibody controls HIV by neutralization, inducing comple ment mediated destruction of infected cells and triggering antibody dependent cell-mediated cytotoxicity (ADCC). We demonstrate that antibodies directed to HIV envelope protein gp120 triggers natural killer (NK) cells to kill autologous primary CD4^+ cells infected with HIV. However, the level of killing of the antibody-coated HIV-infected cells by NK cells was significantly less than killing of an ADCC susceptible cell line. HIV selectively decreases major histocompatibility complex (MHC) class I molecules HLA-A and HLA-B on the infected cells but leaves HLA-C and HLA-E on the surface. Since HLA-C and E may trigger inhibitory receptors on NK cells we wanted to determine whether HLA-C and -E specific inhibitory receptors on
NK cells may prevent the NK cells from mediating ADCC of HIV-infected cells. When we block the interaction between NK cell inhibitory receptors (i.e., NKG2A, CD158a and CD158b) and HLA-C and HLA-E we observed a significant increase in killing of anti-gp120-coated HIV-infected cells by NK cells. Thus, HLA-C and E molecules on infected cells may help facilitate evasion of NK cell mediated destruction of antibody-coated HIV-infected cells.

**IL-10 Controls Inflammation at the Surface of the Diaphragm and in the Pleural Cavity During Muscle Infection with Trichinella spiralis**

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Adult Trichinella spiralis reside in the intestinal epithelium where they release first-stage, or newborn larvae (NBL). NBL enter circulation and, in rodents, a high concentration of the parasite establish in the diaphragm. Given the predilection of T. spiralis for this tissue and the established role of the diaphragm in lymphatic drainage, we sought to characterize the diaphragm-associated and body cavity responses to muscle infection, and to evaluate the influence of IL-10 on these responses. C57BL/6 (WT) and B6.129P2-IL10tm1Cgn (IL-10 -/-) mice were synchronously infected with 15,000-25,000 NBL, administered intravenously. At 0, 5, 10, 20 or 100 days post-infection (dpi), diaphragms and body cavity exudates were recovered. Using standard histochemical, immunohistochemical, and flow cytometric techniques, we found that muscle infection induces an inflammatory response at the surface of the diaphragm as well as in the pleural cavity. In the absence of IL-10, cellular infiltration of the diaphragm was more pronounced, resulting in a 4-fold increase in cells recovered from diaphragms of IL-10 -/- mice compared to WT. Similarly, mice lacking IL-10 expanded the pleural B and T cell populations 6 fold and 9 fold, respectively. In addition, IL-10 controls the expansion of B-2 cells (11-fold) and B-1 cells (4-fold) in the pleural cavity following infection. In the peritoneal cavity, B-2 cells expand only 3-fold in the absence of IL-10, while the B-1 cell population is diminished following infection. Oral infection elicited both a pleural and a peritoneal response. Taken together, these results document a role for IL-10 in controlling a lymphocytic response at the surface of the diaphragm and in the body cavities during muscle infection with T. spiralis.

**Decreased Expression of iNOS in SHP1 Deficient Mice May Contribute to Viral Spread in CNS Glia**

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We have observed a severe defect in the ability of mice lacking SHP1, a tyrosine phosphatase, to mount an effective anti-viral response to Theiler’s murine encephalomyelitis virus (TMEV). These mutant “moth-eaten” mice exhibit greater viral replication in the brain and spinal cord and extensive demyelination following intracerebral inoculation, as compared to normal littermate controls. Using dsRNA exposure of whole glial cultures to mimic infection, we have found that SHP1 deficient cultures showed significantly greater inducible nitric oxide synthase (iNOS) transcripts compared to normal
littermates; however, the amount of protein expressed was significantly less. This decreased expression was also evident in immunohistochemical stains. Furthermore, the activity of NOS in moth-eaten cultures was significantly less. The production of nitric oxide by synthases during a viral infection has been linked to reductions in replication and spread, thereby representing a critical step in the innate immune response. The evidence presented indicates that a lack of iNOS expression in moth-eaten mice may account for the observed increase in TMEV replication.

An alternative, yet related, explanation for increased replication in moth-eaten mice involves the p38 MAP kinase pathway. We have found that TMEV replication is significantly greater in IL1 treated wildtype cultures. The IL1 pathway is activated during CNS viral infections. Interestingly, it has been shown that activation of particular IL1 signaling pathways can promote the replication of an RNA virus. We are currently exploring the possibility that increased p38 MAP kinase activity, in addition to the defect in iNOS expression, in SHP-1 deficient mice is contributing to the spread of the virus in these mice.

The Selective Modulation of MHC Class I Molecules by HIV Protects CD4+ T-cells From Destruction by Natural Killer Cells

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In our current study, we demonstrate that the expression of MHC class I molecules, HLA-A and HLA-B, are decreased on the surface of HIV-infected primary CD4+ T-cells, while the levels of HLA-C and HLA-E remain constant. Moreover, we have observed that HIV induces the expression of HLA-G molecules, which also can decrease NK cell responses. We hypothesize that HIV manipulates MHC class I molecules not only to protect the HIV-infected cells from CTL mediated destruction, but also to protect from NK cell responses. We wanted to determine whether the presence of HLA-C, E and G in the absence of HLA-A and B would account for the inability of NK cells to kill HIV-infected cells. To evaluate this objective, primary CD4+ T-cells were infected in vitro with HIV-1. Autologous NK cells were isolated, and depleted of cells expressing NK cell inhibitory receptors for HLA-C and HLA-E. We demonstrated that NK cells lacking inhibitory receptors for HLA-C and HLA-E had little if any ability to destroy HIV-infected primary T-cells. When the interaction of HLA-G on the surface of HIV-infected primary T-cells with inhibitory receptors on NK cells was blocked, a significant increase in killing was observed over controls. Taken together, these studies indicate that despite the selective surface reduction of HLA-A and HLA-B by HIV-1, NK cells are prevented from killing HIV-infected cells in part, because of HLA-G. Studies to further investigate the role of HLA-C, -E and -G on NK cell responses will be undertaken.

The Role of IFN-g in CD4 Effector Cell Trafficking and Protection in Response to Influenza Infection

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Interferon-gamma (IFN-g) is an important component of the anti-viral response; however, the contribution of CD4 derived IFN-g in the response to influenza remains poorly characterized. Transfer
of CD4 T cell effectors isolated from draining lymph nodes (DLN) or lung of sublethally infected BALB/c mice can confer protection to a subsequent lethal influenza infection. In contrast, transfer of CD4 effectors from the same organs of sublethally infected IFN-g−/− mice do not promote survival after transfer and subsequent lethal infection, suggesting that IFN-g is required for CD4 mediated protection. To further investigate the role of IFN-g in protection against lethal influenza infection, TCR transgenic mice, recognizing the peptide HA126-138 from influenza hemagglutinin, were used. CD4 T cell effectors from TCR transgenic wildtype (WT) or TCR Tg IFN-g−/− mice were generated in vitro in the presence of Th1 polarizing conditions. Both WT and IFN-g−/− effectors localized to the lung upon intravenous adoptive transfer, even in the absence of inflammation. Upon infection with a lethal dose of influenza, the numbers of WT and g−/− effectors in the lung peaked by day 2 post infection (p.i.) and declined rapidly thereafter. Concomitant with the decline of CD4 effectors in the lung was an influx of host CD8 cells to the lung and airways by day 6 p.i. However, there was no difference in the ability of WT and IFN-g−/− effectors to traffic to the lung or to recruit host cell populations early in the response to lethal influenza infection. The ability of WT effectors to be directly cytolytic or to suppress viral replication may be the mechanism by which these cells confer protection while CD4 effectors deficient in IFN-g production do not. Supported by PHS grants T32-AI49823 (DMB) and PO1-HL63925.

Granulocytes Are Actively Recruited to the Spinal Cord during EAE

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) that is mediated by CD4+ Th1 cells. It is widely used as a mouse model of multiple sclerosis (MS). Although past studies have concentrated on the role of lymphocytes and macrophages in EAE, recent data suggests that granulocytes also infiltrate MS lesions and release products that increase blood-brain-barrier permeability. These observations lead us to postulate that ELR+ CXC chemokines, potent chemo-attractants for granulocytes, might be upregulated in the CNS during early phases of autoimmune demyelination and promote leukocyte trafficking to the CNS. Histology revealed that neutrophils are present in the inflamed spinal cords of C57BL/6 mice sensitized against an immunodominant peptide of myelin oligodendrocyte glycoprotein. Flow cytometry confirmed that between 17% and 28% of spinal cord mononuclear cells bore the neutrophil specific markers 7/4 and Ly6G. Neutrophils were even more prominent in the CNS of symptomatic C57BL/6 IFN? deficient mice, which experience a hyper-acute form of EAE. Furthermore, in all instances granulocyte infiltration was associated with CNS upregulation of the ELR+ CXC chemokines KC and MIP-2, and their receptor CXCR2, as measured by RNase protection assays. Our data suggests that granulocytes are actively recruited to the site of CNS inflammation during EAE, and may have an important role in perpetuating the inflammation and/or directly causing damage to myelin and axons. Agents that block ELR+ CXC chemokines and/or antagonize the CXCR2 receptor might be useful for the treatment of CNS autoimmune diseases such as MS.

MALT1/Paracaspase is a Signaling Component Downstream of CARMA1 and Mediates T-cell Receptor-induced NF-κB Activation

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T cell receptor (TCR) induces a series of signaling cascades and leads to activation of multiple transcription factors including NF-κB. Although the mechanism of TCR-induced NF-κB activation is not fully understood, recent studies indicate that Bcl10 and CARMA1, two adaptor/scaffold proteins, play essential roles in mediating TCR-induced NF-κB activation. MALT1/paracaspase is a caspase-like protein that contains an N-terminal death domain and two immunoglobulin-like (Ig) domains, and a C-terminal caspase-like domain. It binds to Bcl10 through its Ig domains and cooperates with Bcl10 to activate NF-κB. Therefore, it has been hypothesized that MALT1 may be involved in mediating TCR signal transduction. In this study, we show that MALT1 is recruited into the lipid rafts of the immunological synapse following activation of the TCR and the CD28 co-receptor (CD3/CD28 costimulation). This recruitment of MALT1 is dependent on CARMA1, since CD3/CD28 costimulation failed to recruit MALT1 into lipid rafts in CARMA1-deficient T cells. In addition, we also found that MALT1 not only binds to Bcl10 directly but also associates with CARMA1 in a Bcl10-independent manner. Therefore, MALT1, Bcl10, and CARMA1 form a tri-molecular complex. Finally, expression of a MALT1 deletion mutant that contains only the N-terminal death domain and the two Ig domains completely blocked CD3/CD28 costimulation-NF-κB, but not TNF-α-induced NF-κB activation. Together, these results indicate that MALT1 is a crucial signaling component involved in TCR signal transduction pathway.

Differential Antigen Presentation Regulates the Changing Patterns of CD8+ T-Cell Immunodominance in Primary and Secondary Influenza Virus Infections

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Previous studies have shown that CD8+ T cells specific for the NP366-374/D^b (NP) and PA224-233/D^b (PA) epitopes of influenza are present in equivalent numbers following primary infection of C57BL/6 mice. During the secondary response, however, T cells specific for the NP epitope are dominant. Using antigen-specific T cell hybridomas, we demonstrate that these epitopes are differentially expressed following in vitro infection with influenza virus. Whereas both dendritic and non-dendritic cells express the NP epitope, the PA epitope is exclusively expressed by dendritic cells. This pattern of epitope expression was also found on cells isolated from influenza virus infected mice. Additionally, we show that naive and memory T cells differ in their capacity to detect antigen on different subsets of antigen presenting cells. Together these studies suggest that differential antigen presentation regulates the changing patterns of CD8+ T cell immunodominance in primary and secondary influenza virus infections. Using PA-pulsed dendritic cells as vaccines, we have increased the number of activated PA-specific T cells in the lungs but there is delayed viral clearance, which has important implications for vaccine development.
Investigating the function of llama heavy-chain antibodies

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Llama heavy-chain antibodies (HCAb) are homodimers of gamma chains that are devoid of light chains. The contribution of these antibodies to immunity is unknown. We set out to make IgG isotype specific monoclonal antibodies (mAbs) in order to investigate the role of HCAb during infection. Seventeen mAbs specific for llama IgG1, IgG2 and IgG3 were produced. We selected six mAbs that showed greatest specificity for their respective llama IgG under experimental conditions. We have applied these reagents in experiments to dissect the IgG response to a parasitic nematode infection in llamas. \textit{Parelaphostrongylus tenuis} (\textit{P. tenuis}), a parasite that parasitizes white-tailed deer, induces an incapacitating neurologic disease in atypical hosts such as llamas. We have documented the kinetics of the total IgG response to \textit{P. tenuis} infection in llamas. By using the mAbs to dissect the IgG response we intend to characterize the Th2 response induced by a parasitic nematode infection in llamas and elucidate the role of HCAb in this type of infection.

The Effects of IL-12 on Adhesion Molecule Expression in EAE

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) that is widely used as an animal model of multiple sclerosis (MS). Myelin-reactive CD4\textsuperscript{+} T cells from immunized donors transfer the disease to naive syngeneic recipients. We and other investigators have previously shown that IL-12p40 monokines play a critical role during the differentiation of EAE-inducing CD4\textsuperscript{+} T cells. However, the mechanism by which IL-12 promotes encephalitogenicity remains to be elucidated. Here we show that IL-12 directly upregulates the expression of the adhesion molecule, P-selectin glycoprotein ligand (PSGL1), on myelin-reactive T cells in association with the acquisition of encephalitogenic properties. IL-12 mediated PSGL1 upregulation follows induction of Fucosyl Transferase VII, an enzyme critical for the function of P-selectin ligand. Cerebrovascular endothelium showed transient expression of P-selectin, the receptor for PSGL1, during acute EAE. Moreover, preliminary results indicate that pre-incubation of IL-12 stimulated myelin-reactive CD4\textsuperscript{+} T cells with a blocking antibody against PSGL-1 prevents the induction of EAE. Collectively our data suggest that IL-12 driven PSGL1 expression is an important step in EAE pathogenesis and that blockade of P-selectin ligand-P-selectin interactions might be ameliorative in autoimmune demyelinating disorders such as MS.


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The involvement of β1 and β2 adrenergic receptors (ADRs) in host resistance to *Listeria monocytogenes* using mice lacking either or both ADR subtypes is being investigated. While an immunologic role for the β2 ADR is described in CD4+ Th1 cell differentiation, the role of the β1 ADR in immune regulation is uncertain. Our results thus far suggest distinct differences between experimental groups subjected to bacterial infection with regards to bacterial burden and Th2 cytokine production. Currently, 50.0%, 33.3% and 16.7% of β1−/−, β2−/− and β1β2−/− mice, respectively, have died by day 3 following a sublethal *L. monocytogenes* infection. No wild type (FVB) mice perished under the bacterial inoculums administered (5.5-6.7 x 10^3/IV injection). Highest splenic bacterial burdens (1.2 x 10^9 CFU/organ) and IL-6 production in both liver and spleen (628.8 pg/mg protein and 3295 pg/mg protein, respectively) were observed in β1-deficient mice, reflecting impaired host resistance to bacterial infection in these mice. Previous studies in our lab using ADR subtype blocking and stimulating agents identified the β1 ADR as a neurotransmitter signaling receptor that inhibits host resistance to infection under acute cold/restraint stress (ACRS). Therefore, studies are ongoing to assess the effects of stress on β1 and β2 and β1/β2 ADR deficient mice. Results of these combined studies may further our understanding of a central role for ADRs in the neuroimmune circuitry inherent to the regulation of host immunity.

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**Can Azithromycin Modulate Murine Immune Responses to a Pneumococcal Vaccine and Infection Model?**

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**Background:** Antibiotics are widely prescribed in clinical practice for the management of bacterial infections, including respiratory tract infections. Over the last decade an increasing interest has developed on their effect on the immune system independently from their antimicrobial properties. We have begun to study the effect of a macrolide antibiotic (azithromycin) on murine immune responses.

**Objective:** To characterize the effect of azithromycin on the murine immune system.

**Design/Methods:** Adult BALB/c mice were given one dose of Azithromycin (50mg/kg) into their peritoneum or phosphate-buffered saline as control. One hour later all mice received one intramuscular injection of a heptavalent protein-conjugated pneumococcal vaccine (Prevnar®) containing 0.2µg of protein-conjugated pneumococcal polysaccharide type 14. Mice were bled seven days later to determine the primary antibody response. The following day the mice were inoculated with *Pneumococcus* type 14. Three days after inoculation, mice were bled again, and euthanized. Nasal washings were done, and organs were harvested including the nasal associated lymphoid tissue (NALT). Nasal fluid was plated overnight for bacterial growth. We performed a super array gene analysis in RNA derived from NALT to evaluate for possible differences for several cytokines/chemokines. The student’s t-test was used for statistical analysis.

**Results:** The group of mice that received azithromycin had a decreased total antibody response when compared to the control group (mean antibody titer of 52.33±52.8 vs. 317±123, p<0.01). Similar results were obtained for IgG1 levels but not for IgG2a. The nasal washings from animals treated with azithromycin grew a higher number of colony forming units (cfu) than the ones obtained from the control mice (1165±961.7 vs. 152.5±141.5, p<0.01). Expression of several cytokines such as macrophage inflammatory protein (MIP-1a), and B lymphocyte chemo attractant (BLC/BCA-1) was markedly reduced in the group of mice that received azithromycin.

**Conclusions:** Azithromycin exerts a modulatory effect on the immune system by decreasing the humoral response in a murine model, and the clearance of *Pneumococcus* type 14 from murine nasal
cavities. It also affects the expression for certain cytokines and chemokines. More studies are needed to determine the molecular bases of the above findings, and how to apply that knowledge for the benefit of patients.

A Role for Interleukin 16 in Cryptococcal Lung Infection

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Cryptococcus neoformans is a fungus that causes mortality in immunocompromised people. In murine cryptococcosis, pulmonary clearance requires concerted action of CD4+ and CD8+ T cells. The mechanism by which CD8+ T cells fight cryptococcal pneumonia is poorly characterized. In this study, at 4 days of infection, CD4+/CD8+ cell ratios in the airway of infected mice were lower than ratios observed in spleens. Subsequently, CD4+/CD8+ cell ratios in airways were about twice those observed in spleens, suggesting that initially, CD8+ T cells are preferentially recruited/retained in the airways. CD8+ T cell-deficient mice had 10-fold fewer CD4+ T cells in airways at 7 days, suggesting that CD8+ T cells are implicated in attraction of CD4+ cells. Interleukin 16 is secreted by airway epithelia, eosinophils, and lymphocytes. In CD8+ T cells only, bioactive IL16 is constitutively secreted. IL16 is a potent chemoattractant for CD4+ T cells. CD8+ T cell-deficient mice given exogenous IL16 had CD4+ T cell numbers comparable to those of CD8+ T cell-replete controls. CD8+ T cell-replete mice given anti-IL16 had significantly fewer CD4+ T cells in airways at days 4 and 8 of infection than did mice given control antibody; by day 12 of infection, there was no difference between groups. Finally, anti-IL16-treated mice more fungus in airways at days 4, 8 and 12 of infection than did control antibody-treated mice. These data suggest that one role of CD8+ T cells is secretion of chemoattractant IL16 which enhances influx of CD4+ T cells in the airways of C. neoformans-infected mice.

Immunobiology of the Prostate; Identification of a Previously Undescribed Lymph Node

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Prostate cancer and prostatitis are two pathologies of the prostate in which the immune system may play a key role. In prostate cancer the failure of the immune system to recognize and destroy malignant cells is one factor that allows the cancer to progress. In contrast, prostatitis results in an excessive immune response leading to immune destruction of healthy tissue. Hence, understanding the immunobiology of the prostate could assist in developing treatments for prostate associated diseases, however little is known about this aspect of the prostate. We sought to better characterize the immune system within the prostate using an imaging technique, which allows detailed visualization of key immunocomponents. Using this technique, we discovered a previously undescribed lymphoid structure in the base of the murine ventral prostate. Further analysis revealed this structure to possess lymph node architecture, and an organized arrangement of lymphocytes usually associated with peripheral lymph nodes. Vascular analysis revealed a complex network of capillary vessels and the presence of lymph node specific high endothelial venules (HEV), as well as a rich network of lymph vessels associated with the prostate lymph node. We concluded that this lymphoid structure possessed essential components usually associated with lymph nodes. Therefore, the presence of this lymph node in the prostate could have implications for the cause of prostatitis and potentially could be targeted for immunotherapy against
prostate cancer. Also, the key components of this node, such as lymphatics and specialized endothelium, could be exploited to prevent the spread of metastasis.

\textit{Mycobacterium bovis} BCG Lipids Induce Proinflammatory Monokines and Promote Granulomagenic Cell Recruitment

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The hallmark of mycobacterium-induced pathology is granulomatous inflammation at the sites of infection. The tuberculoid granuloma is a fascinating example of host-pathogen balance, limiting the dissemination of the pathogen, but allowing persistence of infection. Mycobacterial lipids are potent immunomodulators that contribute to the granulomatous response. We have investigated the granulomagenic nature of cell wall lipids of \textit{Mycobacterium bovis} Bacille-Calmette Geurin (BCG) using a novel model. In this model, BCG-lipids were coated onto polystyrene microspheres, and suspended with bone marrow-derived macrophages in an extracellular matrix gel that was injected intraperitoneally into mice. An experiment was conducted to determine the effect of prior sensitization to heat-killed BCG on response to BCG-lipid, or control phosphatidyl glycerol (PG) granulomas. Cohorts of animals were sacrificed, and the matrix and peritoneal exudative cells (PECs) analyzed at 14 hours, 4 days, 7 days and 12 days. When compared to PG controls, BCG-lipids elicited enhanced cell recruitment of neutrophils, macrophages, eosinophils and lymphocytes. Cytokine secretion from the matrices was assessed by ELISA. BCG-lipids induced higher levels of the proinflammatory monokines; TNF?, IL-1?, IL-1?, and IL-6 than PG. BCG-lipids also induced higher levels of IL-10 and reactive nitrogen intermediates at 14 hours, compared to PG controls. There was no significant difference between BCG-lipids and PG for induction of IL-2, IL-4, or IFN?, irrespective of sensitization of the host to BCG. Interestingly, cells from BCG-lipid bearing matrices were a better source of cytokines than cells in the surrounding peritoneal exudate. In summary, prior sensitization to heat-killed BCG made no appreciable difference in the recruitment of leukocytes or secretion of cytokines in BCG-lipid induced granulomas. This suggests that the granulomagenic nature of BCG-lipids elicits primarily innate responses.

Inducing and Recalling Immune Memory to AgI/II by Mucosal Immunization

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Intragastric (i.g.) immunization with the recombinant chimeric immunogen, SBR-CTA2/B, which consists of the SBR segment of \textit{Streptococcus mutans} AgI/II coupled to cholera toxin (CT) A2/B subunit, induces AgI/II-specific salivary IgA and serum IgG antibodies in mice. To evaluate the ability of SBR-CTA2/B to recall responses after i.g. or intranasal (i.n.) immunization, female BALB/c mice (N = 4-6/group) were immunized with either 100µg (i.g.) or 25µg (i.n.) of SBR-CTA2/B, 3 times at 10-day intervals and rested for 6 months. One, two, or three booster immunizations with SBR-CTA2/B were then administered by the same route, dose, and interval; control mice were not boosted. Saliva and serum were collected 7 days after each boost, and specific antibodies were determined by ELISA.
Proportions of memory and activated T and B cells in lymph nodes and spleen of animals sacrificed on day 10 after each boost were evaluated by two-color immunofluorescent staining and flow cytometry. Agl/II- and CT-specific antibody levels detected in serum (IgG) and saliva (IgA) rose with the number of booster immunizations, and higher levels were recorded from mice immunized i.n. Greater proportions of memory (CD45RB-low) T cells and activated (CD25+/CD69+) B and T cells were also found in lymph nodes of mice primed and boosted i.n. Thus an i.n priming and boosting regime was found preferable for recalling and activating immune memory cells that are involved in generating salivary IgA antibodies for protection against the oral cariogenic bacterium, *S. mutans*. Supported by NIH grant DE06746.

**Effects of Ethanol on CD4 T-Cell Function**

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It has been shown that alcoholics have increased frequency of certain types of infection and several forms of cancer. This implies that ethanol suppresses the immune system. We employed a model using TCR transgenic T cells (specific for chicken OVA323-339) to determine the effect of ethanol on antigen specific T cell function. In vivo studies are being done to determine the direct effect of ethanol on T cell function. In vivo studies will also be done to determine the effect of ethanol on T cells at an organismal level. We have found that 24 hour pre-treatment with ethanol (1.6-2.0%) inhibits the proliferative response of CD4+ T cells. This has been shown to be due to an effect on T cells and not on APC. At the levels of ethanol where proliferation is inhibited, T cells are still viable. Interestingly, ethanol need not be present in the cultures during stimulation with antigen. This suggests that pre-exposure to ethanol may induce a state analogous to tolerance or anergy in T cells. We have also found that ethanol depletes cellular cholesterol levels and that allowing the T cells to rest for 2.5 hours prior to antigen addition restore proliferative ability. The mechanisms by which ethanol disrupts T cell function are unknown. However, a prerequisite to activation is the organization of TCR-associated signaling molecules in the immunological synapse. Signaling molecule organization is facilitated by cholesterol-rich lipid raft domains, and could be disrupted by alterations in membrane fluidity. Since exposure to ethanol depletes choleterol in CD4 T cells, we plan to determine if ethanol treatment impairs lipid raft organization and subsequent TCR signaling.

**The Potential Role of Nef in Inducing Cytokine Expression in HIV Infected CD4+ T Cells**

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Prior to CD4+ T cell depletion, T cell dysfunction may play a profound role in the pathogenesis of HIV infection. It has been proposed that HIV alters the cytokine expression of infected CD4+ T-cells, specifically causing a shift from a T\_H1 cytokine secretion pattern to a T\_H2 cytokine pattern. To investigate this potential shift in cytokine expression, the intracellular expression of IFN-?, IL-2, IL-4, and IL-10 was measured in HIV infected (HIV p24 antigen positive), primary CD4+ T cells following
activation. An overall increase in cytokine expression was observed in the infected cell population as compared to the uninfected cell control, regardless of TH1 or TH2 type. This data suggests that cytokine dysregulation does occur during HIV infection, but it does not specifically lead to a shift in the type of cytokines produced. Nef has recently been described as an enhancer of host cell gene expression. We, therefore, evaluated the potential role of Nef as an inducer of cytokine production in the infected cell with the utilization of an HIV deletion construct that is defective in Nef production. A decrease in overall cytokine production was observed with the deletion construct as compared to the wild type virus, indicating a potential role of Nef as an inducer of cytokine production following infection by HIV.

B-cells are Required for Optimal Resistance to the Gastrointestinal Nematode Heligmosomoides polygyrus

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It is estimated that a billion people worldwide are infected with parasitic nematodes and a million people, mainly in developing countries, die from these infections annually. Acquired resistance to the gastrointestinal nematode Heligmosomoides polygyrus is thought to be largely dependent on the presence of IL-4-secreting CD4+ T cells. The development of optimal immunity to the closely related nematode Trichuris muris requires B cells, however, the role of B cells (and their products) has not been extensively evaluated in H.polygyrus infection. We found that B cell-deficient chimeric mice were significantly impaired in their ability to clear a secondary worm infection whereas worm expulsion in control, B cell-sufficient chimeric mice was efficient. To assess whether CD4 T cell function was impaired in mice lacking B cells, mice were infected and cytokine production following in vitro restimulation with either anti-CD3 or H.polygyrus antigen was analyzed. CD4 T cells from B cell-deficient mice produced significantly lower amounts of type-2 cytokines (IL-4, IL-5, IL-13), whereas T cells from both groups made similar amounts of type-1 cytokines (IL-2 and IFN?). Thus, the inability of B cell-deficient mice to clear worms efficiently may in part be explained by defective CD4 T cell function and reduced production of type-2 cytokines. In addition, B cells may contribute to immunity by production of cytokines and antibody. We will also present results of experiments which utilize chimeric mice to evaluate the role of B cell-derived cytokines (IL-4 and lymphotoxin a) and antibody (class II-deficient B cells) in the development of immunity to H.polygyrus infection.

Diminished CD8+ T cell Repertoire and Response in Aged Mice

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Aged individuals suffer from infectious disease with increased frequency and severity. In particular, influenza viruses represent a major cause of illness among elderly individuals. Given that the protective efficacy of vaccination is greatly reduced in the elderly, it is critical that effective vaccination strategies for the elderly be developed for not only protection from natural yearly influenza infection, but also epidemics. An age-related defect in the activation of naïve CD4+ T-cells as well a diminished primary CD8+ T-cell response to influenza virus infection has been described, although there is less evidence of a functional defect in the aged CD8+ T-cells. It has been suggested that the defective cellular immune
response in the aged is in part due to a loss of diversity in the CD8+ T-cell repertoire. We have compared the overall and functional diversity of the naïve CD8+ repertoire in young and aged mice via DNA spectratype analysis. The data suggests reduced diversity among the aged naïve CD8 population. Currently we are examining the diversity of the CD8+ cellular immune response of aged mice as compared to young mice in response to a primary influenza virus. Together, these studies will determine whether an age associated loss of naïve T-cell diversity affects immune function in the elderly. Given that some vaccination strategies rely on defined viral proteins or epitopes as immunogens and a significantly reduced aged repertoire diversity may result in 'holes' in the T-cell repertoire, these vaccination strategies may prove ineffective in an elderly population requiring alternate approaches.

Repopulation of the Antigen-specific Memory CD8+ T-cell Pool Following Re-exposure to Antigen

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Upon antigenic stimulation, naïve CD8+ T cells become activated effector cells. This population of effector cells later decreases in size and remains as a small pool of resting memory cells, capable of a rapid response on re-exposure to the same antigen. However, new naïve cells undergo an effector response during re-exposure, resulting in new memory cells. The number of circulating memory CD8+ T cells remains constant; therefore, a mechanism for homeostatic control of antigen-specific memory cells must exist. To investigate regulation of memory cells, we utilized two genetically marked strains of mice, both with a transgenic TCR that recognizes the peptide 257-264 of the OVA protein in association with MHC Class I. Naïve transgenic CD8+ T cells from the first donor were transferred with peptide–loaded bone marrow–derived dendritic cells (bmDCs) into syngeneic mice. These T cells expanded, acquired an effector phenotype, then became quiescent memory cells. The same mice then received a transfer of peptide–loaded bmDCs and naïve transgenic CD8+ T cells from the second donor. These cells also become effector, and then memory, cells. The composition of the memory pool in these mice was analyzed following a third challenge with antigen. The recall memory population was composed almost entirely of memory cells generated from the second response, whereas memory cells generated from the first response had been eliminated. We propose that memory cells generated in a subsequent exposure to the same antigen delete existing antigen-specific memory cells, at least under the conditions of these experiments. This may be a mechanism for homeostatic control of memory cell populations.

Immune Responses to Human Papillomavirus Antigens in HLA Class II Transgenic Mice

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Following a viral infection there are multiple factors that influence the immune response elicited to the viral proteins. Some viruses evade the immune system and cause chronic infection and this may
represent a risk factor for the host to develop cancer. A previous study has shown that women who are HLA-DRB1*0401 have an increased susceptibility to develop cervical cancer following infection with high-risk HPV types. The role of HLA and the immune response to HPV could be crucial in clearing the virus before it transforms the host cells. We are utilizing an HLA-DRB1*0401 transgenic mouse model to study the HLA-DRB1*0401 restricted immune response to HPV oncogenic protein E7.

Our hypothesis is that HPV infected HLA-DRB1*0401 patients are more susceptible to developing cervical cancer following HPV infection because they are unable to mount an effective immune response to viral antigens. We are studying the immune response to the protein E7, and overlapping peptides derived from its sequence, in HLA-DRB1*0401 transgenic mice in order to identify potentially immunogenic peptides to be tested in a tumor model.

Our data indicate a decrease in lymphocyte proliferation to E7 in the DR4 transgenic mice following immunization when compared to non-transgenic murine strains C57BL/6, BALB/c, 129J, and SJL. The DR4 transgenic mice make IL-10 and low levels of IFN-g in response to secondary in vitro stimulation with HPV protein E7 as determined by cytokine ELISA.

HLA transgenic mice provide us a unique opportunity to understand the immune response to HPV-16 protein E7 and to identify potentially immunogenic peptides for future vaccine studies.

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**Immunomodulating Gene Fusion Protein CTA1-DD Enhances Both Systemic and Mucosal Immune Response**

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The toxic activity of mucosal adjuvants often conflict with issue of host safety and tolerability. Mucosally active vaccine adjuvants that will prime a full range of local and systemic immune responses against defined antigenic epitopes are much needed. One of the best studied and most effective mucosal adjuvant described is cholera toxin (CT) but the extremely toxic effect of CT precludes its clinical use in vaccination protocol.

Our objective was to evaluate the use of a recently developed novel immunomodulating gene fusion protein, CTA1-DD, that combines the ADP-ribosylating ability of cholera toxin (CT) with a dimer of an Ig-binding fragment, D, of *Staphylococcus aureus* protein A. The CTA1-DD adjuvant was found to be non-toxic and greatly augmented T-cell dependent and independent responses.

We evaluated the ability of CTA1-DD to augment the immune response to the outer membrane protein P6 of non-typeable *Haemophilus influenzae* (NTHI), when adjuvant and antigen were delivered intranasally. In addition, we tested the adjuvant at several concentrations to determine the dose that gave an optimal immune response.

We evaluated both the systemic and mucosal components of the immune response. We measured total antibody titer, the duration of the response and antigen specific immunoglobulin subclass distribution. The presence of high levels of sIgA indicated that the mucosal immune system had been activated, which is an important component for protection against a respiratory pathogen such as non-typeable *Haemophilus influenzae* (NTHI).

Our poster will discuss the significance of our findings.
Crystal Structure of Superantigen *Mycoplasma-arthritidis* Mitogen (MAM) Complexed with Peptide/Major Histocompatibility Complex

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Mycoplasma arthritidis-derived mitogen (MAM) is a soluble bacterial protein that functions like a superantigen (SAG), which binds to major histocompatibility complex (MHC) class II molecules and stimulates the activation of a large fraction of T cells by recognizing specific T cell receptor (TCR) Vß subfamilies. Here we present the crystal structure, at 2.5 Å resolution, of MAM complexed with a human MHC class II molecule, HLA DR1, loaded with a haemagglutinin peptide (HA) of influenza virus. This complex structure reveals a novel fold for MAM composed of two a-helical domains, forming a L-shaped molecule. The N-terminal domain of MAM is a four-helical bundle with a long N-terminal loop of 25 residues wrapping around. In C-terminal domain, one central helix is surrounded by five others. This fold is entirely different from that of the pyrogenic superantigens, consisting of a ß-grasped motif and a ß-barrel. The DR1/HLA/MAM complex structure shows that the N-terminal of MAM mainly binds to the DR1 a1 domain and the bound HA peptide, and to a less extend, to the DR1 ß1 domain. Under crystallization condition, two MAM molecules form a homodimer through their C-terminal domains in an asymmetric fashion. The two MAM molecules in the dimer cross-link two MHC molecules to form a (DR1/HA/MAM)₂ complex. Based on the structure, a hypothetical model of TCR₂MAM₂MHC₂ is proposed.

Assessment of Anti-tumor Immunity Following Local Radiation Therapy

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The initiation and effector phases of anti-tumor immune responses were evaluated in mice after localized tumor irradiation. Tumors were established in C57BL/6 mice using B16F10 melanoma cells expressing ovalbumin (OVA) as a model tumor antigen. Seven days after tumor cell injection mice were treated with either a single radiation dose of 15 Gy or five consecutive daily doses of 3 Gy. Immune responses directed against OVA or the endogenous TRP-2 protein were assessed in all mice on day 14 post tumor injection. Increased numbers of antigen presenting cells (APC) were found within tumor draining lymph nodes (TDLN) that stimulated OVA-specific T cell hybrids in vitro for both radiation doses. In addition, TDLN of irradiated mice contained increased numbers of T cells that secreted IFN-? in response to the OVA peptides 257-264 and 323-339, as well as the TRP-2 peptide 180-188. Activation in TDLN correlated with an increase in infiltrating immune cells in the single dose irradiated tumors compared to non-irradiated tumors. Tumor-infiltrating lymphocytes (TIL) isolated from control and irradiated tumors, had similar capacities to lyse ⁵¹Cr labeled B16/OVA target cells in a class I MHC restricted manner. Although both treatment regimes slowed tumor growth, neither prevented tumor outgrowth. Adoptive transfer studies indicate that effector cells migrate to the treated tumors in greater numbers than to the control tumors. These results suggest that cell death resulting from local tumor irradiation provides antigen that can be processed by APC and stimulate T cells to produce IFN-?. Additionally, local radiation treatment may make tumors more accessible to tumor-specific lymphocytes.
Discordance of iNOS RNA and Protein Expression in Mice Lacking SHP1

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We have observed a striking deficiency in the ability of mice lacking SHP1, a tyrosine phosphatase, to mount an effective anti-viral response to Theiler’s murine encephalomyelitis virus (TMEV). Using dsRNA to mimic infection, we have found an interesting discordance between transcription and expression of inducible nitric oxide synthase (iNOS), an interferon inducible gene, in SHP1 deficient “motheaten” mice. Glial cells of motheaten mice showed significantly higher transcripts of iNOS in response to dsRNA; however, the amount of protein was significantly less. This decreased expression was also evident in immunohistochemical stains. Furthermore, activity of iNOS in motheaten cultures was significantly less. The production of NO by synthases during a viral infection has been linked to reductions in replication and spread, thereby representing a critical step in the innate immune response to viral infection. We are currently analyzing whether dsRNA specifically affects translation or proteolysis of iNOS in an SHP1 dependent manner.

Functional Heterogeneity of IFN-?-Expressing Cells in Response to Influenza Infection

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Infection with the respiratory influenza virus elicits a type 1 polarized immune response characterized by IFN-? production. However, we know little about the location and frequency of IFN-?-expressing cells in vivo and whether these cells are homogeneous with respect to IFN-? production and other effector functions. We used bicistronic IFN-?-eYFP reporter mice (Yeti) and MHC class I tetramers to visualize and functionally characterize IFN-?-expressing cells. Antigen-specific CD8$^+$ T cells were almost exclusively eYFP$^+$ in all organs with fluorescence intensities being highest at the site of infection whilst lowest in the draining lymph nodes. However, the eYFP median fluorescence intensities were heterogeneous in all organs. IFN-? production upon activation ex vivo was restricted to eYFP$^+$ cells and correlated directly with the fluorescence intensity of the sorted population. Despite the expression of the eYFP reporter gene the production of IFN-? was dependent on re-stimulation suggesting a mechanism of post-transcriptional regulation. The secretion of other cytokines and chemokines also correlated with the eYFP brightness and was augmented in cells derived from the lung indicating a generally more differentiated effector status. The functional heterogeneity was not limited to antigen-specific cells of the CD8$^+$ lineage and also found in eYFP$^+$ CD4$^+$ T cells.

Our results demonstrate a functional heterogeneity of IFN-?-expressing T lymphocytes even within an antigen-specific cell population. The identification of cells poised for IFN-? production is highly relevant to assess the functional potential of immunological memory.
The Effect of B-cell Receptor Signaling on the Cell Biology of Endocytosis and Antigen Processing

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While B cell receptor (BCR) signaling is known to result in changes in Major Histocompatibility Complex (MHC) class II expression and trafficking, the effect of BCR signaling on the cell biology of antigen endocytosis and processing is unclear. Therefore using a well controlled system in which we were able to study antigen endocytosis in the presence and absence of BCR signaling, we analyzed the effect of BCR signaling on the cell biology of antigen endocytosis, persistence, processing, and presentation. The results demonstrate that BCR signaling altered neither the global level endocytosis nor the intracellular persistence of internalized antigen. Moreover, while BCR signal did result in an increase in the level of total cell surface MHC class II molecules as well as specific peptide--class II complexes, stimulation failed to alter the fraction of class II molecules loaded with antigen--derived peptide. These results indicate that while BCR-mediated signaling does result in an increase in MHC class II, signaling does not directly change the overall biology of antigen endocytosis and processing. These results also demonstrate that the heightened efficiency of BCR--mediated antigen processing, when compared to fluid phase antigen processing, occurs independent of BCR signaling--induced global alterations in endocytosis, processing and presentation. This finding suggests that other unique aspects of BCR biology, such as the prolonged intracellular persistence of antigen--BCR, may play a key role in the highly efficient processing and presentation of BCR associated antigen.

T-cell Signaling Through the Immunological Synapse

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T cell activation requires a coordinated reorganization of cell surface receptors, cytoskeletal elements and cell signaling lipids and proteins into a spatially and temporally ordered structure termed the immunological synapse. The immunological synapse is thought to play to important roles in T cell function. First, polarization of the microtubule organizing center and associated secretory apparatus toward the antigen presenting cell allows for directional secretion of cytolytic granules and newly synthesized cytokines. Second, the assembly of cell signaling complexes and selective exclusion of phosphatases from the central domain of the immunological synapse provides for sustained signaling that is required for complete T cell activation. We have been examining to role of two cell surface proteins in regulating the formation of the immunological synapse, the specific targeting of signaling proteins to the central region of the synapse, and how these localization events regulate distinct outcomes in T cell activation. We have found that the integrin, LFA-1, which is a well known cell adhesion molecule can facilitate the recruitment of PKCtheta to the immunological synapse, while the costimulatory receptor, CD28, is required to target PKCtheta to the central region. This function of CD28 is mediated by its ability to activate PI-3kinase and results in the nuclear localization of NF-kB and upregulation of IL-2 transcription. In addition, CD28 can transduce PI-3kinase-independent signaling that upregulates IL-2 secretion through post-transcriptional mechanisms. The overall goal of these studies is to understand the specific molecular and cell biological events which contribute to establishing the threshold of T cell activation.
Intestinal Intraepithelial Lymphocytes: Inflammatory and Regulatory Cytokines in a Model of Secondary Immunodeficiency

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Intestinal intraepithelial lymphocytes (iIEL) constitute an unusual immunological compartment being the first immune cell line of defence in the intestine.

Our studies in a model of secondary immunodeficiency in Wistar rats (protein deprived at weaning and refed with a 20% casein diet during 21 days: R21 group) when compared to control animals (C60) have shown in iIEL:

1) An increased percentage of TCR?? cells expressing CD8?? as well as CD25 (Cytometry 41: 115, 2000).
2) The increase of inflammatory cytokines: TNF-? and IFN-? and regulatory cytokines: TGF-?.
3) Co-localisation of TNF-? with TCR?? and CD8?? IEL.
4) Co-localisation of TNF-RII with TCR??.
5) NF-?B is increased in cytosol being diminished in the nuclear fraction. Its activation is TNF? independent.
6) The anti-inflammatory cytokine IL-10 is decreased.

This has been ascertained by immuohistochemistry, flow cytometry, ELISA and western blot.

The oral administration of the immunomodulator Thymomodulin during the refeeding period (R21-TmB group) reverts these findings.

From our results we conclude: 1) that we are in presence of an inflammatory process due to the dextrin contained in the diet that is acting as a luminal antigen accompanied by specific tolerance (Lymphology, 2003, 36 -1-: 26-38). 2) The existence of specific oral tolerance may be due to the observed increase of TGF-?, an immunosuppressor cytokine produced by Tr cells, thus controlling the inflammatory damage. 3) The immunomodulator TmB may act as a therapeutic agent in secondary immunodeficiencies as the one provoked by malnutrition.

IL-27 Signaling Limits Optimal Control of Bacterial Growth in Mycobacteria-infected Mice

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Induction of IFN-? producing CD4 T cells is crucial to the containment of tuberculosis and depends to a large degree on IL-12p70. IL-27 has however recently been implicated in the induction of a Th1 cytokine profile during T cell activation; the role of this cytokine in the protective response to TB has however not yet been investigated. IL-27 is thought to begin the polarization of Th1 T cells by inducing Th1 transcription factor, T-Bet, via T cell cytokine receptor (TCCR or WSX-1)-mediated activation of STAT-1 and by inducing the expression of IL-12R?2 and thus responsiveness of the T cells to IL-12p70 signaling. In the present study, mice lacking the TCCR molecule were infected via the aerosol route with virulent M.tuberculosis and disease development monitored. We show that the number of antigen-
specific CD4 Th1 cells induced during infection was not compromised in the absence of TCCR and that granulomas in the late stages of disease were more lymphocytic in nature than in control mice. This improved granuloma formation correlated with increased ability of the TCCR-deficient mice to limit bacterial growth. Reduced expression of IFN-?NOS2 and IL-17 mRNA was seen in the lung of infected TCCR-deficient animals. We have previously hypothesized that expression of IFN-? within infected tissues can compromise the survival and efficacy of CD4 Th1 effector T cells. We further hypothesize that in the absence of IL-27 there is decreased expression of IFN-? and that this allows improved survival of CD4 effector cells, which thereby mediate improved control of bacterial growth.

Response of CD8+ T Lymphocytes During Influenza Infection: Phenotype and Comparison with CD4+ T Lymphocyte Expansion and Migration

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Naïve CFSE labeled TCR transgenic CD8+ T cells were adoptively transferred into mice subsequently infected with influenza. The kinetics of expansion, division, migration and effector function were followed for 30 days. Transferred CD8+ T cells were recovered from draining lymph nodes (DLN), non-draining lymph nodes (NDLN) and spleen at early time points (day 2-4 post infection (PI)) but are not found in lung and airways until day 5 PI. CFSE monitored division was seen first in the DLN, while cells that reach the lung had undergone more than six divisions. Transferred CD8+ T cells in DLN, NDLN, spleen, lung and airways had high surface levels of CD25, CD43, CD44 and Ly6C and had lost CD62L indicating activated cells. These activated CD8+ T cells produced IFN-? and TNF-? and were cytotoxic ex vivo. The expansion and division of influenza specific CD4+ and CD8+ TCR transgenic T cells were compared by co-transfer into the same host, followed by influenza infection. CD8+ T cells expanded to a higher number than CD4+ T cells in lungs and airways, with comparable numbers in DLN, NDLN and spleen. CFSE monitored division of CD4+ and CD8+ T cells was similar in DLN and spleen. CD4+ T cells were detected in much smaller numbers than CD8+ T cells 30 days post infection. These data provide new insights into CD8+ T cell activation in response to a locally proliferating virus and, show, that CD4+ and CD8+ T cells are regulated differentially during influenza infection. Funding: NIH PO1 HL639525 and T32AI49823 (DMB)

Differential Inhibition of B cells via Fc?RII

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Receptors for the constant region of IgG antibodies (Fc?R) are present on a variety of immune cells. Fc?RIIB is unique among the Fc? receptors in its ability to inhibit B cell activation when Fc?RII is co-crosslinked to the B cell receptor for antigen (BCR). Co-crosslinking the BCR to Fc?RII has been shown to inhibit B cell blastogenesis, proliferation and reduce intracellular calcium flux. In previous studies of B cell inhibition by Fc?RII, we observed a complete lack of a calcium response after treatment with anti-idiotype Ab as compared to treatment with a either polyclonal anti-BCR Ab or immune
complexes. The response was Fc?RII-dependent as a response was observed when cells were pre-incubated with blocking antibody to Fc?RII. This lead to the hypothesis that changes in metrics of B cell activation, as a result of co-crosslinking the BCR to Fc?RII, will vary dependent on the type of cross-linking agent used. In subsequent studies, a concentration of whole polyclonal anti-BCR that induced a minimal calcium response inhibited the B cell response to a second stimulation via the BCR. This observation can not be attributed solely to homologous desensitization of the BCR as the calcium response to the second stimulus after an equimolar amount of F(ab’)2 anti-BCR or whole Ab with an Fc?RII-block pretreatment was similar to a sham first stimulus. These results should further the understanding of the inhibitory role of Fc?RII and could also be therapeutically important for allergic or autoimmune diseases as treatments are designed to exploit the inhibitory function.

Th1 Polarization Occurs in the CNS, But Not in Peripheral Lymphoid Tissues, During EAE

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Experimental autoimmune encephalomyelitis (EAE) is an autoimmune demyelinating disease of the central nervous system (CNS) that is mediated by CD4+ T cells specific for myelin peptides. It is widely depicted as a prototype of Th1-mediated organ specific autoimmunity. In this study, we analyzed the cytokine profile of myelin-specific T cells from lymph node, spleen and CNS during EAE on the single cell level using a highly sensitive Elispot assay. Our studies demonstrate that the peripheral T cell repertoire is more diverse than previously portrayed, with comparable numbers of IL-4, IL-5 and IFN? producers present in lymph nodes and spleen. Fluorispot analysis suggests that IL-4/IL-5, and IFN? are secreted by two distinct subpopulations. IFN? producers co-secrete the pro-inflammatory chemokines MIP-1? and RANTES. By contrast, cells isolated from the inflamed CNS represent a uniform population, exclusively secreting IFN?, MIP-1a and RANTES. Our studies suggest that EAE should be classified as a Th1-mediated disease based on the biological functions of the effector cell repertoire within the target organ as opposed to in the periphery. In future studies we plan to investigate whether Th1 polarization within the CNS results from selective recruitment/retention of IFN? producers or from the active conversion of T cells to a Th1 lineage.

Regulation of T-cell Priming by CD38 in Airway Inflammation

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CD38 regulates inflammation and innate immune responses by controlling neutrophil and dendritic cell (DC) migration to certain chemoattractants. To analyze the role of CD38 in the development of allergic inflammation, we immunized and sensitized CD38 deficient and WT mice with OVA. We found less tissue damage and inflammation in the airways of CD38KO mice compared to WT mice. To analyze whether CD38 modulates the influx of inflammatory cells to the lung airways we administrated IL-13 intranasally in WT and KO mice and measured cell recruitment. The number of neutrophils,
lymphocytes and eosinophils was significantly higher in both WT and CD38KO mice treated with IL-13 compared with the controls groups treated only with PBS. However, the number of inflammatory cells was equivalent between both IL-13 treated groups, suggesting that IL-13 induced inflammation is not dependent on CD38. Next, we analyzed whether T cell priming was defective in CD38 deficient mice by transferring OVA specific transgenic (Tg) T cells into WT and CD38KO mice and then sensitizing the animals with NP-OVA. The expansion of activated Tg cells was significantly lower in CD38KO mice than in WT mice, indicating that normal T cells were inefficiently primed in CD38KO hosts. The antibody response and the recruitment of inflammatory cells like macrophages and eosinophils were also significantly reduced in the CD38KO mice. These results suggest that CD38 play a crucial role in the priming of T cells during allergic airway inflammation. This work was supported by grant AI-43629.

Immunomodulatory Effects of IL-12 on Modifying the Asthmatic Inflammatory Responses in Mice

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The increasing incidence of asthma is a major public health problem that especially afflicts children. The “hygiene hypothesis” suggests that asthma may result from excessive Th2 responses in children not previously exposed to Th1-inducing infections. We tested the hypothesis that induction of Th1 responses in newborn BALB/c mice would suppress subsequent allergic responses (airway hyperresponsiveness, allergic lung inflammation, Th2-type cytokine production). IL-12 is a cytokine known to direct Th cell development into a Th1 direction and to suppress Th2 responses, and therefore offers a means of modifying the asthmatic inflammatory response. BALB/c mice were intranasally administered 0.1 ?g of IL-12 on days 3 and 8 after birth or 0.05 ?g of IL-12 for 10 consecutive days (days 3 to 12 after birth). Mice were sensitized by intraperitoneal injection of 10 ?g of OVA with alum on days 5 and 10 and intranasally challenged with 100 ?g of OVA or PBS on days 29-33. An additional group of mice was treated with 0.1 ?g of IL-12 without allergic sensitization and challenge. On day 34, airway responsiveness was assessed and blood, bronchoalveolar lavage (BAL) fluid and lung tissue were collected for analysis. It was found that all OVA-sensitized and -challenged mice developed characteristic features of asthma, such as airway hyperresponsiveness (AHR), allergic airway inflammation, and elevated total and Ag-specific IgE in serum and BAL. Intranasal administration of IL-12 induced expression of IFN-? in the lung and spleen. Local administration of 0.1 ?g IL-12 twice before sensitization decreased the levels of OVA-specific IgE in serum and total IgE in BAL fluid, compared with OVA-sensitized and -challenged mice. IL-12 treatment also tended to reduce airway responsiveness after allergen challenge, but this effect was not statistically significant. Treatment with 0.1 ?g of IL-12 did not inhibit allergic lung inflammation, as indicated by BAL cell counts and histological evaluation of lungs. In contrast, local administration of 0.05 ?g IL-12 for 10 consecutive days during sensitization exerted opposite effects: enhanced AHR and increased levels of total IgE and OVA-specific IgG1 in BAL fluid. Moreover, administration of 0.05 ?g IL-12 during sensitization significantly increased numbers of inflammatory cells in BAL fluids and inflammatory lung infiltrates. We conclude that neonatal treatment with IL-12 can have suppressive or enhancing effects on development of allergic lung inflammation depending upon the timing and dosage used (supported by NIH grant AI 41765 and a grant from Philip Moris, Inc.).
Lack of IL-27 Signaling Reduces T-cell Loss in Disseminated Progressive Mycobacterial Disease

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The protective strong Th1 immune response that controls mycobacteria can also lead to T cell-mediated immunopathology resulting in significant tissue destruction. Induction of IFN-\(\gamma\) producing CD4 T cells depends to a large degree on IL-12p70. Recently other IL-12 related cytokines such as IL-27 have been implicated in the induction of Th1 cytokines during T cell activation. IL-27 is involved in the initiation of polarization of Th1 T cells by signaling through the T cell cytokine receptor (TCCR or WSX-1). In this study mice lacking the TCCR molecule and therefore unable to mediate IL-27 induced signals were intravenously infected with \textit{M. avium} 724 and the progression of disease monitored by bacterial burden, T cell response and histopathology. Following infection with this virulent mycobacteria in intact mice, CD4 T cell numbers expand but then contract, in contrast this same population in TCCR-KO mice expands and remains high throughout. This enhanced lymphocytic response is also seen in the granulomas of TCCR-KO mice and correlates with improved control of bacteria. The amount of IFN-\(\gamma\) producing cells and the level of IFN-\(\gamma\) mRNA remain similar between the intact and TCCR-KO mice. Our data support the hypothesis that IL-27 signaling is not essential for the induction of Th1 responses but that it does serve to limit the size of the CD4 T cell population during mycobacterial disease.

The Role of Respiratory Antibody in Protection Against \textit{Streptococcus pneumoniae} Infection

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Colonization of the upper respiratory tract by \textit{S. pneumoniae} is a major cause of community-acquired pneumococcal infection. However, the importance of mucosal-derived antibody in controlling colonization is poorly understood. The role of respiratory antibody in preventing colonization by this pathogen has now been examined using polymeric Ig receptor knockout (pIgR\(^{-/-}\)) mice, which lack the ability to actively secrete IgA into mucosal lumen, and infection with serotype 14 \textit{S. pneumoniae}, which causes nasal colonization but does not induce systemic inflammatory responses in mice. Intranasal vaccination with conjugate vaccine (PS14-CRM\(_{197}\)) elicited specific anti-capsular polysaccharide (PS) serotype 14 antibody locally and systemically, and pIgR\(^{-/-}\) mice had more systemic and less nasal IgA antibody than wild-type mice, as expected (~ 5 and 0.16 times, respectively, compared to wild-type mice). Vaccination elicited protection against nasal colonization in wild-type mice \((p<0.05)\). Although total levels of systemic antibody in vaccinated pIgR\(^{-/-}\) mice were similar to wild-type mice, pIgR\(^{-/-}\) mice were not protected against nasal carriage. \textit{In vitro} studies with pIgR\(^{-/-}\) immune serum showed that there was no defect in the ability of serum PS14-specific IgA to bind to bacteria, but in the absence of pIgR it could not be actively secreted into the mucosal lumen. The relative importance of sIgA in host defense was further determined using IgA gene-deficient (IgA\(^{-/-}\)) mice. Intranasally vaccinated IgA\(^{-/-}\) mice were not protected from nasal colonization. Although secretory IgA is important for protection against nasal carriage, it does not have a crucial role in protection against systemic pneumococcus.
infection, because both vaccinated wild-type and pIgR/- mice were protected from lethal systemic infection by serotype 3 strain. The results demonstrate the critical role of secretory IgA in protection against pneumococcal nasal colonization. (Supported by NIH grant AI 41715 and a grant from Philip Morris, Inc.)

**Trichinella spiralis** Glycans Complexed with Monoclonal IgG Isotypes Interact With Mast Cell Fc Receptors

Seana Thrasher
Cornell University

Our aim is to define the role of mast cells in expulsion of *Trichinella spiralis* from the intestinal epithelium during a challenge infection. We hypothesize that host immunoglobulins complexed with parasite antigens interact with Fc receptors on mast cells to promote the rapid expulsion of larvae from the intestine. Although correlative evidence suggests that expulsion is dependent on mast cells, the mechanism of expulsion has not been determined. Passive immunization of rats with glycan-specific monoclonal IgG isotypes causes complete expulsion of *T. spiralis* larvae within 24 hours. This protective mechanism only occurs when rats have been previously infected with an unrelated nematode, such as *Heligmosomoides polygyrus*. This infection likely activates some non-specific factor of innate immunity that cooperates with antibodies to cause expulsion. First, we determined by flow cytometric analysis that IgG1, IgG2a, and IgG2b but not IgG2c immune-complexes bind Fc receptors on RBL-2H3 cells, a mucosal mast cell line. Blocking experiments revealed that IgG1 and IgG2a bind to the high affinity IgE receptor (Fc*RI*) and a low affinity IgG receptor (Fc*RIIb*). IgG2b bound only Fc*RIIb*. Second, we found that IgG2a immune-complexes trigger RBL-2H3 degranulation and IgG2b does not. Even though IgG1 immune-complexes bound Fc*RI*, they did not trigger degranulation. Understanding Fc receptor interactions with protective antibodies will contribute to determining whether initiation of rapid expulsion in the rat depends on mast cell activation via immune-complexes.

**Mechanisms of Humoral Immunity During Murine Infection By Related Ehrlichial Strains**

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Previous work from our laboratory has demonstrated that the administration of specific antibody prior to or during infection protected susceptible SCID mice from lethal infection by the obligate intracellular bacterium *Ehrlichia chaffeensis*. We have hypothesized that protection in our model involved (1) enhanced opsonization and/or (2) cellular activation through antigen-antibody-Fc? receptor (FcR) interactions. We have begun to utilize the closely related ehrlichial strain *Ixodes ovatus* ehrlichia (IOE), which causes fatal infection in immunocompetent mice, to further our understanding of immunity against obligate, intracellular bacteria. Current experimental data indicate that: (1), mice genetically-deficient for Fc?R, specifically Fc?RI*, succumb to sublethal inoculums of IOE, (2) mice genetically-
deficient for B cells are acutely susceptible to IOE infection, and (3) mice experimentally depleted of complement fail to clear a sublethal dose of IOE. Together, these results suggest that, like with E. chaffeensis, protection against IOE is dependent on the presence of functional Fc?R, specifically Fc?RI. However, there is a greater dependence on the presence of functional B cells and a full system of complement with IOE infection compared to E. chaffeensis. Data obtained using both infection models support, directly and indirectly, an important role for antibody in immunity against ehrlichial infection.

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Evidence for a Specific IgA Fc Receptor on Murine Macrophages

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IgA is the most abundant immunoglobulin at mucosal surfaces (Brandtzaeg 1989). Not only it plays a vital role in innate immunity and prevent microorganisms from invading mucosal barriers (Monteiro and Van De Winkel 2003), it also serves as an important immune modulator to control inflammatory responses (Kerr 1990). An IgA specific Fc receptor (CD89) in humans has been found on myeloid-derived phagocytic cells and mediate opsonophagocytosis (Monteiro and Van De Winkel 2003). However, a specific Fc?R in rodents has not yet been identified. This has limited in vivo approaches for the understanding of IgA function in lung immunity. Our preliminary studies have now strongly indicated the presence of a specific Fc receptor for IgA on a mouse myeloid cell line (J774A.1). First, we have observed that immune IgA is capable of mediating phagocytosis of fluorescently labeled S. pneumoniae by J774A.1 cells. Second, free IgA can bind to J774A.1 cells in a dose-dependent manner. Third, other known IgA receptors, including Fc?/?R and pIgR are not expressed by the J774A.1 cells, determined by RT-PCR. Current studies are designed to identify the receptor at the protein and DNA level. The expression profile and the signaling pathway of the novel receptor will also be studied. A murine Fc?R knockout model will be developed to fully characterize the in vivo functions of IgA and its receptor. The study will provide fundamental knowledge for developing IgA-derived therapeutic strategies and mucosal vaccinations in humans. (Supported by NIH grant AI41715 and a grant from Philip Morris, Inc.)
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