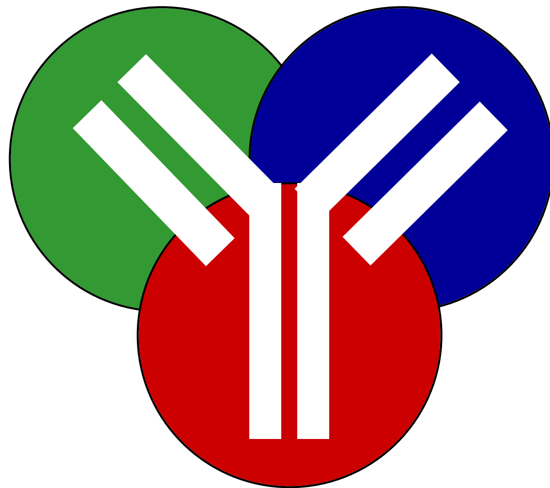
The Fifth Upstate New York Immunology Conference



November 3-6, 2002

The Sagamore
At Bolton Landing, NY

November 3, 2002

Dear Meeting Participant,

Welcome to the Fifth Upstate New York Immunology Conference. This annual meeting began in 1998 to facilitate interactions among immunologists in Upstate New York, including those from Trudeau Institute, Albany Medical College, Wadsworth Center, SUNY Upstate Medical University, the University of Rochester and Cornell University. This year the meeting has expanded to include participants from SUNY at Buffalo and Roswell Park Cancer Institute. We are pleased that you are here to share your research with your colleagues from the upstate area.

We are also happy to welcome our keynote speakers for this year's meeting. Dr. Hildegund Ertl from the Wistar Institute and Dr. Susan Pierce from NIH/NIAID. Dr. Ertl's talk is entitled "Genetic Vaccines". Dr. Pierce will talk on "Lipid rafts and the spatial organization of B cell signaling receptors". In addition, we will have presentations from faculty members, post-docs and graduate students from each of the participating institutions.

We hope you enjoy the meeting.

Sincerely,
The UNYIC Organizing Committee

Laura Haynes, Chairperson, Trudeau Institute
Edmund Gosselin, Albany Medical College
Gary Winslow, The Wadsworth Center

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We would like to thank our sponsors for their generous support. We would also like to thank Susan Richards, Kristin Meadows and Jeannine Light for administrative support and Eric Yager for organizing the meeting web site.

CONFERENCE SCHEDULE

Nov. 3 Sunday

3-5 Arrival and Registration
Conference Center Lobby
Hotel check-in begins at 4 pm

5-7 Dinner *Wapanak*

7-8 **Keynote Speaker**
Dr. H. Ertl, Wistar Institute
Genetic Vaccines

8-10 Vendor Fair & Mixer *Nirvana*

Nov. 4 Monday

7-8 Breakfast *Sagamore*
Dining Room

8-10 **Session 1.** *Wapanak*
Immunity and Infection, Chair: A. Cooper

Andrea Cooper, Trudeau Institute
Protective T cell responses to Tuberculosis
in the murine lung

Gary Winslow, Wadsworth Center
Persistence and turnover of antigen-specific
T cells during Tuberculosis infection

Steve Smiley, Trudeau Institute
Fibrin-mediated protection against infection-
stimulated immunopathology

Troy Randall, Trudeau Institute

Proliferation, V region mutation & affinity selection outside the germinal center in a novel population of B cells responding to influenza

10-10:30 Break

10:30-12:30 **Session 2**, *Wapanak*

Immune Regulation I, Chair: F. Lund

James Clements, Roswell Park Cancer Institute

The regulated expression of SLP-76 contributes to establishment of thymocyte signaling potential

Dorina Avram, Albany Medical College

CTIPs, novel leukemia-associated transcriptional regulators

Frances Lund, Trudeau Institute

Regulation of innate and adaptive immune responses by the ectoenzyme CD38

Wei-ping Zheng, University of Rochester Medical Center

Mechanisms for maintaining differentiation potentials of CD4 T cells

12:30-1:30 Lunch *Nirvana*

1:30-5	**Session 3.**	*Wapanak*

Chair: L. Haynes

Tim Powell, Trudeau Institute
The activation and migration of CD8 T lymphocytes during influenza infection

Deborah Brown, Trudeau Institute
Phenotypic alterations in influenza specific CD4+ T cells correlate with migration to the lung

Diana Albu, Albany Medical College
Induction of mucosal and systemic immune response to HIV-1 glycoproteins

Daniel Potvin, Wadsworth Center
Treatment with exogenous IL-12 protects against lethal coxsackievirus B4 infection

2:50-3:20 Break

Paul Arnaboldi, Albany Medical College
The role of IgA in allergic lung inflammation

Scott Gerber, University of Rochester Medical Center
Visualization & characterization of tumor blood vessels and effects of IL-12

Dawn Jelley-Gibbs, Trudeau Institute
Decreased CD4 memory T cell generation as a consequence of persistent antigen exposure

Andrew Watson, Wadsworth Center
Memory T cell activation

4:40-5 Poster set up *Triuna A & B*

5-6 Posters & Mixer

6-8 Dinner *Nirvana*

8-9 **Keynote Speaker** *Wapanak*

Dr. S. Pierce, NIH/NIAID
Lipid rafts and the spatial organization
of B cell signaling receptors

9-10 Mixer *Triuna A & B*

Nov. 5 Tuesday

7-8 Breakfast *Sagamore Dining Room*

8-9:30 **Session 4**. *Wapanak*

Immune Receptors, Chair: J. Drake

James Drake, Albany Medical College
Lipid raft dependent and independent
B cell receptor functions

Sarah Gaffen, SUNY at Buffalo
Cytokine receptor signaling

Edmund Gosselin, Albany Medical College
Production of enhanced immunity utilizing
Fc R1-specific scFv-Ag conjugates

9:30-10:00 Break

10:00-12:00 **Session 5**. *Wapanak*

Immune Regulation II, Chair: D. Metzger

Dennis Metzger, Albany Medical College
Role of IgA in Control of Innate Immunity

Richard Phipps, University of Rochester Medical Center
Prostaglandins as modulators of immunity

Yasmin Thanavala, Roswell Park Cancer Institute
Immune responses in patients with COPD

Alice Sijts, University of Rochester Medical Center
Expression of the proteasome activator PA28
rescues the presentation of a CTL epitope on melanoma
cells

Benjamin Segal, University of Rochester Medical Center
The role of lymphoid chemokines in autoimmune
inflammation

12:00-1:00 Lunch *Trillium*

1:00-2:00 Meeting to discuss future organization of UNYIC--
all those who are interested are invited to attend

2:00 Depart

Keynote Speakers

Hildegund C.J. Ertl, M.D.
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The Wistar Institute
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A prophylactic vaccine to HIV-1 has to induce potent cellular and humoral immune responses to antigens of HIV-1. HIV-1 is in most cases sexually transmitted and effective prevention of a systemic chronic infection is assumed to require antibodies and memory T cells at the mucosal sites of viral entry. Pursuant to these goals, the laboratory has developed a vaccine regimen in a rabies mouse model, which optimally induces mucosal antibody secretion and systemic cytolytic T cell responses. Priming with a DNA vaccine against a model (rabies) antigen, followed by booster immunization with an intranasally applied, E1-deleted adenoviral recombinant expressing the antigen of interest, induces potent serous antibody responses, as well as a strong, prolonged antibody response at the vaginal mucosa of immunized mice. DNA vaccine priming also reduced the subsequent antibody response to the carrier adenoviral antigens. Further, in mice pre-immune to adenovirus, DNA vaccine priming overcomes the impaired B cell response to rabies antigen expressed by the adenoviral recombinant vaccine. The vaginal B cell response can further be enhanced by interleukin (IL)-4, IL-10, or IL-5, co-administered as genetic adjuvants with DNA vaccine, prior to subsequent booster immunization within recombinant adenoviral vaccine. This type of a vaccine regimen is now being applied to the far more complex HIV system by generating vaccines expressing the env or gag of HIV-1 or SHIV, a chimeric virus of HIV and simian immunodeficiency virus. Plasmid DNA vaccines are being developed as reagents for 'priming' and recombinant adenoviral vaccine for subsequent 'booster' immunizations.

References:

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Vaccine 2000 Jun 15;18(25):2804-7

Susan K. Pierce, Ph.D.
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The B-cell antigen receptor acts during B-cell activation both to initiate signalling cascades and to transport antigen into the cell for subsequent processing and presentation. Recent evidence indicates that membrane microdomains, termed lipid rafts, have a role in B-cell activation as platforms for B-cell receptor (BCR) signalling and might also act in antigen trafficking. Lipid rafts might facilitate the regulation of the BCR during B-cell development by B-cell co-receptors, and during viral infection. So, lipid rafts seem to be an important new piece of the B-cell signalling puzzle.

References:

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Poster

Abstracts

Mechanism involved in idiopeptide protection from lupus nephritis

Jennifer F. Nyland[*], Feng Feng[§*], Jerrie Gavalchin[§*]
[§]Cornell University, Ithaca, NY and [#]SUNY Upstate Medical University, Syracuse, NY.

The female F_1 progeny of the (SWRxNZB) cross (SNF_1) develop a lupus-like nephritis with lifespans of about one year. A peptide derived from amino acids 62-73 (aa62) of the heavy chain variable region of a disease-inducing antibody (Ab) induces proliferation of pathogenic idiotype-reactive T cell clones. Here we tested whether disease could be modulated by administration of aa62 to prenephritic mice by classical routes to induce tolerance or active immunity. Eight-week old female SNF_1 mice received aa62 via gavage or subcutaneous immunization. Survival data indicated increased survival with either aa62 treatment. Kidney pathology was reduced with aa62 by both methods, significantly by 16 weeks. $Id^{LN}F_1^+$ and anti-$Id^{LN}F_1^+$ Ab-producing B cells were decreased and increased, respectively, with both aa62 treatments; this change occurred by 12 weeks of age with vaccination. The ability of B cells from aa62 treated mice to present antigen in proliferation assays was reduced by 10 weeks, but not at later time points. There was an increase in Th1 cytokine production, as well as TNF- in response to aa62 by splenocytes from both aa62 treatment groups *in vitro*, as early as 12 weeks. Together, these data demonstrate that vaccination or oral dosing with a pathogenic idiopeptide from an autoAb can delay spontaneous nephritis in SNF_1 mice. Further, they suggest that the mechanism(s) involves early events that result in modulation of both pathogenic B and T cell populations.

Treatment with Estradiol Induces Autoimmunity in Non-Autoimmune Mice.

F. Feng[§*], F. Jiang[#], J. Nyland[#], D. Hart[#], J, Gavalchin[§#]. [§]Cornell University, Ithaca, NY and [#]SUNY Upstate Medical University, Syracuse, NY.

Previous work has suggested that estrogen (E-2) accelerates autoimmune diseases in mice that are genetically predisposed to their development, such as the lupus-prone mice (SWR X NZB) F_1, or SNF_1 (H-2 $^{d/q}$). Female SNF_1 mice develop spontaneously accelerated nephritis. Male mice develop later disease, however it may be accelerated by treatment with pharmacological doses of E-2. Here, we asked whether E-2 would induce the development of lupus nephritis in non-autoimmune mice, by treating the cross between DBA/1 and BALB/c mice, or DBF_1 (H-2 $^{d/q}$), with -estradiol. We found that E-2 treatment resulted in a significantly ($p < 0.01$) shortened lifespan, and histological examination of kidneys from the E-2 treated mice revealed significant ($p < 0.05$) glomerular damage associated with nephritis. Flow analysis for lymphocyte phenotypes showed that there were significantly ($p < 0.05$) increased numbers of $CD25^+Id^{LN}F_1$-reactive and $CD44^+Id^{LN}F_1$-reactive T cells with E-2 treatment. Furthermore, while the proliferative response to ConA was decreased in the E-2-treated group compared to the oil treated mice, the proliferative response to a pathogenic $Id^{LN}F_1$ peptide, aa62-73, was increased ($p < 0.05$). While E-2 treatment led to significant ($p < 0.01$) increases in both Th-1 and Th-2 cytokine production by Con-A stimulated splenocytes, aa62-73 stimulated lymphocytes produced significantly ($p < 0.03$) less IFN- . Interestingly, ELISPOT analysis showed that there were increases in the numbers of B cells producing pathogenic $Id^{LN}F_1$ antibody or anti-$Id^{LN}F_1$ antibody following E-2 treatment ($p < 0.05$). Taken together, these results suggest that E-2 may induce autoimmunity in mice not normally prone to develop disease.

Trafficking of Antigen-Specific T Cells to the Prostate in the TRAMP Model

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*Departments of Microbiology and Immunology & Urology, SUNY Upstate Medical University, Syracuse, NY, [$]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, and [#]Immunology, Mayo Clinic, Rochester, MN

Prostate cancer is the second leading cause of cancer related death and the most frequent newly diagnosed cancer in men. Because cells of the prostate are androgen dependent, hormone therapy is a common treatment method for advanced prostate cancer. However, the majority of patients have recurrence with an androgen-independent tumor. Androgen ablation causes rapid damage and death of prostate cells via apoptosis, providing potential tumor Ags to the immune system. Consistent with this, T cell and APC infiltration is seen in prostate tumors following androgen withdrawal. This has led to the idea that androgen withdrawal can initiate a cell-mediated immune response directed toward prostatic Ags. With the specific components of T cell activation having been elucidated, manipulations capable of overcoming T cell tolerance and thus tumor immunotherapy are now possible. Androgen ablation itself is rarely effective enough to clear a prostate tumor. However, with additional immune stimuli, total eradication of prostate cancer is a possibility. By using the **TR**ansgenic **A**denocarcinoma of the **M**ouse **P**rostate (TRAMP) model, in which SV40 T Antigen (TAg) serves as the surrogate prostate tumor Ag, we wish to characterize further cellular infiltration into the prostate following androgen withdrawal. By utilizing the transgenic Byron mouse model, in which the TCR is specific for the H-2K^k restricted epitope of TAg, Ag specific T cell infiltration can be studied.

The Role of IgA in Allergic Lung Inflammation

Paul M. Arnaboldi and Dennis W. Metzger. Center for Immunology and Microbial Disease, Albany Medical College, Albany, NY 12208

IgA is the most abundantly produced immunoglobulin at mucosal surfaces and is a potent mediator of immunity at these sites. The goal of this study was to determine the role of IgA in asthma by utilizing a murine model of allergic lung inflammation (ALI) and mice with a targeted disruption of the -heavy chain gene (IgA$^{-/-}$), resulting in an inability to produce any IgA. In addition, we used mice with a targeted disruption of the poly-immunoglobulin receptor (pIgR$^{-/-}$), rendering them unable to transport polymeric immunoglobulins across mucosal epithelium, thus eliminating secretory (sIgA) from mucosal surfaces. This allowed us to determine the contribution of sIgA versus the effect of total IgA on the development of disease. Mice were sensitized on day 0 with 10μg ovalbumin (OVA) in alum, followed 14 days later by five consecutive daily doses of 100μg OVA in saline to induce inflammation, and disease was determined by an eosinophil-rich infiltrate in the lungs and bronchoalveolar lavage (BAL) fluid, Th2 cytokine production, Th2 associated antibody production (IgG1 and IgE), and the presence of airway hyperresponsiveness (AHR). Staining of lung tissue demonstrated that IgA$^{-/-}$ animals had a severely abrogated inflammatory infiltrate limited to a few cells in the peri-vascular regions of the lung, while IgA$^{+/+}$ mice had a robust infiltrate in both peri-vascular and peri-bronchiolar regions. There was no difference in the inflammatory infiltrate of pIgR$^{+/+}$ and pIgR$^{+/+}$ mice. IgA$^{-/-}$ mice had ~50% less inflammatory cells in BAL fluid compared to IgA$^{+/+}$ mice. Additionally, the inflammatory cells isolated from the BAL fluid of IgA$^{+/+}$ mice were comprised of 50-60% eosinophils, with the remainder being neutrophils and small lymphocytes. On the other hand, BAL fluid from IgA$^{-/-}$ mice contained virtually no granulocytes and was comprised mainly of small lymphocytes. Again, there was no difference in the number of infiltrating cells in the BAL fluid of pIgR$^{+/+}$and pIgR$^{-/-}$ mice. Analysis of antibody levels demonstrated that IgA$^{-/-}$ mice had significantly less total and IgG1 OVA specific antibody in BAL fluids, as well as significantly less serum total IgE, compared to IgA$^{+/+}$ mice. No differences in the level of total or IgG1 OVA specific antibody or total IgE were detected between pIgR$^{+/+}$ and pIgR$^{-/-}$ mice. The presence of AHR was determined using whole body barometric plethysmography. Neither IgA$^{+/+}$, pIgR$^{+/+}$ nor pIgR$^{-/-}$ mice developed substantial AHR following the induction of allergic lung inflammation. Interestingly, IgA$^{-/-}$ mice developed significant AHR despite having an abrogated inflammatory response and reduced IgE levels. An extensive analysis of cytokine and chemokine expression was performed using nylon membrane gene arrays (Superarray, Bethesda, MD). Surprisingly, few differences in expression levels were detected during the challenge phase of our model, while differences were detected during the sensitization phase in several cytokines shown to be involved in ALI (IL-4, 5, 9, 13, and 17). The significance of these results is currently being evaluated by real-time PCR, as well as cytokine expression in pIgR$^{+/+}$ and pIgR$^{-/-}$ mice. Our results clearly demonstrate that IgA deficient animals have a greatly diminished level of ALI compared to normal animals, demonstrating a critical role for IgA in the development of this mucosal disease. Furthermore, our results suggest that IgA plays a role in suppressing the development of AHR, lending support to a burgeoning theory that ALI and AHR develop via independent mechanisms. Finally, our results demonstrate that secretory component is not necessary for the development of ALI, as no differences were detected between pIgR$^{+/+}$ and pIgR$^{-/-}$ mice. Current experiments are designed to determine the mechanisms responsible for the influence of IgA on allergic lung inflammation, and whether IgA is necessary for the priming or effector stages of the disease. (Supported by NIH grants AI41715 and HL62120).

The biological outcome of CD40 signaling in B lymphocytes is dependent on the duration of CD154 expression on T cells: reciprocal regulation by IL-4 and IL-12

Byung O. Lee, Laura. Haynes, Sheri. M. Eaton, Susan. L. Swain, and Troy. D. Randall
Trudeau institute, 100 Algonquin Ave., Saranac Lake, NY 12983

Signaling through CD40 is controlled by the regulated expression of its ligand, CD154. Although CD154 is thought to be only transiently expressed on T cells early after activation, we demonstrate that the kinetics of CD154 expression depend on the developmental state of the responding T cell as well as the available cytokines, and costimulatory signals.. We found that CD154 is rapidly induced on activated naive T cells within 6 hours after activation and is reduced to background level within 24 hours. However, we also found that CD154 is re-expressed between 48 - 72 hours after activation. This second phase of CD154 expression was sustained by IL-12 and repressed by IL-4. Furthermore, we found that Th2 memory cells expressed CD154 only at early timepoints after activation, while Th1 memory cells expressed CD154 at both early and late timepoints. However, the sustained expression of CD154 on activated Th1 memory cells is not fixed property of Th1 cells, since IL-4 blocks the second phase of CD154 expression in restimulated Th1 cells. Additionally, we found second phase of expression is greatly depend on costimulatory signals, such as CD28 and ICOS. Finally, we showed that activated T cells can block antibody secretion from B cells through sustained expression of CD154. Thus, prolonged expression of CD154 on Th1 cells, but not Th2 cells, regulates B cell effector functions and likely influences the effector functions of other CD40 expressing cell types.

Chemokine expression in spinal cords during EAE is altered by IFNγ deficiency.

Thaddeus Carlson, Kathy Aligene, Jim Powers and Benjamin Segal.
The University of Rochester School of Medicine and Dentistry.

Experimental autoimmune encephalomyelitis (EAE) is a Th1 T-cell mediated disease. Nonetheless, the Th1 cytokine IFN is not required for disease since INF -/- mice are at least as susceptible, if not more so, as their wildtype counterparts. These knockout mice also have a qualitatively different inflammatory infiltrate in spinal cord lesions characterized by the presence of mast cells and numerous neutrophils. Based on the fact that chemokines are very important in the recruitment of inflammatory cells to EAE lesions we hypothesized that the chemokine profile of IFN -/- mice with EAE is different from that of wildtype mice. EAE was induced in wildtype C57BL/6 mice and IFN -/- (C57BL/6 background) mice by active immunization and RNA was isolated from spinal cords at peak disease. Reverse transcriptase PCR was performed to determine chemokine mRNA expression. mRNA for the IFN -inducible chemokines IP-10 and I-TAC were expressed in wildtype mice with EAE, but naïve mice and IFN -/- mice showed no expression. The receptor for these chemokines, CXCR3 was expressed in wildtype and slightly lower levels in IFN -/- mice. The CXC chemokines MIP-2, LIX, and KC were expressed in both wildtype and IFN -/- mice. Neurotactin and its receptor CX3CR1 were expressed in naïve mice as well as both wildtype and IFN -/- mice with EAE. Based on this data there is a different pattern of chemokine expression in wildtype and IFN -/- mice with EAE. Further studies to assess the functional significance of this difference will include more quantitative measures of chemokine mRNA as well as protein expression, determination of chemokine expression at different time points in the disease process and neutralization studies to determine the physiological role of individual chemokines.

Induction of Mucosal and Systemic Immune Response to HIV-1 Glycoproteins

Diana I. Albu[1], Agnes Jones-Trower[2], Amy Woron[1], Kathleen Stellrecht[1], Christopher C. Broder[2] and Dennis W. Metzger[1]
[1] Center for Immunology & Microbial Disease, Albany Medical College, Albany NY, 12208
[2] Department of Microbiology and Immunology, Uniformed Services University, Bethesda MD, 20814

Characterization of the immune responses to HIV-1 and its components represents a key factor for effective vaccine development. The Envelope glycoprotein (Env) of HIV-1 is the principal antigen to which virus-neutralizing antibodies are directed, and will likely be included as a key component of a successful vaccine formula. Env is cleaved by host cell proteases into the gp120, external, and gp41 transmembrane subunits that remain non-covalently associated. Soluble, truncated forms of oligomeric HIV-1 Env known as gp140, are comprised of the uncleaved gp120 and gp41 ectodomain subunits, while soluble gp120 Env formulations are monomeric. In this study we have investigated the induction of protective mucosal immunity to HIV-1 by intranasal (i.n.) immunization of mice with gp120 and gp140 together with IL-12 and cholera toxin subunit B (CTB) as adjuvants. It was found that both IL-12 and CTB were required to elicit mucosal antibody responses, and that i.n. immunization resulted in increased total, IgG1 and IgG2a anti-HIV-1 antibody levels in serum, increased total, IgG1, IgG2a, and IgA antibody expression in bronchoalveolar lavage (BAL) fluids and increased IgA antibody levels in vaginal washes. Levels of anti-HIV-1 antibodies in both sera and secretions were higher in mice immunized with gp140 compared with those immunized with gp120. However, only gp120-specific mucosal antibodies demonstrated neutralizing activity against HIV-1. Taken together, the results show that IL-12 and CTB act synergistically to enhance both systemic and local antibody responses to HIV-1 glycoproteins and that even though gp140 is a better immunogen than gp120 for i.n. immunization, only gp120-specific mucosal antibodies interfere with virus infectivity.

Phenotypic Alterations in Influenza Specific CD4$^+$ T Cell Effectors Correlate with Migration to the Lung

Deborah M. Brown, Kim Sorrell, Debra Duso, Eulogia Roman and Susan L. Swain.
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The mechanisms involved in the homing properties of CD4$^+$ T cell effectors to inflammatory sites remain poorly understood. It has been proposed that the expression of certain adhesion molecules on CD4 effector cells can dictate their in vivo “address” in response to inflammatory stimuli. In this study, an adoptive transfer system was designed to examine the activation and migration patterns of CD4$^+$ T cells during influenza viral infection. Within 4 days after infection, flu specific CD4 cells upregulate CD43, CD49d (4 integrin) and LPAM (4 7 integrin) concomitant with increasing cell divisions. CD62L expression decreases with proliferation indicating that CD4 cells are activated and transitioning to the effector state. Furthermore, the cells with the highest level of adhesion molecules and lowest expression of CD62L are found in the lung, suggesting that these cells are the most highly activated. P-selectin glycoprotein ligand (PSGL-1 or CD162) was initially high on naïve CD4$^+$ T cells, decreased with proliferation in the draining LN, and subsequently increased on cells that traffic to the lung and airways. Studies are underway to determine whether influenza specific CD4 effectors generated during an active infection can then migrate to other sites of inflammation, or are programmed to remain in the lung to combat future respiratory challenges.

Decreased CD4 memory T cell generation and function as a consequence of persistent antigen exposure.

Dawn M. Jelley-Gibbs and Susan L. Swain.
Trudeau Institute Inc., Saranac Lake, NY 12983.

Constant T cell receptor (TCR) stimulation during expansion of CD4 effectors leads to the differentiation of a population of poorly functional effectors incapable of establishing effective secondary responses or substantial memory populations. Conditions of constant TCR stimulation are established by pulsing cultures every 24 h with fresh Ag/APC. The effectors generated under conditions of constant TCR stimulation are inefficient at producing cytokines. When adoptively transferred into host mice these inefficient effectors fail to establish high frequencies of memory T cells, and the memory cells which do establish have poor cytokine production. Furthermore, adoptive transfer of up to $20x10^6$ poorly functional Th1 effectors fails to protect against lethal infection with PR8 influenza virus, whereas as few as $5x10^6$ fully functional Th1 effectors generated with normal Ag exposure are protective. We have three hypothesis that may explain the failure of effectors generated under conditions of constant TCR stimulation to protect against influenza virus infection. First is that these effectors may not home to the lungs of influenza virus infected mice. Second is that these effectors, although capable of homing to the lungs, may fail to recruit virus-specific $CD8^+$ effectors to the lungs. Third is that these effectors may home to the lung, but direct an inappropriate type 2 polarized $CD4^+$ or $CD8^+$ T cell response in the lungs. Implications of this work indicate a potential mechanism by which chronic or persistent viral infections evade the immune response in otherwise immunocompetent individuals.

The Immunopathology of Mycobacterial Lipids: The role of BCG lipids in cell recruitment and granuloma formation.

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Mycobacterium tuberculosis, the causative agent of tuberculosis, is the leading cause of death attributable to an infectious agent, and currently infects one third of the world's population, The hallmark of mycobacterial infection is granulomatous inflammation at 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, inducing both proinflammatory cytokines and immunosupressive mediators. We have shown that lipids of *Mycobacterium bovis* Calmette-Geurin (BCG) are granulomagenic using a novel granuloma system. In this system, BCG lipid-coated polystyrene microspheres and bone marrow-derived macrophages suspended in an extracellular matrix gel, are delivered intraperitoneally into BCG-sensitized and naïve mice. The model exhibits a granulomatoid pattern of leukocyte recruitment. Monocytes, epitheloid macrophages, dendritic cells, and $^+$ and $^+$ T cells follow initial neutrophil recruitment. BCG-sensitized mice exhibit an accelerated and more intense cell recruitment to the granuloma, compared with naïve controls, which suggests an adaptive immune response to BCG lipids. In adoptive transfer experiments of CMFDA-labeled, T cell-enriched splenocytes, harvested from mice bearing peritoneal granulomas, $CD3^+$ cells were shown to be recruited to the granulomas of recipient mice. Of the CMFDA-labeled $CD3^+$ cells recruited to the peritoneal granuloma, 30%-40% were TCR $^+$. This represented a significant enrichment for $^+$ T cells in the granuloma, compared with the spleen of the recipient, where $^+$ T cells comprised 6-16% of donated cells. The majority of the remainder of the CMFDA-labeled cells in both spleen and granuloma were $^+$ T cells. The results were confirmed in a congenic system of adoptive transfer. Where around 60% of donated cells entering the granuloma were +CD3+. These results demonstrate that BCG lipids actively contribute to cell recruitment in mycobacterial granulomas.

Visualization and characterization of tumor blood vessels and effects of IL-12

Scott Gerber[1], James Moran[1], John Frelinger[1], Jeffery Frelinger[2] Bruce Fenton[1] and Edith Lord[1]
[1]University of Rochester, Rochester, NY; [2]University of North Carolina, Chapel Hill, NC

The formation of new blood vessels, a process known as angiogenesis, is essential for tumor growth and thus limiting this process has been proposed as a treatment for tumors. However, blood vessels are also essential for allowing tumor access by chemotherapy drugs and for the infiltration and egress of cells and molecules of the host immune system. Clearly a better understanding of the angiogeneic process within tumors is needed, but study of vessels in tumors is technically challenging. We have developed a new technique using a whole mount histology procedure and C57BL/6 mice that express green fluorescence protein (GFP) under the control of the MHC class I promotor, that greatly simplifies this process. Cells of the C57BL/6 melanoma, B16, a non-GFP expressing, highly tumorigenic and metastatic tumor cell line were transplanted into these mice, which readily allowed visualization of the GFP^+ host derived blood vessels and infiltrating host cells. Staining of the viable tumor pieces with fluorescent conjugated antibodies allowed further characterization of the host cells and the newly formed vessels. To further examine the importance of the angiogenic process, we used tumors that had been genetically modified to express interleukin-12 (IL-12). This pro-inflammatory cytokine, which activates CTL, NK cells, macrophages, and biases toward Th1 cell development, has also been shown to inhibit angiogenesis. The vessel morphology of these B16/IL-12 expressing tumors was greatly altered resulting in smaller, less branching vessels that coincided with slower tumor growth patterns. To understand the mechanism of how IL-12 elicits its anti-angiogenic effects in tumors, we analyzed the expression of the VEGF family of pro-angiogenic molecules and their receptors. These were examined using RT-PCR, and antibody mediated fluorescent whole mount histology to stain surface marker expression on vessels. Although both parental and IL-12 expressing tumors exhibited message for various VEGF molecules, the vascular growth factor receptor, VEGFR-3, was found to be down-regulated only in vasculature within B16/IL-12 suggesting a role in IL-12's anti-angiogenic effects.

Lineage-specific regulation of naïve $CD8^+$ T cell division in lymphopenic hosts

Roslyn A Kemp and Richard W Dutton
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Naïve $CD8^+$ T cells can proliferate in the absence of antigen presentation in lymphopenic, but not intact, hosts, a phenomenon termed homeostasis-driven division. CFSE labeled naïve $CD8^+$ T cells transferred into lymphopenic hosts are capable of multiple divisions; however, cell recovery is low, indicating that the purpose of this proliferation is not to homeostatically restore T cell numbers. The absence of proliferation in intact animals implies active inhibition of naïve donor cells by host immune cells. In accordance with this hypothesis, transfer of naïve polyclonal $CD8^+$ T cells markedly slows division of naïve transgenic CFSE-labeled $CD8^+$ T cells in lymphopenic hosts. Aged naïve $CD8^+$ T cells can inhibit this division equally well as young naïve $CD8^+$ T cells. Interestingly, neither naïve $CD4^+$ T cells nor B cells can inhibit naïve $CD8^+$ T cell division. Effector or rested effector (memory-like) polyclonal $CD8^+$ T cells are also incapable of inhibiting naïve $CD8^+$ T cell proliferation. These data imply that naïve $CD8^+$ T cell proliferation and subsequent homeostasis is regulated in a lineage-specific fashion and is also dependent upon the activation state of the inhibiting cell.

Multiple domains of PKC-ε are necessary for localization during IgG-dependent phagocytosis: role of DAG in membrane translocation

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PKC- is necessary for efficient phagocytosis of IgG-opsonized particles in macrophages. The current studies address the mechanism of PKC- localization. Treatment of GFP PKC- overexpressors with DAG or PKC inhibitors caused translocation from the cytosol to the plasma membrane. That DAG stimulates phagocytosis, while the other drugs inhibit it, suggests that membrane localization is not sufficient for target ingestion. We propose that catalytic activity and, by extension, localization, is necessary for phagocytosis. Confocal microscopy was used to demonstrate that PKC- accumulates on phagosomes with a residence time of ~2 min. Overexpression of the first variable region (V1) or the entire regulatory domain (RD) of PKC- depressed phagocytosis. This effect was not due to a defect in actin polymerization as RD localized to targets and increased target-associated actin polymerization as did full length PKC- . Thus, the regulatory domain localizes the catalytic region to facilitate target ingestion. Truncation and deletion mutants were used identify region(s) of the regulatory domain necessary for localization. Deletion of the C1B region of the DAG binding domain abrogates PKC- localization in response to IgG-opsonized targets. These results suggest that DAG facilitates PKC- accumulation in phagosomes. Additionally, the DAG antagonist 1 hexadecyl-2-acetyl glycerol (EI 150) blocks the accumulation of PKC- in phagosomes. These results support the hypothesis that DAG, binding to the C1B, is responsible for the localization of PKC- to phagosomes. Pharmacological studies demonstrate that inhibition of phospholipase D-derived phosphatidic acid blocks phagocytosis, consistent with a requirement for PLD-derived DAG in uptake. Chimeras of PKC- and PKC- were used to determine if C1B was sufficient for localization. Chimeric PKC- containing the C1B (_C1B) localized to phagosomes. In contrast, PKC- expressing C1B (_C1B) did not accumulate, suggesting that C1B is necessary, but not sufficient, for localization. It further suggests that some other domain of PKC- , possibly V1, contributes to its phagosomal localization. (National Arthritis Foundation and NIH, MRL, Grants from the Ministry of Education, Culture, Sports, Science and Technology in Japan, NS)

Defining Intracellular Signaling Pathways In Murine Dendritic Cells: A Potential Role For The SLP-76 and ADAP Adaptor Proteins

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The dendritic cell (DC) is the only antigen presenting cell (APC) capable of sensitizing naïve T cells and directing the induction of immunological memory. In an inflammatory context, DCs undergo a maturational process in which the cell is transformed from an antigen capturing to an antigen presenting cell. The unique characteristics possessed by DCs have targeted these cells as potential adjuvants for vaccines. Despite recent advancements in our understanding of DC immunobiology, the intracellular signaling pathways responsible for DC development and function remain largely undefined. Knowledge of how these pathways function to control DC activity may be useful in the optimization of DC-based vaccines and may identify possible targets for manipulation of DC function. We have chosen to focus our initial experiments on two hematopoietic adaptor proteins SLP-76 (SH2 domain-containing Leukocyte Protein of 76 kDa) and ADAP (Adhesion and Degranulation promoting Adaptor Protein) for the following reasons: 1) preliminary data generated in our laboratory indicates that murine bone marrow derived DCs (BMDCs) express SLP-76 and ADAP, 2) SLP-76 and ADAP are known to be critical for the development and function of numerous hematopoietic cell types, 3) DCs obtained from patients deficient for the Wiskott-Aldrich protein (WASP), a SLP-76 associated protein display abnormal polarization, motility and F-actin accumulation, and 4) recently, a role for SLP-76 and ADAP in regulating actin polymerization and cytoskeletal re-organization has been described. Many of the changes that accompany DC maturation and the effector functions executed by these cells are dependent on reorganization of the actin cytoskeleton, including dendrite formation, phagocytosis, directed migration and polarization. Furthermore, T cell receptor mediated intracellular signaling pathways involving these adaptor proteins have been well characterized, providing a useful guide for our initial studies. Given that the hematopoietic adaptor proteins SLP-76 and ADAP are necessary constituents of intracellular signaling pathways in multiple hematopoietic cell types and their implication in cytoskeletal reorganization, **we hypothesize that SLP-76 and ADAP adaptor proteins participate in the biochemical signaling pathways that regulate dendritic cell biological activity.**

Evolution of HSP-Elicited Immunomodulation

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Heat shock proteins (hsps) are highly conserved and evolutionarily ancient intracellular proteins that chaperone peptides through the intracellular milieu. Hsps also play a role in host defense by eliciting adaptive immune responses against a variety of potential invaders including tumors, virus and bacteria. It is postulated that hsps, bound to their chaperoned peptides, are released from necrotic cells, taken up by APCs, and re-presented in the context of MHC. Hsps also stimulate innate immunity independent of the chaperoned peptides. The physiological relevance of hsp-mediated innate immune responses in mammals is not yet well understood, but may be more important to "lower" vertebrates that have less well-developed MHC-dependent immunity.

We have chosen an evolutionary approach to explore the role of hsps in immunity using the amphibian, Xenopus. The immune system of adult Xenopus is remarkably similar to mammals; larvae, however, lack expression of MHC class I. Immunization of adult Xenopus with hsps generates peptide-dependent immune responses against alloantigens and tumor-associated antigens. We have also reported that hsps evoke anti-tumor immunity in Xenopus tadpoles in a MHC-unrestricted, peptide-independent manner.

In the present study, we examined cellular and gene responses in Xenopus that are induced by the hsp gp96. In both larvae and adults, mRNA for IL-1 (innate immunity) and MHC class II (adaptive immunity) was upregulated in splenocytes from gp96-immunized animals. Twenty-four hours after exposure to gp96, C3 mRNA was upregulated in liver of adult frogs. NK killing was enhanced in adult Xenopus following immunization with gp96.

Gp96 regulation in response to an extracellular stimulus was also examined. Adult Xenopus B cells stimulated with LPS or heat-killed E. coli upregulate gp96 mRNA as well as intracellular and cell surface gp96 expression. We propose that this modulated gp96 surface expression may trigger an innate immune response in the absence of soluble gp96 release following necrosis. In this way, hsps would serve as a "danger signal" (mediating an immune response to peptides that may not otherwise be immunogenic) and function as a bridge between innate and adaptive immunity.

Validation of a Phenotypic Drug Susceptibility Assay for Influenza Virus Neuraminidase Inhibitors

JJ MCSHARRY ETAL.

BACKGROUND: SOME CURRENTLY USED PHENOTYPIC ASSAYS DO NOT ACCURATELY DETERMINE THE DRUG SUSCEPTIBILITIES OF INFLUENZA VIRUS CLINICAL ISOLATES FOR THE NEURAMINIDASE INHIBITORS. TO DETERMINE IF ANY PHENOTYPIC ASSAY CAN ACCURATELY DETERMINE EC_{50} VALUES FOR INFLUENZA VIRUS CLINICAL ISOLATES, THREE PHENOTYPIC ASSAYS WERE COMPARED FOR THEIR ABILITIES TO DETERMINE THE DRUG SUSCEPTIBILITIES OF A PAIR OF INFLUENZA VIRUS CLINICAL ISOLATES TO THE NEURAMINIDASE INHIBITORS: RWJ-270201, GS4071 AND GG167.

METHODS: VIRUS PAIR: A/TEXAS/36/91 (H1N1) (WT) AND 35-9 A/TEXAS/36/91 (HA:G225N; NA:H274Y) (MUTANT). DRUG SUSCEPTIBILITIES WERE DETERMINED BY FLOW CYTOMETRY (FACS), VIRUS YIELD, AND NEURAMINIDASE INHIBITION (NAI) ASSAYS.

RESULTS: FOR THE DRUG SUSCEPTIBLE ISOLATE, THE FACS ASSAY YIELDED EC_{50} VALUES FOR THESE THREE NEURAMINIDASE INHIBITORS OF 0.001, 0.002, AND 0.005 μM; THE VIRUS YIELD ASSAY GAVE EC_{50} VALUES OF 0.001, 0.002, AND 0.005 μM, AND THE NAI ASSAY GAVE IC_{50} VALUES OF 0.045, 0.255, AND 0.078 nM, RESPECTIVELY. FOR THE DRUG RESISTANT ISOLATE, THE FACS ASSAY GAVE EC_{50} VALUES OF 0.090 (90X), 2.886 (1443X), AND 0.012 (2.4X) μM; THE VIRUS YIELD ASSAY GAVE EC_{50} VALUES OF 0.02 (20X), >2.0 (>1000X), AND 0.009 (1.8X) μM, AND THE NIA GAVE IC_{50} VALUES OF 51.98 (1148X), >1000 (3920X), AND 3.039 (39X) nM, RESPECTIVELY. FOR THE DRUG SUSCEPTIBLE ISOLATE, THE FACS AND THE VIRUS YIELD ASSAYS GAVE SIMILAR EC_{50} VALUES AND THE NAI ASSAY GAVE IC_{50} VALUES THAT WERE 10 TO 100 FOLD LESS FOR THE NEURAMINIDASE INHIBITORS. FOR THE DRUG RESISTANT ISOLATE THE EC_{50} AND IC_{50} VALUES OBTAINED WITH THE THREE ASSAYS WERE MORE VARIABLE.

CONCLUSION: THESE RESULTS SHOW THAT ALL THREE PHENOTYPIC ASSAYS YIELD SIMILAR RESULTS AND DISTINGUISH BETWEEN A DRUG SUSCEPTIBLE AND A DRUG RESISTANT INFLUENZA VIRUS CLINICAL ISOLATES. THE FACS ASSAY AND NAI ASSAY ARE RAPID AND QUANTITATIVE. HOWEVER, ONLY THE FACS AND VIRUS YIELD ASSAYS CAN DETECT MUTATIONS THAT LEAD TO DRUG RESISTANCE IN BOTH THE HA AND NA GENES. THE NAI ASSAY CAN NOT DETECT INFLUENZA VIRUSES WITH MUTATIONS IN THE HA GENE THAT MAY LEAD TO RESISTANCE TO NEURAMINIDASE INHIBITORS. THE FACS ASSAY IS MORE RAPID AND LESS LABOR INTENSIVE THAN THE VIRUS YIELD ASSAY. THESE RESULTS SHOW THAT THE FACS ASSAY MAY BE SUPERIOR TO THE NAI ASSAY FOR DETERMINING SUSCEPTIBILITIES TO OF INFLUENZA VIRUSES TO THE NEURAMINIDASE INHIBITORS.

Lymphotoxin- -Deficient Mice make CD8 T Cell Mediated Responses in the absence of organized lymphoid tissue.

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Lymphotoxin -/- (LT-/-) mice are thought to be unable to generate effective T and B cell responses. This is attributed to the lack of lymph nodes and the disrupted splenic architecture of these mice. However, despite these defects we found that LT -/- mice and normal wild-type mice infected with influenza A generated similar numbers of influenza-specific CD8 T cells that were able to produce IFN- and kill target cells presenting influenza peptides. Thus, although CD8 immune response was delayed in LT -/- mice by 2–3 days, the cellular and humoral immune response was sufficient to mediate viral clearance in LT -/- mice that were infected with relatively low doses of influenza virus. In addition, we examined another CD8 T cell mediated response, Delayed Type Hypersensitivity (DTH), and found that the LT -/- mice produce a significantly lower amount of IFN- mRNA and the peak response was delayed 4 days compared with wild type mice. These results demonstrate that neither LT nor constitutively organized lymphoid tissues are absolutely required for the generation of effective immunity against the respiratory virus influenza A. However LT , the presence of lymph nodes and a fully organized spleen is needed to mount a DTH reaction.

A Pivotal Role for IFN-γ in Infection Induced Modulation of Experimental Autoimmune Encephalomyelitis

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Establishment of a systemic inflammatory response by prior infection with *Mycobacterium bovis* BCG ameliorates the clinical course of passively transferred EAE in WT C57/B6 mice. This counter-intuitive observation is a manifestation of the long-recognized, yet poorly understood, phenomenon known as "adjuvant immunotherapy". Intriguingly in the absence of IFN− infection significantly exacerbates disease and is associated with an uncontrolled accumulation of CD4+ T cells in the CNS. IFN− is essential to the induction of anti-microbial effector function during BCG infection and regulates the survival of encephalitogenic T cells in the target organ in EAE. These observations have led us to develop a unifying hypothesis wherein the mechanisms employed to arrest bacterial growth also regulate CD4+T cell expansion during infection and limit the development of concurrent responses. Furthermore we propose that this mechanism acts in an Ag-non-specific, activation-dependent fashion. We have tested this hypothesis by tracking the fate of encephalitogenic T cells both in the periphery and the target organ following transfer to infected and uninfected hosts.

Treatment with exogenous IL-12 protects against lethal coxsackievirus B4 infection

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Coxsackievirus infection has been linked to the induction of immunopathology and autoimmune disease. Our lab has developed a murine model of coxsackievirus B4-induced disease consisting of a virulent variant, CB4-V. CB4-V infection causes a severe pancreatitis characterized by inflammation of the exocrine pancreas and acinar cell necrosis that progresses to chronic disease. A previous study suggests that the immune response to CB4-V infection exacerbates disease. CD4 knockout mice survived lethal infection with CB4-V, while wild type BALB/c mice succumbed to infection. This result suggests that modulation of the immune response could alter the outcome of disease. We investigated whether interleukin 12 (IL-12) treatment could modulate the outcome of CB4-V infection. IL-12 treatment protected BALB/c mice from lethal infection with CB4-V. Treatment with IL-12 also protected the exocrine pancreas of CB4-V-infected mice from total destruction. The beneficial effects of IL-12 treatment were not due to decreased viral replication. This result suggests that IL-12 treatment reduces immune-mediated tissue damage associated with CB4-V infection thereby decreasing disease severity. We next examined whether IL-12 treatment altered the immune response to CB4-V infection. Administration of IL-12 increased endogenous interferon-gamma (IFN) production during CB4-V infection. IFN was necessary for IL-12-mediated protection from lethal CB4-V infection. IFN knockout mice succumbed to lethal CB4-V infection despite IL-12 treatment. In addition, treatment with exogenous IFN protected BALB/c mice from lethal infection with CB4-V. Therefore, IL-12-mediated protection of BALB/c mice from lethal CB4-V infection is dependent on the production of IFN . We hypothesize that IFN induced by IL-12 treatment alters the $CD4^+$ T cell response to CB4-V infection leading to a reduction of immunopathology.

The activation and migration of CD8 T-lymphocytes during influenza infection

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This study shows the differentiation, migration and proliferation of naive transgenic CD8+ T-lymphocytes during a primary influenza infection. Upon adoptive transfer of CFSE labeled naïve, influenza HA peptide specific, TcR transgenic T-cells into naïve recipients followed by influenza infection, CD8 T-cells gather initially in the mediastinal lymph nodes where activation and proliferation of these cells can be visualized as soon as four days post infection. CD8 cells begin to enter the airways five days post infection and these cells express CD25, CD44, low levels of CD62L and have lost CFSE indicating a number of cell divisions. The CD8 T-cells that enter the lung, airways and DLN can be stimulated ex-vivo to express IFN and TNF . The peak of the response in terms of CD8 T-cell numbers occurs at day 7-9 post infection and after this the numbers of T-cells in all organs decline. We believe this to be an ideal model to study CD8 T-cell activation after infection with a locally proliferating virus.

Potential Mechanisms for Elimination of Auto-reactive B cells

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Receptors for the constant region (Fc) of IgG antibodies (Fc R) are present on a variety of immune cells. Three subtypes of Fc R, Fc RI, Fc RII, and Fc RIII, have been identified in mice and humans. Fc receptors mediate functions such as endocytosis, phagocytosis, respiratory burst, Ag presentation, the negative regulation of B cells, and Antibody Dependent Cell-Mediated Cytotoxicity (ADCC). Fc RIIB is unique among the Fc receptors in its ability to inhibit B cell activation when antigen/antibody (Ag/Ab) complexes cross-link the B cell receptor for antigen (BCR) with Fc RIIB. Alternatively, if the Fc portion of Ab bound to the BCR does not bind to Fc RIIB, it can bind to Fc R on nearby cytotoxic cells such as neutrophils, NK cell, or macrophages. Recognition of B cells by such effector cells could then result in elimination of the sam B cells through ADCC. Thus, we have attempted to develop an Ag/Ab complex analogue that can mediate inhibition and/or elimination of B cells, in particular those that produce auto-antibodies. The components of this Ag/Ab complex analogue consist of a biotin-binding targeting element (IgG3-Av) and biotinylated functional elements, Protein L (B-PL) and biotinylated BSA derivatized phosphorylcholine (PC-BSA-B). We hypothesize that our immune complex analogue of IgG3-PL will inhibit human B cells by simultaneously binding the BCR and Fc RII, or eliminate them via ADCC. We demonstrate that IgG3-Av can be used to mediate functions associated with Fc receptors specifically, phagocytosis, inhibition of B cell activation and ADCC. Inhibition of B cell activation was examined by monitoring calcium flux and ^{3H}-thymidine incorporation. Using the B cell line Daudi, altered calcium fluxes by IgG3-PL coated beads correlated with reduced proliferation. Furthermore, inhibition was not as great in the presence of antibody that blocks Fc RII. ADCC mediated by IgG3-Av was examined by measuring lactate dehydrogenase release by target cells derivatized with biotin. Fifteen to twenty percent killing was observed in the presence of IgG3-Av. This killing was reduced by 65% when free biotin, which should block the interaction between IgG3-Av and B-PL, was added to the assay. The above studies demonstrate that B cells can be inhibited and/or eliminated using the IgG3-Av immune complex analogue.

We further hypothesized that specific targeting of auto-reactive B cells using the IgG3-Av immune complex analogue will depend on it being monomeric. However, HPLC data from our IgG3-Av construct showed it is not. Thus, we sought to compare the function of multimeric and monomeric Ag/Ab complexes (complex analogues). To accomplish this, functional studies are being conducted using the A20μWT cell line, which expresses surface IgM that is specific for PC. We have measured inhibition of calcium flux using multimeric anti-biotin/PC-BSA-B complexes and anti-idiotype antibody (TC54). Use of TC54 allows us to mimic a monomeric Ag/Ab complex analogue. The most striking result occurred when treating the cells with TC54. There was a complete lack of a calcium flux in A20μWT cells in the presence of TC54. However, a response was observed when cells were incubated with blocking antibody to Fc RII prior to TC54 addition. These results support the idea that a monomeric Ag/Ab complex analogue will have the capacity to inhibit B cell activation. This could be therapeutically important by permitting specific targeting of allergic or auto-immune B cells.

Mycobacterial lipids induce a granulomatous response involving the recruitment of IFN-gamma-producing T cells

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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 also destroys infected tissues. 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 lipids. Cell wall lipids are delivered via polystyrene beads along with bone marrow-derived macrophages in an ECM gel into mice i.p. The matrix and peritoneal exudate, as well as draining lymph nodes and spleen, are retrieved at different times for analyses. Histology revealed a granulomatous pattern of leukocyte recruitment into the matrices. Early influx of granulocytes gave way to a prevalence of monocytes, epithelioid macrophages and lymphocytes by 4 days and sequelae indicative of chronic mycobacterial lesions within 12 days. FACS immunophenotyping confirmed the early recruitment of neutrophils and ensuing arrival of macrophages, dendritic cells, and T cells (TCR $^{+}$ and TCR $^{+}$). Whereas matrices containing phosphoglycerol-coated beads or no lipid tended to float in the peritoneal cavity, matrices containing BCG lipids tended to adhere to fatty tissue in the peritoneum within 7 days. Adherent matrices were vascularized, and leukocytes arrived from the blood, resulting in granulomas that were more cellular and fibrotic. Stimulation of T cells with PMA and ionomycin revealed that a higher percentage of TCR + and TCR + T cells in the granuloma could produce IFN , TNF and IL-4 than T cells in the peritoneal exudate or draining lymph nodes. Taken together, the data from this model show that BCG lipids exert an adjuvant effect, which serves to recruit neutrophils and macrophages as well as creating a cytokine microenvironment, which promotes vascularization and T cell activation.

Specific mucosal immunity and enhanced sinus clearance of nontypeable *Haemophilus influenzae* after intranasal immunization with outer membrane protein P6 and cholera toxin.

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Background: Sinusitis is one of the most common infectious diseases. Nontypeable *Haemophilus infuenzae* (NTHi) is a major pathogen of acute sinusitis and this bacterium is often isolated from sinus effusions. NTHi is increasingly showing antibiotic resistance, and frequent recurrence of acute episodes of sinusitis in children may worsen the course of asthma and other upper respiratory tract diseases. The development of an effective vaccine against acute recurrent sinusitis in sinusitis-prone children is desirable, and the goal of our immunological research on the sinus is to establish a vaccine. Mucosal immunization was shown to induce protective immunity against the spectrum of respiratory diseases caused by NTHi, however, no previous studies investigated the effect of vaccination in sinus mucosa (SM). Recent efforts to develop an effective vaccine candidate against NTHi have focused on P6, an outer membrane protein of NTHi and a common antigen to all strains. In this study, we investigated the potential of intranasal and systemic routes of immunization with P6 and cholera toxin (CT) for induction of mucosal immunity and for enhancing the clearance of NTHi from the maxillary sinuses in a rat model.

Methods: Wistar rats were immunized intranasally (unilaterally) or intraperitoneally with P6 and CT 5 times, on days 0, 7, 14, 21 and 28 and were killed on day 35. Another group of mice was immunized with CT alone as a control. We established a method for isolating lymphocytes from the SM and analyzed the numbers of total or P6-specific antibody-producing cells in SM, as well as in the nasal mucosa (NM) and nasal-accociated lymphoid tissue (NALT) by means of ELISPOT assay. P6-specific antibody titers were determined in sinus wash, nasal wash and serum samples by means of ELISA assay. Histological sections were stained for IgA-, IgG- and IgM-positive cells. We developed a sinus model of NTHi clearance in rats: 7 days after the final immunization a suspension of live NTHi was injected into the sinus and sinus washes were collected 12h and 24h after the inoculation.

Results: By using the isotype-specific ELISPOT assay, we found many antibody-producing cells in SM. The dominant isotype was IgA, followed by IgG- and IgM-producing cells. The P6-specific antibody-producing cells in rats intranasally immunized with P6 and CT were increased in the SM. The sinus and nasal antibody-producing cells comprised essentially IgG- and IgA-producing cells, whereas only few P6-specific IgM-producing cells could be detected. After intranasal immunization there were fewer antibody-producing cells in NALT, which is considered a mucosal inductive site, than in SM or NM. Intranasal immunization with P6 and CT induced P6-specific mucosal IgG and IgA Ab responses in sinus washes, as well as in nasal washes. In contrast, P6-specific mucosal Ab responses were not detected after intranasal immunization with CT alone, or after intraperitoneal immunization. The magnitude of P6-specific responses was similar in both right and left sinuses. A similar magnitude and isotype pattern of immune responses was found both in the maxillary sinus and in the nasal cavity. In serum, significant levels of P6-specific IgG and IgM Ab responses with similar magnitude were induced after intranasal and intraperitoneal immunization with P6 and CT, but not with CT alone; however, P6-specific IgA Ab responses were not detected. Immunohistochemistry showed the number of IgA-, IgG- and IgM-positive cells in the SM, as well as in the NM, of intranasally immunized with P6 and CT rats. Intranasal immunization with P6 and CT enhanced the ability to clear NTHi infection from the bilateral sinuses. At 12 hours postchallenge, the number of NTHi in the left sinus was

88.4% smaller than that of the controls, and the number of NTHi in the right sinus was 86.5% smaller than that of the controls. Animals intranasally immunized with P6 and CT cleared all live NTHi from the SW by 24 h, while the control rats still retained 10^2-10^3 bacteria at that time. However, the enhanced clearance of NTHi from the sinus was not observed in CT-immunized rats. The number of NTHi in the rats immunized intraperitoneally with P6 and CT did not differ significantly from control animals at any point in time.

Conclusions: Intranasal immunization with P6 and CT induces P6-specific sinus mucosal and systemic responses and enhances clearance of NTHi from the maxillary sinus. Systemic administration of the vaccine affected neither SM immunity nor sinus clearance of NTHi. These findings suggest that intranasal immunization might be a useful strategy and more effective than systemic immunization in preventing acute recurrent sinusitis. Moreover, bilateral immune responses could be induced in the sinus by unilateral intranasal immunization.

GM1 lipid rafts function as platforms for antigen processing and presentation independent of the BCR

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Plasma membrane lipid rafts form membrane-signaling regions that are essential for the function of a number of receptors including the BCR. However, the role of lipid rafts in antigen processing and presentation and its relationship to presentation of Ags by the BCR is still not fully elucidated. Compared with presentation by fluid phase endocytosis, we demonstrated that GM1 ganglioside, a major component of rafts, play an important role in processing and presentation of bound ligands. Thus, either native Enterotoxin B subunits or their antigen conjugates bound with high affinity to GM1 on surface of B cells, were targeted to class II-rich compartments, and stimulated antigen specific proliferative and cytokine T cell responses. Non-binding genetic mutant of the toxin and its conjugates were significantly less efficient. We further demonstrated that GM1 bound ligands did not augment nor decreased subsequent kinetics or magnitude of processing and presentation of either BCR-internalized antigen or soluble antigen. Similarly, cross-linking of the BCR had no noticeable effects on presentation of GM1 bound ligand. Moreover, by comparison to the rapid internalization of Ag bound to the BCR, GM1 bound Ag formed a plasma membrane depot detectable for many hours (half life 12 hrs) resulting in significantly delayed processing and presentation. We conclude that GM1-containing rafts function as platforms for antigen processing and presentation by a mechanism independent of the BCR (work published in Int. Immunol. 2001,13:541 and Immunology, 2002, 106: 60).

Immunological Synapse Formation in Memory CD4 T Lymphocytes

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The region of contact between a T cell and an antigen-presenting cell is known as the immunological synapse. The immunological synapse is assembled into spatially segregated supramolecular activation clusters (SMACs). While its complete function is still unknown, the immunological synapse is formed during interactions which ultimately lead to activating CD4 or CD8 T cells or thymic selection. Membrane microdomains, known as lipid rafts, accumulate in the synapse but their exact role in shaping the immune response is still unknown. Whether immunological synapse formation or lipid raft composition is similar between naive and memory T cells is unclear. In the present study we examined immunological synapse formation when naive and memory CD4 T cells undergo antigen-specific cell activation. We studied the intracellular signaling in SMACs and lipid raft composition by immunofluorescence microscopic localization of a number of cell surface and/or signal transduction molecules. We observed a more rapid formation of immunological synapses in memory as compared to naive cells. We also found that the mature synapse was sustained slightly better in memory cells. Examination of lipid rafts showed that CD45 was tightly associated with the TCR within lipid rafts of memory cells but that in naive cells, CD45 was segregated from the lipid rafts while the TCR became associated with lipid rafts only upon cell activation. We propose that the enhanced response of memory cells could be due to a pre-organized immunological synapse where maturation is expedited and critical activation components are localized and recruited to lipid rafts.

Immune Responses to Human Papillomavirus Antigens in HLA Transgenic Mice

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The association of disease susceptibility with specific HLA alleles has been the subject of extensive investigation over the past two decades. Most of the disease associations have been documented with HLA class II genes.

While it is accepted that the development of cervical cancer (following infection with a high-risk Human Papillomavirus (HPV) type) is a multifactorial process, the potential association of specific HLA alleles with disease susceptibility is an important question. In both Hispanic and Caucasian populations, it was shown that HLA-DRB1*04 women infected with HPV have over a two fold increased risk for developing cervical carcinoma.

Our strategy involves the use of an HLA-DRB1*0401 transgenic mouse in conjunction with an immunization method that is designed to facilitate the entry of HPV tumor associated antigen, E7, into the MHC class II processing pathway.

The HLA-DRB1*0401 transgenic mouse is a knock-out for the murine class II IA gene which prevents surface expression of IA. The mouse is a knock-in for human HLA-DRB1*0401and the endogenous mouse IE chain pairs with the DR4 chain to form a functional class II molecule. Amino acids 110 and 139 of the DR4 chain were altered in order to allow for interaction with murine CD4. Prior work performed with this transgenic mouse has clearly established that the HLA class II restricted CD4+ T cells recognize the same peptides as human T cells. The use of transgenic mice expressing a human HLA will allow us to better understand the immune response to HPV proteins and to evaluate the effectiveness of vaccination strategies in preventing (prophylactic) or curing (therapeutic) the growth of HPV E7 expressing tumors.

A study of immune responses to the outer membrane protein P6 of nontypeable *Haemophilus influenzae* following intranasal or systemic delivery

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Nontypeable *Haemophilus influenzae* (NTHI) is a common pathogen that causes repeated cases of otitis media in children, as well as high morbidity in patients with chronic obstructive pulmonary disease (COPD). The outer membrane protein P6 of NTHI can induce an effective systemic immune response when delivered intraperitoneally. In order to elicit both mucosal and systemic immune responses, we are now evaluating intranasal delivery of antigen in conjunction with different mucosal adjuvants. We are comparing the strength of an immune response, along with subclass of antibody produced, when P6 is delivered intranasally in combination with the mucosal adjuvants cholera toxin (CT), heat-labile enterotoxin (LT) IIa, or LTIIb. Preliminary data indicates that intranasal immunization with P6 mixed with CT, LTIIa, or LTIIb results in high titers of P6 specific serum antibody, along with high levels of sIgA. Immunization with P6 and CT, LTIIa, or LTIIb produces a similar immunoglobulin subclass distribution, with small variations. In addition to evaluating the humoral immune response to P6, we are also evaluating T cell responses. By using overlapping synthetic peptides that span the full sequence of the P6 protein, we have determined the identity of a potential immunodominant T cell epitope.

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