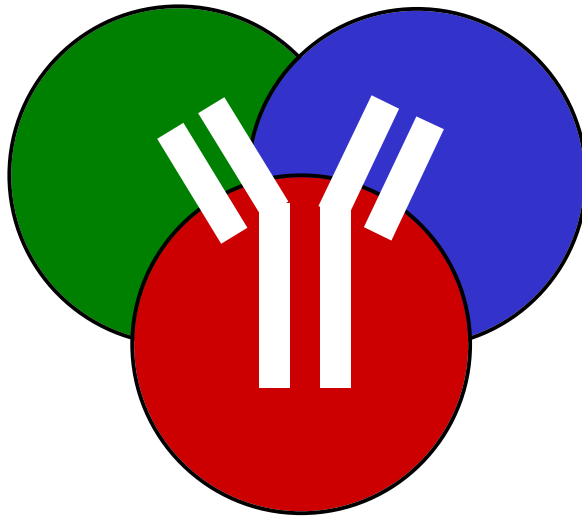


# **The Fourth Upstate New York Immunology Conference**



**November 4-6, 2001**

**The Sagamore  
at Bolton Landing, New York**

November 4, 2001

Dear faculty, students, and visitors:

Welcome to the fourth Upstate New York Immunology Conference. This is an annual conference, begun in 1998, dedicated to building relationships among immunology researchers from upstate New York. Previous year's meetings have involved the Wadsworth Center, Albany Medical College, The Trudeau Institute, SUNY Upstate Medical University, the University of Rochester, and Cornell University. We are pleased that you are here to share your research with your fellow researchers.

We are also happy to welcome three excellent keynote speakers to this year's conference, Dr. Peter Cresswell and Dr. Margaret Hostetter, both from Yale University, and Dr. Frederick Alt from The Howard Hughes Medical Institute, Children's Hospital. Dr. Cresswell will speak on "Redox Reactions in Antigen Processing" and Dr. Hostetter will speak on "Pathogenesis of *Candida albicans* Fungemia: Interactions with the Immune System". The title of Dr. Alt's talk is "Interplay Between Non-homologous End Joining and Cell Cycle Checkpoint Pathways in Normal Development and Tumor Suppression". In addition, representatives from each of the participating institutions have been asked to solicit participation from researchers at their institutions.

We hope you will enjoy this meeting, and, as a result, continue to attend future meetings.

Sincerest thanks,

The UNYIC Organizing Committee

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Gary Winslow, The Wadsworth Center (gary.winslow@wadsworth.org)  
Jim Drake, The Trudeau Institute (jdrake@trudeauinstitute.org)

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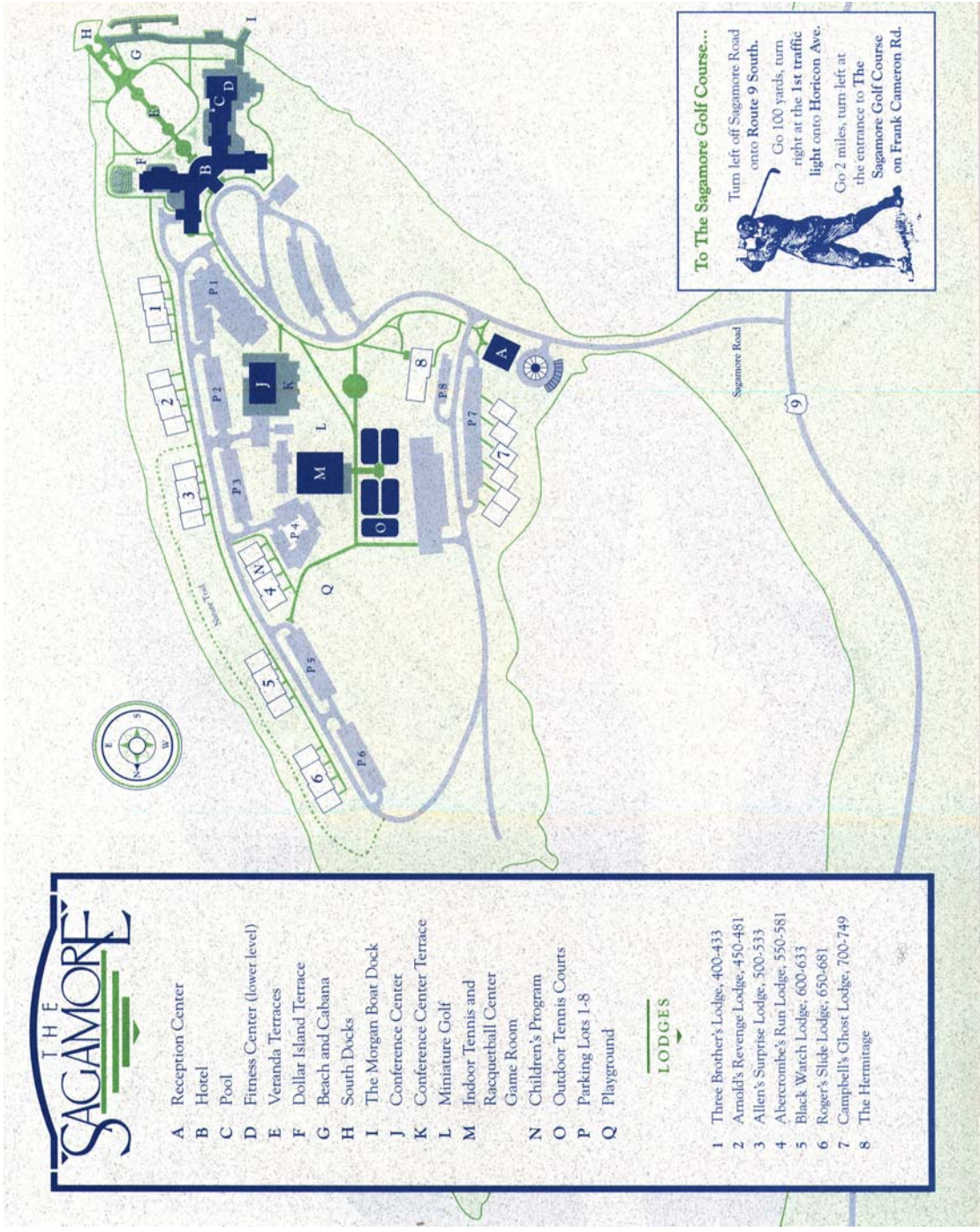
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# **Acknowledgements**

We would like to thank our sponsors for their generous financial support. We would also like to thank Wendy Cragan for administrative assistance, Richard Filkins for handling financial issues, and Eric Yager for organizing the Web page.



**To The Sagamore Golf Course...**

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 Go 100 yards, turn right at the 1st traffic light onto Horicon Ave.  
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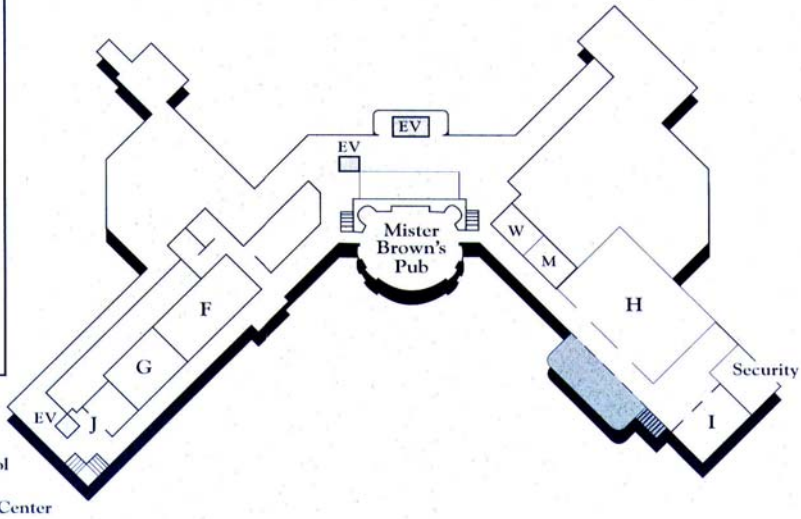
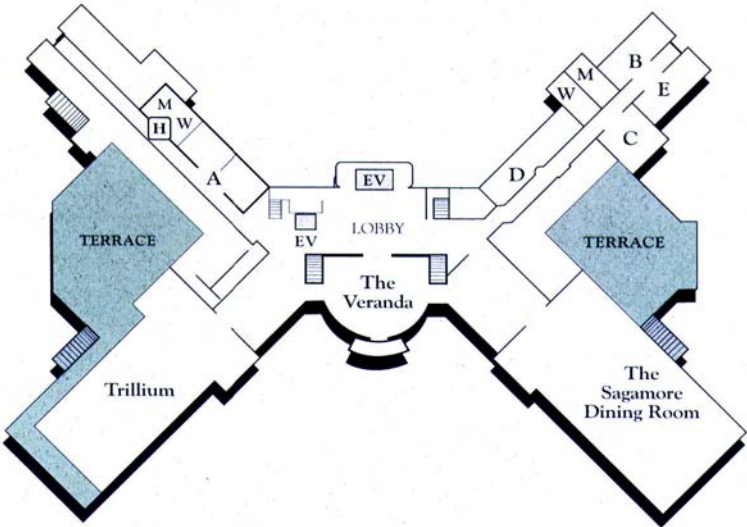
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- B Hotel
- C Pool
- D Fitness Center (lower level)
- E Veranda Terraces
- F Dollar Island Terrace
- G Beach and Cabana
- H South Docks
- I The Morgan Boat Dock
- J Conference Center
- K Conference Center Terrace
- L Miniature Golf
- M Indoor Tennis and Racquetball Center
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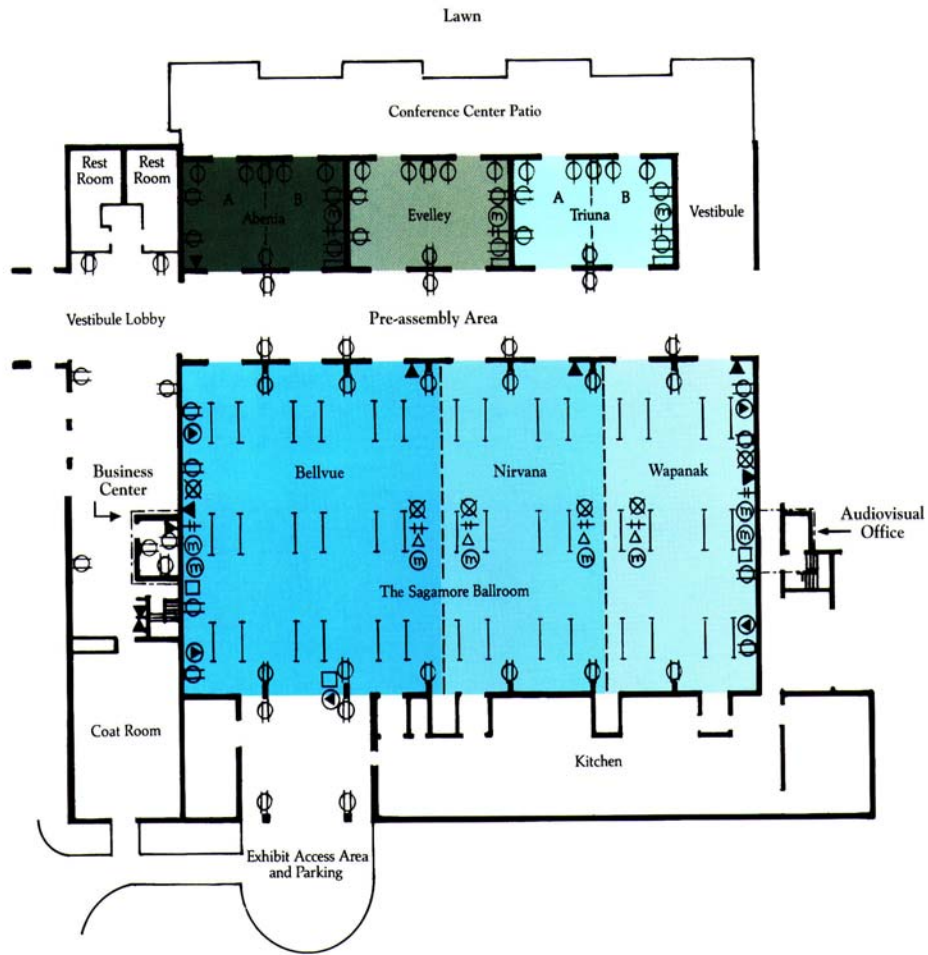
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- 2 Arnold's Revenge Lodge, 450-481
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    - The Sagamore Dining Room
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    - A Crown & Dome Island Rooms
    - B Adirondack Room
    - C Empire Room
  - SHOPS
    - D The Emporium
    - E Hairdresser
- LOWER LEVEL**
- RESTAURANTS
    - Mister Brown's Pub
  - ACTIVITIES
    - F Women's Health Spa
    - G Men's Health Spa
    - Fitness Center & Pool
  - MEETING ROOMS
    - H Dollar Island Rooms
    - I Diamond Island Room
  - SHOPS
    - J Spa Boutique
- M Men's Room  
 W Women's Room  
 H Handicapped Rest Rooms



To Pool and Fitness Center



CONFERENCE CENTER

# Schedule

## *SUNDAY, NOVEMBER 4, 2001*

3:00 – 5:00 P.M.	REGISTRATION	Conference Center
4:00 – 5:00 P.M.	ROOM ASSIGNMENTS	Reception Center
5:00 – 6:30 P.M.	DINNER	Wapanak, Conf. Ctr.
6:30 – 7:30 P.M.	KEYNOTE SPEAKER <b>Peter Cresswell</b> (Yale) – “Redox Reactions in Antigen Processing”	Wapanak
7:30 – 8:30 P.M.	Becton-Dickinson Cytometric Bead Array Assays: A Novel System for Flow Cytometric Analysis	Wapanak
8:00– 10:00 P.M.	VENDOR FAIR AND MIXER (Poster Set-up, Triuna A & B)	Nirvana, Conf. Ctr.

## *MONDAY, NOVEMBER 5, 2001*

7:00 – 8:00 A.M.	BREAKFAST	Sagamore Dining Room
8:00 A.M.– 12:00 P.M.	SESSION I: IMMUNE REGULATION I  Moderator: James Drake	Wapanak
8:00-8:30 A.M.	Troy Randall (T) – “Are lymph nodes required for immune responses?”	
8:30-9:00 A.M.	Nick Crispe (R) – “The role of the liver in systemic immunity”	
9:00-9:30 A.M.	Allen Silverstone (S) – “How dioxins and estrogen affect immune development and function”	

9:30-10:00 A.M.	Break	Foyer, Wapanak
10:00-10:30 A.M.	Jacques Robert (R) – “Are heat shock proteins an evolutionary bridge between innate and adaptive immunity?”	
10:30-11:00 A.M.	Jing-Ren Zhang (A) – “Molecular interaction of poly-Ig receptor with microbial pathogens”	
11:00-11:30 A.M.	Paul Massa (S) – “Specific regulation of MHC I genes in the central nervous system”	
11:45 A.M. – 1:00 P.M.	LUNCH	Nirvana
1:00 – 2:00 P.M.	KEYNOTE SPEAKER <b>Margaret Hostetter</b> (Yale) – “Pathogenesis of <i>Candida albicans</i> Fungemia: Interactions with the Immune System”	Wapanak
2:15 – 5:00 P.M.	SESSION II: IMMUNITY AND INFECTION  Moderator: Timothy Sellati	Wapanak
2:15-2:45 P.M.	Marcia Blackman (T) – “Immune control of gamma-herpesvirus latency”	
2:45-3:15 P.M.	Ed Barker (S) – “Role of NK cells in HIV pathogenesis”	
3:15-3:30 P.M.	Diana Albu (A) – “Induction of a mucosal antibody immune response to HIV-1 gp120”	

3:30-3:45 P.M.	Break	Foyer, Wapanak
3:45-4:15 P.M.	Matt Florczyk (W) – “Genetic response of mycobacteria to intracellular growth within macrophages and low oxygen tension”	
4:15-4:45 P.M.	Tim Sellati (A) – “The role of toll-like Receptor 2 (TLR2) in the immunopathogenesis of Lyme disease”	
4:45-5:00 P.M.	Julia Li (W) – “Properties of antibodies effective during host defense against an obligate intracellular bacterium”	
5:30 – 7:00 P.M.	DINNER	Nirvana
7:00 – 8:00 P.M.	KEYNOTE SPEAKER <b>Fred Alt</b> (Harvard) – “Interplay Between Non-homologous End Joining and Cell Cycle Checkpoint Pathways in Normal Development and Tumor Suppression”	Wapanak
8:00 – 10:00 P.M.	POSTER SESSION AND MIXER	Triuna A & B, Conference Center

***TUESDAY, NOVEMBER 6, 2001***

7:00 A.M. – 12:00 P.M.	CHECKOUT (\$75 penalty after 12 Noon)	Reception Center
7:00 – 8:00 A.M.	BREAKFAST	Sagamore D. R.
8:00 – 11:45 P.M.	SESSION III: IMMUNE REGULATION II  Moderator: Gary Winslow	Wapanak
8:00-8:30 A.M.	Mark Dobrzanski (T) – “Therapeutic effects of tumor-reactive type 1 and type 2 CD8 T cell subpopulations in established pulmonary metastases”	

8:30-9:00 A.M.	Andres Melendez (A) – “Redox-dependent regulation of IL-1 alpha and metastasis”	
9:00-9:30 A.M.	David Topham (R) – “Expression and function of collagen-binding integrins on memory CD8 T cells”	
9:30-10:00 A.M.	Break	Foyer, Wapanak
10:00-10:30 A.M.	Bill Lee (W) – “Activation and anergy in murine CD4 memory T cells”	
10:30-11:00 A.M.	Andras Perl (S) – “Metabolic control of apoptosis in the immune system”	
11:00-11:15 A.M.	Jen Smith-Nyland (S) – “Mechanisms involved in idiotype-peptide induced protection from lupus nephritis”	
11:15-11:30 A.M.	Michael Turner (R) – “Exploiting cross-presentation for the characterization of T cell epitopes”	
11:30 –11:45 A.M.	BUSINESS MEETING	Wapanak
11:45 A.M. – 1:00 P.M.	LUNCH	Sagamore Dining Room
1:00 P.M.	DEPARTURE	

- (A) - Albany Medical Center
- (S) - SUNY Upstate Medical Center
- (T) - The Trudeau Institute
- (W) - The Wadsworth Center

# Keynote Speakers

## **Dr. Peter Cresswell:**

Dr. Cresswell is professor of Immunobiology and investigator for the Howard Hughes Medical Institute, Yale University School of Medicine.

The major interest of Dr. Cresswell's laboratory is in the mechanisms regulating antigen processing. MHC class I molecules in the endoplasmic reticulum (ER) bind peptides translocated from the cytosol by the Transporters associated with Antigen Processing (TAP). The assembly of a class I- $\beta$ 2 microglobulin dimer involves two chaperones, calnexin and calreticulin, and the thiol oxido-reductase, ERp57. Calreticulin- and ERp57-associated class I molecules physically associate with TAP molecules, with an MHC encoded glycoprotein, tapasin, serving as a bridge. The functions of this complex are currently under investigation.

MHC class II molecules form a nine chain complex in the ER, with three alpha-beta dimers associated with a trimer of the invariant chain. This complex is targeted to the endosomal pathway where the invariant chain is degraded. A residual invariant chain fragment is catalytically eliminated by an MHC-encoded glycoprotein, HLA-DM, liberating the peptide binding site of the class II molecule. The role of a gamma interferon-inducible lysosomal thiol reductase (GILT) in peptide generation is being investigated. Other work in the laboratory centers on the role of tetraspanins in MHC function and on antiviral mechanisms of proteins inducible by Type 1 and Type 2 interferons.

## **References:**

Phan, U.T., Arunachalam, B., Cresswell, P. Gamma-interferon-inducible lysosomal thiol reductase (GILT): Maturation, activity and mechanism of action. *J. Biol. Chem.* 275(34):25907-25914, 2000.

Cannon, K.S. and Cresswell, P. Quality control of transmembrane domain assembly in the tetraspanin CD82. *EMBO J.* 20(10):2443-2453, 2001.

## **Dr. Margaret Hostetter:**

Dr. Hostetter is professor of Pediatrics and Microbial Pathogenesis and director of Yale Child Health Research Center (Microbiology). She received her B.A. at Denison University in 1970, and M.D. at Baylor College of Medicine in 1975.

The focus of the Hostetter laboratory is to understand how pathogenic organisms evade host defenses to cause infection and injury. *Streptococcus pneumoniae*, the leading cause of death from respiratory infections the world over, encodes surface proteins that bind or degrade the third component of complement, the primary opsonin for pneumococci in lung and blood. These novel proteinases, unlike any others in the database, are highly conserved in pneumococcal serotypes and protective in a variety of animal models of pneumococcal infection. The yeast *Candida albicans* is the most frequent cause of life-threatening fungal infection in patients with handicapped immune systems: premature newborns, post-operative surgical patients, people with HIV infection, and neutropenic cancer or transplant patients. The gene INT1, identified in her laboratory, is unique to *Candida albicans* and is the first gene reported to link epithelial attachment, formation of invasive hyphae, and virulence. T lymphocytes are the target of Int1p's effects. Molecular screens have been devised to identify candidal proteins that augment the virulence of Int1p in blood, kidney, and intestinal tract.

### **References:**

Hostetter MK. Integrin-like proteins in *Candida* spp. and other microorganisms. *Fungal Genetics and Biology* 28:135-145, 1999.

Madsen M, Lebenthal Y, Cheng Q, Smith BL, and Hostetter MK. A pneumococcal protein that elicits IL-8 from pulmonary epithelial cells. *J. Infect. Dis.* 181:1330-1336, 2000.

Zhang Y, Masi AW, Barniak V, Mountzouros K, Hostetter MK, Green BA. Recombinant PhpA protein, a unique histidine-motif containing protein from *Streptococcus pneumoniae*, protects mice against intranasal pneumococcal challenge. *Infect. Immun.* in press, February 2001.

## **Dr. Frederick Alt:**

Dr. Alt is Charles A. Janeway Professor of Pediatrics at the Children's Hospital, Boston, and Professor of Genetics at Harvard Medical School, as well as Senior Investigator at the Center for Blood Research at Harvard Medical School. He obtained his undergraduate degree in biology from Brandeis University and his Ph.D. degree in biological sciences from Stanford University,

Dr. Alt is interested in the molecular mechanisms involved in the rearrangement and expression of antigen receptor genes. He is also interested in elucidating potential roles of proteins involved in antigen receptor gene rearrangement, particularly their influence on genomic stability and cancer.

The ability of the immune system to respond specifically to a vast array of antigens relies on genetic (DNA) rearrangements that create new genes during the development of B and T lymphocytes. To date, lymphocytes are the only known mammalian cells that undergo specific gene rearrangements. His lab is working to elucidate mechanisms involved in the de novo assembly of these genes. In addition, they are attempting to determine potential roles of proteins involved in lymphocyte gene rearrangement in other processes, including maintenance of genome stability in various cell types.

### **References:**

Sekiguchi J, Ferguson DO, Chen HT, Yang EM, Earle J, Frank K, Whitlow S, Gu Y, Xu Y, Nussenzweig A, Alt FW. Genetic interactions between ATM and the nonhomologous end-joining factors in genomic stability and development. *Proc Natl Acad Sci U S A*. 2001 Mar 13;98(6):3243-3248.

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Gartner F, Alt FW, Monroe RJ, Seidl KJ. Antigen-independent appearance of recombination activating gene (RAG)-positive bone marrow B cells in the spleens of immunized mice. *J Exp Med*. 2000 Dec 18;192(12):1745-54.

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*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 in the Human Resources Department or Student Affairs.*

**POSTER**

**ABSTRACTS**

**Poster No. 1**

**Manipulation of T Cell Activation for the Immunotherapy of Prostate Cancer**

Michael Anderson, Jennifer Herzog, and Arthur Hurwitz  
SUNY/Upstate

Tumors are notorious for escaping the immune system. This may be due to tolerance to self-Ags expressed by the tumor or the inability of tumors to provide a costimulatory signal and thus induce clonal anergy of potentially tumor-reactive T cells. We hypothesize that the activation of tumor-specific CD4<sup>+</sup> T cells may be a critical step in generating an effective anti-tumor response. To test this hypothesis, we will utilize the Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model, which carries the SV40 T/t (TAg) oncogene under a prostate-specific promoter. In this model, TAg serves as both a "self" Ag as well as the surrogate tumor Ag. Our preliminary data show TAg expression within the thymus, confirming the possibility of central tolerance. We will test the efficacy of a cell-based vaccination scheme, with or without additional immune stimuli, in generating an anti-TAg immune response in mature TRAMP male mice. The adoptive transfer of TAg-specific CD4<sup>+</sup> T cells into TRAMP mice at the time of vaccination will be utilized to provide additional CD4<sup>+</sup> T cell help. We will also test whether the manipulation of T cell activation signals can enhance tumor immunity by the use of an anti-CD40 stimulating Ab as well as an anti-CTLA-4 blocking Ab.

## Poster No. 2

### The Defective Anti-viral State of SHP-1 Deficient Mice

Kathryn L. Beuler and Paul T. Massa  
SUNY/Upstate

We have observed a striking defect in the ability of a mutant mouse lacking SHP1, a critical regulator of cytokine signaling, to mount an effective anti-viral response to Theiler's murine encephalomyelitis virus (TMEV), a naturally occurring murine pathogen. These mutant mice exhibit rapid viral spread and extensive demyelination following intracerebral inoculation with the virus, as compared to normal littermates. We have found that in response to infection and cytokine stimulation, there is a differential activation of STAT molecules governing interferon inducible genes that leads to a shift from anti-viral to a pro-apoptotic/inflammatory response, which ultimately leads to the significant and rapid demyelination observed in these mice.

TMEV replicates in both the brain and spinal cords of SHP-1 deficient "motheaten" mice to a 20 fold higher titer versus normal littermates. This phenomenon is visible as early as 4 days post infection and points to an inability to control viral replication. Motheaten mice exhibited elevated levels of activated/tyrosine phosphorylated STAT1 in response to both IFN $\gamma$  and IFN $\beta$ . It is possible that this hyperactivation may lead to greater STAT1 homodimer versus STAT1/2 heterodimer formation thus altering the type of genes that are induced during viral infection. This theory is supported by EMSA analysis of GAS and ISRE regions of IFN $\gamma$  treated oligodendrocytes, which revealed a higher GAF to ISGF3 ratio in motheaten mice. This suggested a shift toward inflammatory versus anti-viral gene induction. A similar pattern was observed in gel shift analysis of the IRF1/GAS following 48hr TMEV infected mixed glial cultures, despite equal induction of IFN $\gamma$  in normal and motheaten mice. Therefore, we believe the data suggests that dysregulation of the STAT system in SHP1 deficient causes a defect in the expression of innate anti-viral genes in response to TMEV.

### Poster No. 3

#### **HIV-1 Tat Enhances Expression of MHC Class I Molecules on Infected Primary CD4+ Cells.**

Matthew Bonaparte and Edward Barker  
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The Tat protein of HIV is a potent transactivator of transcription from the viral promoter and is an essential component of virus replication. Tat has been reported to modulate the expression of a variety of host cell genes. Here we wanted to determine if the *tat* product may also play a role in modulating the expression of major histocompatibility complex (MHC) class I molecules. To evaluate this objective, we isolated primary CD4+ cells from the blood of HIV uninfected donors and infected them *in vitro* with a lentivirus vector which was made to stably express both HIV-1 *tat* together with a marker protein GFP. MHC class I molecules were increased on CD4+ cells expressing *tat* but not in cells expressing GFP alone when compared with untransduced controls. As expected we observed a decrease in MHC class I expression on primary CD4+ cells stably transduced with HIV-1 *nef*. We also demonstrated that an HIV strain with wild-type *tat* in which *nef* was removed and replaced with the GFP gene enhanced the expression of MHC class I molecules on CD4+ cells. These studies indicate that *tat* expression in HIV-infected primary CD4+ cells increases MHC class I molecules on the cell surface.

## Poster No. 4

### **Sympathetic Nervous System Plays a Major Role in Acute Cold/Restraint Stress Inhibition of Host Resistance to *Listeria Monocytogenes***

L Cao, N M Filipov and D A Lawrence  
Wadsworth Center

Multiple studies have demonstrated that both glucocorticoids and norepinephrine (NE) can modulate immune responses *in vitro* and *in vivo*, and it is well known that the sympathetic nervous system innervates both primary and secondary lymphoid organs. Here, we show that acute cold/restraint stress (ACRS) significantly lowers host resistance to *Listeria monocytogenes* (*LM*) in BALB/c mice, and the involvement of stress hormones corticosterone (CORT) and NE is evaluated. CORT and NE were investigated by pretreating mice with the CORT synthesis inhibitor metyrapone and the chemical sympathectomy drug 6-hydroxydopamine (6-OHDA), respectively. *LM* burdens were determined 3 days post-infection. 6-OHDA significantly decreased the *LM* burden in both control and stressed animals. 6-OHDA also completely blocked the stress effects observed in spleens while only partially affecting the liver. The 6-OHDA-uptake inhibitor desipramine confirmed that peripheral sympathetic adrenergic nerves and NE depletion and not the nonspecific 6-OHDA toxicity were responsible for the enhanced host defense. In contrast, metyrapone-treated animals had further decreased host resistance to *LM* after ACRS. The results suggest that the peripheral sympathetic nervous system (SNS) postganglionic neurotransmitter NE plays a major role in *LM* host resistance, but there are significant tissue-dependent effects after ACRS, while CORT provides a potential protective effect after ACRS. Altogether, stress hormones play important roles in stress-modulated host resistance and NE is a major hormone involved in ACRS-induced suppression of host resistance in the spleen but not the liver. (Supported by NYSDOH and NIEHS ES15-3506A).

## Poster No. 5

### Cytokine Induced Apoptosis in Oligodendrocytes

Karen L. Fecenko and Paul T. Massa  
SUNY/Upstate

We have previously described the expression of the protein tyrosine phosphatase SHP-1 in myelin forming oligodendrocytes of the CNS. Interestingly, SHP-1 deficient mice exhibit decreased myelin formation compared to normal littermates. This may relate to the function of SHP-1 in controlling the response of oligodendrocytes to IL-6 family cytokines. Previous studies of demyelinating diseases have indicated the presence of the proinflammatory cytokines IL-6 and apoptotic bodies within demyelinating plaques. We propose that SHP-1 protects oligodendrocytes from detrimental activities of IL-6 that occurs in immune responses in the CNS.

Using SHP-1 deficient mice, we have studied the effects of IL-6 exposure in oligodendrocytes. We show that IL-6 stimulates STAT 1/3 homo/heterodimers as downstream signal transducers to activate specific genes, such as IRF-1 and caspase 1, in oligodendrocytes and that SHP-1 controls this activation. We show data that lack of SHP-1 in oligodendrocytes leads to increased apoptosis, which can be blocked with the general caspase inhibitor zVAD. Therefore, SHP-1 may be a crucial regulator of the oligodendrocyte response to IL-6 during immune responses in the CNS.

## Poster No. 6

### **Visualization of Vessel Morphology and Host Cell Infiltration in Cytokine Transfected Tumors grown in Transgenic GFP+ Mice**

Scott A. Gerber, James P. Moran, John G. Frelinger, Jeffrey A. Frelinger,  
and Edith M. Lord  
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B16 melanoma tumors were grown in C57BL/6 mice expressing green fluorescent protein (GFP) under the control of the major histocompatibility complex (MHC) class I promotor. A new method using whole tissue mounts was developed which minimized tissue manipulation and allowed observation of highly GFP positive blood vessels and host leukocytes within the growing tumors. These studies revealed that the vessels within the tumor tissue were much more irregular and tortuous compared to those within surrounding normal muscle. Cytokines play important roles in the growth and function of host immune cells and can also affect their interactions with malignant cells.

Furthermore, previous studies had shown increased vascularization in a mammary tumor in which IL-2, a T cell growth promoting cytokine, was produced locally. In contrast, IL-12, which also promotes T cell function, has been shown to have an anti-angiogenic effect. For these reasons, B16 tumors were stably transfected with genes for either IL-2 or IL-12 and blood vessels along with host cell infiltration studied. Vessel morphology was greatly altered in cytokine expressing tumors, especially in those expressing IL-12 in which smaller, less branching vessel morphology coincided with slower tumor growth patterns. In addition, the local production of cytokines by tumors changed the number and pattern of infiltrating host cells. CD4<sup>+</sup> and CD8<sup>+</sup> T cells were found in much greater abundance in IL-2 and IL-12 expressing tumors. Interestingly, in the IL-2 transfected tumors, the T cells, although present in large numbers, were generally found quite near the vessels with relatively little infiltration into the actual tumor tissue. In contrast, the IL-12 expressing tumors possessed increased numbers of T-cells, but they were more dispersed and found at greater distances from blood vessels. Both IL-2 and IL-12 induced the upregulation of the adhesion molecule VCAM on blood vessels within the tumors, which may contribute to the increased T-cell infiltration. The expression of additional adhesion molecules is currently being examined to determine what is responsible for the altered pattern of T cell infiltration between the IL-2 and IL-12 expressing tumors.

Supported by NIH grant CA28332

**Poster No. 7**

**Effect of IL-7 on the Regulation of CD4 T cell Memory Development and Maintenance**

JiChu Li and Susan Swain  
Trudeau Institute

*In vitro* generated CD4 effectors, give rise to a long lived population of memory cells when they are transferred to an adoptive host, but the role of survival factors (gc cytokines) played in this transition and in maintaining memory T cells *in vivo* are not yet clear. Here we show evidence that, stimulated with suitable amount of IL-7, Th1 and Th2 effectors became death resistant, progressively more resting, and acquire a phenotype more associated with memory. The data suggest that survival factors play an important role in the regulation of CD4 T cell memory development and maintenance.

## Poster No. 8

### **Antibodies Effective in SCID Mice during Infection by the Obligate Intracellular Bacterium *Ehrlichia chaffeensis* are of Picomolar Affinity and Exhibit Preferential Epitope and Isotype Utilization**

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Humoral immunity is generally believed not to be effective in host defense against intracellular bacteria. However, our previous studies have shown that antibodies, in the absence of lymphocytes, can protect SCID mice from lethal infection by the obligate intracellular bacterium *Ehrlichia chaffeensis*. To address the mechanism(s) whereby antibodies protect against this intracellular bacterium, a panel of monoclonal antibodies was generated and examined to identify characteristics that were essential for *in vivo* efficacy. These monoclonal antibodies were characterized based on the following properties: antigen specificity, isotype utilization, and binding affinity. Our results indicated that all of the effective antibodies recognized epitopes in the first hypervariable region (HVR1) of the *E. chaffeensis* outer membrane protein-1g (OMP-1g). Only IgG antibodies were found to be effective, and the following hierarchy of protective efficacy was observed: IgG2a>>IgG3=IgG2b. The highly effective antibodies also exhibited high picomolar affinity and very long binding half-lives. The *in vivo* efficacy was positively correlated with binding affinity of the antibody. Among the effective antibodies, IgG2a, the most effective isotype, exhibited 10<sup>3</sup>-fold higher affinity than IgG3 and IgG2b. The characteristics of effective antibodies are indicative of a type I antibody response with highly focused antigen specificity. Therefore, for antibody immunity to be effective during host defense against intracellular bacterial infection, it may be necessary to generate specific antibodies with appropriate isotypes and very high binding affinity against the intracellular bacterium.

## Poster No. 9

### Protective Efficacy of Pneumococcal Conjugate Vaccine is Enhanced in Mice Treated with Exogenous IL-12

Joyce M. Lynch, Bernard P. Arulanandam, Dennis W. Metzger  
Albany Medical College

Interleukin-12 (IL-12) is a potent mucosal adjuvant that can modify the immune response to encapsulated organisms by inducing B cells to switch to  $\gamma 2a$  and  $\gamma 3$  isotypes and by stimulating the production of interferon- $\gamma$  (IFN $\gamma$ )<sup>1,2</sup>, IL-12 can also promote the expression of SIgA<sup>3</sup>. In this study, the ability of IL-12 to enhance the efficacy of intranasal immunization with pneumococcal conjugate vaccines and to increase protection against mucosal and systemic infection by these encapsulated organisms was investigated in BALB/c mice. It was found that mice treated with IL-12 demonstrated increased expression of lung and splenic IFN- $\gamma$  and interleukin-10 mRNA, and increased protection against infection with *S. pneumoniae*. After intranasal vaccination and treatment with IL-12, followed by intraperitoneal challenge of adult mice with Type 3 pneumococci, there was 75% (6/8) survival compared to 37.5% (3/8) survival among mice that received Type 3 conjugate vaccine alone. Protection against Type 14 carriage was also examined in mice immunized with Type 14 vaccine conjugate and treated with IL-12. The IL-12 treated mice possessed fewer Type 14 colonies ( $p < 0.05$ ) in the upper respiratory tract when compared to mice that received the vaccine alone. Interleukin-12 treated mice also elicited higher antibody titers, IgG2a being significantly increased when compared to mice that received vaccine alone. We conclude that i.n treatment with IL-12 is able to enhance the immune response to pneumococcal conjugate vaccines administered i.n., thereby increasing both systemic and mucosal protection against invasive infection by *S. pneumoniae*.

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## Poster No. 10

### Rapid Phenotypic Drug Susceptibility Assay for HIV Clinical Isolates

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With the advent of HAART therapy for the treatment of HIV infected patients and its subsequent failure in some patients, there is a need for rapid phenotypic drug susceptibility testing. Currently available phenotypic drug susceptibility assays are too time consuming and labor intensive to be clinically relevant. The currently licensed homologous recombination assays only monitor mutations in the protease and reverse transcriptase (RT) genes missing mutations that occur outside of these genes that may render antiretroviral therapy less effective. To remedy this situation, we developed a rapid, phenotypic drug susceptibility assay for HIV clinical isolates that utilizes genetically engineered cell lines that express green fluorescent protein (GFP) when infected with lymphotropic, macrophage tropic, or dual tropic HIV laboratory strains and clinical isolates. GFP expressing cells were obtained from the NIH AIDS Research and Reference Reagent Program. The cells were infected with HIV-1<sub>LAI</sub>, HIV-1<sub>LAI-M184V</sub>, and some clinical isolates in the absence and presence of various concentrations of AZT, 3TC, abacavir and delavirdine. After incubation for 48 hr, the cells were harvested and the percent of cells expressing GFP was determined by flow cytometry. The IC<sub>50</sub> values for wild type virus were  $0.06 \pm 0.04 \mu\text{M}$  AZT,  $1.76 \pm 0.09 \mu\text{M}$  3TC,  $0.05 \pm 0.02 \mu\text{M}$  delavirdine, and  $1.60 \pm 0.06 \mu\text{M}$  abacavir. The 3TC resistant HIV isolate had IC<sub>50</sub> values of  $0.04 \pm 0.01 \mu\text{M}$  AZT and  $>20 \mu\text{M}$  for 3TC. The drug susceptible clinical isolates had IC<sub>50</sub> values for these drugs similar to those of the wild type virus. Equivalent results were obtained with these drugs when the GFP cells were infected with H9 cells chronically infected with HIV<sub>IIB</sub>. These results demonstrate the use of this rapid, phenotypic drug susceptibility assay for determining IC<sub>50</sub> values for RT inhibitors of HIV replication. The assay should be particularly useful for drugs that block early events in the HIV replication cycle, such as compounds that block virus attachment.

## Poster No. 11

### Naive to Memory CD4 T Cell Differentiation in the Aged Lymphoid Microenvironment Leads to a Defective Population of Memory Cells

James N. Mittler and William T. Lee  
Wadsworth Center

Aging is associated with a progressive decline in protective immunity to foreign pathogens. Many aspects of protective immunity decline, particularly the response of CD4 T cells. CD4 T cell activation, clonal expansion, and differentiation to effector CD4 T cells can be influenced by various cell-associated and soluble factors present in the lymphoid microenvironment in vivo. To investigate whether the aged lymphoid microenvironment can support CD4 T cell responses we have established an adoptive transfer model in which young (i.e. functional) ovalbumin (OVA)-specific CD4 T cells from DO11.10 mice are transferred into aged host mice. Using this model we have previously demonstrated that the primary response to OVA immunization by functional naive DO11.10 CD4 T cells is not impaired in the aged lymphoid microenvironment. In the present study we have analyzed the recall response of the young DO11.10 CD4 memory T ( $T_M$ ) cells that had developed in the aged microenvironment. Young and aged (18 month) BALB/c mice were transferred with CD4 T cells from young DO11.10 mice, and subsequently immunized with OVA. One month after the primary immunization, host mice were re-challenged with OVA and the response of the DO11.10  $T_M$  cells was monitored. While phenotypic markers of activation were similar in both hosts after re-challenge with OVA, the absolute number of DO11.10 T cells was reduced in the aged hosts, indicating that clonal expansion was impaired. Additionally, analysis of cytokine production revealed that, in the aged hosts, fewer DO11.10  $T_M$  cells produced lymphokines after re-challenge with OVA than those in young hosts. In vitro, purified DO11.10  $T_M$  cells from aged host mice displayed fewer rounds of cell division following restimulation with OVA when compared to those isolated from young hosts. However,  $T_M$  cells purified from young DO11.10 mice and adoptively transferred into naive host mice, exhibited comparable clonal expansion in both young and aged recipients following immunization with OVA, thus indicating that the aged lymphoid microenvironment can support the growth of functional CD4  $T_M$  cells. These results suggest that antigen-specific  $T_M$  cells that develop in the aged lymphoid microenvironment are rendered less functional in terms of clonal expansion and effector function upon a secondary encounter with antigen.

## Poster No. 12

### **The Mechanism by which Locally Produced Interleukin-12 Mediates its Anti-Tumor Activity Varies Depending on Tumor Phenotype**

J.P. Moran, S.A.Gerber, J.G. Frelinger and E.M. Lord  
University of Rochester

We examined the anti-tumor effects of IL-12 in two different murine tumor models, EMT6 mammary carcinoma (of BALB/c origin) and the K1735 melanoma (C3H origin). These cell lines were transfected with the p35 and p40 subunit genes of murine IL-12 and clones were selected that secrete 1-30 ng/ml/106cells. IL-12 expressing EMT6 tumors (EMT6/IL-12) placed i.m. in the leg grew to about 10 mm in diameter and then regressed. Interestingly, the IL-12 expressing K1735 tumors (K1735/IL-12) maintained a lag phase of non-measurable tumor growth for over two weeks, followed by tumor outgrowth with kinetics similar to parental tumors. Tumor outgrowth was found to be associated with a loss of IL-12 production *in vivo* by this cell line. Mice that rejected EMT6/IL-12 tumors were protected against challenge with parental EMT6 cells. Mice bearing K1735/IL-12 tumors neither rejected their tumors, nor were protected from further challenge with parental cells. In addition, tumor-infiltrating lymphocytes (TILS) isolated from EMT6/IL-12 tumors effectively lysed chromium labeled EMT6 target cells, whereas, TILS from K1735/IL-12 tumors displayed no lytic activity. Both IL-12 expressing tumor models, however, grew progressively from the time of injection in nude mice indicating an important role for T cells in each case. A likely role for T cells is the production of IFN- $\gamma$  in response to IL-12. EMT6 cells grown *in vitro* in media containing 20ng/ml of IFN- $\gamma$  displayed greatly decreased growth kinetics compared to untreated cells, whereas, K1735 cells grown under similar conditions were relatively unaffected. IFN- $\gamma$  strongly induced the expression of the anti-angiogenic chemokine IP-10 by both cell lines. Interestingly, only the EMT6 cell line was able to secrete the pro-angiogenic molecule VEGF in response to low oxygen conditions. These results indicate that locally produced IL-12 likely mediates the rejection of EMT6 tumors through tumor cell lysis by host immune cells and direct cytotoxic effects of IFN- $\gamma$ , whereas, its anti-angiogenic potential may be counterbalanced by strong induction of VEGF by hypoxic tumor cells. In contrast, IL-12 does not induce immunity to K1735 tumors, however, tumor growth may be controlled through an anti-angiogenic mechanism.

This work was funded by NIH grants CA28332 and AI07285.

## Poster No. 13

### Mechanisms Involved in Idiotype-Peptide Protection from Lupus Nephritis

Jennifer Nyland, Feng Jiang, Feng Feng, and Jerrie Gavalchin  
SUNY/Upstate

The F1 progeny of the (SWR x NZB) cross (SNF1) develops a lupus-like disease with high serum titers of anti-DNA antibodies, severe immune complex-mediated renal glomerulonephritis, and increased frequency and severity in females. A peptide derived from amino acids 62-73 of the variable region of the heavy chain of one pathogenic antibody has previously been shown to induce proliferation of idiotype-reactive T cell clones nearly as well as its parent antibody. Additionally, we have previously shown that vaccination with this peptide prolongs the life of recipient prenephritic SNF1 females. We have found that aa 62-73 vaccination results in rapid changes in immune system phenotype and function, leading to a delay in the onset of nephritis. Clinical indicators of nephritis are significantly reduced by 20 weeks of age with vaccination. Kidney pathology is significantly reduced beginning at 16 weeks. Phenotypic analyses of Ig-producing B cells suggests that IdLNF1+ Ab-producing B cells are decreased and anti-IdLNF1+ B cells are increased by 12 weeks of age. There is a switch from a Th2 to Th1-type cytokine profile at 12 weeks. Phenotypic analyses of splenic T cells indicate a reduction in the ratio of CD4+ to CD8+ cells at 10 weeks and a reduction in activation and memory IdLNF1 T cells at 20 weeks. Proliferative responses to antigen implicate a reduction in the population of idiopeptide-presenting cells. Together these data suggest that the events involved in the mechanism(s) by which aa 62-73 vaccination delays the onset of SNF1 lupus nephritis may occur as early as 10 weeks, and involve modulation of both the B and T cell populations.

## Poster No. 14

### Analysis of Mouse TNF- $\alpha$ Gene Expression Using a Novel Reporter System

Phyllis M. O'Donnell, Isabel Alvarez de Mora, and Steven M. Taffet  
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Bacterial lipopolysaccharide (LPS) induces a rapid and transient increase in transcription of the TNF- $\alpha$  gene in cells of monocyte/macrophage lineage. Fully understanding both the induction and attenuation of TNF- $\alpha$  at the molecular level remains controversial and incomplete. This study examined the intronic enhancer (iE) which was first described in our laboratory. The iE is located in the third intron of the TNF- $\alpha$  and composed of the tandemly duplicated ETS binding site 5' GGA<sup>A</sup>/T. The transcriptional activity of the iE was LPS-inducible when placed in a CAT reporter and transfected into RAW264.7 cells. Using extracts of either RAW264.7 murine macrophage-like cells or BMDM, the major DNA-binding activity was identified to be GABP, a member of the ETS family of transcription factors; however, DNA-binding activity appeared unaltered in the presence of LPS. Together these data suggest that post-translational modifications may contribute to iE activation in response to LPS. Mutations altering both GGAA half-sites resulted in a reduction of functional activity and a loss of DNA-binding activity. The same is true when the downstream or more 3-prime GGAA was mutated to CGAA leaving the upstream or more 5-prime GGAA intact. Interestingly, when the upstream GGAA was mutated leaving the downstream GGAA intact, both functional and DNA-binding activity persisted.

The majority of studies investigating potential transcriptional elements utilize reporter plasmids such as CAT or luciferase. However, artificial reporter plasmids can only provide an intimation of the transactivation potential of a particular site. The absolute significance of a particular site in the context of the natural gene still remains unknown. To address this uncertainty, we developed a system that could more accurately assess the transcriptional activity of a potential enhancer in the mouse TNF- $\alpha$  gene. In order to determine the role of potential regulatory regions in context of the entire TNF- $\alpha$  gene, we produced HA-tagged genomic TNF- $\alpha$  constructs that contain various mutations. Total RNA from transfected RAW264.7 cells was analyzed by RNase-protection (RPA). A HA-tagged genomic TNF- $\alpha$  construct that contains a deletion of the entire iE or a mutation of the downstream GGAA displayed a decrease in LPS-induced mRNA production compared to the wild-type. Cells transfected with other TNF- $\alpha$  constructs containing deletions of the proximal promoter, the distal 5-prime flanking region NF- $\kappa$ B sites, and AU-Rich element were also analyzed for comparison. Results indicate that all of these sites are important for the induction of TNF- $\alpha$ . These data suggest an essential role for the iE in the induction of TNF- $\alpha$  gene transcription in the murine macrophage.

This project was funded by a grant from the American Heart Association.

## Poster No. 15

### Inhibition of Human B cells via Fc $\gamma$ RII using Immune Complex Analogues

Mark Preissler and Edmund Gosselin  
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Fc $\gamma$ RIIB is unique among the receptors for IgG antibody in its ability to inhibit B cell activation. B cell responses can be down-modulated when antigen/antibody (Ag/Ab) complexes cross-link the B cell receptor for antigen (BCR) with Fc $\gamma$ RIIB. Fc $\gamma$ RIIB1 is the only receptor for IgG on murine B cells. In contrast, human B cells may express any of three Fc $\gamma$ RII isoforms, Fc $\gamma$ RIIA, Fc $\gamma$ RIIB1 and Fc $\gamma$ RIIB2. An Ag/Ab analogue that co-aggregates the activating BCR with Fc $\gamma$ RIIB in an antigen-independent manner could not only be a useful tool in studying Fc $\gamma$ RII receptor biology but also in controlling auto-immune and allergic diseases. Our Ag/Ab complex analogue consists of a biotin-binding targeting element (IgG3-Av) that binds receptors for IgG and a biotinylated functional element, Protein L (B-PL). Protein L is a surface protein of *Peptostreptococcus magnus* that binds to the light chain of antibodies and thus, it can mimic Ag binding to B cells in a polyclonal manner. We hypothesize that our immune complex analogue of IgG3-PL will inhibit human B cells by simultaneously binding the BCR and Fc $\gamma$ RII. Biotinylated Protein L was created by isolating the Protein L gene from *P. magnus* and inserting it into the PinPoint vector system from Promega. The PinPoint vector system allows for the expression and purification of endogenously biotinylated proteins. The ability of B-PL to bind avidin and antibodies was determined by ELISA. ELISA analysis showed increased antibody responses only in wells coated with avidin. This indicates that B-PL is indeed binding the antibody. B cell inhibition was measured by monitoring proliferation via tritiated thymidine incorporation. B cells were incubated with a constant amount of *Staphylococcus aureus* Cowan I, which aggregates the BCR, in the presence or absence of IgG3-PL complexes. Preliminary results using commercially available B-PL confirm that Protein L is not mitogenic to human B cells. They also suggest that IgG3-PL complexes may be inhibitory but that the ratio of functional and targeting elements may be important to the level of inhibition. Future experiments include evaluating the ability of IgG3-PL and IgG3-Ag complex analogues to inhibit the activation of Ag-specific and autoimmune B cells as well as the mechanisms involved. IgG3-Ag complex analogues could also be a novel approach to specifically treating autoimmune and allergic diseases.

**Poster No. 16**

**CD4<sup>+</sup> T Cells and NK Cells Collaborate to Reject Glial Tumors**

Lucas P. Williams and Benjamin M. Segal  
University of Rochester

Gliomas are the most common and most lethal primary brain tumors of adults. They are refractory to conventional treatments largely due to their infiltrative growth patterns. Immunotherapy offers an attractive therapeutic approach against these malignancies since it has the potential to selectively target migrating glioma cells while sparing adjacent healthy brain tissue. Here, using a subcutaneous model of glioma cell growth in mice, we demonstrate that CD4<sup>+</sup> T cells and NK cells, but not CD8<sup>+</sup> T cells, play a critical role in tumor rejection following vaccination with irradiated glioma cells. NK cells directly lyse glioma cells in vitro and we speculate that they may deliver the lethal hit in vivo as well. The chemokine, IP-10, and apoptosis-related molecules, Fas and Fas ligand, are preferentially expressed in tumors undergoing rejection. We propose that glioma-specific CD4<sup>+</sup> T cells either produce or induce IP-10 that in turn attracts and/or stimulates NK cells to kill glioma targets in the tumor bed via a Fas dependent pathway.

**Poster No. 17**

**Heterogeneity of IFN-gamma production and target cell lysis by CD8 T cells revealed by a novel single cell cytotoxicity assay, the Lysispot**

Jennifer Snyder, William J. Bowers, Alexandra M. Livingstone, F. Eun Hyung Lee, Howard J. Federoff, Tim R. Mosmann  
University of Rochester

Antigen-specific T cells express several potent effector functions during immune responses. Direct killing of infected cells is crucial for clearing viruses and other intracellular pathogens, but it has been difficult to measure the frequency of cytolytic cells. We have now developed a single cell assay to measure the number of cytotoxic cells in a population, using an HSV amplicon vector to express *E. coli*  $\beta$ -galactosidase in mouse or human target cells, and an Elispot to detect release of  $\beta$ -galactosidase from killed target cells. This antigen-specific, perforin-dependent Lysispot assay has been combined with a cytokine Elispot in a two-color assay to confirm that cytotoxicity and IFN $\beta$  secretion are regulated independently. The simultaneous enumeration of cytokine secreting and cytotoxic cells should be invaluable for *ex vivo* analysis of immune responses during infection and autoimmunity.

## Poster No. 18

### Exploiting Cross-presentation for the Characterization of T Cell Epitopes

Michael J. Turner, C. Siddiq Abdul-Alim, Edith M. Lord and John G. Frelinger  
University of Rochester

The analysis of T cell epitopes is critical for the study of T cell responses against pathogens, self-proteins and tumors. To assist in these studies we have developed a novel method for identifying T cell epitopes using a one step procedure for isolating recombinant antigen, and using it directly for antigen presentation to T cells. To do this we have exploited a bacterial expression system in which 6x-Histidine tags are added to the amino terminus of the recombinant protein allowing purification by affinity chromatography using Ni/chelate magnetic beads and magnetic separation. Once conjugated with protein, these beads can be directly cultured with APC for presentation of the antigen to class I and class II restricted T cell hybridomas. The feasibility of this approach was demonstrated using previously characterized ovalbumin specific T cell hybridomas; B3Z a class I (Kb) restricted hybridoma, and BDZ, a class II (I-Ad) restricted hybridoma. We have subsequently applied this system to the analysis of T cell epitopes of prostate specific antigen (PSA). Epitope mapping was performed by generating several recombinant PSA C-terminal deletion constructs by PCR. These constructs were expressed as N-terminal 6x-His tagged proteins and conjugated to Ni/chelate magnetic beads directly from bacterial lysates. By culturing these PSA deletion construct loaded Ni/chelate beads with APC and PSA specific T cell hybridomas, it was possible to identify class I and class II restricted T cell epitopes of PSA. Further analysis using synthetic peptides demonstrated that the epitopes identified using this novel method contribute significantly to the PSA specific immune response *in vivo*. This technique has been further developed to identify altered peptide ligands, with increased T cell recognition, which may assist in the generation of more potent PSA specific responses for use in the treatment of prostate cancer.

Supported by NIH grants CA-70218 and AI07285

## Poster No. 19

### Targeting Antigen to Fc $\gamma$ RI – A Modular Approach to Vaccines

Mary Walsh, Jeff A. Banas, Stan Mudzinski, Mark T. Preissler, Bob Graziano, and Edmund J. Gosselin  
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Antigen processing and presentation is an important event in the initiation of an immune response. This job is carried out by a group of specialized cells known as antigen presenting cells (APC), which include B cells, macrophages and dendritic cells. These cells engulf antigen, break it down, and present it to CD4<sup>+</sup> T cells, thereby initiating the adaptive immune response. By targeting antigen to APC, this response can be significantly enhanced without the use of traditional adjuvant.

It has been shown that targeting antigen to monocytes/macrophages (M $\phi$ ) and dendritic cells (DC) through Fc gamma receptor type I (Fc $\gamma$ RI), antigen processing and presentation, and thereby antigen-specific T and B cell responses, can be significantly enhanced *in vitro* and *in vivo*. Utilizing recombinant DNA technology, we have created a unique two component system which delivers biotinylated antigen to Fc $\gamma$ RI on M $\phi$  and DC. The first component, the targeting element, consists of an scFv of mAb H22 (specific for Fc $\gamma$ RI) fused to a subunit of streptavidin. The second component, the functional element, is composed of biotinylated antigen. The biotinylated antigen used to characterize this system is tetanus toxin. Thus far, we have shown that the targeting element can simultaneously bind both biotin and Fc $\gamma$ RI. Furthermore, the targeting element and functional element bind to each other, and when in conjugate form, enhance antigen specific T cell responses *in vitro*. Ultimately, this strategy may be applied as a universal system for delivery of biotinylated proteins to any target of interest in a number of applications. Such applications include: cancer therapy, tumor imaging, immunoassays and vaccinations. Thus, this two-component strategy has the potential significantly advance vaccine and therapeutic technologies.

## Poster No. 20

### **Anergy in Memory CD4<sup>+</sup> Murine T Lymphocytes**

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The immune system has evolved to respond quickly and vigorously to foreign pathogens while not responding to self-components. Autoimmunity arises when there is a failure of self-recognition. Primary and secondary immune responses differ, as secondary responses (mediated by memory lymphocytes) occur more quickly and are more intense than primary responses (mediated by naive lymphocytes). Differences in activation sensitivities suggest that these cells might also possess unique regulatory processes which control self/non-self recognition. We have previously shown that naive and memory T cells could respond to the same stimuli differently. Specifically, memory cells appeared to be anergic or nonresponsive to the stimuli SEB and anti-CD3, respectively, which induced strong activation and proliferation by naive cells. In contrast, both cell types proliferated when stimulated with a peptide antigen, such as of ovalbumin (OVA). In the current study we have examined whether SEB induces anergy or promotes the differentiation from a responsive (naive) to a non-responsive (memory) cell. Further, it was unclear whether the exposure of memory cells to either SEB or anti-CD3 leads to the same biological and signaling consequences. Our data show that even though memory cells do not proliferate upon primary exposure to either SEB or anti-CD3, they become anergic only in response to SEB. The kinetics of anergy induction follow the same kinetics as robust cell proliferation, indicating that in the same time frame cells are either committed to proliferate or become anergic. We further report that TCR-mediated signal transduction is similar in naive cells responding to SEB, anti-CD3, and peptide antigen, and in memory cells responding to peptide antigen. In contrast, signal transduction is defective in memory cells exposed to SEB and anti-CD3. Interestingly, we have observed that memory cell anergy is associated with increased activity of the src kinase, fyn. Thus, qualitative signaling differences are related to the nature of the stimulus and the differentiation state of the responding cell.

## Poster No. 21

### ***Borrelia burgdorferi* Infection in CD14 Deficient Mice Results in More Severe Lyme Disease Manifestation**

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There now exists a large body of *in vitro* evidence supporting the notion that the Lyme disease (LD) spirochetal bacterium *Borrelia burgdorferi* possess lipoproteins capable of activating innate immune cells via the pattern recognition receptor (PRR) CD14. Moreover, the fact that these same lipoproteins are immunodominant antigens during LD suggests that they also are critically involved in the adaptive immune response to the spirochete. Given the ability of innate immune responses to “set the stage” for subsequent adaptive immune responses, we hypothesize that (i) lipoproteins interact with CD14 to trigger innate immune responses which contribute to the clinical manifestations of LD, and that (ii) these innate responses to borrelial lipoproteins modulate adaptive immunity during LD. To explore this possibility we have assessed the clinical course and severity of disease in wild-type and CD14-deficient C3H/HeN mice following infection with *B. burgdorferi* via the deer tick, *Ixodes scapularis*. Given the inability of macrophages from CD14-deficient mice to respond to spirochetes we were quite surprised to discover that natural infection in these animals results in more, rather than less, severe disease. Histological evaluation of hearts and tibiotarsal joints revealed that inflammation in the CD14-deficient background was characterized by greater myocarditis and more extensive synovitis, tendonitis, and bone destruction when compared with that seen in wild-type mice. Finally, activation of innate immunity by microbial constituents is considered a prerequisite for, and even “directs”, the development of the slower adaptive responses that are often essential for pathogen elimination. Thus, it was of interest to determine whether the absence of CD14 resulted in alteration of the humoral immune response to *B. burgdorferi*. In this regard, we found no significant differences in IgM, IgG1, IgG2a, IgG2b or IgG3 reactivity between wild-type and CD14-deficient animals. Improved understanding of the role CD14 plays in LD immunopathogenesis will lead to the development of therapeutic strategies to reduce or ameliorate the destructive consequences of borrelial infection.