6. Progress Report

6A. Period Covered 09/15/2007 – 08/31/2012

6B. Accomplishments of the training program

Trainees. During years 31-35 of this training grant, the covered period, 23 pre-doctoral and 16 postdoctoral trainees have been supported, with 36 of 39 continuing as faculty members, teachers in K-12 education, administration or research staff positions (academic staff), and/or continued academic training **(Figure 3)**. Of the 30 mentees pursuing additional academic training, 21 are continuing various training paths at UAB. The nine remaining mentees are pursuing training at the La Jolla Institute of Allergy and Immunology, Medimmune, University of Pennsylvania, Northwestern, Tufts, Johns Hopkins, NIH, Baylor, and Duke. [The three post-doctoral trainees who are engaged in other activities include one pharmacologist at FDA/CDER, one inspector for the FDA and one scientific writer.] Our trainees in this period have been 41% male and 59% female; and have included 18% under-represented minorities (15% African American, 3% Native American) (Table 11). This compares to the previous five year period where the trainees were 60% male and 40% female, and 9% under-represented minorities (6% African American and 3% Hispanic American).

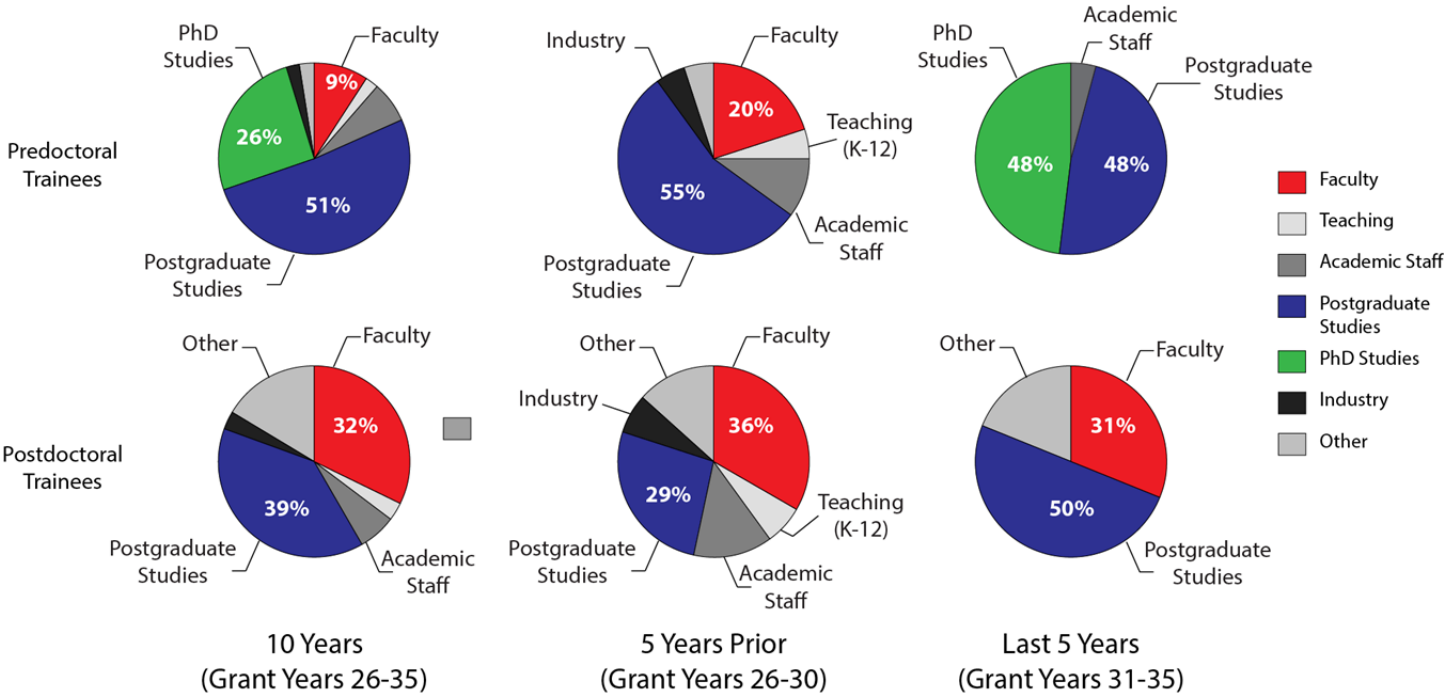

Figure 3. Training outcomes. Shown in the top row are outcomes for our pre-doctoral trainees over the past 10 years, and also separated into those who obtained funding for grant years 26-30, and those who obtained funding for grant years 31-35 (the most recent). Shown in the bottom row are the statistics for post-doctoral trainees. Some of our trainees have achieved university faculty rank, are teaching at the K-12 level, or are functioning on the research staff or the administration (academic staff) at a university level. Some are pursuing post-graduate training; others are still in the process of completing their PhD studies. A small percentage is in industry. A scattering of trainees have pursued non-academic, although still research or science based, careers (other). None have dropped out of science or technology completely.

Over this same period, our trainees have trained with 31 different primary or co-mentors from five different departments (Cell, Developmental & Integrative Biology, Medicine, Microbiology, Pathology and Pediatrics) and 10 divisions. We believe that this interdisciplinary breadth provides a rich investigative training experience for trainees and faculty alike.

Over the period covered, our trainees have published 110 peer-reviewed manuscripts (Table 6A/6B). Twelve of these have two or more trainees as co-authors. Of these, ~20% were published in journals such as

Science, Nature, Nature Immunology, Immunity, the *Journal of Experimental Medicine,* the *Journal of Biological Chemistry,* the *Proceedings of the National Academy of Sciences USA, Annual Reviews of Immunology, Nature Reviews in Immunology, Current Opinion in Immunology, Trends in Immunology,* and *Seminars in Immunology.*

Of the 23 predoctoral trainees, twelve have completed graduate studies and earned a PhD. The eleven remaining trainees are still pursuing predoctoral studies, with most planning to graduate within the next two years. All twelve of the students who have earned their PhD published at least one first author paper, with the group averaging 2.0 first author papers each and 3.8 papers as either first or middle authors. Of the 16 postdoctoral trainees, thirteen have completed their training on the T32. Of these thirteen, five (38%) now hold faculty positions. This group of thirteen has published an average of 2.7 papers from work supported by this training grant, with eleven (85%) publishing at least one paper as first or middle author, and 9 (69%) publishing at least one paper as first author.

All our trainees are expected to work with their mentors to prepare, and highly encouraged to submit, at least one grant seeking extramural research support. Over the period covered, **seven trainees (Deshane, Huff, Kin, Maynard, Meares, Ramos and Williams) succeeded in receiving their own extramural funding while on this training grant.**

Mentors. To enhance our emphasis on both immunologic diseases and basic immunology, we have added 26 new faculty (Drs. Almeida, Brown, Cron, Deshane, DeSilva, Fujihashi, George, Goepfert, Harrington, Hatton, Hughes, Hsu, Kabarowski, Katz, Lefkowitz, Li, Lund, P Mannon, R Mannon, Pasche, Randall, Schwiebert, Shalev, Smythies, Steele and Tse). This represents a net increase of 20 faculty members since the last competitive renewal, with eight previous mentors having either retired, left the institution, or changed their area of research. These 26 new faculty support new and enhanced areas of emphasis within the broad field of immunology, reflecting our commitment to rigorous research training in these areas and new opportunities provided by extramurally funded grants and program projects. The newly recruited faculty has provided increased programmatic emphasis on interdisciplinary research to effectively interpret the impact of our new knowledge of basic immunology on the pathogenesis of a wide range of immunologic diseases, including bioinformatics.

Major efforts have also been made to enhance gender equity and to recruit and retain faculty from under-represented groups. The representation of women among the faculty has increased from 16% in 2006 to 30% in 2012. Three faculty members, Drs. Katz, Lopez and Schroeder (Melendez) (5% of the faculty), are Hispanic-Americans.

Training Experience. Our expanded faculty have facilitated the development of a formal co-mentorship program designed to broaden the training experience through a translational perspective and to provide content focused on the immunologic diseases. Our goal is to bring together mentors with and without clinical training. Examples of co-mentorship between previous and new faculty include the co-mentorship of Dr. Ben Christmann by Drs. Elson (Medicine/Gastroenterology) and Tse (Microbiology), of Dr. John Anderson by Drs. Chaplin (Microbiology) and Schwiebert (Cell, Developmental & Integrative Biology), of Mr. Michael Edwards by Drs. Katz (Pediatric Dentistry) and Michalek (Microbiology), and of Dr. Tracy Hwangpo and Ms. Ewa Szymanska by Drs. Schroeder (Medicine/Clinical Immunology & Rheumatology) and Brown (Public Health/Epidemiology).

In 2008, Dr. Schroeder, the Director of this training program, was also named Director of the UAB Program in Immunology. This led to a tighter integration between the UAB Program in Immunology and this T32. Our trainees now serve as official hosts and meet for lunch and a literature discussion/mentoring session with on-campus visitors coming for the Program in Immunology Seminar Series. They also serve as trainee representatives in the major administrative subgroups of the UAB Program in Immunology, which provides them with a broader perspective of the opportunities for service and leadership that are an integral part of the academic experience.

Our program has increased its emphasis on the formal mentoring of post-doctoral trainees. This includes structured practice with feedback in oral and poster presentations, in grant writing, in manuscript preparation and in career planning. Following the lead of the National Postdoctoral Association, we instituted the use of individual development plans (IDPs) for post-doctoral trainees in 2011. These plans provide a mechanism to help the trainees identify and clarify their professional development needs and their career objectives; and to facilitate and structure communication between the post-doctoral trainees and their mentors. The goals of the IDPs are to help each trainee identify and seek out the tools and training necessary to achieve their long-term career interests and to help the trainees and their mentors come to a common understanding of how best to maximize the benefits of their current efforts. This process parallels that expected for pre-doctoral trainees through their dissertation committees.

Program Administration. In addition to our T32 Internal Advisory Committee, we have created a separate External Advisory Committee. The instructions for this application specifically prohibit reporting the membership of this newly constituted EAC. However, the three members of the EAC have provided written evaluations of the current program that have been made available to the T32 Advisory Committee and to all Program Faculty.

6C. Specific effects of this training program on curriculum and/or research directions

Trans-disciplinary UAB-based coursework. Recognizing the inter-relatedness of studies ranging from molecules to man, training program faculty have initiated several new graduate courses emphasizing the development of knowledge from bench to bedside and back. Experimenting with different teaching and participation formats and intentionally creating student work groups of pre-docs, PhDs, and MD/PhDs, our training faculty have emphasized this inter-relatedness (e.g. MIC778-Primary Immune Deficiencies, MIC740/CB745-Protective and Pathogenic T cell Responses, GBS 743-Innate Immunity, and MIC700 Advanced Course in Autoimmunity).

Development of the T32-specific course MIC741, “Topics in Professional Development”. This course, established in 2009 and coordinated by the Program Director and Associate Director, meets for two hours weekly in the evening after the Program in Immunology Seminar. In Year 1, the course focused on Training in Biostatistics and on weekly interactions with University Leaders, such as the Dean of the School of Medicine and the Chairs of the various Departments to which the Program Faculty belong. These leaders provided enrichment through the discussion of topics such as how to become a reviewer or editor of a journal, how to network and participate on a national level through a scientific organization, how do private foundations work and how do they fund science, etc. *The response to the Biostatistics course led to the establishment of a similar course for all GBS trainees.*

In Year 2, we worked on grant preparation with trainees writing five page grants focused on hypothesis, significance, innovation and approach. After an oral presentation and questions, each grant was then thoroughly discussed by the class in the form of a study section. Trainees were given the opportunity to rebut on the week following. The ultimate expectation is for each trainee to submit their grant proposal for external funding.

In Year 3, we worked on oral presentations, with each trainee tasked with a 15 minute presentation again focused on hypothesis, significance, innovation and approach. After each presentation and questions, each presentation was then thoroughly discussed by the class in the form of a study section. Trainees were then given the opportunity to rebut on the week following. The second half of the year attention turned to more practical aspects of preparation for presentations. The training grant purchased Adobe Illustrator, Acrobat and Photoshop, and the group completed five Lynda.com training classes focused on gaining expertise with these programs.

As a result of these shared activities, this training grant has achieved a special identity within the graduate program at UAB, with its students achieving a special *esprit de corps*. It has also allowed the Program Director

and Associate Director to individually counsel each trainee in their research projects. This approach has strengthened the training program's ability to engage in ongoing evaluation of goals and progress in order to establish achievable benchmarks of success for advancement.

6D. Benefits to the training program from the funds provided under Training Related Expenses.

Over the covered period, funds provided under Training Related Expenses were used to defray the cost of health insurance for the trainees. In addition, in 2007, funds were used to support an 11th pre-doctoral position for Mr. Don McGuire. From 2008 to the present, these funds were used to partially defray the salary of Ms. Paula Robinson, who was responsible for assistance in the administration of the training program, in the coordination of meetings both for trainees and for program faculty, and in the preparation of the annual reports; to purchase textbooks, scientific software such as Sigmaplot and End Note, presentation software such as Adobe Illustrator, Acrobat and Photoshop, and laptop computers for the trainees; to cover speaker fees for the Program in Immunology Seminar Series; for renting poster boards for the Poster Session of the Topics in Professional Development course (MIC741); and to provide honoraria for our external advisors.

6E. Trainee Research Summary (for the past 5 years).

Postdoctoral Trainees

1. John Anderson, MD **07/01/2010 – 06/30/2011**

(Mentor: David Chaplin, MD/PhD; Co-mentor: Lisa Schwiebert, PhD)

Asthmatic airway inflammation correlates to increased levels of inflammatory mediators (chemokines and cytokines), increased free radical (super oxide, O2.-, nitirc oxide, NO) production, and the increased presence of inflammatory cells. However, the inflammation is not uniform throughout the airway. Dr. Anderson's work has been to characterize the different inflammatory profiles of the proximal or large airway compared to the distal or small airway in human asthmatic subjects. He has shown an increase in NO and its metabolites localized to the distal airway correlating with an increased monocyte population capable of NO production. This finding contrasts sharply to the uniform presence of O2.- producing cells found throughout the airway. Additionally, he has observed unique cytokine and chemokine profiles of the proximal and distal airway, further emphasizing regional variability in asthmatic inflammation. Dr. Anderson's work will continue to focus on the developing techniques to better characterize proximal vs. distal airway, and characterizing the cellular source for the different inflammatory profiles.

Dr. Anderson received less than two years of research training because he transitioned into an Assistant Professor faculty position at UAB after just one year of training.

2. Benjamin S Christmann, PhD **03/01/2009 – 02/28/12**

(Mentor: Charles Elson, MD; Co-mentors: Hubert Tse, PhD and Chad Steele, PhD)

Dr. Christmann's project examines the interplay between the adaptive immune system and the gut microbiota. The focus is the presence of serum IgG that recognize a set of antigens cloned from the murine microbiota that are also present in the human microbiome. Over the past year, he has used a protein microarray to analyze human serum samples provided by several international collaborators and has uncovered shared patterns of human reactivity to the gut microbiota. He has shown that this baseline reactivity differs in different hyper-inflammatory and autoimmune diseases such as Crohn's disease, Ulcerative Colitis, Rheumatoid Arthritis, and Celiac disease. This work has been presented at the annual FOCIS meeting in 2012, and is currently being prepared for two separate publications to be submitted by the end of the year. It has spurred requests for additional collaboration both inside and outside of UAB.

Dr. Christmann completed three years of training on this T32 training grant in February of 2012, and is continuing his studies as a postdoctoral fellow in the Division of Gastroenterology in the UAB Department of Medicine.

3. Jessy Deshane, PhD **07/01/2007 – 07/31/2009**

(Mentor: David Chaplin MD/PhD)

Dr. Deshane has been pursuing her long standing interest in free radicals and their critical role in modulating inflammatory responses. For her postdoctoral program, she explored the potential of specialized innate immune cells termed myeloid-derived regulatory cells as significant sources of immunoregulatory free radicals and as critical regulators of the inflammatory response during allergic airway inflammation.

Dr. Deshane transitioned into an Assistant Professor faculty position in the Division of Pulmonary, Allergy and Critical Care Medicine after completing her training.

4. Kari Dugger, PhD **07/01/2009 – 06/30/2010**

(Mentor: Lisa M Schwiebert, PhD)

Exercise profoundly influences the immune system; such effects include an exercise-mediated redistribution of T lymphocytes in vivo. Increasing evidence demonstrates that T lymphocytes play a central role in the pathogenesis of asthma. Dr. Schwiebert has reported previously that repeated bouts of aerobic exercise at a moderate intensity ameliorate asthma-related responses, including airway inflammation and hyperresponsiveness. No published studies to date, however, have determined the effects of exercise on T lymphocyte migration within the asthmatic lung. Dr. Dugger hypothesized that aerobic exercise training at a moderate intensity alters T cell migration within the asthmatic lung.

Dr. Dugger was a trainee on the T32 for less than two years prior to accepting a faculty position at the University of South Alabama, however she was a postdoctoral fellow in Dr. Schwiebert's laboratory for more than two years in total.

5. David T Glover, PhD **12/1/2007 – 11/30/2008**

(Mentor: David E Briles, PhD)

Dr. Glover was interested in characterizing the novel *S. pneumoniae* antigen PcpA, which is a major target for vaccines. He has shown that immunization with PcpA elicits protection against lung infection and fatal sepsis in murine models of infection. He then explored the possibility that immunization with PcpA and other *S. pneumoniae* antigens will confer greater protection against infection. He also examined the effect of immunization with PcpA in combination with a mutant form of Pneumolysin, as well as characterized the immune response generated in mice immunized with PcpA.

Dr. Glover was a trainee on the T32 for only one year. He continued his training as a postdoctoral fellow at Emory University through 2011. He is currently serving as an Inspector for the FDA.

6. Whitney Helms, PhD **01/01/2006 – 09/30/2008**

(Mentor: Casey Weaver, MD)

Dr. Helms focused on investigating the role of IL-23 in T cell function. At the time, the dogma stated that while IL-23 is important in the maintenance of the Th17 lineage, the cytokine is dispensable for the development of these cells. *In vivo*, however, mice lacking IL-23 fail to development many Th17 dependent autoimmune disease phenotypes. She worked to develop a mouse model to conditionally knockout the IL-23 receptor in various cell types to better explore where and when IL-23 signaling is important in the development of autoimmune disorders. She used a recombineering strategy to target exons in the *il-23r* gene and successfully replaced an endogenous *il-23r* allele in a mouse embryonic stem cell line.

Dr. Helms is currently serving as a pharmacologist for FDA/CDER.

7. Brantley Herrin, PhD **09/01/2005 – 01/31/2008**

(Mentor: Max Cooper, MD)

The extant agnathans, lamprey and hagfish, possess a distinct anticipatory immune system consisting of lymphocyte-like cells expressing variable lymphocyte receptors (VLRs) composed of somatically rearranged leucine-rich repeat (LRR) subunits, rather than the immunoglobulin subunits utilized by the adaptive immune systems of jawed vertebrate species. Computational analysis of VLR cDNA and genomic sequences derived from sea lamprey predicts a potential repertoire of >1014 receptors. Although the diversity of the VLRs has been well documented, relatively little is known about the structure and function of VLR proteins. Plasma from lamprey immunized with human red blood cells (RBCs) agglutinates RBCs in vitro, and that VLRs in the lamprey plasma are responsible for the agglutination. The agglutination reaction suggests that secreted VLR proteins are multivalent. Dr. Herrin's studies demonstrated that secreted VLRs are composed of disulfide-linked VLR subunits that form a multivalent protein complex, similar to mammalian secretory IgM. Further analysis of VLRs from lamprey plasma was hindered by the variable length and composition of VLR lamprey larvae. Fortunately, HEK-293 cells transfected with VLR cDNAs secrete and assemble VLRs into multimers of approximately equivalent molecular weight as those detected in lamprey plasma. Furthermore, the HEK-293 expression system served as the basis for a screening strategy that has resulted in the identification of antigen specific VLR clones. Site-directed mutagenesis was conducted to identify the amino acids necessary for secretion and assembly of VLR multimers, as well as the antigen binding site of antigen specific VLR clones.

After completing his training, Dr. Herrin was recruited as an Assistant Professor to the faculty of the Department of Pathology at Emory.

8. Tracy Hwangpo, MD/PhD **07/01/2011 - Present**

(Mentor: Harry W Schroeder, MD/PhD; Co-mentor: Elizabeth Brown, MPH PhD)

In the Adult Primary Immunodeficiency Clinic in the Southeastern United States, clinicians see patients with Common Variable Immune Deficiency (CVID), patients with recurrent sinopulmonary infections with normal serum immunoglobulins (RESPI), and other types of immunodeficiency such as IgA Deficiency. Dr. Hwangpo is in the process of developing a formal database to characterize the phenotypic and genetic profile of these patients to examine the factors that contribute to the development of CVID. She is also accumulating data regarding B cell numbers of these patients which will be compared to disease phenotype, age, and HLA haplotype to examine the relationship of various B cells to disease severity. Dr. Hwangpo is also involved in an ongoing project to characterize the MHC domain of CVID, RESPI, and control patients via SNP analysis. She is collaborating with Dr. Tom Rothstein at the Feinstein Institute for Medical Research to evaluate B-1 cell numbers in CVID and RESPI, and with Dr. Mary Carrington at the NCI to evaluate KIR haplotypes in CVID and RESPI.

Dr. Hwangpo presented her work this year at the annual meetings of the American Federation for medical Research SSCI/SAFMR, at the American Academy of allergy, Asthma and Immunology, and at the American College of Allergy, Asthma and Immunology.

10. Shannon M Kahan, PhD **07/01/12 – Present**

(Mentor: Allan Zajac, PhD)

CD8 T cell responses are vital for the control of many intracellular pathogens and tumors. The initial activation of these cells steers their development into effector and memory T cell populations. The goals of Dr. Kahan's project encompass understanding how cellular and cytokine signals regulate the functional properties of the responding T cells, and whether tactically changing these parameters can tailor responses that confer superior immunological protection against specific pathogens. To address this she will use innovative dual cytokine reporter mice to dissect functional T cell subsets, as well as utilize unique systems that permit manipulation of adhesion molecules and cytokine levels. Overall, these studies are likely to define both the functional

attributes of CD8 T cells, which signify their ability to combat acute and chronic viral infections, and how these qualities can be regulated by practical intervention strategies.

Dr. Kahan's goal is to pursue an academic career. She just began her studies in July of 2012.

10. Nicholas Kin, PhD **12/01/2007 – 02/16/2009**

(Mentor: John F Kearney, DDS PhD)

Marginal Zone (MZ) B cells play an important role in the clearance of blood-borne bacterial infections via rapid T-independent IgM responses. The Kearney laboratory has previously demonstrated that MZ B cells respond rapidly and robustly to bacterial particulates. To determine the MZ-specific genes that are expressed to allow for this response, MZ and Follicular (FO) B cells were sort-purified and analyzed via DNA microarray analysis. Dr. Kin identified 181 genes that were significantly different between the two B cell populations. 99 genes were more highly expressed in MZ B cells while 82 genes were more highly expressed in FO B cells. To further understand the molecular mechanisms by which MZ B cells respond so rapidly to bacterial challenge, idiotype positive and negative MZ B cells were sort-purified before (0 hour) or after (1 hour) i.v. immunization with heat killed Streptococcus pneumoniae, R36A, and analyzed via DNA microarray analysis. Dr. Kin identified genes specifically up regulated or down regulated at 1 hour following immunization in the idiotype positive MZ B cells. These results give insight into the gene expression pattern in resting MZ vs. FO B cells and the specific regulation of gene expression in antigen-specific MZ B cells following interaction with antigen.

Dr. Kin left the T32 training program after receiving an individual NRSA F32 fellowship. He is currently an Assistant Professor at Jefferson State University in Birmingham, AL.

11. Craig Maynard, PhD **07/01/2011 – 12/31/11**

(Mentor: Charles Elson, MD; Co-mentor: Robin Hatton, PhD)

The overall goal of Dr. Maynard's research project is to investigate the cell intrinsic and cell extrinsic factors that support the induction and maintenance of T cell-mediated intestinal immune regulation. Specifically, he is trying to determine how the commensal microbiota, by modulating expression of specific metabolites, contributes to the development and maintenance of intestinal T regulatory (Treg) cells. In addition to this, Dr. Maynard is also trying to identify molecular pathways that cooperatively control the expression of regulatory molecules such as interleukin-10 (IL-10) by intestinal Treg cells.

After receiving an individual Career Development Award from the CCFA, Dr. Maynard left the T32 training program to continue his post-doctoral studies under his own funding.

12. Gordon Meares, PhD **09/01/2008 – 08/31/2009**

(Mentor: John Corbett, PhD)

Type 1 diabetes is an autoimmune disease characterized by inflammation in and around pancreatic islets followed by the selective destruction of β-cells. Loss of insulin producing β-cells leads to a lifelong dependency on exogenous insulin as well as numerous secondary complications. Cytokines produced by the infiltrating inflammatory cells have been implicated in the destruction of β-cells by mechanisms that included β-cell production of nitric oxide. While nitric oxide is responsible for β-cell death, it also activates a program that promotes functional recovery of β-cells if the insult is removed. Based on preliminary data showing that the AMP-activated protein kinase (AMPK) is activated by cytokines and nitric oxide, Dr. Meares hypothesized that AMPK is a primary regulator of a protective program that facilitates functional recovery of β-cells from cytokine and nitric oxide-mediated damage. His research was focused on elucidating the molecular mechanisms of IL-1-induced activation of AMPK and the influence of AMPK on potential downstream targets such as PGC1.

After he received an individual F32, Dr. Meares left the T32 training program to continue his post-doctoral studies under his own funding. More recently, he has also received an individual NMSS fellowship.

13. LaToya Mitchell, PhD **08/01/2009 – 06/25/2011**

(Mentor: David Briles, PhD)

Streptococcus pneumoniae (SP) is one of the leading causes of invasive bacterial disease in the world. There are more than 93 SP serotypes. Current polysaccharide vaccines against the 13 most virulent SP serotypes in children have been effective. However, serotype replacement, decreased vaccine efficacy and cost are developing issues. One solution is a protein vaccine against a SP virulence factor, protecting across all serotypes. The cholesterol-dependent cytolysin, pneumolysin (Ply), is one candidate. With the help of a collaborator, Dr. Mitchell utilized three, non-toxic recombinant Ply variants that differ in their ability to bind cholesterol or form a lytic pore. They are CBM Ply (cholesterol binding mutant L460D), Monomer Ply (binds cholesterol but does not form oligomers) and Prepore Ply (binds cholesterol but does not form a complete pore). Dr. Mitchell hypothesized that the three, individual Ply variants are effective vaccine candidates against invasive disease in mice caused by SP and immune responses against these variants are TLR4 independent. Dr. Mitchell performed protection studies with the three vaccine candidates as well as evaluating the toxicity of WT ply in a primary response. The various effects on body temperature, pathological effects, proinflammatory cytokine response and immune signaling pathways that may be involved were also investigated.

Dr. Mitchell accepted a position as medical writer at Wolters Kluwer's in Science Communications Group.

14. Lakisha Moore-Smith, PhD **01/01/2012 – 06/30/12**

(Mentor: Boris Pasche, MD/PhD)

TGFBR1*6A is a 3-alanine mutation within exon 1 of the TGFBR1 gene which has been associated with increased cancer risk in breast, colon and lung cancer mouse models. The Pasche laboratory has developed three novel transgenic strains, a knock-in of human TGFBR1*6A, a knock-in of human TGFBR1*9A (Ozgene, Australia) and a haploinsufficient TGFBR1 mouse. Previous studies have suggested that TGF-b signaling through TGFBR1 is required for differentiation of both Th17 and Treg T cells. Using a haploinsufficient TGFBR1 colon cancer model, Dr. Moore-smith has detected a decrease in CD3 positive immune infiltration suggesting that TGFBR1 haploinsufficiency may contribute to altered differentiation and infiltration of Th17 and Tregs in the tumor microenvironment. A second ongoing study is a test of the role of TGFBR1haploinsufficiency on the development of lung fibrosis. We have found an increase in $CD3^+$ T cell numbers in the haploinsufficient mice in the lungs of bleomycin treated mice, which are a model for pulmonary fibrosis. The presence of these T cells suggests that inflammation is occurring even though the process of fibrosis is inhibited.

Dr. Moore-Smith was accepted into the training program on September 1, 2011. However, she delayed entry until January 1, 2012 due to her mentor's funding considerations. She has decided to pursue training in clinical medicine, as well, with the goal of pursuing a translational research career in oncology. She was accepted to the UAB School of Medicine as a medical student in April of 2012. She intends to continue her research studies as time permits.

15. Colleen Winstead, PhD **08/01/09 – 07/31/12**

(Mentor: Casey T Weaver, MD; Co-mentor: Robin Hatton, PhD)

The question of whether T cell memory migration and pathogen tropism coincide is a long-debated and controversial one. An enormous amount of research focused on T cell homing to peripheral tissues. Site specificity in expression of T cell growth factors (interleukins-2, -7, -15, -21) suggests that distribution of memory cells to non-lymphoid peripheral sites following pathogen clearance should be an important, focused goal of vaccination. Likewise, the potential for establishment of peripheral T cell memory through delivery of mucosal vaccines is underappreciated. During the course of her training on this T32, Dr. Winstead created several complementary cytokine reporter mouse models that allow the identification of cells competent to express IL-2, IL-21, and IFN-γ in response to *in vivo* infection (peripheral and mucosal) with a gram-positive

bacterial pathogen (*Listeria monocytogenes*). Using strains of *Listeria monocytogenes* engineered to express foreign MHCII peptides and peptide-loaded MHCII tetramers, Dr. Winstead has been able to track and assess the fate of endogenous, antigen-specific CD4 T cells in infected mice. Preliminary data suggests IL-2-'competent' and 'incompetent', antigen-specific CD4 T cells differ in their ability to traffic to peripheral tissues following an acute, systemic infection, resulting in a potential functional, as well as proliferative and survival advantage for competent cells over those acutely activated, but incompetent for IL-2 expression.

Dr. Winstead is in the process of preparing manuscripts reporting her findings. She plans to continue her post-doctoral training for one more year before seeking an academic position..

16. Carlene Zindl, PhD **05/01/2010 - Present**

(Mentor: Casey T Weaver, MD, Co-mentor: David D. Chaplin, MD PhD)

Dr. Zindl has focused on testing the role of IL-22 production by colonic neutrophils on providing protection against colitis. She has developed a novel IL-22 conditional knockout/reporter mice to allow evaluation of the protective function of colonic neutrophils *in vivo.* Using a dextran sodium sulfate (DSS)-induced mouse model of acute colitis, she observed an IL-23-dependent up-regulation of IL-22 in the middle and distal colon at the onset of epithelial cell damage. The transfer of IL-22-competent neutrophils to IL-22-deficient mice protected the colonic epithelium from DSS-induced damage. She found that IL-22—producing neutrophils targeted colonic epithelial cells to up-regulate the antimicrobial peptides. This study established a novel role for neutrophils in providing IL-22—dependent mucosa' epithelial support that contributes to the resolution of colitis. In a complementary study, she has targeted a human CD4 (hCD4) reporter into the endogenous IL-22 gene, behind an internal ribosome entry site (IRES) element and immediately downstream of the stop codon in the fifth exon. Using a mouse model of transmissible colitis induced by the rodent intestinal bacterial pathogen, *Citrobacter rodentium,* we has observed a linear correlation between hCD4 reporter expression and intracellular IL-22 levels in colonic Th17 and Th22 cells. She is in the process of characterizing reporter activity in innate colonic populations. In addition, loxP sites have been engineered into the IL-22 locus and she has begun generating IL-22 conditional knockout mice by crossing her IL-22/hCD4 reporter/conditional knockout homozygous mice with Cre transgenic mice under control of different promoters (eg, *Lys-Cre* for macrophage/neutrophil-specific deletion), thus permitting cell-specific deletion of the IL-22 gene. The overall goal to generate IL-22 conditional knockout/reporter mice is manifold: develop a novel mouse model that lacks IL-22-producing cells *in vivo,* to mark cells that express IL-22, and to permit *in situ* detection and recovery of these cells in intestinal and lymphoid tissue sites.

Dr. Zindl has submitted one manuscript reporting her findings, and is preparing a second. She plans to continue her post-doctoral training for one more year before seeking an academic position..

Status of continuing trainees.

- Due to their dates of appointment, Drs. Hwangpo, Kahan and Zindl will continue being funded by this T32 from eight to ten months after the official end date of training grant.

Predoctoral Trainees

1. George Atkinson, MD/PhD **09/01/2005 – 07/31/2009**

(Mentor: Etty (Tika) Benveniste, PhD)

Mr. Atkinson was interested in the mechanisms by which cytokines involved in immune function can contribute to tumorigenesis. He was particularly interested in glioblastoma (GBM), the most frequent and malignant primary brain tumor in adults. While new therapeutic regimens have marginally improved overall patient life spans, the lethality and aggressive nature of GBM warranted further investigation into its underlying biology. The mammalian NF-κB family includes five members: p65 (RelA), RelB, c-Rel, p50/p105 and p52/p100. In the

unstimulated state, these proteins exist in the cytoplasm bound to the IκB inhibitory complex. Upon stimulation, IκB is degraded and NF-κB dimers translocate to the nucleus where they activate a number of pro-inflammatory target genes, such as IL-8 and MMP-9. The NF-κB pathway, particularly activation of p65, is aberrantly activated in GBMs. A protein that recently has been shown to play a regulatory role in the NF-κB pathway is peptidyl-prolyl isomerase (PPIase) Pin1. Pin1 is the only member of the PPIase family which has been shown to preferentially bind phosphorylated serine or threonine residues immediately preceeding proline residues (pSer/Thr-Pro) and promote isomerization of this bond. Mr. Atkinson hypothesized that Pin1 may retain activated p65 in the nucleus, fostering increases in NF-κB signaling. To study the role of Pin1 in NF-κB signaling, he created a stable, tet-inducible siRNA glioma cell line that specifically knocks down Pin1 expression. Mr. Atkinson used this cell line to investigate the role of Pin1 in NF-κB signaling. Using immunofluorescence and western blots, he probed the effects of Pin1 on p65 localization and a number of important sites of phosphorylation on p65, such as S276. He has also used microarray technology to look at the broad effects of Pin1 on NF-κB regulated genes. One of these genes, IL-8, shows significant inhibition in Pin1 knockdown cells that have been stimulated with TNFα. This inhibition has also been confirmed by qRT-PCR and ELISA.

Dr. Atkinson is pursuing a residency in Pediatrics at Tufts University, and plans an academic career.

2. Allison Brady, MPH **09/01/2011 – 08/31/12**

(Mentor: Moon Nahm, MD/PhD)

Streptococcus pneumoniae (pneumococcus) is a Gram-positive diplococcus that commonly colonizes the mucosa of the nasopharynx (NPX), but can spread to normally sterile sites to cause diseases, such as: otitis media (OM), pneumonia, bacteremia, and meningitis. The major virulence factor of pneumococcus is its capsular polysaccharide (CPS). It is not clear why some capsule types are more likely to cause disease, while others rarely progress beyond carriage state. One possible explanation may be the interaction of pneumococcus with innate immune opsonins, such as Ficolin-2. Ms. Brady and colleagues recently found that serotype 11A CPS is bound by Ficolin-2, whereas serotype 11E CPS is not. Serotype 11A and 11E CPS structures differ only by the 0-acetylation (OAc) of the 6 carbon of the galactose residue in their repeating units. This difference is attributed to the capsular polysaccharide synthesis *(cps)* locus gene *wcjE.* The *wcjE* allele encodes a putative transmembrane 0-acetyl transferase and is intact in serotype 11A, but genetically inactivated in 11E. Serotype 11A may become 11E after colonization in order to evade host innate immune opsonin Ficolin-2. The *wcjE* allele is found in the *cps* loci of 15 serotypes that express O-acetylated epitopes similar to serotype 11A. To determine whether Ficolin-2 binding of WcjE-mediated epitopes plays a broad role in pneumococcal infections, Ms. Brady tested the ability of Ficolin-2 to bind wcjE-containing serotypes. Sje found that Ficolin-2 binds most wcjE-containing serotypes, but does not bind to any of the serotypes tested thus far that lack *wcjE.* Disruption of *wcjE* in serotype 20A leads to loss of Ficolin-2 binding, which in turn appears to be CPS-dependent. Ms. Brady is currently constructing other wcjE-deficient strains to show loss of Ficolin-2 binding, further testing the hypothesis that Ficolin-2 binding of *S. pneumoniae* is wcjE-dependent.

Ms. Brady is continuing her graduate training in Dr. Nahm's laboratory.

3. Maureen Cox **09/01/2010 – 08/31/12**

(Mentor: Allan J Zajac, PhD)

CD8 T cell responses are critical for protection from intracellular pathogens, and one hallmark of effective CD8 T cell responses is the development of a memory T cell pool which can rapidly respond to reinfection. Interactions mediated by intercellular adhesion molecule 1 (ICAM-1) have been suggested to be critical in the formation of memory CD8 T cells, as these interactions enhance both antigenic and cytokine signals. We have investigated the effect of ICAM-1 on cellular immune responses to acute infections. Surprisingly, elevated numbers of virus-specific CD8 T cells were maintained in ICAM-1 null mice following LCMV infection, and this increase in the virus-specific T cell pool represents enhanced retention of "effector-like" CD8 T cells

(CD127I0KLRG-1 hi IL-2) into the memory phase of the response. The development of memory phenotype CD8 T cells (CD127hI KLRG-1'° IL-2 producing) however, was not impaired by ICAM-1 deficiency. Moreover, ICAM-1 LCMV immune mice were able to efficiently control secondary infections despite proliferative defects observed in their memory T cells. The enhanced maintenance of effector phenotype cells appears to be the result of ICAM-1 deficiency on non-T cells subsets, suggesting a role for ICAM-1 mediated interactions with the APC in programming efficient contraction of effector and proliferative potential of memory CD8 T cells.

Ms. Cox will be graduating this fall and will then start post-doctoral studies with Dr. Tak Mak at Princess Margaret Hospital in Toronto.

4. Michael Edwards **09/01/2009 – 08/31/12**

(Co-mentors: Suzanne M Michalek, PhD; Jannet Katz, DDS PhD)

Mr. Edwards seeks to understand the role of the mTOR signaling pathway in *Francisella tularensis* (Ft) LVS infection. He has studied the interplay between the mTOR downstream effectors and the signaling molecules induced upon Ft LVS infection, as well as the role played by the Ft LVS agonist TLR2 in this process. As second focus is the elucidation of the regulatory role of mTOR on the production and expression of cytokines, on the induction of costimulatory molecules, and on the involvement of transcription factors in this modulation. A third area of study is the role played by mTOR on apoptotic factors. Mr. Edwards has completed the studies on Akt regulation in mTOR signaling, the rictor immunoprecipitation assay, and the PLCγ1 siRNA experiments. A manuscript describing his initial findings has been submitted for publication.

Mr. Edwards plans to graduate in the summer of 2013.

5. Dalia Gaddis, PhD **08/01/2004 – 09/30/2009**

(Mentors: Allan J Zajac, PhD; Suzanne M Michalek, PhD)

Ms. Gaddis' research was focused on understanding the role of the innate and adaptive immune response following *P. gingivalis* infection, a Gram-negative bacterium that is one of the causative agents of periodontal diseases. Her research included studies designed to determine the role of Toll-like receptor expression and dendritic cell activation in deriving a successful immune response to one of the virulence factors of *P. gingivalis*, the hemagglutinin B (HagB). Results from these experiments showed that HagB is a TLR4 agonist that activates dendritic cells, leading to upregulation of co-stimulatory molecules, pro-inflammatory cytokine production and activation of different signaling pathways including the MAP kinases and NF-κB. This activation also requires CD14 and adaptor molecules MyD88 and TRIF. Her results also show that immunization with HagB leads to the development of a specific memory CD4 T cell response towards HagB as manifested by the production of IFN-γ, IL-4 and IL-17. This cytokine production is differentially regulated over time by TLR4 since the absence of TLR4 results in augmentation of IL-4 production accompanied by a decrease in both IFN-γ and IL-17. More recently, the Michalek lab obtained preliminary results indicating that IL-10 may be of importance in inhibiting the development of optimal immune responses towards *P. gingivalis*. A second aspect of Ms. Gaddis' research was focused on understanding how T cell responses are structured following infection with *F. tularensis*, another Gram-negative bacterium classified as a category A biodefense agent. Her results show that TUL4, a lipoprotein expressed by the bacterium, plays an important role in IFN-γ production by CD4 T cells. In the absence of TUL4, T cells from infected mice are less activated, but have a better secondary proliferation response. In addition, the absence of TUL4 results in a more immunodominant IFN-γ production by CD8 T cells.

Dr. Gaddis is pursuing post-doctoral training at the La Jolla Institute of Allergy and Immunology.

6. Christopher Haga, PhD **01/01/2004 - 05/31/2008**

(Mentor: Max Cooper, MD)

Mr. Haga's research was focused on elucidating the signaling pathway for FCRL5 in B cells. FCRL5 contains one ITAM-like consensus sequence and two ITIM consensus sequences in its intracellular domain. His studies indicated that FCRL5 acts as an inhibitory molecule. Upon stimulation and co-ligation with the B cell receptor, FCRL5 binds SHP-1 via ITIM1 and ITIM2, subsequently inhibiting both calcium flux as well as overall cellular phosphorylation. He next evaluated the role of FCRL5 in the MAP kinase signaling pathway, used an extracellular FCRL5-Fc IgG fusion construct to probe for potential ligands, and attempted to crystallize FCRL5 in order to determine the structure.

Dr. Haga is completed post-doctoral studies at Emory University, and is now a Research Associate at Scripps Research Institute in La Jolla, CA.

7. Matthew Halpert, PhD **12/01/2007 – 09/30/2011**

(Mentor: Louis Justement, PhD)

TREM-like Transcript 2 (TLT2) is a transmembrane receptor expressed on B cells, macrophages and neutrophils in mouse and humans, making it the only TREM family member to date to be expressed on both the myeloid and lymphoid lineage. As of yet, an endogenous ligand has not been identified and the function of TLT2 remains to be elucidated. Because neutrophils are a primary mediator of the host innate immune response to bacterial infection, the analysis of factors that control neutrophil function is of great interest. Mr. Halpert showed that TLT2 potentiates multiple neutrophil functions, including the respiratory burst, degranulation, and chemotaxis, and does so only in a synergistic fashion with appropriate secondary stimuli. Additionally, Mr. Halpert has shown that TLT2 ligation on macrophages can induce the secretion of several cytokines and growth factors ex vivo, the most notable being MIP-2, KC, and G-CSF. Importantly, this production is without the addition of a secondary stimulus, indicating that TLT2 ligation is sufficient to drive this response. Of note is that TLT2 ligation does not activate the macrophage in a generalized manner, as evidenced by a lack of change in CD69, CD80, and CD86 expression after treatment, as well as the fact that phagocytosis of E. Coli or Zymosan is not affected by TLT2 treatment. A similar upregulation of these cytokines is seen within mere hours after intravenous, intraperitoneal, or intratracheal injection of TLT2 mAb, and the ensuing infiltrate of neutrophils is rapid and directed to the site of injection. Given that the predominant cytokines produced by TLT2-treated macrophages signal through GPCRs, it is not surprising that the recruited neutrophils in the TLT2-treated mouse express higher levels of CD11b than the neutrophils recruited in the FMLP- or LPS-treated mouse. Therefore, it appears that TLT2 may play an important role in both the initiation and enhancement of neutrophil recruitment during acute inflammation.

Dr. Halpert is pursuing post-doctoral studies at Baylor.

8. Kayci Huff, PhD **07/01/2005 – 06/30/2007; 06/01/2008 – 03/31/2010**

(Mentor: Phillip D Smith, MD)

Ms. Huff was interested in homeostasis of the intestinal mucosa. In order to define the role of the stroma in mucosal homeostasis, she determined whether stroma-associated products in normal human mucosa down-regulate T cell function and whether the presence of pro-inflammatory cytokines in the stroma of Crohn's mucosa block this down-regulation. To recapitulate the lamina propria microenvironment in vitro, she used human blood and intestinal lamina propria mononuclear cells and lamina propria stroma (after removal of cells). Stroma-conditioned media (S-CM) derived from normal mucosa (normal S-CM) profoundly inhibited CD3/CD28- and mitogen-induced T-cell proliferation and pro-inflammatory (IFN-γ) cytokine production. In sharp contrast, S-CM derived from inflamed Crohn's disease mucosa (Crohn's S-CM), permitted substantial CD3/CD28- and mitogen-stimulated T-cell proliferation and IFN-γ production. To address this dichotomy, she first showed that equivalent levels of TGF-β and IL-10 are present in normal S-CM and Crohn's S-CM, whereas the level of IL-6 was significantly greater in the Crohn's S-CM compared to normal S-CM. Consistent with higher levels of IL-6 in Crohn's S-CM, co-localization studies revealed that IL-6 was produced by lamina propria mast cells which were increased in number in Crohn's mucosa, whereas IL-6-producing cells were

absent in normal mucosa, despite the presence of mast cells. Moreover, the addition of rhIL-6 to normal S-CM reversed the down-regulation of T-cell function mediated by normal S-CM, mimicking the effect of Crohn's S-CM, and pre-incubation of Crohn's S-CM with neutralizing IL-6 antibodies increased the capacity of Crohn's S-CM to down-regulate pro-inflammatory T-cell function, especially with co-addition of neutralizing IL-1β antibodies. In conclusion, these novel findings indicated that intestinal stroma/extracellular matrix contributes to mucosal homeostasis through cytokine regulation of effector T cell proliferation and cytokine release.

Dr. Huff, an MSTP trainee, returned to medical school to complete her medical training.

9. Kate Kosmac **09/01/2009 – 08/31/12**

(Mentor: William Britt, MD)

Ms. Kosmac has developed a mouse model of CMV infection that has allowed investigation of the molecular mechanisms of viral infection that contribute to brain injury. Developmental abnormalities within the cerebellum coincide with a robust inflammatory response, which is likely a result of the innate immune response to viral replication within the CNS. Previous studies in Dr. Britt's laboratory had shown that CNS inflammation in CMV infected animals parallels a decrease, within the cerebellum, in granule neuron proliferation. Ms. Kosmac has shown that the robust inflammatory response in the CNS present during CMV infection leads to the decrease in granule neuron proliferation, which contributes to cerebellar maldevelopment.

Ms. Kosmac plans to graduate in the fall of 2012. A first author paper describing her work has been submitted and a second is in preparation.

10. Travis Laver, PhD **08/01/2004 – 07/31/2008**

(Mentor: Etty (Tika) N Benveniste, PhD)

Mr. Laver was interested in the role of interleukin-8 (IL-8) in the innate immune response. In addition to immune functions, IL-8 is known to contribute to the pathogenesis of a number of diseases, including cancer. Interferon-β (IFN-β), a Type I interferon, inhibits the expression of IL-8, but the details of this effect are not known. He investigated transcriptional control of the IL-8 gene and the mechanism by which IFN-β exerts inhibitory effects on IL-8 expression. He found that stimulation of U87-MG glioma cells with phorbol 12-myristate 13-acetate (PMA) results in a rapid recruitment of NF-κB p65 to the IL-8 promoter. Additionally, he found that the IL-8 promoter is constitutively acetylated on histones 3 and 4, indicating that the gene is accessible to transcription factors. Positive regulators of gene transcription such as the histone acetyltransferase (HAT) p300 were rapidly recruited to the IL-8 promoter along with RNA polymerase II. At the same time, negative regulators such as the histone deacetylases (HDACs) 1 and 3 that are constitutively present at the IL-8 promoter were dismissed. Upon treatment with IFN-β, however, there were rapid decreases in acetylation of histones 3 and 4 along with decreased levels of NF-κB p65 and RNA polymerase II present at the IL-8 promoter. He showed that these promoter effects resulted in decreased IL-8 promoter activity, mRNA levels and protein levels when cells were treated with IFN□β. His work defined the promoter-specific events necessary for induction of IL-8 expression in malignant astrocytoma cells, and the mechanistic requirements for inhibition of IL-8 by IFN-β in these cells.

Dr. Laver is pursuing training in veterinary medicine at the University of Pennsylvania. He intends an academic career.

11. Tamer Mahmoud, PhD **08/01/2004 – 07/31/2009**

(Mentor: John F Kearney, DDS PhD, Co-mentor: Harry W Schroeder Jr, MD PhD)

Mr. Mahmoud was interested in understanding antibody responses to polysaccharides associated with pathogenic microorganisms, important for improving vaccine design, especially in neonates that respond poorly to these types of antigens. He investigated the role of the lymphoid specific enzyme TdT in generating B cell clones responsive to α 1→3 Dextran (DEX). TdT is a DNA polymerase that plays a major role in generating

diversity of lymphocyte antigen receptors during V(D)J recombination. He showed that the DEX-specific antibody response is lower in TdT-/- mice than wild type BALB/c mice and that the dominant DEX-specific J558 idiotype (Id) was not detected in the sera of TdT-/- mice. Nucleotide sequencing of heavy chain CDR3s of sorted DEX-specific plasma cells post-immunization showed that TdT-/- mice generate a lower frequency of the predominant adult molecularly-determined clone J558. Complementation of TdT expression in TdT-/- mice by early forced expression of the short splice variant of TdT restored WT numbers of J558 Id+ clones but abrogated the development of the minor M104E Id+ clones. J558 Id V(D)J rearrangements are detected as early as 7 days after birth in IgM negative B cell precursors in the liver and spleen of WT and TdT transgenic mice but not in TdT-/- mice. These data suggest that TdT is essential for the generation of the higher affinity DEX-responsive J558 clone. Mr Mahmoud was also able to show that mice that are restricted to DH segment usage in reading frame 2 are capable of mounting an increased antibody response to DEX.

Dr. Mahmoud is pursuing post-doctoral training at Med-Immune in Gaithersburg, MD.

12. Donald McGuire **08/01/2008 – Present**

(Mentor: Chander Raman, PhD)

Mr. McGuire's research has focused on CD5 associated signaling pathways in CD4 T cells. CD5 attenuates TCR signaling and provides important survival signals through CK2. CD5 mutant mice demonstrate reduced severity of experimental autoimmune encephalomyelitis and altered T cell polarization. He has shown that CD5 enhances IL-6, IL-10, Interferon gamma, and Interferon alpha receptor STAT phosphorylation. Due to possibility of multiple relevant signaling processes, he has begun to utilize flow cytometry to determine the signaling strength of multiple STATs during *ex vivo* polarization of T cells. This has allowed him to determine whether CD5 enhancement of IL-6 signaling is responsible for the inhibition of Th1 and enhancement of Th17 polarization.

Mr. McGuire plans to graduate in the fall of 2013.

13. Sarah Mollo **09/01/2009 – 08/31/12**

(Mentor: Laurie E Harrington, PhD)

Ms. Mollo is studying the role of B cells in the establishment and maintenance of CD4 T cell memory, as well as their impact on the CD4 T cell recall response. Using B cell-deficient mice, she has shown that B cells are important for the formation of CD4 effector and memory T cells during *Listeria monocytogenes* infection. By depleting B cells with anti-CD20 prior to infection with *Listeria monocytogenes,* she examined the requirement of B cells during priming for the generation of CD4 T cell responses and found a reduced frequency and number of antigen-specific CD4 T cells capable of producing IFNγ and IL-2 at effector and memory time-points. Importantly, the CD4 T cell response was further diminished in B cell-deficient mice suggesting that the effect of B cells on splenic architecture can impact CD4 T cell function. Due to the differential activation requirements between naïve and memory cells, she analyzed the recall response of CD4 memory T cells in the absence of B cells. Similar to naïve CD4 T cells, she found that CD4 memory cells require B cells in order to generate an optimal secondary response.

Ms. Mollo plans to graduate in the summer of 2013.

14. Lindsey Padgett **08/01/2010 – Present**

(Mentor: Hubert Tse, PhD)

Aspergillus fumigatus (A.f.) is the cause of invasive aspergillosis (IA) which has become a leading cause of death in immunosuppressed populations in medical centers worldwide. There is currently no completely effective treatment or cure for IA, so it is critically important to pursue new avenues to achieve this goal. A.f. shares a common sialic acid-associated derivative, neuraminic acid, with Group B streptococci, thus she

hypothesized that some of these capsular polysaccharide epitopes found on various serotypes of GBS would be conserved on both GBS and A.f. She found that a monoclonal antibody (mAb) specific for the GBS typeIb (GBSIb) capsular polysaccharide associated oligosaccharide, sialyllacto-N-tetraose (s-LNT), also binds to A.f. conidia and hyphae. Based on previous studies implicating sialic acids as major virulence factors for GBS, she hypothesized that s-LNT specific mAbs recognizing sialic acid associated epitopes on the surface of A.f. and would be protective in a murine model of IA. The goal of her continuting project are to identify the epitope on the surface of A.f. which is recognized by anti-s-LNT antibodies, to determine whether anti-sLNT antibodies protect in a murine model of IA, and to determine the mechanism by which anti-sLNT antibodies affect IA disease progression.

Ms. Padgett plans to graduate in the fall of 2013.

15. Theresa Ramos **09/01/2009 – 08/31/12**

(Mentor: Scott Barnum, PhD)

With a fatality rate of 15-30% and with 10% of survivors suffering permanent neurological sequelae, cerebral malaria (CM) is one of the most severe clinical complications of *P. falciparum* malaria. Previous studies had indicated that mice deficient in complement component C5 were protected from cerebral complications. To determine if prevention of earlier pathway activation could foster similar protection, she has examined mice deficient in pathway specific complement components C4 (classical and MBP pathways and Factor B (alternative pathway) as well as C3 due to the cleavage products forming the C5 convertase. She has found no significant improvements in survival between wild type mice and complement mice deficient, C4-1- mice and Factor 13-1-, mice. However, moderate protection in C3-/- mice was observed, suggesting that blocking C5 convertase formation is more important in preventing disease then blocking activation of a specific pathway. To determine the protective mechanism of C5 deficient mice, she then examined mice deficient in the anaphylatoxin receptors (C3aR1- and C5a11-/-) and found no protection in these mice, suggesting protection from ECM in C5-/- mice is not mediated through C5a-induced inflammation as previously reported. Given these data she tested whether protection could be attributed to inhibition of C5b pairing with other complement proteins to form the MAC (C5bC6-C9). She treated wild type mice with an anti- C9 antibody specific for mouse C9 and found a significant increase in survival of treated mice. These results suggest that the pathological effects of complement in ECM are primarily mediated at the level of C5 through the terminal pathway of complement. She has also demonstrated that anti-C9 antibody treatment significantly delays the development of ECM giving additional support for the contribution of the terminal complement pathway in ECM development.

Ms. Ramos has obtained her own individual F31 funding and plans to graduate in the spring of 2013.

16. Daniel Schreeder, MD/PhD **12/01/2007 – 11/30/2009**

(Mentor: Randall Davis, MD)

Mr. Schreeder was interested in the role of the human Fc Receptor-like 6 (FCRL6) molecule in the immune response. The Fc receptor-like molecules (FCRL) are a recently described family of type I transmembrane receptors with extracellular immunoglobulin (Ig)-like domains and cytoplasmic tails possessing tyrosine-based signaling capability. Despite their evolutionary relationship to Fc receptors (FcR), Ig-binding studies have failed to show an Fc receptor function for the FCRLs and therefore these molecules are orphan receptors. In humans, FCRL1-5 are primarily expressed by B lineage cells and are capable of potent immunoregulatory function by means of their cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs) or inhibition motifs (ITIMs). Human FCRL6, the most recently identified FCRL member, is positioned apart from the other FCRL genes on chromosome 1q21-23 near the Fc receptor for IgE (FcεRI) and encodes three extracellular Ig-like domains, an uncharged transmembrane domain, and a cytoplasmic tail with a consensus ITIM or a noncanonical ITAM. Like the other FCRL molecules, studies have failed to show an Ig-binding function for FCRL6. Mr. Scheeder's research focus was on answering three specific questions about FCRL6: (i) What is

the cellular expression pattern of FCRL6? (ii) What is the ligand for FCRL6? (iii) What is the function of FCRL6?

Dr. Schreeder is pursuing a residency in Internal Medicine at Johns Hopkins and intends to pursue an academic career.

17. Elizabeth Staley, PhD **08/01/2005 – 07/31/2009**

(Mentor: Robinna Lorenz, MD/PhD)

Ms. Staley was interested in the role of P-glycoprotein (P-gp) in the immune response. She generated colonic epithelial cell lines stably expressing P-gp targeting short hairpin RNAs (shRNAs). She has shown that P-gp deficiency is integral to the epithelial responses to TLR9 associated CpG stimulation, and TLR4 associated LPS stimulation. She also showed that while stimulation with these ligands does affect the cellular inflammatory response, it does not seem to affect barrier integrity or localization of the barrier protein ZO-1. Animals deficient in P-gp expression have been generated on the C57BL/6 background. These animals are resistant to the development of spontaneous colitis. C57BL/6.mdr1a-/- mice are susceptible to colitis induction via epithelial barrier disruption using dextran sodium sulfate (DSS), although they are resistant to colitis induction through using *Helicobacter bilis*, or piroxicam treatment. A second area of interest was the role of TLRs and TLR ligands in murine development. She studied the effects of the intestinal microbiota on innate immunity at specific time points in murine development. This information was critical to advance understanding of the role of P-glycoprotein in mucosal immune responses.

Dr. Staley is in medical training at UAB and intends to pursue an academic career.

18. Ewa Szymanska **08/01/2009 – 09/30/12**

(Mentor: Harry W Schroeder, MD/PhD; Co-mentor: Elizabeth Brown, MPH PhD)

Common Variable Immunodeficiency (CVID), the most common primary immune deficiency under the care of clinical immunologists, defines patients suffering with serum immunoglobulins (sIg) deficiencies. Family members of CVID patients often present with selective IgA deficiency (IgAD); as well as with recurrent sino-pulmonary infections (RESPI) who present similarly to their CVID relatives, but with normal sIg levels. Ms. Szymanska has studied a pair of identical female twins, where one suffers with severe infections and depressed immunoglobulin levels (CVID), and the other has milder infections and normal immunoglobulin titers (RESPI). Phenotypic characterizations of peripheral blood B cell subpopulations by FACS has revealed higher numbers of immature B cells in the CVID twin, but progressively lower numbers of transitional, mature, memory IgD+/IgD-, and plasmacytes when compared to the RESPI twin. Deep sequencing of immunoglobulin transcripts from the transitional, memory IgD+/ IgD-, and plasmacyte fractions has revealed a consistently significant lower prevalence of tyrosine in the CDR-H3 loops of the CVID patient than an age and sex-matched control. Her RESPI twin, in contrast, has higher levels . Our findings suggest that in addition to a progressive block in mature B cell differentiation starting from transitional B cells, both CVID and RESPI may be associated with an altered development of the antibody repertoire, which may help explain why, in spite of the presence of IgG, both patients suffer with infections. A comprehensive analysis of the B cell receptor in CVID may help extend the disease definition and help identify the mechanisms that underlie the immune deficiency in of CVID.

Ms. Szymanska plans to graduate in the summer of 2013.

19. Melissa Thal, PhD **08/01/2004 – 09/30/2009**

(Mentor: Christopher Klug, PhD)

Ms. Thal was interested in early B cell fate choice. Over the last few years, several studies have identified critical transcription factors and signaling components that influence fate specification from uncommitted common lymphoid progenitor cells in adult bone marrow. However, very little mechanistic data have been offered to explain how these factors might positively regulate specification. Ms. Thal investigated how the

activation of Early B cell factor (Ebf1) functions as the primary initiating event in B-cell fate choice. Ebf1 accomplishes this by potently down-regulating expression of the E2A inhibitory factors, Id2 and Id3, and by increasing expression of E2A mRNA. Down-regulation of both Id2 and Id3 is essential in that overexpression of either factor in wild-type bone marrow cells blocks B cell development at the onset of specification. By inducing higher expression and presumably activity of E2A, EBF advances the differentiation pathway and promotes B cell specification.

Dr. Thal is pursuing postdoctoral studies at Northwestern University.

20. Rebekah Wharton **08/01/2010 – Present**

(Mentor: John Kearney, DDS, PhD)

Aspergillus fumigatus (A.f.) is the cause of invasive aspergillosis (IA) which has become a leading cause of death in immunosuppressed populations in medical centers worldwide. There is currently no completely effective treatment or cure for IA, so it is critically important to pursue new avenues to achieve this goal. A.f. shares a common sialic acid-associated derivative, neuraminic acid, with Group B streptococci, thus Ms. Wharton hypothesized that some of these capsular polysaccharide epitopes found on various serotypes of GBS would be conserved on both GBS and A.f. She found that a monoclonal antibody (mAb) specific for the GBS typeIb (GBSIb) capsular polysaccharide associated oligosaccharide, sialyllacto-N-tetraose (s-LNT) also binds to A.f. conidia and hyphae. Based on previous studies implicating sialic acids as major virulence factors for GBS, she has hypothesized that s-LNT specific mAbs recognizing sialic acid associated epitopes on the surface of A.f. and would be protective in a murine model of IA. Experiments to determine whether anti-sLNT antibodies protect in a murine model of IA and to determine the mechanism by which anti-sLNT antibodies affect IA disease progression are ongoing.

Ms. Wharton plans to graduate in the summer of 2013.

21. LaTonya Williams **08/01/2009 – 06/30/2010**

(Mentor: Paul Goepfert, MD)

Despite efforts to understand the immune mechanisms that regulate containment of HIV-1, the specific qualities of anti-viral CD4+ and CD8+ T cell responses required to achieve durable control of HIV-1 remains largely unknown. In the majority of patients, HIV-1-specific CD8 T lymphocytes (CD8-TL) gradually become less functional and persist in an exhausted state, unable to effectively eradicate infected targets. The Goepfert laboratory has previously focused on the quality of the response and have found that polyfunctional CD8 TL, capable of cytokine secretion, proliferation, and degranulation are generated in primary infection and are maintained in HIV-1 controllers. However, it remains ill-defined which factors are responsible for the maintenance of these polyfunctional CD8-TL. Several lines of evidence suggest that the early CD4+ T cell-mediated CD8-TL priming events may be crucial for the programming of vigorous effector and memory CD8-TL responses. Mouse models of chronic viral infection demonstrate a vital role for IL-21, in the induction, quality, and longevity of anti-viral CD8-TL responses. Miss Williams's preliminary studies demonstrate that HIV-1-specific CD4+ T cells are compromised in their ability to produce IL-21 during chronic infection. Interestingly, she demonstrates that HIV-1-specific CD8-TL are also capable of producing IL-21 and are even better associated with viral control than their CD4+ T cell counterparts. In light of the altered IL-21 production in HIV-1 and its proposed importance in the generation of effective virus-specific effector CD8-TL, she hypothesizes that the loss of polyfunctional CD8-TL in chronic HIV-1 infection is a consequence of the functional abrogation of IL-21-producing HIV-1-specific CD4+ and CD8+ T cells. Utilizing polychromatic flow cytometry, this project proposes to elucidate the quantitative and qualitative requirements of the latter cells in contributing to the generation of protective, long-lived HIV-1-specific CD8-TL responses in HIV-1 controllers. Understanding the molecular mechanisms accounting for the link between CD4+ T cell help and CD8+ T cell functional quality and anti-viral efficacy will provide new insights for recapitulation into an effective HIV-1 vaccine.

Ms. Williams obtained F30 funding and has continued her graduate work under the mentorship of Dr. Goepfert and plans to graduate in the fall of 2012.

22. Jillian Adams Wholer, PhD **12/01/2007 – 05/31/2009**

(Mentor: Scott R Barnum, PhD)

Ms. Wholer (maiden name Adams) was interested in the role of the b2-integrins on T cell subsets, particularly as it relates to the development and progression of experimental autoimmune encephalomyelitis (EAE) and the ligands for these receptors in the same system. Members of the β2-integrin family of adhesion molecules, CD11a, CD11b, and CD11c, have all been shown to play a role in the pathogenesis of EAE. CD11d had yet to be studied in demyelinating disease and its functions remained unclear. Ms. Wholer found that CD11d is the only member of the β2-integrin family of adhesion molecules that fails to protect against the development of EAE. Surprisingly, the EAE studies suggested that CD11a, CD11b, and CD11c were all contributing to T cell activity during disease development by mechanisms beyond the migration of these cells into the CNS. However, the contributions of individual T cell subsets to the overall phenotypes seen were unclear. Earlier studies showed that over the course of EAE a higher proportion of γδ T cells express the β2-integrins when compared to αβ T cells. Given this, she hypothesized that the β2-integrin family was important to the functions of γδ T cells that contributed to the development of EAE. However, she showed that even though expression is enriched in this T cell subset, the β2-integrins on γδ T cells for do not seem to be required for disease development. The β2-integrin family has also been implicated in regulatory T cell function and homeostasis. Studies using transfer EAE with CD11a-/- mice have suggested these mice may have regulatory defects. Therefore she investigated the role CD11a plays in regulatory T cell biology. Ms. Wholer found that CD11a-/- mice have reduced Treg populations throughout the secondary lymph tissue and that this reduction may be due to a reduced capacity to generate peripheral Tregs. She also found that CD11a is critical to Treg function in vitro, but does not seem to be as important in vivo. Importantly CD11a appears to be mediating its immunosuppressive effects independently of interactions with ICAM-1 on target T cells. Overall, the studies provided further evidence that the β2-integrin family of adhesion molecules functions in many aspects of T cell biology aside from cellular migration and that these functions differ between T cell subsets.

Dr. Wholer is currently pursuing post-doctoral studies at the NIH.

23. John Yi, PhD **09/01/2008 – 08/31/2010**

(Mentor: Allan J Zajac, PhD)

Mr. Yi was interested in understanding how CD4 T cells provide help to CD8 T cells. One proposed mechanism of help by CD4 T cells is through the production of interleukin 21. IL-21 is a member of the g chain cytokine family, which includes IL-2, IL-4, IL-7, and IL-15, and it is well appreciated that CD4 T cells are the main producers of IL-21. The focus of his project was to delineate the role of IL-21 in anti-viral immune responses, with an emphasis on CD8 T cells. Provocative data showed that IL-21 is vital for the clearance of the natural mouse pathogen lymphocytic choriomeningitis virus. His findings also showed that the targeted deletion of IL-21 is associated with reduced polyfunctional CD8 T cell responses and a more severely exhausted phenotype. Mr. Yi performed studies to delineate the direct versus indirect effects of IL-21 on effector, memory, and exhausted CD8 T cell responses, evaluate the impact of IL-21 in promoting polyfunctional anti-viral CD8 T cells, and determine the therapeutic benefits of IL-21 administration or ablation on immunity and viral control. Collectively, these studies advanced our fundamental understanding of anti-viral defense mechanisms and may provide the foundation for translational studies to develop new immune based therapies for controlling viral infections.

Dr. Yi is pursuing postdoctoral training at Duke University.

<u>Status of continuing trainees.</u>

- Due to their date of appointment, Don McGuire, Lindsey Padgett, Eva Szymanska and Rebekah Wharton will continue being funded by this T32 from one to four months after the official end date of training grant. All four have all been admitted into candidacy and completed the necessary coursework for their PhD degrees. All four anticipate defending their dissertation research by the end of the 2013.

6F. Distribution of training grant positions.

The T32 Executive Committee has made an effort to keep all the training positions filled (Table 11) and to distribute the trainee positions among the numerous mentors associated with the training grant. Months of support from year 35 were left unspent only because, as a result of the grant ending on 08/31/12, partial-year appointments due to irregular start and end-dates did not leave sufficient months of support to allow new appointments. As summarized in Table 12A, there have been 43 predoctoral trainees from 26 different mentors on this T32 in the past 10 years of support. Of the 26 mentors, 15 have had only 1 trainee funded by the T32, 4 mentors have had 2 trainees, 5 mentors have had 3 trainees, 1 mentor has had 4 trainees, and 1 mentor has had 5 trainees (this includes being a co-mentor to 1 trainee). The predoctoral trainees associated with the training program average 5.0 years in the program and receive an average of 2.7 years of support per trainee during that period. A similar distribution of postdoctoral trainee positions is depicted in Table 12B. In the past 10 years of support, this T32 has funded 31 postdoctoral trainees from 21 different mentors, with an average of 1.9 years of support per individual from the T32. Twelve of these mentors had only 1 postdoctoral trainee, 6 mentors had 2 postdoctoral trainees, and 3 mentors have had 3 postdoctoral trainees placed on training grant. In total, there have been 74 trainees from 38 different mentors, highlighting the diverse group of individuals associated with the training program.

6G. Recruitment of individuals from underrepresented groups during the previous funding period.

Recruitment and retention of underrepresented minorities among predoctoral trainees. For the period 2008-2012, recruiting activities in the participating departments and programs resulted in a total of 665 predoctoral minority applicants (Table 7A). Of these, 107 applicants were offered positions, 44 enrolled, and 39 remain in training. Of the 23 predoctoral trainees supported by this training program in the last five years, one is URM.

Recruitment and retention of underrepresented minorities among post-doctoral trainees. For the period 2007-2011, the years for which data is complete, recruiting activities in the participating departments and programs resulted in a total of 1121 postdoctoral minority applicants (Table 7B), Of these, 179 were offered positions and accepted the offer. Of these, 101 postdoctoral minority trainees are still at UAB. Of the 16 postdoctoral trainees supported by this training program in the last five years of support, six are URM.

Recruitment and retention of women and underrepresented minorities among the mentors. The ITP also seeks to enhance gender equity and to recruit and retain faculty from under-represented groups. We have increased the representation of women among the faculty from 16% in 2006 to 30% in 2012. Three faculty members, Drs. Katz, Lopez and Schroeder (Melendez) (5% of the faculty), are Hispanic-Americans.

Recruitment and retention of individual with disabilities among trainees. The ITP seeks to accommodate and retain individuals with disabilities by working with the UAB Office of Disability Support Services (DSS). Of the 15 self-identifying individuals with disabilities clearly associated with the training program for the past five years (Table 10), one was supported by this T32.

6H. Research instruction and training.

Didactic Coursework (Pre- and Postdoctoral trainees, required). Pre-doctoral trainees are accepted into this training program only after having completed their first year graduate courses. Post-doctoral trainees are also expected to participate in those modules of the graduate curricula pertinent to their mentored project and research interests. This occurs during the first or second research years. Both pre- and postdoctoral trainees are also expected to take the graduate course in biostatistics. All of the trainees (pre- and postdoctoral) are required to take GRD 717, Principles of Scientific Integrity, and are expected to take a course in bioinformatics, genomics, computational biology or systems biology. In 2009, we implemented a new course, “Topics in Professional Development” (MIC741) which is required of all trainees participating in the training program.

This class has worked with the T32 trainees on poster and grant preparation, and exposed them to leading Immunologists and senior administrators at UAB. This course will include an overview of new technologies and approaches, including an introduction to bioinformatics, genomics, computational biology or systems biology.

The faculty and the administration at UAB are fully cognizant of the need for training to encompass advances in computational technology and changes in scientific emphasis. In January 2011, the Department of Pathology created a new Division of Informatics, currently headed by Dr. Jonas Almeida; and the Department of Microbiology focused additional support on its long-standing (Biomedical Informatics) unit, headed by Dr. Elliot Lefkowitz. Both of these distinguished academicians have ongoing collaborations with faculty mentors and their trainees, and both have joined our training program as mentors. A number of educational and training opportunities have been developed in these and other departments. These include GBS722: GGS Bioinformatics, which is focused on learning how to use large-scale, generic databases; GBS 755: Integrative Bioinformatics, which is focused on practical uses of semantic web and cloud computing technologies and resources; BST 676: Statistical Bioinformatics, which is focused on analysis of data generated by high throughput genomic technologies; CIS 640: Bioinformatics I and CIS 641: Bioinformatics II, which are focused on computational methodologies in bioinformatics; the CCTST-BMI Summer Series: CTSA Informatics Competencies, which is focused on key topics in biomedical and health informatics for clinical and translational science researchers; and an NHGRI short course which is focused on statistical methodologies and algorithms used to evaluate next-generation sequencing databases. A more complete description of these courses can be found in Appendix C4. Our trainees are expected to attend at least one of these courses.

UAB is making major efforts to provide additional opportunities for trainees to develop and certify training in the developing fields of genomics, proteomics, and translational studies. A translational science course for post-doctoral scholars is taught by Drs. Chaplin and Schwiebert, both of whom are long-standing mentors of this training program. Other courses include GBS 724: Principles of Genetics; MGE 725: Advanced Medical Genetics; GBS720: Genomic Structure and Function; GBS 721: Genetic Epidemiology; GBS 723: Model Systems for Genetic and Epigenetic Analysis; and GMB/PHR 744: Proteomics and Mass Spectroscopy. A certificate program in Translational and Molecular Sciences is open to all students in a PhD or MD/PhD program. The HHMI program is another avenue available to allow trainees to gain expertise in translational approaches to biomedical studies. Finally, the Analytic Imaging and Immunoreagent Core provides training in the latest imaging techniques and approaches.

Interdisciplinary Enrichment Program (Pre- and Postdoctoral trainees, required). A unique value-added activity for our T32 trainees is a biweekly luncheon with visiting scholars for the Program in Immunology (Appendix D2). This series of visiting professors provides an important opportunity for informal exchange of research ideas and questions. We have received exceptionally positive feedback from our trainees, as well as the visiting scholars, regarding the impact of this forum.

Research in Progress (Pre-doctoral and Postdoctoral trainees, required). Students in training participate in weekly Research in Progress meetings that are sponsored by the various individual departments and divisions that make up this T32 training program.

Journal Clubs, Seminars, and Conferences (Pre-doctoral and Postdoctoral trainees, required). Throughout the year there is a seminar program sponsored by the Program in Immunology which is required for all T32-associated trainees to attend (Appendix C2), as well as additional seminars supported by other Centers and Departments within the University that trainees are encouraged to attend**.** In addition, there are a number of specific topically focused journal clubs, including Allergy and Clinical Immunology, Autoimmunity, Cellular and Molecular Immunology, Inflammation, Neuroimmunology and Mucosal Immunology. Trainees are required to participate in at least one journal club, selected with the advice and counsel of their mentor. Trainees are also encouraged to attend and present at regional and national scientific meetings, such as the American Association of Immunologists National Scientific meeting and the Federation of Clinical Immunology Societies meeting.