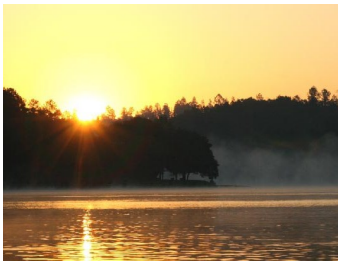
22nd Annual Microbiology Research Retreat

October 18 - 20, 2013



Alabama 4H Center

Columbiana, AL

AGENDA

FRIDAY, OCTOBER 18TH

1:00 PM	**Registration**	Guthrie Lodge
2:15 PM	**Fran Lund** Welcome	Auditorium
2:25 PM	**Pete Burrows** Introduction	
2:30 PM	**Venkata Yeramilli**[PD] Role of Aryl Hydrocarbon Receptor in B Cell Development and Function	Kearney Lab
2:45 PM	**Tyler Stewart**[NI] Aberrant *O*-glycosylation of IgA1 in IgA Nephropathy (IgAN) and the Role of Sialyl-Tn Antigen	Novak Lab
3:00 PM	**Preeyam Patel**[NI] Anti-Phosphorylcholne Antibodies Modulate House Dust Mite Allergy in Mice	Kearney Lab
3:15 PM	**Stewart New** [NI] Modulation of Autoimmune Diabetes by Antibodies Specific for N-acetyl-D-glucosamine	Kearney Lab
3:30 PM	**Lindsey Padgett**[GS] Ablation of Reactive Oxygen Species Intensifies Diabetogenic CD4 T Cell Effector Responses	Tse Lab
3:45 PM	**Ashley Burg**[NI] Superoxide Production Mediates Anti-Viral Responses to Diabetogenic Viruses	Tse Lab
4:00 PM	Break	
4:15 PM	**Hao Li**[GS] Type I Interferons-induced Follicular Translocation of Lymphotoxin-Expressing Marginal Zone B cells Initiates Lupus	Mountz Lab
4:30 PM	**Shannon Kahan**[PD] The Role of Early IL-2 and IFN-γ Production in Determining the Fate of Newly Activated Anti-viral CD8 T Cells.	Zajac Lab
4:45 PM	**Rakesh Bakshi**[PD] IL-2 Production by CD8 T Cells Forecasts the Formation of Immunological Memory	Zajac Lab
5:00 PM	**Yuan Tian**[NI] The Role of IL-21 in Memory CD8 T Cell Differentiation and Maintenance	Zajac Lab
5:15 PM	**Break**	
5:30 - 7:00 PM	**Poster Session**	Seminar Room
7:30 PM	**Dinner**	Cafeteria
8:30 PM	**Campfire**	

SATURDAY, OCTOBER 19

7:30 - 8:30 AM	**Breakfast**	Cafeteria
8:30 AM	**Julio Ayala-Figueredo**[NI] Regulation of *Vibrio Cholerae* Biofilm Formation by H-NS Repression and Anti-Repression	Benitez Lab
8:45 AM	**Stephanie Garcia**[GS] Dispersion of *Streptococcus mutans* Biofilms by a Novel Small Molecule	Wu Lab
9:00 AM	**Haley Echlin**[GS] Two Glycosylation-Associated Proteins Protect the Third Protein from Degradation through Interaction with a Peptidase System in *Streptococcus parasanguinis*	Wu Lab
9:15 AM	**Beth Walters**[NI] Single Amino Acid Mutations in Ribosomal Protein S25 Significantly Affect Internal Ribosome Entry Site Translation	Thompson Lab
9:30 AM	**Shane Kelly**[GS] Autophagy is Regulated by 5'-3' mRNA Decay in *Saccharomyces cerevisiae*	Bedwell Lab
9:45 AM	**Break**	
10:00 AM	**Gwendolyn Gunn**[NI] Long-Term Nonsense Suppression Therapy with NB84 Moderates MPS I-H Disease Progression	Bedwell Lab
10:15 AM	**Jeffery Vahrenkamp**[GS] Conditions Governing the Fate of Reiterative Transcripts	Turnbough Lab
10:30 AM	**Jocelyn Hauser** [GS] Aeration-dependent Regulation of Capsule in *Streptococcus pneumoniae*	Yother Lab
10:45 AM	**Jiri Vlach**[PD] Structure of the Complex between Calmodulin and HIV-1 Matrix Protein	Saad Lab
11:00 AM	**Abdellah Akil**[PD] Mouse Models of Hepatitis C Virus Replication and Tumorigenesis	G. Luo Lab
11:15 AM	**Session ends**	
11:20 AM	**Group Picture** (Prevelige)	TBA
11:30 AM	**Lunch**	Atrium
12:00 – 6:30 PM	**Free Time** (Activities include climbing wall, swinger, basketball, putt-putt)	
6:30 PM	**Dinner**	Auditorium
7:00 PM	**Kimberly Benton** Division of Cellular and Gene Therapies at the Center for Biologics Evaluation and Research, FDA Scientific Careers in the Food and Drug Administration	Auditorium
8:30 PM	**Campfire**	

SUNDAY, OCTOBER 20TH

(Check out by 9am)

8:00 - 9:00 AM	**Breakfast**	Cafeteria
9:00 AM	**Dmitry Shayakhmetov** University of Washington Sensing Pathogen Entry into Cell	Auditorium
9:30 AM	**Mengxi Jiang** University of Michigan BK Polyomavirus and the Host DNA Damage Response	
10:00 AM	**Charles Elson** Adaptive Immunity to the Microbiota in Humans	
10:30 AM	**Pete Burrows** Awards	
11:00 AM	**Pete Burrows** Closing remarks	

2013 Poster Presentations

Constance Agamasu[NI]

Eugene J Becker[GS]

Allison M Brady[GS]

Kyle M Brawner[NI]

Victor Yimin Du[GS]

Sarah Dulson[NI]

Vojtech Franc[GS]

Aaron Geno[PD]

Kristopher Genschmer[PD]

Rosie L Hill[GS]

Nicolas Edouard Roger Maillard[PD]

Nicholas R Potochick[NI]

Travis Ptacek[PD]

Shannon Romano[NI]

Brady L Spencer[NI]

Sara L Stone[NI]

Jian Tao[NI]

Arthur H Totten[NI]

Arthur J VanValkenburg[GS]

Matthew Weaver[NI]

Xiaojiao Xue[GS]

GS – Graduate Student (Year 4 and up)

NI – New Investigator (Year 1-3)

PD – Postdoctoral Fellow

ORAL ABSTRACTS

Role of Aryl Hydrocarbon Receptor in B Cell Development and Function

Venkata Yeramilli and John Kearney

The aryl hydrocarbon receptor (AhR) plays important roles in the immune system. The contributions of AhR ligands to the differentiation and functions of Th17/Treg cells are relatively well understood. In contrast, little is known about the role of AhR in B cell differentiation and function. Microarray and quantitative PCR analysis of marginal (MZ) and follicular (FO) B cells revealed that resting MZ B cells expressed higher levels (4-5 fold) of AhR compared to FO B cells. Based on these data, we hypothesized that AhR plays a role in the development and function of MZ B cells. Consistent with our hypothesis, we found fewer MZ B cells, but not FO and B1 B cells in $AhR^{-/-}$ compared to $AhR^{+/+}$ mice. Following in vivo LPS treatment, MZ B cells from $AhR^{-/-}$ mice expressed higher levels of CD86 than their wild-type (WT) counterparts, indicative of a hyperactive phenotype. When immunized with *Streptococcus pneumonia,* compared to WT mice, $AhR^{-/-}$ mice mounted a robust immune response with significantly high titers of anti-phosphorylcholine IgM. In contrast, upon immunization with a model T cell-dependent antigen, Trinitrophenyl Keyhole Limpet Hemocyanin (TNP-KLH), $AhR^{-/-}$ mice had anti-TNP titers that were comparable to those in $AhR^{+/+}$ mice. These findings suggest that AhR plays a role in regulating T cell-independent but not T cell-dependent humoral response. Further studies are underway to elucidate the role of AhR in the development and activation of innate-like B cells.

Aberrant *O*-glycosylation of IgA1 in IgA Nephropathy (IgAN) and the Role of Sialyl-Tn Antigen

Tyler J. Stewart, Hitoshi Suzuki, Milan Raska, Kazuo Takahashi, Koshi Yamada, Milada Stuchlova Horynova, Matthew B. Renfrow, Jan Novak

Background: In IgAN aberrant *O*-glycosylation leads to galactose deficient IgA1 hinge region (HR) forming an auto antigen. Previous data has demonstrated increased α2,6 sialylation of *O*-linked GalNAc of IgA1 HR from patients suggesting a role of ST6GalNAcII in IgAN. Using several complementary approaches, we tested whether premature sialylation by ST6GalNAcII can inhibit the addition of β1,3 galactose to IgA1 HR by C1GalT1.

Methods: In vitro reactions of purified recombinant ST6GalNAcII and C1GalT1 with IgA1 HR synthetic glycopeptides (previously GalNAcosylated by GalNAc-T2) or desialylated IgA1 were characterized using high-resolution mass spectrometry (MS) or ELISA. Relative MS quantification was based on the total ion counts of various glycoforms were analyzed to characterize sialylation based inhibition of galactosylation at the molecular level. Lectin ELISA was used to assess availability of terminal GalNAc.

Results: Using lysates from IgA1-secreting cells from IgAN patients and healthy controls as the sources of ST6GalNAc-II and C1GalT1 enzymes, we performed in vitro enzymatic sialylation of desialylated IgA1 followed by galactosylation. Assessment of glycosylation by lectin ELISA revealed that sialylation of IgA1 by ST6GalNAc-II prevented effective galactosylation of IgA1 by C1GalT1. We confirmed these findings by using recombinant enzymes. We have now produced recombinant ST6GalNAcII and C1GalT1 and demonstrated that both enzymes are active and quantified relative amounts of various HR glycoforms after single glycosyltransferase reactions. Furthermore, results from dual enzyme in vitro reactions support inhibition of C1GalT1 by α2,6 sialylation.

Conclusion: Overproduction of sialyl-Tn antigen prior to galactosylation could result in galactose-deficient IgA1 HR, the phenotype associated with IgAN.

Anti-phosphorylcholne Antibodies Modulate House Dust Mite Allergy in Mice

Preeyam Patel, John Kearney

Currently, an estimated 20% of the population worldwide exhibits allergies and approximately 80% of these individuals suffer from respiratory allergens. House dust mite (HDM), *Dermatophagoides pteronyssinus*, are one of the most common worldwide respiratory allergens and unlike most other respiratory allergens, HDM contain phosphorylcholine (PC) moieties.

The prevalence of asthma and allergic disease has been seen mostly in industrialized countries. HDM have evolved away from their parasitic nature to reside mostly in pillows, mattresses, and carpet, all comforts of developed industrial countries. The "hygiene hypothesis" correlates this increase with reduced exposure to microbes and decreased childhood infections. Without repeated microbial exposure to establish a normally dominant Th1 response, individuals with asthma and allergy mostly exhibit a Th2 response.

In order to understand the link between early microbe exposure and protection against allergy and asthma, mice were neonatally immunized with R36A, a phosphorylcholine-bearing strain of *Streptococcus pneumoniae*. In comparison to naïve mice, these mice generate significantly higher anti-phosphorylcholine (PC) IgM antibodies. *In vitro* these anti-PC antibodies decrease uptake of HDM allergen by antigen-presenting cells. When passed through a HDM allergy model, mice neonatally immunized with R36A exhibit dampened allergy as demonstrated by significantly decreased cellular influx of eosinophils, neutrophils, and T cells, into the bronchoalveolar lavage fluid and lung parenchyma. This demonstrating a protective role for anti-PC antibodies against HDM allergy and opens doors for further study of early microbial exposure and protection against allergy and asthma.

Modulation of Autoimmune Diabetes by Antibodies Specific for N-acetyl-D-glucosamine

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

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

Ablation of Reactive Oxygen Species Intensifies Diabetogenic CD4 T Cell Effector Responses

Lindsey E. Padgett* and Hubert M. Tse

In Type 1 diabetes (T1D), infiltrating leukocytes generate reactive oxygen species (ROS) and pro-inflammatory cytokines that collectively participate in beta-cell destruction and enhance autoreactive T cell responses. Our laboratory previously demonstrated that superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are T1D-resistant partly due to skewed T cell responses. To further dissect the role of ROS on autoreactive T cell responses, our laboratory generated the NOD.BDC-2.5.*Ncf1*^{m1J} mouse, possessing diabetogenic CD4 T cells unable to synthesize superoxide. We hypothesized that similar to NOD.*Ncf1*^{m1J}, dampening ROS would diminish NOD.BDC-2.5 diabetogenic T cell responses. Interestingly, in contrast to NOD.*Ncf1*^{m1J}, IFN-gamma was upregulated 2-fold by N OD.BDC-2.5.*Ncf1*^{m1J} CD4 T cells compared to NOD.BDC-2.5 upon polyclonal stimulation (295.2 ±12.4ng/mL vs. 156.0 ±4.8ng/mL; *p*=0.0045). Synthesis of the pro-inflammatory cytokine TNF-alpha was also increased 1.5-fold by superoxide-deficient CD4 T cells compared to NOD.BDC-2.5 (*p*=0.0006). Adoptive transfer of pre-activated NOD.BDC-2.5.*Ncf1*^{m1J} CD4 T cells was more diabetogenic, as 63% of NOD.*scid* recipients (n=11, *p*=0.0662) by 9 days post transfer were hyperglycemic, in contrast to only 17% of wild-type-transferred mice (n=7). Providing mechanistic evidence for the enhanced pro-inflammatory profile and diabetogenicity in the absence of superoxide synthesis, activated NOD.BDC-2.5.*Ncf1*^{m1J} CD4 T cells displayed 1.5- and 2-fold enhanced phosphorylation of redox-sensitive tyrosine kinases within the T cell receptor (TCR) signaling cascade, lymp hocyte-specific protein tyrosine kinase (P-Lck (Y505)) and linker for activation of T cells (P-LAT (Y191)), respectively, compared to NOD.BDC-2.5. Future experiments will determine the redox-sensitive cues that modulate TCR activation within NOD.BDC-2.5.*Ncf1*^{m1J} T cells and potentially define novel targets for T1D prevention.

Superoxide Production Mediates Anti-Viral Responses to Diabetogenic Viruses

Ashley R. Burg and Hubert M. Tse, PhD

Viral infections are thought to be a trigger for Type-1 diabetes (T1D) onset in human patients and murine models. Virus-induced diabetes can be mediated through the synthesis of damaging pro-inflammatory cytokines and reactive oxygen species (ROS) by islet-infiltrating leukocytes. We recently demonstrated a critical role for ROS production in T1D pathogenesis, as superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are highly T1D-resistant. Interestingly, bone marrow-derived macrophages (BMM Φ) from these mice have reduced responses to the viral dsRNA-mimic, poly(I:C). Therefore, we hypothesized that superoxide deficiency will alter the diabetogenicity of viral infections through diminished innate anti-viral responses. To test this hypothesis, we performed *in vitro* infection studies with diabetogenic Encephalomyocarditis strain D (EMCV-D) and Coxsackie B4 (CB4) viruses. We found EMCV-D- and CB4-induced levels of RIG-I were 1.7- and 2-fold lower, respectively, in NOD.*Ncf1*^{m1J} BMMΦ than NOD. CB4-induced TLR3 levels were also diminished 1.4-fold in NOD.*Ncf1*^{m1J} BMMΦ as compared to NOD. Cytokine analysis revealed that EMCV-D-induced IL-1β synthesis in NOD.*Ncf1*^{m1J} BMMΦ was reduced by 1.9-fold compared to NOD (6.27 ±0.04 vs. 11.91 ±0.19 pg/mL; *p*0.0001), albeit no detectable levels with CB4. NOD.*Ncf1*^{m1J} BMMΦ also showed a 1.2-fold decrease in TNF&a lpha; synthesis upon EMCV infection compared to NOD (4968.0 ±68.7 pg/mL vs. 6011.0 ±154.5 pg/mL; *p*0.0035), and a 2-fold decrease upon CB4 infection (12.44 ±0.91 pg/mL vs. 24.74 ±0.66 pg/mL; *p*0.0005). These findings highlight the importance of superoxide synthesis and redox-dependent signaling on anti-viral responses. Future studies will further define the role of superoxide on innate immune responses that culminate in viral-induced diabetes.

Type I Interferons-induced Follicular Translocation of Lymphotoxin-Expressing Marginal Zone B cells Initiates Lupus

Hao Li , Hui-Chen Hsu, Qi Wu, PingAr Yang, Laurence Morel, Yang-Xin Fu, Jun Li, Bao Luo, Hideo Yagita and John D. Mountz

Systemic lupus erythematosus (SLE) is characterized by elevation of type I interferon signature genes and long time type I IFNs exposure does increase the incidence of lupus. But how type I IFNs integrate the early immune events to initiate the disease remains poorly understood. Here we provided evidence that type I IFNs produced by endogenous apoptotic debris activated plasmacytoid dendrit ic cells played a crucial role to initiate lupus via follicular translocation of membrane lymphotoxin (mLT)-expressing marginal zone (MZ) B cells in the spleen of lupus prone mice. The mislocation of mLT^{+} MZ B cells induces two important pathogenic effects. First, it initiates the deteriorations of marginal zone macrophages (MZMs) in the MZ which permit the initial exposure of the adaptive immune system to the autoantigens released from uncleared apoptotic cells. Secondly, it activates the Follicular dendritic cells (FDCs) by providing both self-antigen and lymphotoxin stimuli. Activated FDCs serve as a cellular network on which responding immune cells survey antigen bearing processes during T-dependent humoral responses to self-antigens. The disease initiation was efficiently abrogated and all abnormal features did not appear in BXD2-*Ifnar*$^{-/-}$ mice. The present study reveals an innate immune cell coordination mediated by type I IFNs which initiates the lupus.

The Role of Early IL-2 and IFN-γ Production in Determining the Fate of Newly Activated Anti-viral CD8 T Cells

Shannon M. Kahan, Rakesh K. Bakshi, Rita Luther, Laurie E. Harrington, Casey T. Weaver, and Allan J. Zajac

The clearance and control of many viral infections rely on the development of an efficacious CD8 T cell response. Activated CD8 T cells can develop into phenotypically and functionally distinct subsets; however what determines their fate commitment is not fully understood. The purpose of this study is to test the hypothesis that the developmental fate of a CD8 T cell is shaped early after activation, both by its intrinsic functional properties as well as by its perception of extrinsic signals. Using novel double cytokine reporter mice we are dissecting the early CD8 T cell response based upon two prominent functional features that influence T cell fate and function, IL-2 and IFN-γ secretion. Using this unique system we have determined that (1) upon activation, naïve CD8 T cells produce a transient burst of IL-2 and IFN-γ and (2) distinct functional populations secreting both IL-2 and IFN-γ, only IL-2 or IFN-γ, or neither cytokine are evident within hours of activation *in vitro* and *in vivo*. Furthermore, we have shown that this early functional heterogeneity is influenced by the proinflammatory cytokines, IL-12 and Type I Interferon, which sway the functional response away from IL-2 production toward IFN-γ secretion. Ongoing studies are underway to further dissect the features of these early functional populations, establish how these functional characteristics influence the fate and function of the cell, and determine whether changing these attributes can transform the composition of the responding CD8 T cell pool.

IL-2 Production by CD8 T Cells Forecasts the Formation of Immunological Memory

Rakesh K. Bakshi, Rita Luther, Casey T. Weaver, Laurie E. Harrington and Allan J. Zajac

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

The Role of IL-21 in Memory CD8 T Cell Differentiation and Maintenance

Yuan Tian, Maureen A. Cox and Allan J. Zajac

Populations of memory CD8 T cells develop in response to infections with many intracellular pathogens. These cells can be further subdivided into central memory (T_{CM}), effector memory (T_{EM}) and tissue-resident memory (T_{RM}) cells, which cooperate to protect the host from reinfection. Interleukin-21 (IL-21) is a cytokine that has been shown to sustain CD8 T cell responses, especially during chronic viral infections. Therefore, we hypothesized that IL-21 is required for the differentiation and maintenance of specific subsets of effector and memory CD8 T cells. To address this we used mixed bone marrow chimeras to compare the development of IL-21 receptor (IL-21R)$^{+/+}$and IL-21R$^{-/-}$ CD8 T cells in the same host. We discovered that IL-21Rdeficiency resulted in the defective accumulation of CD44^{hi}CD62L^{low} effector/T_{EM} CD8 T cells. Conversely, expression of the IL-21R permitted the formation of effector/T_{EM} phenotype CD8 T cells, as confirmed by increased expression of KLRG1, and decreased levels of CD122 and CD127. Notably, IL-21R$^{-/-}$CD8 T cells were disproportionately reduced in both lymphoid and nonlymphoid tissues compared to their IL-21R$^{+/+}$counterparts. Although less abundant, IL-21R$^{-/-}$CD8 T cells present in the small intestine epithelium had similar expression of T_{RM}markers including CD103 and CD69 compared to IL-21R$^{+/+}$ cells, and theIL-21R$^{-/-}$ T_{RM} phenotype cells expressed lower levels of granzyme B. Taken together, these findings suggest that IL-21 regulates both systemic and local CD8 T cell responses and provides new information regarding how the composition and distribution of the CD8 T cell pool is controlled by IL-21.

Regulation of *Vibrio Cholerae* Biofilm Formation by H-NS Repression and Anti-Repression

Julio C. Ayala, Hongxia Wang, Anisia J. Silva and Jorge A. Benitez

Biofilm formation plays an important role in the persistence of *Vibrio cholerae* in the aquatic environments and in cholera transmission. The genes responsible for the biosynthesis of the *Vibrio* polysaccharide (VPS) extracellular matrix, a major component of biofilms, are located in two operons, in which *vpsA* (UDP-N-acetylglucosamine 2-epimerase) and *vpsL* (glycosyltransferase) are the first genes of operons I and II, respectively. Cyclic diguanylate (c-di-GMP) is a bacterial second messenger that coordinates motile-to-sessile transitions. A current model of c-di-GMP signaling in *V. cholerae*, involves the LuxR-type regulator VpsT, which binds c-di-GMP to positively regulate the transcription of the *vps* genes by a mechanism that is not well understood. In a previous study, we showed that the histone-like nucleoid structu ring protein (H-NS) directly represses the expression of *vps* genes. Here we used a chromatin immunoprecipitation (ChIP) assay to demonstrate that VpsT antagonizes H-NS occupancy at the *vpsA* promoter. We show that VpsT binds to the promoter of *vps* operons I and II in a c-di-GMP-dependent manner. Using primer extension and DNase I footprinting, we were able to map the transcriptional start sites and the H-NS and VpsT contact regions at the *vpsL* and *vpsA* promoters respectively. We found that the VpsT binding sites overlap some of the long oligomerization stretches of H-NS along the *vps* promoters. Overall, the results suggest the novel hypothesis that c-di-GMP enhances biofilm formation in *V. cholerae* by diminishing H-NS repression at *vps* promoters.

Dispersion of Streptococcus Mutans Biofilms by a Novel Small Molecule

Sandra Stephanie Garcia, Hui Wu

Dental caries is a costly disease characterized by the demineralization of enamel, otherwise known as tooth decay. Despite advances in science, dental caries is the most common infectious disease worldwide and is increasing in incidence among young children. The etiologic causative agent of dental caries is *Streptococcus mutans.* Not only can *S. mutans* form biofilms readily on the tooth surface, but this bacterium rapidly produces lactic acid from dietary sugars. With the goal of developing a therapeutic agent that is species specific for *S. mutans*, we constructed a diverse library of small molecules based on the structural motifs of bromoageliferin, a marine sponge product with antibiofilm and antibacterial properties.

By screening our library of small molecules with a biofilm dispersion assay, we identified 3F1 as a novel small molecule that selectively disperses *S. mutans* biofilms. While 3F1 dispersed approximately 50% of *S. mutans* biofilm, it did not disperse biofilms formed by commensal species *Streptococcus sanguinis* or *Streptococcus gordonii*. Surprisingly, the effect of 3F1 can be negated with the addition of copper ions. However, 3F1 does not act similar to known copper chelators. We hypothesize that our small molecule induces the dispersal of *S. mutans* biofilms by interacting with a potentially unknown copper-binding molecule, which is necessary for biofilm maturation, structure, or maintenance. Identification of the target through techniques such as RNA deep-sequencing will could potentially become a therapeutic target for the prevention or treatment of dental caries, while maintaining the commensal populations.

Two Glycosylation-Associated Proteins Protect the Third Protein from Degradation through Interaction with a Peptidase System in *Streptococcus parasanguinis*

Haley Echlin, Fan Zhu, and Hui Wu

Streptococcus parasanguinis is a primary colonizer of the oral cavity and adheres to the tooth surface using long fimbriae. The major constituent of these fimbriae is Fap1, a serine-rich repeat glycoprotein (SRRP); SRRPs are important conserved bacterial adhesins. Although the exact mechanism of Fap1 biogenesis remains a mystery, we have shown that Gap1, Gap2, and Gap3 are involved. These three proteins form a complex and are required for biogenesis of mature Fap1, with direct impact on adhesion and fimbriae biogenesis. Gap1 stabilizes both Gap2 and Gap3; Gap2 augments Gap1's function of stabilizing Gap3. To investigate the mechanism of how Gap1 and Gap2 stabilize Gap3, we analyzed Gap3 levels by creating double mutants of *gap1* or *gap2* and several *clp* genes, including that of the protease ClpP and four ATPases, ClpC, ClpE, ClpL, and ClpX. We found that *gap1*/ *clpP* and *gap2*/ *clpP* mutants restored Gap3 levels, suggesting that Gap3 is protected by Gap1 and Gap2 from ClpP degradation. The double mutants of *clpC*, *clpL*, and *clpX* did not restore Gap3. However, the *gap1*/*clpE* and *gap2*/ *clpE* mutants did restore Gap3, similar to the *clpP* double mutants. We conclude that both ClpP and ClpE play a role in Gap1/ Gap2 protection of Gap3, but ClpC, ClpL, and ClpX do not. The interactions between these proteins may play an important role in the formation of the stable Gap1/2/3 complex, with direct effect on Fap1 biogenesis. Understanding these interactions will give us insights into bacterial pathogenesis and potential targets for drug development.

Single Amino Acid Mutations in Ribosomal Protein S25 Significantly Affect Internal Ribosome Entry Site Translation

Beth A. Walters and Sunnie R. Thompson

The vast majority of eukaryotic mRNAs are translated through a cap-dependent mechanism. However, under cellular stress cap-dependent translation is down-regulated and mRNAs are translated using an alternative translational mechanism. One such cap-independent mechanism requires an internal ribosome entry site (IRES) located in the 5' UTR, which recruits the ribosome internally to the mRNA in the absence of a 5' cap. We have previously shown that ribosomal protein S25 (Rps25) is required for IRES-mediated translation. Importantly, deletion of RPS25 does not significantly affect cap-dependent translation, global protein synthesis, or ribosome biogenesis suggesting that it does not have a significant role in ribosome function apart from its role in IRES-mediated translation. We have performed a mutational analysis of Rps25 in order to better understand its function in IRES-mediated translation. Interestingly, we have identified single amino acid changes that result in a significant decrease in cricket paralysis virus intergenic region (CrPV IGR) IRES activity. These mutations are located in regions that have been shown to be in contact with the CrPV IGR IRES, specifically, the unstructured N-terminal domain of Rps25 that contacts the ribosomal P-site and the surface exposed region of Rps25. In addition, mutations in the C-terminal domain that are predicted to disrupt folding of the head domain resulted in reduced IRES activity. Further analysis of these key Rps25 residues using in vitro assays will reveal their role in IRES binding to the 40S ribosome and their effect on positioning of the 40S ribosome on the IRES.

Autophagy is Regulated by 5'-3' mRNA Decay in *Saccharomyces cerevisiae*

Shane P. Kelly and David M. Bedwell

Autophagy is the process of making nutrients bioavailable during periods of starvation. To do this, the cell sequesters cytosolic contents into autophagosomes that fuse with the mammalian lysosome/yeast vacuole where the contents are degraded and recycled for starvation specific functions. The inhibition of autophagy during nutrient rich conditions has largely been attributed to the Target of Rapamycin (TOR). The molecular mechanisms of autophagosome formation have been dissected in detail, however, little is known about how autophagy is controlled at the mRNA level. The steady state mRNA level is largely proportionate to protein expression and steady state mRNA is the consequence of the difference between the rates of transcription and decay. We have observed the rapid degradation of the mRNA decapping factor, Dcp2p, during nitro gen starvation. Dcp2p is the catalytic subunit of the only mRNA decapping complex known to exist in yeast. Dcp2p degradation is dependent on the proteasome and TOR inhibition, suggesting that the TOR pathway inhibits its proteasomal degradation during nutrient-rich conditions. Furthermore, autophagy is partially upregulated in the *dcp2Δ* strain even during nutrient-rich conditions when TOR should inhibit autophagy. Also, several autophagy related (ATG) mRNAs are partially upregulated in the *dcp2Δ* strain. We show that *ATG8* mRNA (Atg8p is involved in autophagosome expansion) is degraded by multiple decapping enzymes, specifically by the 5'-3' mRNA decay machinery, and ATG8 mRNA half-life increases significantly during nitrogen starvation, suggesting a functional role for the proteasomal decay of Dcp2p. These data indicate that mRNA decay i s a novel post-transcriptional regulatory mechanism of autophagy.

Long-Term Nonsense Suppression Therapy with NB84 Moderates MPS I-H Disease Progression

Gwen Gunn, Yanying Dai, Ming Du, Valery Beklakhov, Jeyakumar Kandasamy, Trenton Schoeb, Timor Baasov, David M. Bedwell, Kim M. Keeling

Nonsense suppression therapy utilizes compounds that suppress translation termination at premature termination codons (PTCs) to restore partial protein function. We hypothesize that suppression therapy utilizing the designer aminoglycoside NB84 can moderate progression of the lysosomal storage disease Mucopolysaccharidosis type I-Hurler (MPS I-H), the severe form of a-L-iduronidase deficiency. a-L-iduronidase participates in glycosaminoglycan (GAG) catabolism and its insufficiency causes progressive GAG accumulation and onset of the MPS I-H phenotype, which consists of multiple somatic and neurological defects. 60-80% of MPS I-H patients carry a nonsense mutation in the *IDUA* gene.

We previously showed that 2-week treatment with the designer aminoglycoside NB84 restored enough a-L-iduronidase function to reduce tissue GAG accumulation in the *Idua*^{tm1Kmke} MPS I-H mouse model, which carries a PTC homologous to the human *IDUA-W402X* nonsense mutation. In our current study we administered NB84 for 28 weeks to evaluate the ability of suppression therapy to sustain enzyme activity and alleviate progressive phenotypes while monitoring toxicity. Here we report that long-term NB84 administration maintains a-L-iduronidase activity and GAG reduction in *Idua*^{tm1Kmke} mice throughout a 28-week treatment period.

Examination of more complex MPS I-H phenotypes in *Idua*^{tm1Kmke} mice following 28-week NB84 treatment revealed significant moderation of the disease in the brain, heart, and bone, which are resistant to current MPS I-H therapies. We employed a diverse set of assays to evaluate multiple phenotypic aspects of the disease. This is the first study to demonstrate that long-term nonsense suppression therapy can moderate progression of a genetic disease.

Conditions Governing the Fate of Reiterative Transcripts

Jeffery Vahrenkamp and Charles Turnbough Jr.

During a transcription cycle, one DNA base in the RNA polymerase active site base pairs with a ribonucleotide. After the formation of a phosphordiester bond between this RNA base and the nascent transcript, the RNA and DNA strands move upstream one step together exposing a new DNA base in the active site, and the cycle repeats. This leads to a strand of RNA with a sequence identical to the non-template DNA. However; when a homopolymeric tract (HPT), a stretch of three or more of the same nucleotide, is present in t he initially transcribed region, the nascent RNA can slip upstream independent of the DNA strand. This exposes the previously used DNA base in the active site once more, allowing it to code for more than one base in the transcript. This slippage reaction can occur repeatedly and is known as reiterative transcription.

After reiterative transcription has occurred, two fates await the transcript. The RNAP can escape the reiterative transcription cycle, leading to a full length transcript with additional bases on its 5' end, or it can abort the reiterative transcript and release it. Examples of both fates exist in nature, but the rules governing these fates were unknown. We determined that 5' HPTs allow for easy entry into and exit from the reiterative transcription cycle, while internal HPTs require a strong pause on the HPT to enter the reiterative transcription cycle. We have also determined that internal T HPTs are unique in their ability to exclusively produce abortive reiterative transcripts.

Aeration-dependent Regulation of Capsule in *Streptococcus pneumoniae*

Jocelyn R. Hauser and Janet Yother

Streptococcus pneumoniae typically resides in the aerated environment of the nasopharynx but encounters less aerated environments when causing diseases such as pneumonia, sepsis, and meningitis. Reduced aeration enhances production of the polysaccharide capsule, which provides protection against complement-mediated opsonophagocytosis. Under aerobic growth, *S. pneumoniae* produces large quantities (>1 mM) of hydrogen peroxide (H_2O_2) due primarily to the activity of SpxB, a pyruvate oxidase. SpxB is an important virulence factor but little is known about its direct function in pathogenesis. We hypothesized that generation of the oxidizing agent H_2O_2 may serve as a regulatory signal for capsule production, as biosynthetic and accessory enzymes responsible for capsule production respond to oxidizing and reducing conditions. To begin to test our hypothesis, we generated an *spxB* mutant and cha racterized capsule production. Deletion of *spxB* reduced H_2O_2 production >90% and increased capsule production under both aerated and non-aerated growth as compared to the parent D39 strain. The addition of exogenous H_2O_2 (0.5 mM) in the culture medium restored capsule production in the mutant to near parental levels. For the parent strain, the addition of exogenous H_2O_2 decreased capsule levels under non-aerated growth but did not alter the capsule levels under aerated growth, due apparently to the already high concentration of endogenous H_2O_2. In colonization experiments, the SpxB mutant exhibited a 10-fold decrease in the number of bacteria recovered from the nasopharynx of mice. Our data suggest H_2O_2 production may serve as a posttranslational mechanism by which *S. pneumoniae* responds to aeration and modulates capsule production.

Structure of the Complex Between Calmodulin and HIV-1 Matrix Protein

Jiri Vlach, Alexandra Samal and Jamil Saad

A successful replication of human immunodeficiency virus 1 (HIV-1) relies heavily on its interactions with numerous cellular proteins, which are often proteins central to critical cellular functions. Calmodulin (CaM) is a ubiquitous calcium-binding protein that regulates diverse cellular processes. HIV-1 proteins Nef, Tat, Env and the matrix (MA) domain of Gag have been shown to interact with CaM but the functional role of these interactions remains unknown.

Detailed knowledge of structural properties of protein–protein complexes is helpful in understanding the requirements of the interactions. Our lab is interested in elucidating the structural basis for HIV–host interactions and we focus on how the Gag protein is trafficked in the cytoplasm of the host cell and the subsequent assembly on the plasma membrane. Here, we present a three-dimensional NMR structure of CaM bound to the minimal binding domain of MA (residues 8–43). The interaction between the N-terminal α-helix of MA (E14–R20) and the C-terminal domain of CaM is stabilized mainly by insertion of large W16 and I19 side chains into the hydrophobic groove of CaM. The C-terminal α-helix of MA (L31–L40) is docked on the N-terminal domain of CaM and this interaction is stabilized by favorable contacts between MA residues Y29, L31, I34 and V35 and hydrophobic CaM residues. The MA:CaM complex is also stabilized by salt bridges between basic MA and acidic CaM residues. We will discuss possible implications of our findings in the context of previous genetic studies pointing the mentioned regions to various events during viral replication.

Mouse Models of Hepatitis C Virus Replication and Tumorigenesis

Abdellah Akil, Kyung-Don Kang, Qunfeng Wu, Joshua Justice, and George Luo

Small animal model is the major barrier for hepatitis C virus (HCV) research. We have demonstrated that HCV can replicate in mouse embryonic fibroblasts (MEF) as well as hepatocytes but at much lower efficiency compared to human hepatocytes. We have successfully constructed stable mouse hepatoma (Hepa 1-6) cell lines that harbor replicating HCV RNA genomes. Characterization of these stable HCV-replicating mouse hepatocyte cell lines in vitro and in vivo revealed several important findings. First, cellular microRNAs (miRNAs) were found to also play important roles in HCV RNA replication in mouse hepatocytes. Second, adaptation of HCV in vitro and in vivo remarkably enhanced HCV RNA replication. We are working to determine the underlying molecular mechanism for viral adaptation in mouse hepatocytes. More importantly, it appeared that HCV replication enhanced the proliferation of mouse hepatoma cells in vitro and in vivo. Additionally, we have made transgenic mice either expressing a full-length RNA genome of genotype 2a HCV or the human receptors important for HCV infection. These animal models will facilitate our studies on the underlying molecular mechanism of HCV replication, virus-host interaction, pathogenesis, and carcinogenesis in vivo. More significantly, mouse models of HCV infection and/or replication will facilitate the development of effective vaccines and antiviral therapies.

Highlighting the Diverse Role of Cytoskeleton in Retroviral Assembly: Structural Studies of the Interactions Between Gag Domains and Cytoskeleton Components

Constance Agamasu, Ruba H. Ghanam, Alexandra B Samal, Jamil S Saad

Retroviruses are small intracellular parasites that have been associated with an array of diseases ranging from malignancies to AIDS. All retroviruses encode a polyprotein called Gag that contains all the structural elements required for virus assembly. A critical step in the late stage of replication is the transport of Gag from the cytoplasm to the Plasma Membrane (PM) for assembly. However this mechanism of transport remains largely unknown. The cortical actin cytoskeleton has been implicated in Gag transport since it is located underneath the PM and provides either a track or a diffusion barrier to facilitate directed intracellular movements. Two prominent cytoskeleton regulators, Filamin A (FLNa) and IQGAP1 have been shown to bind to different domains of Gag to facilitate Gag trafficking to the PM. FLNa has been shown to co-localize with Human Immunodeficiency Virus (HIV)-1 Gag during virus assembly. Alternatively, IQGAP1, another prominent cytoskeleton regulator, was found to play an important role in murine leukemia virus (MLV) production, but not HIV-1 production. Collectively, the mechanism of transportation of Gag to the plasma membrane is rather complex and may involve multiple factors within the cytoskeleton structure. We hypothesize HIV-1 and MLV Gag proteins directly interact with FLNa and IQGAP1, respectively, to facilitate trafficking to the PM for assembly. We have employed NMR, biochemical and biophysical methods to understand how HIV and MLV Gag protein interact with cytoskeleton components**.** Our preliminary data may suggest different pathways for HIV and MLV Gag assembly and also highlight the diverse roles of the cytoskeleton components and their interacting partners in virus assembly.

FCRL5 Promotes T cell-independent Innate-like B cell Responses and Counter-regulates Tolerance Through the Lyn/SHP-1 Circuit

Eugene Becker Jr., Zilu Zhu, Woong Jai Won, Shanxiang Zhang, Riccardo Dalla-Favera, and Randall S. Davis

The earliest phases of humoral immune defense are predominated by specialized subpopulations known as splenic marginal zone (MZ) and body cavity-derived B1 B cells. These frontline responders have been termed “innate-like” given their broadly-reactive natural antibody repertoires and capacity for rapid responses to T cell-independent (TI) antigens. Although their ability to produce self-reactive Ig affords widespread host protection during pathogen challenge, it also implicates a key role for innate-like B cells in maintaining the balance of tolerance. Despite their essential contributions in primary responses, why these cells possess such prompt responsivity to innate-stimulation is poorly understood. The discovery of a family of Fc receptor-like (FCRL) molecules with homology to the classical FCRs for IgG and IgE, complex tyrosine-based activation and inhibitory function, and preferential B cell expression has introduced a new level of B cell regulation. Although their *in vivo* functions have not yet been investigated, human FCRL (hFCRL) members are becoming increasingly appreciated for their associations with multiple immune-related diseases. In particular, hFCRL3 has emerged as a generalized risk factor in autoimmunity. To better understand the role of hFCRL3 in B cells, we investigated its closest relative mouse FCRL5 (mFCRL5). This molecule shares homologous extracellular domains, binary SHP-1/Lyn-dependent tyrosine-based signaling, and innate-like B cell expression. Here we demonstrate that *Fcrl5* deficiency impairs humoral responses to TI antigens and differentially modulates autoimmunity in lupus-prone SHP-1 and Lyn mutant mice. These data indicate a critical role for mFCRL5 in regulating innate-like B cell function at the interface of protection and tolerance.

Blood Collection Tubes Influence Serum Ficolin-1 and Ficolin-2 Levels

Allison M. Brady, Brady L. Spencer, Ann R. Falsey, and Moon H. Nahm

The ficolins are a recently discovered family of host innate opsonins that can activate the lectin pathway of complement. The ficolins bind many ligands, though are typically described as binding acetylated sugars. Ficolin-1 and ficolin-2 are known to bind *Streptococcus pneumoniae* serotypes 19C and 11A, respectively. While studying the ficolins binding to pneumococci, we found variation in ficolin-2 binding amongst sera collected in different types of blood collection t ubes. Plastic tubes, which contain a silica clot activator, yielded sera with reduced ficolin-2 binding and apparent ficolin-2 levels. We found that silica clot activator eluted from plastic red top tubes can inhibit ficolin-2 ligand binding, while other related proteins, like MBL and ficolin-1, were not affected. These tube types did not affect the concentrations of other related opsonins: C1q, MBL, or ficolin-3. Interestingly, we also found ficolin-1 levels were increased two to three fold in plastic SST tubes compared to other tube types. These findings have implications for future ficolin-1 and ficolin-2 studies, as proper sample collection and handling are essential.

Altered Gastric Microbiota in H. pylori-infected Children Versus Adults

Kyle Brawner, Casey Morrow, Ranjit Kumar, Paul Harris, and Phillip Smith

The effect of *Helicobacter pylori* colonization on the composition of the human gastric microbiota is a matter of debate. The few studies that address this topic have focused on adults; we are unaware of any report that has examined the impact of *H. pylori* on the gastric microbiota in children. In the current study, we recruited a human cohort of 90 people residing in Santiago, Chile, where *H. pylori* is endemic. This cohort was divided into 4 groups: *H. pylori*-infected and non-infected children and *H. pylori*-infected and non-infected adults. We collected gastric fluid from these patients by endoscopy and isolated DNA from these samples. The V4 region of the bacterial 16S rRNA gene was PCR-amplified, and PCR products were sequenced by next-generation sequencing to assess the composition of the gastric microbiota of all 4 gro ups. Although the *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria* phyla dominated in all groups, *H. pylori*-infected children harbored significantly more non-*Helicobacter Proteobacteria* and significantly less *Firmicutes* than *H. pylori*-infected adults. The overall intra-subject diversity of the gastric microbiota of a given *H. pylori*-infected child was on average higher than that of a given *H. pylori*-infected adult, while inter-subject diversity analysis revealed the gastric microbiota of *H. pylori*-infected children were more similar to each other than to the gastric microbiota of *H. pylori*-infected adults. These results may shed light on why children display less severe stomach inflammation than adults in response to *H. pylori* infection.

HLA-I Associated HIV Adaptation Compromises Acute CD8 T Cell Responses: Implications for Vaccine Design

Victor Y. Du, Anju Bansal, Jonathan Carlson, Jesus F. Salazar-Gonzalez, Malinda Schaefer, William Kilembe, John Sidney, Sonya Heath, Eric Hunter, and Paul A. Goepfert

HIV CD8 T cell escape mutations can be predicted based on the human leukocyte antigen class I (HLA-I) alleles of the infected population, whereby an HIV-1 epitope adapts to HLA-I restricted pressure to form an adapted epitope. We would like to assess whether these epitopes can induce CD8 T cell recognition in acute infection. We predicted the number of HLA-I restricted non-adapted (NAE) and adapted epitopes (AE) encoded by each person's infecting virus, using their HLA-I alleles and their viral sequences. By stimulating patient PBMC with peptides matching these NAE and AE in an IFN-γ ELISPOT assay, we compared the immunogenicity of the epitopes. Furthermore, an NAE and its counterpart AE-specific CD8 T cell-mediated killings of infected targets w ere assessed. CD8 T cell responses were induced more frequently to NAE. Also, the proportion of NAE encoded by an infecting virus correlates with greater breadth of responses. However, the few immunogenic AE observed exhibited higher HLA binding affinity than the non-immunogenic ones. Comparing side by side of an NAE-specific CD8 T cell line with that of its AE counterpart, we found the NAE induced more killing of infected targets. Overall, our overall study indicates reduced targeting and quality of AE and AE-specific responses, respectively, during acute infection. While efforts have revolved around incorporating this type of epitope in a vaccine, it is likely that AE would compromise T cell responses. NAE should therefore be the center of focus for future T cell-based vaccines.

Ureaplasma DNase Activity and Neutrophils

Sarah Dulson and Li Xiao

Two species of ureaplasma, *U. parvum* and *U. urealyticum*, are known to infect the human urogenital tract.About 20 known or hypothetical nucleases are found in the genomes of each species. Our research aims to elucidate mechanisms by which ureaplasma species evade host immune system, focusing on the ureaplasma DNase activity and neutrophil function. To avoid background DNase activity from regular 10B medium, we modified mycoplasma serum-free media (SFM) for *Ureaplasma* spp. *U. urealyticum* and *U. parvum* were cultured in SFM and growth curves were obtained. DNase activity of supernatant and bacterial pellets from the mid-log phase culture was tested by incubation with human genomic DNA and visualization in agarose gel. The growth curves di ffered between the two *Ureaplasma* species. The log phase for *U. urealyticum* showed exponential growth starting at hour 13 and continuing for 9 hours until hour 22 where it reached its peak concentration of 2.5×10^6 CFU/mL. The lag phase for *U. parvum* also lasted for 13 hours and the log growth was seen between hours 13 and 15 with a peak concentration of 8.5×10^5 CFU/mL. Gel electrophoresis showed significant DNAse activity in both *U. urealyticum* and *U. parvum* supernatant samples. DNA degradation was enhanced with Ca^{2+}/Mg^{2+} and blocked by EDTA/EGTA. Negligible degradation was obtained with samples from re-suspended pellets for either species. The observation that ureaplasma species secrete nucleases is significant because it suggests that these microorganisms could evade innate immune responses by degrading NETs. Studies are ongoing to characterize the nucleases and their roles on NETs and host defense system.

Protocol Optimization for Analysis of Changes in N-glycosylation Profiles of Immunoglobulins from Different Sources by Using Separation Techniques and Mass Spectrometry

Vojtěch Franc, Audra H. Laube, Stacy Hall, Qing Wei, Zina Moldoveanu, Zhi-Qiang Huang, Sonya L. Heath, Michael S. Saag, Matthew B. Renfrow, Jan Novak

Altered glycosylation of IgG and/or IgA occur in many autoimmune and chronic inflammatory or infectious diseases. Analysis of glycosylation changes of immunoglobulins from different sources can elucidate some of pathogenic mechanisms in these disorders. Mass spectrometry (MS) is indispensable for analysis of N-glycosylation, especially when combined with separation methods, such as capillary electrophoresis or liquid chromatography. Many purification and fractionation procedures using basic principles of reversed-phase liquid chromatography have been developed during recent years. In this study, the sample preparation procedure involved in-gel digestion of polymeric and monomeric IgA1 model proteins or IgG from healthy controls and HIV-infected persons) combined with optimized cysteine alkylation prior to SDS-PAGE (for IgA1) to avoid signal splitting of N-glycopeptides in MS. Purification and fractionation of (glyco)peptide mixtures w as achieved by reverse-phase liquid chromatography using a microgradient device. Subsequent LC-MS analysis of fractionated samples was performed by using nano-LC system coupled to LTQ Orbitrap Velos ETD mass spectrometer combined with different fragmentation techniques (CID, ETD, HCD). The acquired MS and MS/MS spectra were interpreted manually and by means of a PinpointTM Software (ThermoFisher Scientific), which allows relative quantification of targeted N-glycopeptides and monitoring of changes in N-glycopeptide profiles between samples from different sources. Our data revealed high degree of microheterogeneity of IgA1 N-glycosylation in the CH3 "tail" glycans, including the presence of high-mannose glycans. Moreover, a label-free approach allowed relative quantification of IgA1 and IgG N-glycopeptides.

Analysis of Invasive Nontypeable *Streptococcus pneumoniae* Isolates Reveals Renetically Intact Capsule Biosynthetic Loci with Inactivating Mutations

In Ho Park, **K. Aaron Geno**, Bernard W. Beall, and Moon H. Nahm

Capsular polysaccharide (CPS) of *Streptococcus pneumoniae* has been considered an absolute requirement for invasive illness by the organism. Previous characterization of nontypeable (NT) *S. pneumoniae* isolates has revealed that some, termed Group I NTs, retain intact capsule loci (*cps*). Others, termed Group II NTs, have exchanged their *cps* loci for novel genes potentially compensating for the loss of CPS, including *aliC, aliD,* and *pspK*. Because previous studies used low-resolution genetic methods (e.g., PCR) to study *cps* loci of Group I NTs, it is unclear whether these strains express capsule at all or in low levels undetectable by Quellung reaction. We examined 103 NT clinical strains isolated from patients with invasive pneumococcal disease and found that while seven belonged to Group II, possessing *aliC* and/or *aliD*, most (89) were Group I NTs. Twenty-six of these were serotype 8, which was significantly overrepresented compared to other serotypes when compared to its relative prevalence in invasive disease. When these isolates were examined, a majority had various defects in the initiating glycosyltransferase *cps8E* that would abolish CPS production. Each isolate's mutation was different, suggesting no shared mechanism for disruption of CPS synthesis in this manner. Further studies are being performed, but these results suggest that hosts may exert selective pressures against serotype 8 CPS expression and that capsular serotype 8 may be biased towards inactivation of CPS expression as a means to escape this pressure.

Using the Modified Surface Killing Assay (MSKA) to Screen Protective Antibodies to Pneumococcal Surface Protein A (PspA) and to Identify Protective Regions of PspA

Kristopher Genschmer and David Briles

Pneumococcal surface protein A (PspA) has been shown to be immunogenic in mice and confer protection from lethal challenge of *Streptococcus pneumoniae.* PspA has been considered a good candidate for a protein based vaccine against pneumococcal infection. There have been recent developments in assays that can serve as *in vitro* surrogates for protection to test PspA immune serum. One such assay, the Modified Surface Killing Assay (MSKA), has been used to predict the abilities of mouse monoclonal antibodies to PspA to protect mice from lethal challenge. In this study, we test multiple monoclonal antibodies to PspA using the MSKA and mouse passive protection studies. Additionally, we created seven 100-AA overlapping fragments of PspA from pneumococcal strain RX1 and examined the binding region of each antibody used in these studies. The results revealed further credence to the ability of the MSKA to be used as a possible surrogate assay of protection, as well as this study revealing portions of the PspA protein that seem to be less protective than others. While shorter portions of the proline rich region can be protective, and the coiled-coiled domain is very immunogenic and protective, not all antibodies that bind to the coiled-coiled region confer protection.

Molecular Piracy: Derepression and Encapsidation of *Staphylococcus aureus* Pathogenicity Islands by Helper Phages

Rosie Hill, Keith Manning, Altaira D. Dearborn, Erin A. Wall, Gail E. Christie, and Terje Dokland

Staphylococcus aureus pathogenicity islands (SaPIs) are mobile genetic elements that encode virulence genes. SaPIs depend on a helper phage for their excision and encapsidation. Recently the phage 80a dUTPase (Dut) was reported as a derepressor of SaPIbov1. We previously showed that *S. aureus* Newman phage phiNM1 is also able to derepress SaPIbov1, even though phiNM1 Dut is highly divergent from 80a's and is predicted to belong to a different family of dUTPases. Our objective is to identify the structural determinants for derepression of SaPIbov1 by type 2 Dut. Our data suggests that phiNM1 and 80a Dut both drerepress SaPIbov1 via a conserved GVSS motif.

Once SaPIs are derepressed they replicate their DNA. Eventually they pirate the helper phage's capsid protein to form their own mini-headed virions. The 80a capsid and scaffolding proteins are cleaved during capsid assembly. We have hound that the proteins are cleaved by *S. aureus* YsxB, a host cysteine protease that also cleaves ribosomal protein L27. Another objective of this study is to determine when cleavage occurs and the structure of YsxB while bound to substrate.

Proteomic Analysis of Engineered IgA1-IgG Immune Complexes Reveals Association with Activated Complement C3

Nicolas Maillard, LeeAnn J. Boerma, Stacy Hall, Zhiqiang Huang, Michal Mrug, Zina Moldoveanu, Bruce A. Julian, Matthew B. Renfrow, Jan Novak

In IgA nephropathy(IgAN), C3 colocalizes with IgA in mesangial deposits. Deletion of CFHR1,3 genes has been recently described to protect from the occurrence of IgAN, highlighting the contribution of complement alternative pathway(AP). Using a model of polymeric galactose-deficient(Gd) IgA1 with recombinant antiglycan IgG derived from an IgA N patient in immune complexes (ICs) formed in the presence of serum, we identified and characterized by proteomic analyses complement C3 products associated with the complexes.

ICs were formed overnight at 4°C, using purified Gd-polymeric IgA1(pAle) myeloma protein, recombinant antiglycan IgG(r1123), and IgA- and IgG-depleted serum of a healthy control. High-molecular-mass fractions (HMMFs;700 kDa) were isolated by size-exclusion chromatography. The fractions that stimulated proliferation of cultured human mesangial cells were pooled, concentrated, and fractionated (SDS-PAGE). After silver staining, protein bands were excised, digested by trypsin, and analyzed by high-resolution tandem mass spectrometry (LTQ OrbitrapVelos;MS/MS), and proteins were identified (MASCOT and SEQUEST algorithms).

Proteomic analysis revealed that C3 α and β chain elements were present in the active ICs and only low amount of βchain was in the corresponding fractions in negative control (serum only). Amino-acid sequence obtained by MS/MS combined with information on the molecular mass of corresponding bands from SDS-PAGE identified iC3b, C3c, and C3dg(confirmed by anti-C3 immunoblotting). The presence of these low-molecular-mass products in HMMFs can only be explained by their binding to ICs. These results thus provide evidence that biologically active IgA1-ICs activate complement C3 and associate with C3 degradation fragments. iC3b, C3c, and C3dg are the products of the action of factor I and H, suggesting a critical role of regulators in the activation of complement AP in IgAN.

Soluble engineered IgA1–IgG ICs can bind C3 elements in vitro.

Towards Understanding the Role of Ribosomal Protein S25 in Translation

Nicholas Potochick, and Sunnie R Thompson

Ribosomal Protein S25 (RPS25) is a highly conserved protein of the 40S subunit of eukaryotic ribosomes that is essential for the translation of viral and cellular internal ribosome entry sites (IRESs). However, canonical cap-dependent translation, and other ribosomal functions are not significantly affected while global protein synthesis is downregulated 15% compared to wild type. Nonetheless, knockout of RPS25 in yeast results in a slow growth phenotype. However, IRES-mediated translation is unlikely to occur in Saccharomyces cerevisiae growing under normal conditions. This suggests that RPS25 may be playing a role in the cell independent of its role in IRES-mediated translation. Towards an understanding of what this role may be, we have tested several translational inhibitors to determine if *rps25aΔbΔ* yeast strains exhibit increased sensitivity over wild type yeast in growth assays. *rps25aΔbΔ* yeast has demonstrated increased sensitivity to cyclohexamide, a translation elongation inhibitor, suggests that translation elongation may be effected by loss of RPS25. Cyclohexamide specifically inhibits translocation by binding the E-site of the 60S ribosome. Interestingly, several other translation inhibitors that work by a variety of mechanisms failed to show increased sensitivity. This may indicate that there is a specific step in translation elongation process in which RPS25 plays an important role. Currently, a genetic screen for *rps25aΔbΔ* suppressor mutants is being developed.

A Novel Variant of FCGR2B is a Risk Factor for Systemic Lupus Erythematosus

Ptacek, T.S., Mukhtar, S., Redden, D.T., Li, X., Ji, C., Absher, D., Edberg, J.C., Kimberly, R.P.

SLE is an autoimmune disease characterized by auto-antibodies and immune complex formation. Fc-gamma Receptors (FCGRs) on leukocytes recognize the Fc domains of IgG participate in antibody-mediated regulation of immune responses. The low affinity FCGRs reside within a 1q23 gene cluster. Copy number variation (CNV) of one the genes, *FCGR3B*, was implicated in SLE. Although CNV has been reported for other genes in the cluster, it has not been reported for t he only inhibitory receptor in the cluster, *FCGR2B*, despite the genomic structure of the gene cluster suggesting its possibility. Prior studies examining functional SNPs of *FCGR2B* establish the gene's importance in autoimmunity and SLE. We hypothesized that CNV of *FCGR2B* may exist, and that it would have significance in the SLE phenotype. Previously, we used pyrosequencing assays to determine CNV across the FCGR gene cluster in a large SLE case control cohort. In addition to observing previously reported CNVs, we identified individuals that appeared to have duplications of *FCGR2B*. Statistical analysis showed that this variant is associated with SLE, and that this effect is independent of *FCGR2B* functional alleles and CNVs of other genes. Other assays suggested that this variant did not include the cytoplasmic tail. We recalled individuals with variant 2B, and genetically matched controls, and are currently characterizing gen omic and mRNA sequence. We were unable to find evidence for a truncated *FCGR2B* protein, although we did gather evidence that the variant may involve other loci. Future studies will begin with establishing the genomic sequence of the *FCGR2B* variant.

Evidence that Estrogens Regulate Heart Rate via a G-protein Coupled Estrogen Receptor

Shannon Romano, Muhan Hu & Daniel Gorelick
Dept of Pharmacology and Toxicology, Microbiology Graduate Theme, Medical Scientist Training Program

Estrogens regulate gene expression directly by binding to estrogen receptors (ERα, ERβ), ligand-dependent transcription factors. It has recently been shown that estrogens can modulate cell function independent of gene expression via a membrane-associated G-protein coupled estrogen receptor (GPER, also known as GPR30). Chronic exposure to tamoxifen, a selective estrogen receptor modulator, has been associated with decreased heart rate. A metabolite of tamoxifen, 4-hydroxytamoxifen, acts as an ERa antagonist and a GPER agonist. The goal of this study was to investigate whether other estrogen receptor ligands affect heart rate and by what receptor. We found that 30-minute exposure to formestane, a steroidal aromatase inhibitor that reduces estradiol synthesis, reduced heart rate in zebrafish embryos. This effect was rescued by the ER/GPER agonist 17b-estradiol (E2) and by the ER antagonist/GPER agonist ICI182,780. However, 4-hydroxytamoxifen treatment failed to rescue formestane-dependent reduction in heart rate. For all treatments, normal heart rate was restored within 30 minutes following incubation in untreated water. Additionally, E2, ICI or 4-hydroxytamoxifen treatment alone increased heart rate. The rapid regulation of heart rate by formestane, E2 and ICI is consistent with non-genomic estrogen receptor signaling. The fact that E2 and ICI182,780 increase heart rate strongly implicates GPER (and excludes ERα and ERβ signaling) because ICI182,780 is an antagonist of nuclear ERs. Furthermore, we conclude that acute exposure to 4-hydroxytamoxifen increases heart rate via an estrogen-receptor independent mechanism. Identifying how estrogen signaling via GPER regulates heart rate could uncover a novel function of the G-protein coupled estrogen receptor.

Inactivation of a Membrane-bound *O*-acetyltransferase WcjE is Commonly Achieved by Mutations in Transmembrane Domains

Brady L. Spencer, Juan J. Calix, Allison M. Brady, Moon H. Nahm

Streptococcus pneumoniae, an important but opportunistic human pathogen, normally resides in the nasopharynx, but can occasionally invade deeper tissues to cause disease. Most pathogenic pneumococci express capsular polysaccharide which shields pneumococci from host immunity and is critical for pneumococcal virulence. Our laboratory discovered that upon invasion, serotype 11A, a common nasopharyngeal colonizer, can undergo micr o-evolution into serotype 11E through the inactivation of *wcjE*. The *wcjE* allele encodes a membrane-bound *O*-acetyltransferase that *O*-acetylates C6 of β-galactose of serotype 11A polysaccharide. TMHMM analysis predicts that WcjE has 10 transmembrane regions, which presumably form a channel through which an acetyl group is exported to be used for an external acetylation of serotype 11A capsular polysaccharide. Interestingly, each 11E isolate has different genetic alterations such as deletions, mutations, or insertions. Moreover, we have also discovered 11A variant (11Av) strains that harbor mutations in *wcjE*, but retain trace amounts of acetylation.

To identify critical domains for *O*-acetyltransferase activity, we mapped the molecular changes of *wcjE* in 21 different 11Av and 11E strains. We identify mutations primarily found in transmembrane regions, with occasional mutations found in loops 2 and 5. Further studies in *wcjE* may provide key insight to *O*-acetyltransferase function in both gram positive and negative bacteria. WcjE is similar to WecH (*Escherichia coli*), *O*atA (*Staphylococcus aureus*), *O*afA (*Salmonella typhimurium*), and the *Shigella spp.* bacteriophage-encoded *O*acT which are involved in O-acetylation of lipopolysaccharide, peptidoglycan, and capsules. WcjE is therefore a relevant protein model for study of antigenic variation, bacterial virulence, and host recognition.

T-bet and IFNγR Signaling Regulates Germinal Center Responses and Long-lived Plasma Cell Development in an Influenza Model

Sara Stone, Andre Ballesteros-Tato, Betty Mousseau, Frances Lund

Bcl6 and Blimp1 are transcription factors that can mutually repress one another. The fate decision of a germinal center B cell (GCB) is controlled by the opposing actions of Bcl6, which sustains GCB cells, and Blimp1, which specifies commitment to the long lived plasma cell (LLPC) pool. To date, it is unclear how these opposing transcription factors are regulated in GCB cells. In activated T lymphocytes, modulation of the Blimp1/Bcl6 ba lance by the transcription factor T-bet regulates memory versus effector fate decisions. Therefore, we hypothesized that T-bet may also control LLPC differentiation from the GCB progenitors by altering the balance between Blimp1 and Bcl6 in GCB cells. To test this hypothesis, we produced chimeric animals in which B cells, but not other cell types, were T-bet or IFNγR deficient. We infected the chimeras with influenza and then analyzed the transcription profile of the GCB cells, enumerated the flu-specific LLPCs in bone marrow and measured the systemic flu-specific antibody response. Following infection, GCB cells expressing CXCR3, a chemokine receptor regulated by T-bet, expressed higher levels of Blimp1 and IRF4, consistent with plasma cell fate commitment. GCB cells that were Tbet-/-, in our chimeric model expressed lower levels of Blimp1 and IRF4. Additionally, flu-specific antibody titers were attenuated and influenza-specific antibody secreting cells were decreased in chimeric animals with Tbet-/- or IFNγr-/- B cells compared to control chimeras with WT B cells. Together, these results suggest that T-bet expression in GCB cells favors Blimp1 expression over Bcl6 and thus supports LLPC development.

Inhibition of Hepatitis C Virus Infection by Mouse Serum

Jian Tao, Stacy D. Hall, Matthew B. Renfrow, Jan Novak, and George Luo

Research on hepatitis C virus (HCV) and development of antiviral drugs and HCV vaccine have been hampered by the lack of robust small animal models. We have demonstrated that HCV can replicate in mouse hepatocytes. Mouse models of HCV infection and replication will be a desirable alternative to determine the importance and underlying molecular mechanisms of cellular genes in HCV replication, pathogenesis, and carcinogenesis as well as to the discovery and development of safe and efficacious antiviral agents and vaccines against HCV infection. The work derived from Dr. Charlie Rice's group at Rockefeller University demonstrated that humanized mice expressing key HCV receptors are susceptible to HCV infection. The transgenic HCV mice have also been constructed in our lab. To facilitate animal studies of HCV infection and replication, we examined the effect of mouse serum on HCV infectivity. Surprisingly, we found that mouse serum could potently block HCV infection in cell culture. To a lesser extent, human serum is also able to inhibit HCV infection. Mechanistic studies demonstrated that mouse serum blocked HCV attachment to hepatocytes. Fractionation of mouse serum in conjunction with mass spectrometry suggested that mouse lipoprotein particles are likely involved in the suppression of HCV infection. Currently, we are testing this hypothesis using MTP inhibitor to block lipoprotein assembly and secretion. If confirmed, we will cross-breed our HCV transgenic m ice with apolipoprotein B-deficient mice. Our ultimate goal is to develop a robust mouse model for efficient HCV infection and replication.

Detection of *Mycoplasma pneumoniae* in Fresh Human Lung Using Reverse Transcriptase-quantitative PCR to Detect 16S rRNA Transcripts

Arthur H. Totten; Li Xiao; Donna M Crabb; Amy Ratliff; Ken B. Waites, and T. Prescott Atkinson

Mycoplasma pneumoniae (Mpn) is a common cause of infections in humans. The organism can often be detected for months after antibiotic treatment, and some PCR-based studies have suggested that the organism may be present chronically at higher frequencies in asthmatics. We hypothesized that in asymptomatic chronically infected subjects, the organism may be difficult to detect if it is present at low levels. Using cDNA from whole RNA extracted from fresh human lung fragments, we evaluated an assay developed for use in experimental infection in mice for the presence of Mpn 16S rRNA transcripts (RT-qPCR), since those are present in higher abundance than genomic DNA (gDNA) by two orders of magnitude.

Optimization of the 16S rRNA RT-qPCR assay showed that positives were reproducible despite contaminating human genomic background. The detection limit was 0.01 CFU/µl in the PCR mixture. There were no cross reactivities with any other mycoplasma or bacterial species except for *M. genitalium*. Analysis of lung fragments from 24 lung cancer patients revealed the presence of Mpn in two (8.3%), neither of which were detected by routine genomic PCR. Both 16S rRNA positive samples were confirmed with 23S rRNA analysis, showing no macrolide resistance at commonly mutated nucleotides. Detection of *Mpn* in fresh human lung is feasible using RT-qPCR for rRNA transcripts. Further work is ongoing to determine the relative sensitivity of this assay compared to routine qPCR using gDNA and to develop an estimate of the prevalence of this organism in lung samples from patients with different disease states.

An Analysis of NF-kappaB Dependent Regulation of the Adaptor Protein Hematopoietic Src Homolgy Domain 2

Arthur VanValkenburg and R. Glenn King

The Hematopoietic Src Homology 2 (HSH2) adaptor protein is differentially expressed in mature B cells and appears to be involved in regulating antibody class switching and terminal differentiation. Enforced expression of HSH2 in a transgenic mouse model was shown to enhance B cell survival in vitro in response to antigen receptor signaling (Herrin, 2005), but impairs isotype class switching, leading to decreased antibody production for most isotypes in vivo (King, 2011). HSH2 is not expressed in developing B lymphocytes until they reach transitional stages 1 and 2 in the periphery. Different subsets of B cells express HSH2 at different levels, with mature follicular B cells and germinal center B cells expressing low levels of HSH2 and B1 B cells expressing high levels of HSH2 (King, 2011). HSH2 expression is upregulated in response to a range of TLR and TNF family receptors via what appears to be an NF-kappaB dependent pathway (Herrin, 2006). Conversely, factors that promote cell death through apoptosis lead to decreased expression of full-length HSH2 whereas factors that promote B cell survival upregulate HSH2 expression (Herrin, 2006). Here we show that HSH2 stability at the protein level is correlated to NF-kapaB activity in a non-transcriptionally dependent manner.

Differential Stability of ATG8 mRNA Contributes to Control of the Autophagic Response

Matthew T. Weaver, Shane P. Kelly, David M. Bedwell

Autophagy is a mechanism by which cells sequester proteins and organelles from the cytoplasm and degrade them in the vacuole (lysosome) to remove damaged components or redirect resources during nutrient scarcity. Autophagy has been found to have a role in some cancers, neurodegenerative diseases, and removal of pathogens.

Atg8p is a ubiquitin-like protein that plays a central role in the construction of the autophagosomal membrane and is used as a marker to determine the magnitude of autophagy. Previous reports have concluded that an increase in ATG8 transcription during nitrogen starvation is responsible for the increase of Atg8p. Consistent with this premise, our lab has observed a 5 to 10-fold increase in the abundance of ATG8 mRNA during nitrogen starvation. However, only a 2-fold increase in transcription from the ATG8 promoter was observed. This indicates induction of transcription represents only a fraction of the overall increase in mRNA abundance (Kelly and Bedwell, unpublished data). We found that mRNA stabilization also plays an important role in the overall accumulation of ATG8 mRNA during the induction of autophagy.

We are currently investigating whether this mechanism is specific to ATG8 or if it regulates other autophagy genes as well. Previous work has established that differential mRNA decapping can regulate mRNA stabilization. The Igo1/2 proteins have been identified as adapters that prevent decapping of nutrient-regulated mRNAs during glucose starvation and in the presence of rapamycin. We will report our findings on their potential role in stabilization of ATG8 mRNA during nitrogen starvation.

Synthetic Aminoglycosides Efficiently Suppress CFTR Nonsense Mutations and Are Further Enhanced by the CFTR Potentiator Ivacaftor

Xiaojiao Xue, Venkateshwar Mutyam, Li Ping Tang, Silpak Biswas, Ming Du, Laura A. Jackson, Yanying Dai, Valery Belakhov, Moran Shalev, Fuquan Chen, Jochen Schacht, Robert Bridges, Timor Baasov, Jeong Hong, *David M. Bedwell and *Steven M. Rowe

New drugs are needed to enhance premature termination codon (PTC) suppression to treat the underlying cause of cystic fibrosis (CF) and other genetic diseases caused by nonsense mutations. We tested new synthetic aminoglycoside derivatives expressly developed for PTC suppression in a series of complementary CF models. Using a dual-luciferase reporter system containing the four most prevalent CFTR nonsense mutations (G542X, R553X, R1162X and W1282X) within their local sequence contexts (the three codons on either side of the PTC), we found that NB124 promoted the most readthrough of G542X, R1162X and W1282X PTCs. NB124 also restored full-length CFTR expression and chloride transport in FRT cells stably transduced with a CFTR-G542X transgene, and was superior to gentamicin and other aminoglycosides tested. NB124 restored CFTR function better than gentamicin and to ~7% of wild type activity in primary human bronchial epithelial (HBE) CF cells (G542X/ delF508), a highly relevant preclinical model with endogenous CFTR expression. Efficacy was further enhanced by addition of the CFTR potentiator ivacaftor (VX-770) to airway cells expressing CFTR PTCs. NB124 treatment also rescued CFTR function in a CF mouse model expressing a human CFTR-G542X transgene; efficacy was superior to gentamicin and exhibited favorable pharmacokinetic properties. NB124 was less cytotoxic than gentamicin in explants of the mouse organ of Corti, a tissue-based model for ototoxicity. These results provide strong evidence that NB124 and other synthetic aminoglycosides provide a promising treatment for a wide array of CFTR nonsense mutations and a 10-fold improvement in therapeutic index over aminoglycoside antibiotics such as gentamicin.

THE UNIVERSITY OF
ALABAMA AT BIRMINGHAM

Knowledge that will change your world