24th Annual
Microbiology Research Retreat

November 13-15, 2015 | The Chattanoogan Hotel | Chattanooga, TN

UAB SCHOOL OF MEDICINE
Department of Microbiology

24th Annual Microbiology Research Retreat
November 13 – 15, 2015
The Chattanoogan Hotel

Friday, November 13th

1:30 PM EST **Registration** (Amphitheater Foyer)

2:45 PM **Fran Lund** (Amphitheater)
Welcome

2:55 PM **Pete Burrows**
Introductory Remarks

3:00 PM **Arthur VanValkenburg**[GS] (Justement)
Regulation of HSH2 adaptor protein turnover via a novel NF-κB-dependent mechanism is involved in determining the nature of the class switched antibody response

3:15 PM ***Victor Y. Du***[GS] (Goepfert)
HIV-1-specific CD8 T cells are poorly cross-reactive during acute infection

3:30 PM **Joshua Justice**[NI] (Jiang)
MutSα is essential for BK polyomavirus replication and host genome stability

3:45 PM ***Norberto González-Juarbe***[PD] (Orihuela)
Pore-forming toxins induce macrophage necroptosis during acute bacterial pneumonia

4:00 PM ***Tyler Stewart***[GS] (Novak)
Using LCMS to define micro and macro heterogeneity of clustered glycosylation by GalNAc-Ts

4:15 PM ***Beth Walters***[GS] (Thompson)
Ribosomal protein S25 plays a role downstream of ribosome recruitment during non-canonical initiation

4:30 PM **Yuan Tian**[GS] (Zajac)
A context-dependent role for IL-21 in modulating the differentiation, distribution, and abundance of effector and memory CD8 T cell subsets

4:45 PM **Kyung-Don Kang**[PD] (Luo)
Cellular factors important for HCV replication in mouse hepatocytes

5:00 PM **Break & Hotel Check-in** (N. Refreshment Island)

5:30 – 7:00 PM **Poster Session** (Rose Room)

7:30 PM **Dinner** (Off-Site)
Assemble in the Broad Street Lobby Entrance (7:15pm)

Saturday, November 14th

7:30 - 8:30 AM **Breakfast** (N. Refreshment Island)

8:30 AM **Shannon Romano**[GS] (Gorelick)
The G protein-coupled estrogen receptor is required for the establishment of basal heart rate

24th Annual Microbiology Research Retreat
November 13 – 15, 2015
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8:45 AM	**Arthur Totten**[GS] *Allergic airway sensitization impairs bacterial specific IgG and correlates with increased Mycoplasma pneumoniae persistence*	(Atkinson)
9:00 AM	***Preeyam Patel***[GS] *CD36 and platelet activating factor receptor (PAFR) orchestrate the development of house dust mite-induced allergic disease*	(Kearney)
9:15 AM	**Ashley Burg**[GS] *Superoxide deficiency protects against Coxsackievirus-induced T1D through dampened antiviralresponses by M2-skewed macrophages*	(Tse)
9:30 AM	**Rosanne Hill**[GS] *The φNM1 type 2 dUTPase initiates mobilization of Staphylococcus aureus bovine pathogenicity island 1*	(Dokland)
9:45 AM	***Mikhail Pavlenok***[GS] *The MspA nanopore as a sensor for DNA sequencing*	(Niederweis)
10:00 AM	**Cameron Crawford**[NI] *The copper dependent effects of pyrazolopyrimidines on Staphylococcus aureus*	(Wolschendorf)
10:15 - 10:30 AM	**Break**	(N. Refreshment Island)
10:30 AM	**Sara Stone**[GS] *T-bet regulates memory B cell and long-lived plasma cell development in an influenza model*	(Lund)
10:45 AM	**Bi Shi**[PD] *Foxp1-deficient $CD4^+$ T cells augment germinal center B cell response with faster kinetics*	(Hu)
11:00 AM	**Stephanie Garcia**[GS] *Anti-caries activity by a novel small molecule against Streptococcus mutans biofilms*	(Wu)
11:15 AM	**Jocelyn Hauser**[GS] *Streptococcus pneumoniae hydrogen peroxide production as an oxygen-dependent signal for capsule regulation*	(Yother)
11:30 AM	**Brady Spencer**[GS] *Additional capsular diversity exists among pneumococci expressing serogroup 33 capsules*	(Nahm)
11:45 AM	**Jiri Vlach**[PD] *Structural studies of the cytoplasmic tail of HIV-1 envelope protein*	(Saad)
12:00 PM	**Group Picture**	TBA
12:15 PM	**Lunch**	(Broad Street Grille)
12:30 PM	**Free Time**	
5:30 – 6:30 PM	**Social Hour**	(Rose Room)
6:30 PM	**Dinner**	(Rose Room)

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7:00 PM **Flavius Martin** (Rose Room)
Amgen
Unlocking the potential of biology for patients

Sunday, November 15

8:30 – 9:00 AM **Breakfast** (N. Refreshment Island)

9:00 AM **Ming Du** (Amphitheater)
Characterization of new nanoluc luciferase-based readthrough/nonsense-mediated mRNA decay (RT/NMD) reporters

9:30 AM **Michael Niederweis**
The necrosis-inducing toxin of Mycobacterium tuberculosis

10:00 AM **Mark Walters**
Using synthetic antibodies to probe IFN receptor function

10:30 AM **Pete Burrows**
Awards

11:00 AM **Fran Lund**
Closing Remarks

2015 Poster Presentations

Constance Agamasu[GS]
Krishna Chinta*
Alex Dalecki[NI]
Rhea Derke[NI]
Kathryn Doornbos[GS]
Junfen Fan*
Huahao Fan*
Jianlin Geng[PD]
Audra Hargett[NI]
Jyoti Jyoti[NI]
Shannon Kahan[PD]
James Kizziah[NI]
Keith Manning[GS]
Ruth McDowell[PD]
Avishek Mitra[PD]
Nadine Morgan[NI]
Richard Murphy[NI]
Saman Najmi[NI]
J. Stew New[GS]
Jessica Peel[NI]
Benghao Peng[GS]
Nick Potochick[GS]
Luhua Qiao[PD]
Vineel P Reddy[PD]
Michael Schultz[NI]
Anukul Shenoy[NI]
Cathy Sung[GS]
Jacob Switzer*
Uday Tak[NI]
Brandy Verhalen[PD]
Olga Viktorovskaya[PD]
Harong Wei[PD]
Xiaojiao Xue[GS]
Venkata Yeramilli[PD]
Dingguo Zhang[NI]

GS - Graduate Student (Year 4 and up)
NI - New Investigator (Year 1-3)
PD - Postdoctoral Fellow or those with terminal degrees who are within **5** years (Ph.D.) and **10** years (M.D.) of receiving their degree

Everyone is encouraged to participate, but awards are restricted to trainees. Trainees are considered Ph.D. graduate students, UAB appointed postdocs and those with terminal degrees who are within **5 years (Ph.D.) and **10** years (M.D.) of receiving their degree*

Regulation of HSH2 adaptor protein turnover via a novel NF-κB-dependent mechanism is involved in determining the nature of the class switched antibody response.

Arthur J. VanValkenburg, R. Glenn King, Louis B. Justement

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

The Hematopoietic Src Homology 2 (HSH2) adaptor protein is differentially expressed in mature, peripheral B cell subpopulations; exhibiting high-level expression in B1 and marginal zone B cells versus low-level expression in germinal center B cells, independent of gene transcription. HSH2 plays an important role in regulating the production of class switched antibodies through its effect on B cell class switching and terminal differentiation. Mouse models revealed that over expression of HSH2 in the B cell lineage attenuates the class switched antibody response, whereas reduced expression accelerates the class switched response. Thus, it is of significant interest to delineate the molecular mechanisms by which expression of HSH2 is controlled. Upregulation of HSH2 expression in response to TNF receptor family and TLR agonists occurs in an NF-κB-dependent manner, yet does not involve increased transcription of the HSH2 locus based on analysis of HSH2 mRNA levels. Additionally, maintenance of HSH2 expression is dependent on NF-κB signaling. Inhibition of NF-κB results in the rapid down regulation of HSH2 with a half-life of 1 hour, which is in contrast to the results obtained following treatment with cycloheximide or actinomycin D demonstrating that mRNA for HSH2 is stable and the protein itself has a half-life greater than 12 hours. These data support the conclusion that HSH2 protein stability is regulated post-transcriptionally via a novel NF-κB dependent mechanism.

HIV-1-specific CD8 T cells are poorly cross-reactive during acute infection

Victor Y. Du, Anju Bansal, Jonathan Carlson, Jesus F. Salazar-Gonzalez, Kristin Ladell, Stephanie Gras, Sonya Heath, David Price, Jamie Rossjohn, Paul A. Goepfert

Background: HIV-1 has a high capacity to mutate so elucidating HIV-1 epitope-specific CD8 T-cell responses that can efficiently target epitope variants is important for effective vaccine development. We therefore evaluated the extent of T-cell cross-reactivity by analyzing samples from acutely HIV-1 infected patients with known sequence of their infecting virus.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from 11 acutely infected patients. Autologous epitopes were designed based on each patient's viral and HLA-I information. CD8 T cells targeting autologous epitopes (N=18) were evaluated for cross-reactivity by stimulating PBMC with epitope variants containing commonly occurring mutations in an IFN-γ ELISPOT assay. Cytotoxicity of cross-reactive T cells was determined by co-culturing epitope pulsed target cells with effector CD8 T-cell lines in a 7-AAD flow-based killing assay. In addition, effector/cytokine polyfunctionality, HLA-I binding affinity, T-cell avidity, epitope/HLA structural analysis and TCR clonotyping assays were performed.

Results: Only 5 of the 18 autologous responses cross-recognized one or more epitope variants. Cross-reactive CD8 T cells exhibited poor target cell killing. This impaired killing was not driven by polyfunctionality or HLA-I binding affinity but reduced T-cell avidity. Structural and TCR clonotype analyses of an autologous/variant epitope pair revealed that amino-acid changes in the TCR contact site affect epitope variant detection and cytotoxicity of a cross-reactive response.

Conclusions: Cross-reactive CD8 T cells appear compromised during acute HIV-1 infection, associated with impaired TCR recognition. These results suggest a polyvalent vaccine approach aimed at inducing broad autologous T-cell responses would be a more promising vaccine strategy.

MutSα is Essential for BK Polyomavirus Replication and Host Genome Stability

Joshua L. Justice and Mengxi Jiang

Human polyomaviruses (PyVs) are quiescent human pathogens with a small, circular DNA genome. Within this family, BKPyV is associated with severe genitourinary disease initiated by viral reactivation in immunocompromised individuals. Due the small size of the PyV genome, and thus limited coding capacity, this family of viruses relies heavily on host factors to replicate and have been pursued as a model of eukaryotic DNA replication. We employed quantitative proteomic analysis of BKPyV infection of primary kidney cells to characterize the impact of BKPyV infection on the host cellular environment. This analysis revealed the eukaryotic mismatch repair (MMR) system as a highly upregulated cellular pathway during BKPyV infection. This prompted us to investigate the relationship between BKPyV replication and MutSα, a DNA damage sensing complex in the MMR pathway. SiRNA knockdown studies revealed that MutSα is essential for BKPyV productive infection. Additionally, MutSα mediates BKPyV-driven DNA damage response (DDR) activation, which is required for BKPyV replication. Furthermore, we found that the absence of MutSα during BKPyV infection drives rapid accumulation of host DNA damage. These findings are one of the first to identify events leading to DDR activation by PyVs and will greatly expand our current understanding of BKPyV interaction with the host DNA damage sensing and repair pathways.

Pore-forming toxins induce macrophage necroptosis during acute bacterial pneumonia

Norberto González-Juarbe, Ryan Paul Gilley, Cecilia Anahí Hinojosa, Akinobu Kamei, Geli Gao, Peter Herman Dubé, Molly Ann Bergman, and Carlos Javier Orihuela.

Necroptosis is a highly pro-inflammatory mode of cell death regulated by RIP1 and RIP3 kinases and mediated by the effector MLKL. We report that pore-forming toxins (PFT) produced by diverse bacterial pathogens induce necroptosis of macrophages. Macrophages pretreated with inhibitors of RIP1, RIP3, and MLKL were protected against death following challenge with *Serratia marcescens, Staphylococcus aureus, Streptococcus pneumoniae, Listeria monocytogenes*, uropathogenic *Escherichia coli* (UPEC), and purified pneumolysin. Macrophages transfected with RIP3 and MLKL siRNA were also protected against *S. marcescens* and pneumolysin induced death. Caspase inhibition had no impact on macrophage death and caspase-1 and -3/7 were determined to be inactive despite the detection of IL-1β in supernatants. Bone marrow derived macrophages from RIP3 KO, but not caspases-1/11 or -3 KO mice, were resistant to PFT-induced death. During *S. marcescens* pneumonia, alveolar macrophages from MLKL KO mice were protected against death in comparison to controls. PFT induction of necroptosis required the loss of ion homeostasis at the plasma membrane, mitochondrial damage, ATP depletion, and the generation of reactive oxygen species. Treatment of mice with necrostatin-5, an inhibitor of RIP1, GW806742X, an inhibitor of MLKL, and necrostatin-5 along with co-enzyme Q_{10} (N5/CoQ_{10}), which enhances ATP production, reduced the severity of *S. marcescens* hemorrhagic pneumonia in a mouse intratracheal challenge model. N5/CoQ_{10} protected alveolar macrophages, reduced bacterial burden, and lessened hemorrhage in the lungs. We conclude that necroptosis is the major cell death pathway evoked by PFT producing bacteria in macrophages and the necroptosis pathway can be targeted for disease intervention.

Title: Using LCMS to define micro and macro heterogeneity of clustered glycosylation by GalNAc-Ts.

Authors: Tyler Stewart, Petr Kosztyu, Kazuo Takahashi, Qi Bian, Zhi-qiang Huang, Milan Raska, Matthew B. Renfrow, Jan Novak

GalNAc-transferases, initiators of mucin-type *O*-glycosylation, add *O*-glycans to protein substrates with high density. This is particularly true for glycosylation of mucins and IgA1 and is termed clustered *O*-glycosylation. Part of this clustered activity is believed to be due to a unique lectin domain at the C-terminal part of the enzyme that facilitates follow-up glycosylation by the catalytic domain through binding to previously incorporated glycans. To better understand clustered glycosylation by GalNAc-Ts, we analyzed a disease-relevant glycosylation by GalNAc-T2 of IgA1 hinge-region. In IgA nephropathy (IgAN), altered initiation of clustered glycosylation has been shown to affect formation of the auto-antigen epitopes. We performed time-course reactions of recombinant GalNAc-T2 with a hinge-region peptide (sHR; VPSTPPTPSPSTPPTPSPSCCHPR), containing 9 potential sites of *O*-glycosylation. Using high-resolution LCMS, we optimized clustered-glycosylation conditions and then, quantified the relative amounts of various glycoforms produced during the reaction time course. Our data demonstrated up to 8 additions of GalNAc to the peptide by GalNAc-T2. The reaction kinetics slowed considerably after the addition of 4-5 GalNAc residues, consistent with glycosylation of human IgA1. In addition to tracking the glycoforms produced we analyzed the site specific preference of GalNAc-T2. We identified preferential sites of glycosylation initiation on the sHR peptide, consistent with the hypothesis of amino acid motif-driven glycosylation initiation, as the sHR peptide contains 2 GalNAc-T-consensus motifs. We will use this technique to compare the activity GalNAc-T14, a disease-associated isoform in IgAN, with the activity of GalNAc-T2 towards IgA1 hinge region, to assess potential isozyme-specific functions in IgAN.

Ribosomal protein S25 plays a role downstream of ribosome recruitment during non-canonical initiation

Beth Walters and Sunnie R. Thompson
Department of Microbiology, University of Alabama at Birmingham, Birmingham AL 35294

During cellular stress cap-dependent translation is down regulated. Therefore, cells must use non-canonical mechanisms of translation initiation to synthesize proteins required during cellular stress. One such mechanism uses an internal ribosome entry site (IRES) to recruit the 40S ribosomal subunit internally to the message, bypassing the cap requirement. We have shown that ribosomal protein S25 (eS25) is required for structurally and functionally diverse IRESs, however the role of this protein in IRES-mediated translation is not fully understood. A series of alanine mutations in conserved residues were generated throughout eS25. Mutations in each of its three domains (N-terminal, head, and C-terminal) reduced IRES activity suggesting that each domain of eS25 is important for IRES-mediated translation. Cap-dependent translation initiation requires a complex of initiation factors that facilitate: 40S subunit joining, mRNA channel opening, positioning of mRNA in the mRNA channel, mRNA channel closing, and 60S subunit joining. However, IRESs manage to accomplish all of these steps with only a few or none of these initiation factors. We have used biochemical and genetic approaches to dissect the role of eS25 in these processes. Our studies suggest that eS25 is required for at least one step that is common to all IRESs that is downstream of 40S recruitment.

A context-dependent role for IL-21 in modulating the differentiation, distribution, and abundance of effector and memory CD8 T cell subsets

Yuan Tian, Maureen A. Cox, Shannon M. Kahan, Jennifer T. Ingram, Rakesh K. Bakshi, and Allan J. Zajac

The activation of naïve CD8 T cells typically results in the formation of effector (T_E) cells as well as memory cells that are retained over time. Memory CD8 T cells can be further subdivided into central memory (T_{CM}), effector memory (T_{EM}) and tissue-resident memory (T_{RM}) subsets, which cooperate to confer immunological protection. Using adoptive transfer studies and mixed bone marrow chimeras in which CD8 T cells either do or do not express the IL-21 receptor (IL-21R), we discovered that under homeostatic or lymphopenic conditions IL-21 acts directly on CD8 T cells to favor the accumulation of T_E/T_{EM} populations. The inability to perceive IL-21 signals resulted in reduced accumulation of CD8 T cells in various tissues including the small intestine intraepithelial lymphocyte compartment. The reduced fraction of IL-21R$^{-/-}$ CD8 T_{RM} cells in the small intestine also displayed markedly lower levels of the cytolytic effector molecule granzyme B. Notably, IL-21 signaling was associated with the expression of the chemokine receptor CX3CR1 and the integrin α4β7 on circulating CD8 T cells in the mixed bone marrow chimeras as well as on CD8 T cells differentiated in vitro. Acute lymphocytic choriomeningitis virus infection overcame the necessity for IL-21 to establish CD8 T_E/T_{EM} and T_{RM} subsets. Overall, this study demonstrates that IL-21 modulates the differentiation, accumulation, and function of effector-phenotype CD8 T cells in a context-dependent manner.

Cellular factors important for HCV replication in mouse hepatocytes

Kyung-Don Kang and Guangxiang (George) Luo

Hepatitis C virus (HCV) causes a serious global health problem, infecting approximately 170 million people worldwide. Although direct acting antiviral agents (DAAs) are highly efficacious to treat hepatitis C, there is no HCV vaccine available to prevent HCV infection. The study of HCV pathogenesis and development of HCV vaccines have been hindered by the lack of a robust small animal model. In this study, we sought to determine cellular factors important for HCV replication in mouse hepatocytes. First, we used a mouse hepatoma cell line, Hepa 1-6, to the efficiency of a subgenomic HCV RNA replication by colony formation assay. The HCV-replicating Hepa1-6 cell clones were subject to miRNA profiling. As a result, we have identified two miRNAs: miR-122 and miR-194, which were highly upregulated in the HCV-replicating Hepa1-6 cells. HCV replication was significantly suppressed by inhibitors of miR-122 and miR-194. Synthetic miR-122 and miR-194 were able to enhance HCV RNA replication. Similarly, miR-122 and miR-194 expressed from recombinant adenoviruses could promote HCV replication in mouse cells. More importantly, miR-122 and miR-194 were found to synergistically increase HCV RNA replication. In addition, our studies demonstrated that knockdown of cellular Xrn1 endonuclease resulted in a significant increase of HCV replication in mouse hepatocytes. More significantly, miR-122 expressed from a recombinant adenovirus was able to enhance HCV replication in the transgenic HCV mouse model by more than 100-fold. These findings will lead to the development of more robust mouse models of HCV infection and replication for the studies of viral pathogenesis and HCV vaccine development.

The G Protein-Coupled Estrogen Receptor is Required for the Establishment of Basal Heart Rate

Shannon N Romano[1,2] and Daniel A Gorelick[1]

Department of Pharmacology and Toxicology[1], Microbiology Graduate Theme[2], University of Alabama at Birmingham

Blood flow in embryos is required for the proper development of the cardiovascular system. Heart rate is a critical determinant of blood flow, yet how basal heart rate is established during embryonic development is not well understood. Here we demonstrate that the G protein-coupled estrogen receptor (GPER) is required for the establishment of normal embryonic heart rate. Estrogens regulate gene expression by binding to the nuclear hormone receptors ERα and ERβ, which are ligand-dependent transcription factors. Estrogens can also modulate cell function rapidly, independent of gene expression, via membrane-associated GPER. Two-day-old zebrafish embryos exposed for one hour to formestane, a small molecule that inhibits estradiol synthesis, exhibited reduced heart rate. This effect was rescued by co-administration of the GPER agonist ICI182,780. Exposure to 17β-estradiol, ICI182,780 or the GPER agonist G1 alone increased heart rate. The effects of ICI182,780 were abrogated by co-incubation with the GPER antagonist G36. In a complimentary approach, we used CRISPR-Cas technology to generate *gper* maternal zygotic mutants. Consistent with our pharmacological data and the hypothesis that endogenous estrogens regulate heart rate via GPER, *gper* mutant embryos exhibited reduced basal heart rate. To determine whether GPER-dependent heart rate influences blood flow, we examined expression of *klf2a*, a transcription factor activated by fluid shear stress that regulates many genes important for blood vessel tone and hematopoiesis. Compared to wildtype embryos, *gper* mutant embryos exhibited reduced expression of *klf2a*, indicating that GPER is required for the expression of blood-flow dependent genes. Together, our results suggest that estrogens regulate embryonic heart rate via GPER, a novel role for GPER during embryonic development.

Allergic Airway Sensitization Impairs Bacterial Specific IgG and Correlates with Increased *Mycoplasma pneumoniae* Persistence

A. H. Totten, D. Luo, L. Xiao, D. M. Crabb, K. B. Waites, T. P. Atkinson

Rationale : *Mycoplasma pneumoniae* (*Mpn*), an atypical human pathogen, has been associated with both asthma initiation and exacerbation. Asthmatics have been reported to have higher carriage rates of *Mpn* compared to non-asthmatic controls. We hypothesized that allergic airway sensitization would impair host immune responses to *Mpn* infection in a murine model.

Methods : BALB/cJ mice were sensitized and challenged with ovalbumin (OVA) to induce allergic airway disease and then infected with *Mpn*. Immune parameters were studied by analysis of cellular infiltrates in bronchioalveolar lavage fluid (BALF), ELISA of specific serum antibody levels for *Mpn* and OVA, and cytokine transcript levels in total lung RNA. Bacterial burden was determined by detection of bacterial 16S rRNA with qPCR.

Results : *Mpn*-specific IgG antibody levels were negatively correlated with OVA-specific IgE levels ($r = -0.6732$, $p < 0.05$). OVA-specific IgE and IgG levels were enhanced following *Mpn* infection, suggesting peripheral Th2 cytokine enhancement with infection ($p < 0.001$). Strikingly, *Mpn*-specific IgG antibody levels during allergic airway sensitization were negatively correlated with *Mpn* latency at 21 days post-infection ($r = -0.6668$, $p < 0.01$).

Conclusions : Prior allergic sensitization results in impaired *Mpn*-specific antibody production and pathogen clearance following subsequent infection. *Mpn* infection augments allergen-specific IgE production in sensitized mice.

TITLE: CD36 and Platelet Activating Factor Receptor (PAFR) Orchestrate the Development of House Dust Mite-Induced Allergic Disease

AUTHOR(S): Preeyam S Patel* and John F Kearney*

ABSTRACT:

There is an alarmingly high rate of asthma that occurs among children born in developed countries. Approximately 89% of these asthmatic children demonstrate sensitivity to house dust mite (HDM) allergen. HDM is composed of many pathogen-associated molecular patterns (PAMPs) such as LPS, chitin, and β-glucan. Upon inhalation, these epitopes are capable of ligating receptors such as TLR2, TLR4, Mannose receptor, and Dectin-2 on dendritic cells (DCs) in the lung to initiate the development of allergic disease. Blockade of these receptors has greatly enhanced our understanding of the mechanism of allergic disease but it has not lead to the generation of any viable therapeutics. Our recently published works have demonstrated that HDM contains phosphorylcholine (PC) epitopes, and for this reason, we hypothesized that PC receptors such as CD36 and platelet activating factor receptor (PAFR) were important in modulating the development of allergic disease. Mice deficient in CD36 or PAFR develop a significantly attenuated form of HDM-induced allergic disease compared to C57BL/6 mice sufficient for both receptors. We further demonstrate that PC-specific antibodies bound to HDM prevented the interaction of the allergen with APCs in the lung by inhibiting their interaction with CD36 and PAFR in the lung. Thus, we have indicated that engagement of CD36 and PAFR by HDM plays a critical role in the development of allergic disease and that inhibiting the engagement of HDM with these receptors via treatment with anti-PC antibodies can significantly attenuate the development of allergic disease.

Superoxide deficiency protects against Coxsackievirus-induced T1D through dampened anti-viral responses by M2-skewed macrophages.

Ashley R. Burg, B.S.[1,2], Shaonli Das, Ph.D., Lindsey E. Padgett, B.S. [1,2], Hubert M. Tse, Ph.D. [1,2,3]

Department of Microbiology[1]
Comprehensive Diabetes Center[2]

Viral infections are highly-suspected environmental triggers in the initiation of Type 1 diabetes (T1D), an autoimmune disease targeting insulin-producing pancreatic β-cells. Many innate anti-viral factors, including IFN-α/β, TNF-α, CXCL10 and CCL5, are also involved in the pathogenicity of T1D. We recently demonstrated a crucial role for reactive oxygen species production in T1D pathogenesis, as Non-Obese Diabetic (NOD) mice deficient in NADPH oxidase (NOX)-derived superoxide (NOD.*Ncf1*^{m1J}) are highly resistant to T1D development, partly due to impaired Toll-like receptor (TLR) inflammatory responses by NOD.*Ncf1*^{m1J} macrophages. Therefore, we hypothesized that loss of NOX-derived superoxide will dampen diabetogenic anti-viral macrophage responses, providing protection from virus-induced diabetes. Indeed, upon infection with a suspected diabetogenic virus, Coxsackie B3 (CB3), NOD.*Ncf1*^{m1J} mice remained protected from diabetes while infected NOD mice displayed accelerated disease. This was accompanied by significant decreases in circulating CXCL10 and CCL5 serum levels, and a decrease in both TNF-α- and IL-1β-producing pancreas-infiltrating macrophages. *In vitro* CB3 infections of macrophages revealed decreases in TNF-α, IFN-β, CXCL10 and CCL5 at the mRNA and protein level. This corresponded to significant decreases in transcription and protein expression of viral RNA sensors, MDA5 and TLR3. Similar to previous results demonstrating a skewed M2 phenotype during spontaneous T1D, CB3-infected NOD.*Ncf1*^{m1J} macrophages displayed enhanced phosphorylated STAT6 and *Arg1*, *Ccl17* and *Retnla* mRNA accumulation. Overall, we show that loss of NOX-derived superoxide leads to protection against CB3-induced diabetes, which is afforded by dampened anti-viral responses by M2-like macrophages.

The ϕNM1 type 2 dUTPase initiates mobilization of *Staphylococcus aureus* bovine pathogenicity island 1

Rosanne L. L. Hill and Terje Dokland

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Staphylococcus aureus pathogenicity islands (SaPIs) are genetic elements that are mobilized by specific helper phages. The initial step is the derepression of the SaPI by the interaction of a phage protein with the SaPI master repressors, Stl. Stl proteins varies between different SaPIs and respond to different phage-encoded derepressors. SaPIbov1 is derepressed by the dUTPase (Dut) of bacteriophage 80α ($Dut_{80\alpha}$) and its phage ϕ11 homolog. We previously showed that SaPIbov1 could also be mobilized by phage ϕ NM1, even though its *dut* gene is not homologous with that of 80α. Here, we show that ϕ NM1 *dut* encodes a type 2 dUTPase (Dut_{NM1}), which has an α-helical structure that is distinct from the type 1 trimeric, β-sheet structure of $Dut_{80\alpha}$. Deletion of dut_{NM1} abolishes the ability of ϕ NM1 to mobilize SaPIbov1. Like $Dut_{80\alpha}$, Dut_{NM1} forms a direct interaction with SaPIbov1 Stl both *in vivo* and *in vitro*. This work provides novel insights into the diverse mechanisms of genetic mobilization in *S. aureus*.

The MspA nanopore as a sensor for DNA sequencing.

Mikhail Pavlenok and Michael Niederweis.

Nanopore sequencing is a novel single molecule DNA sequencing method which is inexpensive, fast, capable of long reads and retains epigenetic information. In this method ionic current is measured while single-stranded DNA (ssDNA) is electrophoretically translocated through a nanometer-scale pore. Each passing nucleotide blocks current with a characteristic amplitude and duration which are used to identify the DNA sequence.

The ***M****ycobacterium* ***s****megmatis* **p**orin **A** (MspA) is an octameric, channel-forming protein with a short and narrow constriction zone which is ideal for nanopore DNA sequencing. However, wild-type MspA does not translocate DNA. We identified key positions in MspA and constructed MspA mutants capable of ssDNA translocation. Due to octameric symmetry of MspA, precise control of any amino acid residue in the functional pore is challenging. Therefore, we constructed a single-chain *mspA (scmspA)* gene where all eight subunits are connected by regions encoding a peptide linker. Linking subunits does not change the channel-forming properties of MspA. Further, we improved membrane insertion of scMspA by introducing additional phenylalanines in the membrane insertion loop of the pore. In conclusion, construction of scMspA enables precise control of the chemistry and the subunit composition of the MspA nanopore and represents an efficient platform for fine-tuning DNA translocation.

The Copper Dependent Effects of Pyrazolopyrimidines on *Staphylococcus aureus*

Author: Cameron Crawford and Dr. Wolschendorf

The usable pool of antibiotics is quickly being eroded due to the ability of bacteria to gain and transfer resistance. As this arms race continues, we will be unable to keep up with the rate at which bacteria acquire resistance mechanisms if we cannot produce new inhibitors. Recent screening efforts within our lab have uncovered the promise of copper complexing compounds against *Staphylococcus aureus*. Herein, we describe two compounds (PZP-915 and PZP-716) containing a pyrazolopyrimidine core that selectively inhibit *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in the presence of copper. These compounds form a complex with copper in a 2:1 ratio as determined by mass spectrometry. The activity of these compounds is attributed to copper complexation, whereas when other transition metals, such as zinc and iron, are added, there is little to no increase in inhibition. In addition, PZP-915 and PZP-716 have low toxicity towards human cell lines when physiologically relevant levels of copper are added. Mutants deficient in copper regulatory systems showed no increase in susceptibility, suggesting that these copper dependent compounds act outside of the mechanisms commonly associated with copper accumulation. Instead, these compounds may work by a target-specific mode of action in *S. aureus*. These compounds illustrate the importance of harnessing metal associated inhibitors that take advantage of the antimicrobial capability of these elements, targeting them specifically towards bacteria.

TITLE: T-bet regulates memory B cell and long-lived plasma cell development in an influenza model

Authors: Sara Stone, Andre Ballesteros-Tato, Frances Lund

ABSTRACT:
Memory B cells (Bmem) and long-lived antibody-secreting cells (LL-ASCs) arise from germinal center B cell precursors (GCB). The transcription factor Bcl6 is required for GCB cell survival and the development of Bmem. Bcl6 also inhibits LL-ASC development by repressing the ASC lineage commitment transcription factor, Blimp1. Although we do not understand how these opposing transcription factors are regulated in GCB cells, in T lymphocytes, the transcription factor T-bet modulates the balance between Blimp1 and Bcl6. Based on our data showing that Th1-mediated naïve B cell differentiation into ASCs *in vitro* requires T-bet and IFNγ, we hypothesized that B cell T-bet expression would be necessary for the development of LL-ASCs from GCB cells. Following infection of mice with influenza A, we found that a subset of GCB cells and ASCs expressed T-bet. In chimeric mice in which 50% of cells are T-bet$^{-/-}$, ASCs were preferentially derived from WT, rather than T-bet$^{-/-}$ B cells and T-bet$^{-/-}$ GCB cells expressed lower mRNA levels of Blimp1. Additionally, we found reduced numbers of flu-specific ASCs and lower flu antibody titers in chimeric animals with T-bet$^{-/-}$ B cells compared to control chimeras with WT B cells. Though we observed no impairment in Bmem formation when all B cells were T-bet$^{-/-}$, in chimeric mice in which 50% of cells are T-bet$^{-/-}$, significantly fewer T-bet$^{-/-}$ B cells were found in the Bmem compartment compared to WT B cells. Collectively, these results support a role for T-bet in determining fate of GCB cells following influenza infection.

Foxp1-deficient CD4$^+$ T cells augment germinal center B cell response with a faster kinetics

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Abstract

Germinal center (GC) B cell response is critical for the generation of high-affinity antibodies. Studies have shown that high cell number of single B cell clone leads to intra-clonal competition, which in turn inhibits the generation of high-affinity antibodies. It has also been shown that boosting Tfh cell number has limited impact on the GC B cell formation and antibody generation. Previously we have shown that Foxp1 deficiency leads CD4$^+$ T cells to preferentially differentiate into Tfh cells. Here we report that in an adoptive co-transfer model system, Foxp1-deficient Tfh cells helped the generation of an accelerated GC B cell response: upon antigen challenge, Foxp1-deficient Tfh cells helped B1-8 B cells proliferate more and differentiate into GC B cells with a faster kinetics. Excitingly, we also found that in the recipient mice with Foxp1-deficient Tfh cells, both transferred B1-8 B cells and endogenous B cells continued to have high frequencies of somatic mutation and high levels of high-affinity antibodies. Our studies suggest that in addition to Tfh cell number, Foxp1-deficient Tfh cells may possess some unique helper functions to boost GC B cell responses.

Anti-caries Activity by a Novel Small Molecule Against *Streptococcus mutans* Biofilms

Sandra S. Garcia, Suzanne Michalek, Christian Melander, Casey Morrow, Hui Wu

Dental caries, commonly known as tooth decay, is the most prevalent infectious disease albeit being highly preventable. Tooth decay is a multifactorial disease, caused by the right combination of sugar intake, poor dental hygiene, and cariogenic bacteria. *Streptococcus mutans* is the most associated with tooth decay which is attributed to the ability of *S. mutans* to metabolize various dietary sugars into lactic acid and to produce sticky glucans for biofilm formation by glucosyltransferases (Gtfs). Fluoride is commonly used to prevent dental caries but it's broad-spectrum antimicrobial properties may be destroying the native oral microbiome. To date, there has not been any success in the production of a species-specific therapy able to efficiently prevent dental caries. We identified a *S. mutans*-specific small molecule and characterized its mechanism in order to identify new biofilm-related targets for the development of more potent and effective therapies.

We utilized a biofilm dispersion assay under different conditions and with single-gene knock-out mutants to elucidate the mechanism of the small molecule. The activity of the small molecule *in vivo* was characterized in a rat caries model.

We identified a small molecule (3F1) capable of selectively dispersing *S. mutans* biofilms *in vitro*. Biofilms were dispersed independent of Gtfs. In the rat caries model, the small molecule prevented dental caries similar to fluoride and the composition of the rat oral microbiome treated with the small molecule did not cause a disturbance in the native microbiome.

The lack of involvement of biofilm-essential Gtfs suggests a potentially unique mechanism for 3F1. Ongoing studies are focusing on identifying 3F1-specific target(s). Identifying such a unique target in *S. mutans* will allow us to design more selective small molecules for the inhibition of species specific biofilm while ultimately keeping the oral microbiome intact.

Streptococcus pneumoniae Hydrogen Peroxide Production as an Oxygen-Dependent Signal for Capsule Regulation

Jocelyn R. Hauser and Janet Yother

Streptococcus pneumoniae, which causes diseases such as pneumonia and otitis media, typically resides in the oxygen rich environment of the nasopharynx. Under this condition, production of its capsular polysaccharide is decreased, allowing colonization. This decrease may be due to the high amount of H_2O_2 (up to 1 mM) produced by *S. pneumoniae* primarily as a result of pyruvate oxidase (SpxB) activity during aerobic growth. An *spxB* deletion mutant exhibits increased capsule production compared to the parent under both high and low oxygen conditions which can be restored to near parental levels with exogenous H_2O_2 in the growth medium (0.5 mM). We hypothesize that generation of H_2O_2 may serve as a regulatory signal for capsule production, as capsule enzymes respond to oxidizing and reducing conditions. Here we found that protein levels of these enzymes were unaffected by the *spxB* mutation. However, *in vitro* activity of the initiating glycosyltransferase, Cps2E, was increased in the *spxB* mutant compared to the parent following growth under high oxygen. Cps2E activity appears to be altered in part through a redox mechanism. We hypothesize that under high oxygen conditions, H_2O_2 production increases, thus causing an increase in enzymatic activity, thereby increasing capsule production. Our data demonstrate a posttranslational mechanism by which *S. pneumoniae* modulates capsule production in response to its environment.

Title: Additional Capsular Diversity Exists among Pneumococci Expressing Serogroup 33 Capsules

Authors:
B. L. Spencer, J.S. Saad, K. A. Geno, M. H. Nahm

Since anti-capsular antibodies confer serotype-specific protection against *Streptococcus pneumoniae* (pneumococcus), understanding capsular diversity is important for anticipating emerging serotypes following vaccine usage. One mechanism driving capsular diversity is O-acetylation, which alters epitopes and may allow evasion of vaccine-elicited antibodies. Serotype 33A has two O-acetyltransferase genes: *wciG* and *wcjE*. A serotype 33A variant, 33F, is *wcjE*-deficient and occurs naturally, but no *wciG*-deficient variants have been reported. Serotype 33F is to be included in the upcoming pneumococcal conjugate vaccine, and we hypothesize that vaccine pressure will select for *wciG*-deficient variants in nature.

We created two 33A variants by inactivating *wciG* or *wcjE* in the serotype 33A strain TIGR33A. Sequencing confirmed O-acetyltransferase gene inactivation. Both variants are viable and have serologically-distinct capsules based on rabbit antisera and Hyp33FG1 (monoclonal antibody) binding. ^{1}H-NMR confirmed that TIGR33A capsule has one WciG- and two WcjE-mediated O-acetyl additions (2.14 ppm and 2.10, 2.18 ppm, respectively) and that TIGR33AΔ*wcjE* (functionally 33F) capsule lacks both WcjE-mediated O-acetyl additions. Finally, ^{1}H-NMR showed that TIGR33AΔ*wciG* lacks WciG-mediated O-acetylation (2.14 ppm) and one peak associated with WcjE-mediated O-acetylation (2.18 ppm).

We conclude that TIGR33AΔ*wciG* is viable, expresses a novel serotype (termed 33X1), and could be present in nature. Hyp33FG1, which recognizes WciG-mediated O-acetylation, can distinguish such isolates. Serotype 33X1 lacks WciG-mediated O-acetylation and one peak associated with WcjE-mediated O-acetylation, indicating that WciG may be required for WcjE target recognition. This study describes a possible mechanism of capsular diversity via O-acetylation and highlights the importance of sensitive serological tools in uncovering novel variants.

Structural studies of the cytoplasmic tail of HIV-1 envelope protein

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The surface envelope (Env) protein of human immunodeficiency virus 1 has a paramount role in mediating the infection of a host cell. The main body of research has been directed at elucidating the mechanisms of Env binding to host receptor, membrane fusion, and evasion of recognition by the immune system. These functions are mediated by the ectodomains of the envelope (gp120) and transmembrane (gp41) parts of Env. The C-terminal cytoplasmic tail of gp41 (gp41CT) plays additional roles in viral replication, such as mediating Env intracellular trafficking and its incorporation into assembling virions. Gp41CT interacts with various cellular and viral components but the purpose of many such interactions remains unknown. There is strong evidence that Env incorporation is mediated by interactions between the matrix (MA) domain of Gag and gp41CT. A high-resolution structure of the 150-amino acid gp41CT domain in the free state or in complex with MA is still unavailable. Structural characterization of gp41CT has been very limited due to the challenges faced during recombinant protein production. We devised new approaches to obtain stable and functional gp41CT protein constructs. Here, we present preliminary results of our investigation of the biochemical and structural properties of gp41CT by NMR and other methods. Our data revealed that N-terminal 45 residues of gp41CT are hydrophilic and lack a regular secondary structure, while the C-terminal 70 residues form a long amphipathic α-helix that is only stable when bound to a membrane mimetic. We have identified key protein-lipid interactions that are implicated in the biological function of the gp41CT. These findings pave the way for full characterization of the interactions between MA and gp41CT during virus assembly, which is critical for Env incorporation into nascent HIV particles.

Molecular Basis for Heme Acquisition and Utilization by *Mycobacterium tuberculosis*

Avishek Mitra, Alex Speer and Michael Niederweis

Mycobacterium tuberculosis (Mtb) is the second leading cause of death worldwide resulting in 1.4 million deaths annually. Mtb is primarily transmitted through aerosols that enter the host lung. Mtb proliferates in alveolar macrophages by inhibiting macrophage maturation and formation of the phagolysosome. An essential aspect of Mtb survival and virulence is the acquisition of iron within the macrophage. Even though iron is abundant in the human host it is sequestered by transport proteins such as transferrin, lactoferrin or stored in ferritin. Mtb overcomes this iron deficiency through production of siderophores called mycobactins, which capture iron from host transferrin. However, heme and hemoproteins account for 90-95% of host iron, making them the major iron source in humans. Recent studies have shown that Mtb can utilize heme as a sole source of iron. In Mtb, the protein MhuD has been clearly established as the heme degrading enzyme, but knowledge on the other components of heme acquisition is limited. To this end, we have constructed a high density transposon library of Mtb with app. 70,000 mutants and isolated transposon mutants with defects in heme utilization. Characterization of selected transposon mutants will enable us to reveal components of the heme uptake system of Mtb. We will use a similar approach to characterize siderophore acquisition by Mtb. Our overarching goal is to understand iron uptake by Mtb and, based on this information, to design new intervention strategies in tuberculosis chemotherapy.

Cell surface reduced thiols level are elevated on activated autoreactive CD4+ T cells

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Reactive oxygen species (ROS) have a role in controlling T cell autoreactivity in the development of autoimmune disease including Type 1 diabetes (T1D). A recent study using superoxide-deficient rats, showed that an increase in the number of cell surface reduced thiols lead to an increased susceptibility in developing autoimmune arthritis. This suggest that ROS regulation of cell surface thiols can influence T cell activation, and the development of autoimmune disease. Our lab has recently demonstrated that the loss of NADPH oxidase (NOX)-derived superoxide enhance diabetogenic CD4 T effector response in T1D. How cell surface reduced thiols are regulated by ROS during autoreactive $CD4^+$ T cell immune responses during T1D is unclear. We hypothesized that activated $CD4^+$ T cells from superoxide-deficient NOD mice will display increased cell surface reduced thiol levels and a concomitant increase in T cell effector responses. To detect cell surface reduced thiols on activated $CD4^+$ T cells, we used fluorescein-5-maleimide (F5M) to measure the level of cell surface free thiols on diabetogenic NOD.BDC-6.9 CD4 T cells following stimulation with their cognate antigenic peptide (hybrid peptide). We demonstrated that after 72hour hybrid peptide-stimulated NOD.BDC-6.9 CD4 T cells displayed a 8.6-fold increase in the percentage of $CD44^+F5M^+$ cells, 3.2-fold increase in the percentage of $CD25^+F5M^+$ cells, and 9-fold increase in the percentage of $CD69^+F5M^+$ cells, respectively, in contrast to unstimulated NOD.BDC-6.9 CD4 T cells. For future studies, we will determine whether activated $CD4^+$ T cells from superoxide-deficient NOD mice have a higher level of cell surface reduced thiols.

Inducing toxicity in the blood fluke *Schistosoma mansoni* by inhibiting the NAD salvage pathway

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Nicotinamide adenine dinucleotide (NAD) is critical for cell survival and plays a role in many cellular processes, including glycolysis and mitochondrial respiration. In mammals and most other organisms, two major pathways of NAD synthesis are *de novo* synthesis from amino acid precursors and the salvage pathway in which nicotinamide-containing precursors are recycled into NAD. A comparative genome analysis of *S. mansoni,* the parasite that causes Schistosomiasis, enabled the assembly of a putative NAD biosynthetic pathway. Interestingly, only orthologues of NAD salvage-specific genes were identified and their expression was confirmed by polymerase chain reaction. This suggests that NAD biosynthesis in *S. mansoni* is dependent on salvaging of NAD precursors. Hence, we hypothesized that pharmacological inhibition of NAD biosynthesis through the salvage pathway in adult *S. mansoni* parasites results in diminution of NAD pools and subsequent cytoxicity. Indeed, our data show that inhibition of *Schistosoma mansoni* NAD-catabolizing enzyme (SmNACE), an active NAD-degrading enzyme, decreased NAD levels. Pharmacological blockade of NAD biosynthesis also reduced the metabolic function and viability of the parasite as measured by the redox indicator Alamar Blue. A high-throughput screening campaign and structural similarity searches of chemical databases have identified several small-molecule inhibitors of recombinant SmNACE. The selectivity of each compound, and their effects on metabolism and viability of *S. mansoni* is currently being assessed. Our preliminary data suggest that blockade of NAD biosynthesis is detrimental to *S. mansoni*, and that targeting the SmNACE-mediated NAD salvage pathway is a promising therapeutic approach for the treatment of Schistosomiasis.

Environmental Regulation of TNT Secretion in *Mycobacterium tuberculosis*

Uday Tak and Michael Niederweis

Mycobacterium tuberculosis (Mtb) remains the most prolific bacterial pathogen globally, having established latent infection within one-third of the world's population. The immune system's attempt to fight infection with Mtb results in the containment of the bacillus within lung granulomas. After phagocytosis, Mtb prevents maturation of the phagosome allowing it to survive inside macrophages. Mtb induces necrotic cell death in infected macrophages allowing it to gain access to the nutrient-rich cytoplasm and escape to the extracellular milieu. The recently discovered toxin TNT (Tuberculosis Necrotizing Toxin) causes necrosis in host cells and is the main cytotoxicity factor of Mtb in macrophages. TNT hydrolyzes the essential cofactor NAD+, leading to metabolic collapse of host cells. TNT is the C-terminal passenger domain of the outer membrane protein CpnT (Channel Protein with Necrosis-Inducing Toxin). CpnT is localized to the outer membrane of Mtb, and translocates TNT to the cell surface where it is cleaved by an unknown mechanism, and released into the extracellular milieu of the macrophage. TNT is highly toxic to both bacterial and host cells, thus expression is tightly regulated. Mtb encounters a range of environmental stressors within the granuloma such as low pH, oxidative and nitrosative stress, nutrient starvation, and hypoxia. We hypothesized that *cpnT* expression is induced by one or several of these stressors. In this study we exposed Mtb to various stress conditions and discovered that hypoxia alone induces expression of *cpnT*. This represents the first step towards understanding the function of TNT *in vivo*.

Mitochondrial bioenergetics of immune cells in a murine model of Type 1 diabetes

Ruth E. McDowell, Ashley Landuyt, Hubert M. Tse

Type 1 diabetes (T1D) is a polygenic autoimmune disease in which an inflammatory leukocytic infiltrate in pancreatic islets results in the destruction of insulin-producing β-cells. Redox signaling via reactive oxygen species (ROS) is an important component of T1D since it plays a critical role in inflammation and immune activation. The major sites of ROS synthesis are the NADPH-oxidase (NOX) complex and mitochondrial electron transport chain. Our lab has previously confirmed that NOX-produced superoxide is critical for development of T1D in the Non-Obese Diabetic (NOD) mouse since the NOD.*Ncf1*^{m1J} strain, which is unable to produce functional NOX, is T1D-protected. However, the role of mitochondrial ROS (mROS) in T1D susceptibility is not well-studied, even though T-cell activation requires mROS for adaptive immune maturation and effector responses. Here we show that mitochondrial bioenergetics differs between CD4 T-cells from diabetic NOD and pre-diabetic NOD mice. CD4 T-cells from diabetic NOD mice have a larger spare respiratory capacity, indicating a greater ability to produce both ATP and mROS. We also observed a greater spare respiratory capacity in NOD.*Ncf1*^{m1J} macrophages compared to NOD macrophages. The differences in spare respiratory capacity in immune cells like macrophages and CD4 T-cells may affect innate and adaptive immune maturation in the progression of T1D. In addition, identifying distinct mitochondrial profiles from diabetogenic immune cells could be exploited as a T1D biomarker. This would be useful as autoantibody synthesis is a poor T1D predictor since the majority of β-cells are destroyed once autoantibody seroconversion is observed.

Structural and Biophysical Characterization of the Interactions Between Calmodulin and the Pleckstrin Homology Domain of Akt

Constance Agamasu, Ruba H. Ghanam, and Jamil S. Saad

The translocation of Akt, a serine/threonine kinase, to the plasma membrane (PM) is a critical step in the Akt activation pathway. It is established that membrane binding of Akt is mediated by direct interactions between its pleckstrin homology domain (PHD) and phosphatidylinositol-(3,4,5)-trisphosphate ($PI(3,4,5)P_3$). There is now evidence that Akt activation in many breast cancer cells is also modulated by the calcium-binding protein, calmodulin (CaM). Upon epidermal growth factor (EGF) stimulation of breast cancer cells, CaM co-localizes with Akt at the PM to enhance activation. However, the molecular details of Akt(PHD) interaction with CaM are not known. In this study, we employed nuclear magnetic resonance (NMR), biochemical, and biophysical techniques to characterize CaM binding to Akt(PHD). Our data show that CaM forms a tight complex with the PHD of Akt (dissociation constant = 100 nM). The interaction between CaM and Akt(PHD) is enthalpically driven and the affinity is greatly dependent on salt concentration, indicating that electrostatic interactions are important for the complex . The CaM-binding interface in Akt(PHD) was mapped to two loops adjacent to the $PI(3,4,5)P_3$ binding site, which represents a rare CaM-binding motif and suggests a synergistic relationship between CaM and $PI(3,4,5)P_3$ upon Akt activation. Elucidation of the mechanism by which Akt interacts with CaM will help in understanding the activation mechanism, which may provide insights for new potential targets to control the pathophysiological processes of cell survival.

Identification of Cellular Factors Required for Efficient Amplification of Dengue and Yellow Fever Viruses.

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All viruses utilize cellular proteins, termed host factors, at various steps of their life cycle to ensure efficient viral growth. Our knowledge of these host factors as well as their roles during viral pathogenesis, is limited. Therefore, we used an innovative method, TUX-MS (thiouracil cross-linking mass spectrometry), which allows for identification of the proteins that directly bind to the viral RNA during a live infection in cell culture. Using two related human pathogens, dengue virus (DENV) and yellow fever virus (YFV), we identified and characterized novel host factors and their functions during infection. We have shown that individual siRNA knockdown of ten host factors identified by TUX-MS (DDX5, DDX17, NONO, hnRNP-M, hnRNP-F, XRCC6, RBMX, RACK1, HMCES and eS25) results in a significant decrease in viral titers. Furthermore, based on the levels of viral RNA in cells following knock down for each of these proteins, eS25, RACK1, DDX5, hnRNP-F, XRCC6, RBMX and HMCES appear to be involved in the early steps of the viral replication cycle. The remaining three proteins: DDX17, NONO and hnRNP-M appear to affect later stages downstream of viral RNA synthesis. In addition, we demonstrate that PCBP2 manifests antiviral activity towards dengue and yellow fever in contrast to its known proviral functions in the replicative cycles of other viruses, such as picornaviruses. Identification and determination of the role of these host factors in dengue and yellow fever viral amplification will provide insight into the biology of the virus, and has the potential to identify antiviral targets for these pathogens.

The Ribosomal Protein S25 Plays a Role in Translation Elongation

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Ribosomal Protein S25 (eS25) is a highly conserved protein on the 40S subunit of eukaryotic ribosomes essential for the translation of viral and cellular internal ribosome entry sites (IRESs). Canonical cap-dependent translation and other ribosomal functions are not significantly affected; yet global protein synthesis is reduced 15% when eS25 is knocked out. Consistent with this, knockout of eS25 in yeast results in a slow growth phenotype. However, IRES-mediated translation is not known to have a significant role in *Saccharomyces cerevisiae*. Suggesting eS25 may be playing a role in either translation elongation or termination. Our data shows both yeast and mammalian ribosomes lacking eS25 run-off the mRNA more slowly indicating a defect in translation elongation or termination. Furthermore, yeast strains that have eS25 knocked out exhibit increased sensitivity to drugs that inhibit the translocation step of translation elongation. This indicates eS25 has a role in translation elongation. The translation elongation rate in cells is regulated through phosphorylation of eukaryotic elongation factor 2 (eEF2) under certain stress conditions. We determined if loss of eS25 could cause ribosomal stress and phosphorylation of eEF2, and indirectly affect elongation rates. No increase in phosphorylated eEF2 and no change in total eEF2 levels were observed with eS25 knocked down. Our data suggests eS25 acts to increase the rate of elongation directly in both mammalian cells and yeast. These studies identify a novel role for eS25 in translation elongation, presenting an opportunity to determine whether the role eS25 plays is related to its function in IRES-mediated translation.

A multi-level control of T follicular helper cell differentiation by transcription factor Foxp1

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Abstract

$CD4^+$ T follicular helper (Tfh) cells are essential for germinal center responses and the generation of high-affinity antibodies. Recently we have identified transcription factor Foxp1 as a novel critical negative regulator of Tfh differentiation, yet the underlying mechanism is still not clear. Here we report that upon antigen challenge, naive $CD4^+$ T cells deficient of Foxp1 had a faster and higher degree of CCR7 down-regulation. In addition, higher number of activated Foxp1-deficient $CD4^+$ T cells migrated into the B cell follicles at the early time points, suggesting that Foxp1 controls the migration kinetics of activated $CD4^+$ T cells during the initial stage of Tfh cell differentiation. Meanwhile, even in the complete absence of ICOS-ICOS ligand interaction, Foxp1-deficient $CD4^+$ T cells still differentiated into Tfh cells, entered B cell follicles and helped B cells form the germinal centers and generate high levels of high-affinity antibodies. Taken together, our studies suggest that Foxp1 exerts a multi-level control in programming Tfh cell differentiation.

IL-2 production by activated naïve and effector CD8 T cells predicts memory formation.

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Although CD8 T cells display differences in their portfolios of effector functions, dissecting this ensemble has been impeded by the lack of effective methods for segregating, tracking, and defining these distinct subsets based upon their cytokine production profiles. To address this, we utilized novel IL-2 and IFN-γ double cytokine reporter mice. We demonstrated that within hours of activation, naïve CD8 T cells undergo a burst of cytokine production and populations are detectable that produce only IL-2, only IFN-γ, co-produce IL-2 and IFN-γ, or do not produce either cytokine, and these patterns of cytokine production can be swayed by changes in the inflammatory milieu. As effector and memory CD8 T cells develop a shift towards IFN-γ production occurs, with a smaller subset capable of co-producing IL-2 and IFN-γ, and the IL-2 only population typically not detectable. By harnessing the cytokine reporter system to separate IL-2 and IFN-γ producing CD8 T cell subsets and conducting a series of adoptive transfer studies, we discovered that recently activated naïve CD8 T cells that do not produce IL-2 preferentially develop effector traits and, conversely, subsets that do produce IL-2 predominately attain memory attributes. Furthermore, IL-2 producing effector CD8 T cells transition into memory populations that rapidly and vigorously proliferate upon secondary challenge. However, IL-2 producing and non-producing cells isolated during the memory phase mount similar recall responses. Taken together, these studies illustrate the functional complexity of CD8 T cell responses, and how this shapes the induction of successful effector and memory pools.

Hidden Treasures: Uncovering unrecognized staphylococcal inhibitors through exploiting copper toxicity.

Author: Alex Dalecki
Mentor: Frank Wolschendorf, Ph.D.

Despite a technological revolution, advanced high throughput antimicrobial screening methods have failed to fulfill their promise. Of sixteen clinically used antibiotic classes, all but two were discovered prior to the 1970s. A growing chorus advocates returning to natural inspirations, from which almost all antibiotics previously arose. Among these inspirations lies metal-mediated innate immunity, by which macrophages unleash a copper burst to destroy pathogens. Although historically medicinally unattractive, the recently described phenomenon of copper-dependent inhibitors has galvanized research exploring the use of ligand complexes to harness copper's antibacterial properties. Here, we describe the results of the first high throughput screening effort to identify copper-dependent inhibitors of *Staphylococcus aureus*. A standard library of 10,000 compounds was assayed for anti-staphylococcal activity both with and without added copper, with hits defined as those compounds exhibiting inhibition in the presence of copper, but impotent in its absence. 53 copper-dependent hit molecules were uncovered, nearly doubling the overall hit rate of the traditional campaign conducted in parallel. Most prominent was a hit family with an extended thiourea core structure, termed the NNSN motif. This motif resulted in copper-dependent and copper-specific *S. aureus* inhibition, while simultaneously being uninhibitory toward eukaryotic cells. A subsequent chemoinformatic meta-analysis of the ChEMBL chemical database confirmed the NNSNs as an unrecognized staphylococcal inhibitor, despite the family's presence in many chemical screening libraries. Thus, our copper-biased screen revealed potent molecular inhibitors even in previously exhausted chemical libraries, potentially offering an economically attractive solution for future drug discovery efforts.

Promotion of hepatitis C virus infection by phosphatidylserine receptor TIM1

Luhua Qiao, George Luo

T-cell immunoglobulin and mucin domain 1 (TIM-1) and its family members were recently identified as phosphatidylserine (PtdSer/PS)-mediated virus entry enhancing receptors (PVEERs), which were found to promote infection of several different enveloped viruses. However, their roles in hepatitis C virus (HCV) infection have not been experimentally examined. Here we successfully constructed the stable human hepatoma cell lines (Huh-7.5) with TIM-1-gene knockout using CRISPR/Cas9-mediated gene-knockout technology. The TIM1-gene knockout cell line was confirmed by DNA sequence analysis and Western blot. The knockout of TIM-1 remarkably reduced HCV and dengue virus (DENV) infection and their attachment to the surface of hepatocytes. The levels of HCV proteins and positive-strand RNA were remarkably decreased in the HCV-infected TIM1-knockout cells. More importantly ectopic expression of TIM1 protein was able to fully restore the capacity of HCV infection in the TIM1-knockout cells, excluding possible off-target effects. Additionally, HCV infection could be inhibited by phosphatidylserine (PS)-containing liposomes but not control lipids. Collectively, our study suggests that TIM1 protein play an important role in HCV infection.

Role of B cells in cutaneous Immunity

Venkata Yeramilli and John Kearney

Our understanding of the skin immune system has increased exponentially in recent years; however, the role of B cells in cutaneous immunity is poorly defined. To address this, we characterized B cells in mouse skin under steady state conditions and following inflammation. We found skin B cells are a heterogeneous population that is distinct from lymph node B cells, with more large B cells that express high levels of CD11b and IgM (B1) and a $CD11b^-$ population (B2). In contrast, the skin-draining lymph nodes comprised predominantly of $CD11b^-$ B2 B cells. Using parabiosis and in-vivo labeling experiments, we found most of the skin B cells are recirculating, in stark contrast to skin $CD4^+$ T cells that resident. Consistent with CD103 expression as a marker for resident cells, most $CD4^+$ T cells were $CD103^+$ while B cells were $CD103^-$. In an impetigo model of bacterial skin infection, we found a large accumulation of B1 cells, but not B2 cells at the site of infection as well in distal non-inflamed skin. From these pilot studies so far we conclude that B cells have the potential to play a central role in skin immune homeostasis. Further studies are underway to functionally characterize the role of skin B cells.

Gp44, an uncharacterized capsid protein of Bacteriophage 80α

Keith Manning and Dr. Terje Dokland, PhD

Bacteriophage 80α is a prototypical bacteriophage that infects *Staphylococcus aureus*. *Staphylococcus aureus* Pathogenicity Islands (SaPIs), are one of the mobile genetic elements present in *S. aureus* that contain virulence factors. Bacteriophage 80α is able to mobilize many different SaPIs and the SaPIs in turn are able to hijack the assembly of 80α virions creating SaPI particles containing SaPI DNA. The capsid head of 80α is composed of 4 major proteins: gp42 (portal), gp44 (minor capsid), gp46 (scaffold), and gp47 (major capsid). Deleting gp42, gp46, or gp47 blocks maturation of the virion. However, deleting gp44 leads to a fully packaged virion that is unable to infect. The function of this protein is still unknown. SaPIs are able to overcome this defect as well in an unknown manner. Understanding the role of gp44 and the ability of SaPIs to infect without gp44 will aid in future studies. This defect does not appear to be related to DNA ejection, as previously thought, as shown by *in vitro* DNA ejection by NaI, heat, or incubation with lysed *S. aureus* remnants. This suggests that gp44 plays a different role in infectivity, possibly as an ejection protein that is seen in other phages. Co-purification of gp44 with portal and scaffold proteins in *Escherichia coli* suggests a relationship between gp44 and scaffold protein. Both proteins exist in the procapsid of 80α, however the scaffold protein exits before maturation while gp44 remains. This signifies that perhaps this interaction is transient and necessary for gp44 incorporation into the phage.

Abstract

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Cytomegalovirus (CMV) is the most common viral infection transmitted from pregnant women to their developing fetuses with an incidence approaching 5/1000 live births. It is a significant cause of abnormal brain development and long-term neurological damage including mental retardation, cerebral palsy, and hearing loss. To understand in the pathogenesis of CMV infection within the CNS, we have developed a mouse model showing features of congenital HCMV infection including focal encephalitis, hypoplasia, and **delayed proliferation of granular neuron precursor cell (GNPC) in mice cerebellum**.

Sonic hedgehog (SHH) is a secreted glycoprotein that binds to its receptor and activates downstream intracellular signaling transduction allowing transcription factors, Glioma-associated oncogene family and N-myc, to traffic into the nucleus further regulating the transcription of SHH-responsive genes. N-myc is a proto-oncogene that is known to act downstream of SHH signaling and regulate genes that induce GNPC proliferation *in vivo* and *in vitro*. Previously, our lab has shown that N-myc transcription level increases during MCMV infection in mice cerebellum. However, there is no evidence that N-myc mediated SHH signaling pathway modulates the outcome of MCMV-induced cerebellar deficits. Thus, we hypothesize that **the alterations of N-myc-mediated SHH signaling pathway during MCMV infection in mice contribute to cerebellar deficit affecting GNPC proliferation during cerebellar development.** Our finding suggests that MCMV infection increases SHH and N-myc protein expression levels as well as alterations in N-myc phosphorylation status in mice cerebellum. This raises further questions such as which phosphorylation sites are affected during MCMV infection and whether this is accounted for the delayed GNPC proliferation during cerebellar development.

Novel HIV-1 cryptic epitopes capable of inducing viral escape mutations.

Binghao J. Peng

Mentor: Dr. Paul Goepfert

Conventional HIV-1 proteins are thought to be derived from open reading frames (ORF). In addition to ORFs, HIV has the potential to transcribe and translate from five additional reading frames termed ARFs or alternate reading frames. Transcription and translation from ARFs has been shown to occur commonly during HIV, SIV, and other viral infections. As a result of ARF usage, antigenic peptides termed cryptic epitopes (CE) are generated, becoming a potential source of HLA presented peptides. It is still unknown whether CE induced immune responses can significantly impact viral fitness at a population level. We therefore hypothesize that CE are able to induce potent effector responses capable of driving HIV escape mutations. To test this hypothesis, peptides were generated from a predicted list of cryptic epitopes derived from acute stage infection sequences of a cohort of Zambian linked transmission pairs. These peptides were then used to screen for T-cell responses in cryopreserved PBMC samples of chronically infected patients.

IFNγ ELISpot assay revealed HIV-1 CE specific responses at the peptide pool level in 8/32 (25%) individuals tested. Such responses were not seen in the 16 HIV seronegative donors. Interestingly, all 10 immunogenic CEs used in this study were mapped specifically to reverse frame 3 of HIV-1 *pol*. These CEs were capable of eliciting both CD8 and CD4 responses, as indicated by both CD8 depletion assay, and flow cytometric analysis. Overall, our data shows CEs can be targeted by T-cells despite their infrequent expression, creating new revenue for HIV vaccine development.

Selection and Receptor Editing in Developing Peripheral B Cells

Jessica Peel, James S. New, Rodney G. King, and John Kearney

Mechanisms underlying peripheral B cell tolerance that regulate the maturation of transitional B cell subsets remain unclear. As it has been established that IgH transgenic B cells are at competitive disadvantage relative to their endogenous counterparts, IgH transgenic B cell systems offer a unique model to further examine peripheral B cell selection. We chose to study the effects of forced expression of an auto reactive BCR on clonal representation using the C57BL/6.HGAC39 mouse, which express a B cell receptor heavy chain transgenic derived from a GlcNAc-specific hybridoma. We report here, that despite successful utilization of IgH transgenes in bone marrow B cell precursors of C57BL/6.HGAC39 mice, IgH transgenic B cells and antibodies are inefficiently represented in peripheral B cell compartments and the circulating antibody pool, respectively. In the spleens of adult C57BL/6.HGAC39 mice we observe a preponderance of B cells not detected by IgH transgene-allotype- and idiotype-specific reagents, suggesting the potential reversion to endogenous Ig-alleles. Furthermore, we observe an accumulation of $CD19^{+}IgM^{lo/-}$ B cells in the spleens of these mice, suggesting a role for negative selective pressures on the acquisition of anergic phenotypes in GlcNAc-specific Tg B cells. We propose that receptor editing mechanisms occurring during progression through transitional B cell stages are responsible for the loss of IgH transgene-encoded antibodies, and that these processes may be central in controlling the representation of various GlcNAc-specific B cell clonotypes, of differing fine-specificities for autologous antigen, in the mature B cell repertoire.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF THE AMINO ACIDS INSERTED AT CFTR PREMATURE STOP CODONS DURING NONSENSE SUPPRESSION IN MAMMALIAN CELLS

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Premature termination codons (PTCs) account for ~11% of all mutations that cause human genetic diseases. Some compounds can suppress PTC and allow synthesis of full-length protein. Little is know about the identity of the amino acids inserted during PTC suppression in mammalian cells. In addition, whether the full-length protein obtained through nonsense suppression is functional despite insertion of near cognate amino acids remains unclear.

In this study, we utilized a CFTR as a disease model to investigate the two questions above. We designed a system that allowed us to purify full-length proteins induced by PTC suppression and identify the amino acid(s) inserted using tandem mass spectrometry (MS-MS). We found context around PTC affected the species and frequencies of amino acids inserted at PTCs. Then we built CFTR constructs containing each of the amino acid variants to study the functional consequences. Many CFTR variants showed reduced cAMP-stimulated chloride channel activity, which correlated with reduced CFTR maturation. Moreover, we found that both the CFTR corrector lumacaftor (VX-809) and the potentiator ivacaftor (VX-770) enhanced CFTR activity in many of these variants. In addition, the combination of nonsense suppression drug G418, corrector and potentiator showed synergistic increases in CFTR function for both the *CFTR-G542X* and *CFTR-W1282X* nonsense alleles.

Our results suggest that nonsense suppression frequently results in the restoration of partial CFTR function that can be further augmented by corrector-potentiator therapy. We conclude that combination therapy with multiple CFTR modulators may be beneficial for the treatment of CF patients with nonsense mutations.

TITLE: Sequence diversity of the proline-rich domain of PspA of *Streptococcus pneumoniae.*

AUTHORS: Jacob Switzer, Reshmi Mukerji, Kristopher Genschmer, David E. Briles

ABSTRACT:
Pneumococcal surface protein A (PspA) is a virulence factor of *Streptococcus pneumoniae*. The α-helical domain (αHD) of PspA elicits antibody that can protect mice from invasive infection. PspA exhibits sequence diversity, which has traditionally been classified based on the amino acid sequence of 1/3rd its αHD, the clade-defining region (CDR). The CDR can partially predict cross-protection by the αHD. The proline-rich domain (PRD) of PspA also elicits protection against pneumococci. The PRD, like the αHD, is variable in sequence but protective epitopes in the PRD are linear sequences, whereas those of the αHD are highly conformational. Our goal is to map the protection-eliciting epitopes of diverse PRD and construct a PRD analog that can elicit protection against the diverse PspAs of all pneumococci. We have analyzed 126 unique PspA sequences out of 221 gene sequence gathered from the BLAST server. The PRD sequences were aligned and phylogenetic trees generated using the unweighted pair group method with arithmetic mean (UPGMA) on Geneious version 7.1.7. There were three distinct allelic "groups". PspAs of each PRD group covered the range of diversity of families and clades as determined by the CDR. Thus, the diversity of PRD groups is largely independent of αHD diversity. This work paves the way for further studies on cross-protection between the different PRD groups. An ideal vaccine could elicit protective antibody against all αHDs and all PRDs, thus redundantly covering all PspAs and offering the best chance of producing a vaccine relatively protected from anti-PspA mediated selection.

Glycosylation Signatures on HIV-1 Envelope Glycoprotein

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HIV-1 gp120 is a subunit of the envelope glycoprotein (Env) spikes on the HIV virion. Env glycans play a key role in virus entry into host cells and as a shield against neutralizing antibodies. gp120 glycosylation is difficult to characterize due to the mutability of HIV-1 and heterogeneity of glycans. To elucidate the extent of glycosylation at each *N*-glycosylation site (NGS) of gp120 variants, we applied our high resolution mass spectrometry workflow that utilizes MS and MS/MS (CID & ETD fragmentation methods) to identify *N*-glycopeptides. By determining the relative abundance of each glycoform for each NGS and comparing them across gp120 variants and mutants, we are able to observe glycosylation pattern signatures. Furthermore, we can modify these signatures by altering NGS and perturbing the *N*-glycosylation pathways with a mannosidase inhibitor (DMJM). Our data led us to begin looking at how glycan signatures at specific NGS affect virus infectivity and CD4 binding. Using a library of known HIV-1 neutralizing antibodies, we were able to corroborate our observed changes in site-specific heterogeneity with biological data that allow us to make novel conclusions regarding the role of shifting *N*-glycan heterogeneity in the overall structure of the gp120 trimer that will have broader impact on the goal of designing vaccines for HIV. We demonstrate a process of interpreting the dense amount of *N*-glycan heterogeneity data and show how the heterogeneity changes under different conditions in the context of the protein's structure and binding.

Identifying the Structure of Human INF-α6

Rhea Derke, Mark R. Walter

INF-α is a family of interferons that is represented by thirteen homologous proteins in humans. Each INF-α has been found to exhibit unique activity profiles, which are related to the innate immune system and are key factors in protection mechanisms against viral infections. Although the direct mechanisms of action of each type of INF-α have yet to be established, the therapeutic possibilities of α-interferons are expected to be wide-reaching. This illustrates the importance of identifying the structure of these proteins, which has been historically difficult via x-ray crystallography. To produce a form of INF-α6 that is readily crystallized, a fusion protein was designed to include T4 Lysozyme (T4L) at the c-terminal end of INF-α6. To date, large-scale expression in *Escherichia coli* of the fusion protein has been accomplished, which resulted in an insoluble product. This product was extracted from inclusion bodies and subsequently refolded. After successful purification via a N-terminal affinity tag, the correct conformation of the INF-α6 portion of the fusion protein was validated through specific binding with a INFAR1-R2-Fc complex. Current efforts aim to optimize purification in order to yield concentrations appropriate for crystallization. The eventual goal is to identify the structure of INF-α6 and the interaction with its receptor.

Submission for Annual Microbiology Retreat, August 2016.
Topic: B Cell Development
Title: Effects of Neonatal Antigen Exposure on Natural Antibody Repertoire Development
Authors: J. Stewart New, R. Glen King, and John F. Kearney
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Abstract:

Antibodies to N-acetyl-D-glucosamine are universally represented in mammals, and play important immunomodulatory roles in the contexts of autoimmunity and allergic airway disease. These dually protective functions are the result of the conservation of GlcNAc epitopes between mammals and microorganisms. Within mammalian systems, GlcNAc motifs are the product of aberrantly truncated post-translational glycan modifications, while exogenously GlcNAc is commonly associated with cell wall antigens of microorganisms including components of the normal microflora. How the integration of endogenous and exogenous GlcNAcylated antigens influences clonal development of the GlcNAc-specific B cell pool, however, is poorly understood. Neonatal immunization with GlcNAc-bearing Group A Streptococcus (GAS) significantly expands the pool of GlcNAc-specific B cells in the peritoneal cavities and spleens of mice relative to non-immunized controls. Intriguingly, neonatal immunization also shifts the dominant GlcNAc-specific B cell phenotype from that of a B-1b cell in naïve mice, to a $CD5^+$ B-1a phenotype; correlating with increased representation of a GlcNAc-specific B cell clonotype expressing IGVH7-3 immunoglobulin heavy chains. This clonotype contributes significantly to secondary responses in adulthood, in contrast to immunization of adult animals, which preferentially expands IGHV 6-3 clones. In gnotobiotic mice we observe log-reductions in the number of GlcNAc-specific B cells as well as significantly altered clonal frequencies compared to SPF animals. Collectively, these data demonstrate the formation of an efficacious natural antibody repertoire results from a complex interplay of signals derived from endogenously- and exogenously-derived antigens. Given the protective properties of neonatally elicited GlcNAc-specific in autoimmune and allergic disease contexts that we have previously reported, these observations have major implications for the development of B cell clonotype-specific immunotherapies.

Foxp1 controls naive CD8$^+$ T cell quiescence by simultaneously repressing cellular metabolism and cell cycle progression

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Abstract

Previously we have shown that transcription factor Foxp1 plays an essential role in maintaining naïve T cell quiescence; in the absence of Foxp1, mature naïve CD8$^+$ T cells proliferate directly to homeostatic cytokine IL-7. Here we report that the deletion of Foxp1 in naïve CD8$^+$ T cells led to enhanced activation of PI3K/Akt/mTOR signaling pathway and its downstream cell growth and metabolism targets in response to IL-7. In addition, we found that the deletion of Foxp1 in naïve CD8$^+$ T cells resulted in increased expression levels of *E2f1*, *E2f2* and *E2f3*, the critical components for cell cycle progression and cell proliferation, in a retinoblastoma protein (Rb) phosphorylation-independent manner. Taken together, our studies suggest that Foxp1 enforces naïve CD8$^+$ T cell quiescence by simultaneously repressing both cellular metabolism and cell cycle progression.

Hepatocyte-like Cells Derived from Human Embryonic Stem Cells as a Robust Model for Hepatitis C Virus Infection

Junfen Fan, Huahao Fan, Ali Zamani, Kyung-Don Kang, and Guangxiang (George) Luo

Hepatitis C virus (HCV) chronically infects 170 million people worldwide, resulting in chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Research into the biology and intervention of HCV requires an in vitro cell-culture system that supports the infection of human hepatocytes. Primary human hepatocytes and the widely used hepatoma cell line Huh7 and its derivatives have some limitations because of poor accessibility or insusceptibility to clinical HCV isolates. Hepatocyte-like cells differentiated from pluripotent stem cells provide an attractive alternative as they not only overcome these shortcomings but can also provide an unlimited source of non-cancer cells for both research and cell therapy. In this study we used the chemically defined media under stepwise fashion to differentiate human embryonic stem cells (hESCs) into hepatocyte-like cells with high efficiency and reproducibility. What's more, the differentiated human hepatocyte-like cells (DHHs) can support the entire life cycle of HCV and the DHHs can express known HCV receptors, including CD81, SR-BI, claudin-1 and occludin. We also found that DMSO can catalyze hepatic differentiation and the Janus kinase (JAK) inhibitor (JAKi) can enhance HCV replication. Our study establishes a new noncancerous and renewable cell-culture system for HCV infection, thus lay the foundation to explore new host factors for HCV infection and to study the pathogenesis of HCV- related diseases.

Polyomavirus T antigens functionally upregulates APOBEC3B

Brandy Verhalen, Gabriel Starrett, Reuben Harris, Mengxi Jiang

Polyomaviruses (PyVs) are small DNA viruses that establish a latent infection in healthy individuals. These viruses can reactivate, primarily in immunocompromised patients, by unknown mechanisms and lead to severe diseases. While only Merkel cell carcinoma has been directly linked to a polyomavirus (Merkel cell polyomavirus, MCPyV), BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV) have also been correlated to various cancers under immunocompromised settings. The apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3) family of cytosine deaminases are important host restriction factors for viruses including both HIV and human papillomavirus. Recent studies have also shown that APOBEC3B (A3B), in particular, is a major source of genomic uracil mutagenesis in multiple cancers and is upregulated during HPV infection. Here, we pose the question as to the effects of polyomavirus infection on APOBEC3 expression. By investigating the mRNA and protein levels of BKPyV infected renal proximal tubule epithelial cells, we have identified that APOBEC3B (A3B) and no other APOBEC3 family member is upregulated during BKPyV infection. The increase of A3B expression yields an increase in deaminase activity. Through ectopic expression of BKPyV, MCPyV and JCPyV T antigens, we have identified that the T antigen protein alone from all three polyomaviruses is sufficient to upregulate A3B protein levels. Future studies will aim to identify the role of A3B in host restriction of BKPyV infection as well as possible mutagenic effects of upregulated A3B on host genome.

Ferritin H chain is a key determinant in tuberculosis disease progression and susceptibility

Vineel P Reddy, Vikram Saini, Travis D Hull, Krishna C Chinta, Subhashini Bolisetty, Joel N Glasgow, James F George, Anupam Agarwal, Adrie JC Steyn.

Iron is an essential factor in host regulatory mechanisms and for the growth and virulence of *Mycobacterium tuberculosis* (*Mtb)*. Ferritin heavy (H) chain is a major host iron storage protein that reduces free iron levels following *Mtb* infection. We hypothesized that perturbations in host iron storage mechanisms alter the outcome of *Mtb* infection. To test our hypothesis, we used knockout mice lacking ferritin H chain expression (FtH LysM$^{-/-}$) in myeloid-derived cell populations to study *Mtb* disease progression. Following *Mtb* infection, we observed significantly increased bacillary burden in the lungs and spleens of FtH LysM$^{-/-}$ mice, dissemination of *Mtb* into the brain and eyes and significantly reduced survival compared to FtH$^{+/+}$ mice. Iron levels in the lungs and spleens of FtH LysM$^{-/-}$ mice were significantly decreased compared to FtH$^{+/+}$ mice, which correlated with increased expression of ferroportin, an iron exporter. Compared to FtH$^{+/+}$ mice, FtH LysM$^{-/-}$ mice had higher levels of iron in the feces as well as decreased ferroportin levels in the duodenum, suggesting that upon *Mtb* infection FtH LysM$^{-/-}$ mice are compromised in iron uptake. Compared to FtH$^{+/+}$ mice, a stronger Th-1 response was generated in FtH LysM$^{-/-}$ mice upon *Mtb* infection. In addition, we observed significantly increased numbers of neutrophils and monocytes in FtH LysM$^{-/-}$ mice compared to FtH$^{+/+}$ mice. We conclude that ferritin H chain is required for resistance to *M. tuberculosis* infection in a mouse model of tuberculosis.

Syndecan Proteoglycans as Attachment Receptors for Hepatitis C Virus and Dengue Virus infection

Huahao Fan, Junfen Fan, and George Luo

Heparan sulfate (HS) is known to covalently attach to core proteins to form heparan sulfate proteoglycans (HSPGs) on the cell surface. HSPGs have been shown to bind to a wide variety of microbial pathogens, especially viruses. The HSPG core proteins include the membrane-spanning syndecans (SDCs), the lycosylphosphatidylinositol-linked glypicans (GPCs), the basement membrane proteoglycan perlecan (HSPG2), and agrin. Our previous studies demonstrated that SDC1 and SDC2 are co-receptors for HCV infection by using small interfering RNA (siRNA). However, another group claims that SDC4 is important for HCV attachment to the cell surface. Recently we have constructed SDC1 and SDC2 knockout Huh7.5 cell lines using CRISPR/Cas9 system. Our studies found that HCV attachment to Huh-7.5 cells is significantly reduced in the SDC1 and SDC2 knockout Huh7.5 cells. Also, SDC2 knockout resulted in a remarkable decrease of Dengue virus (DENV) attachment to cells, suggesting that SDC2 serves as the major attachment receptor for DENV. Taken together, our results confirmed previous findings that SDC1 and SDC2 are HCV attachment receptors and provided new insights into HSPG functions during DENV entry.

Structural features of phage capsid size redirection and cell infection by *Staphylococcus aureus* pathogenicity islands

James L. Kizziah, Altaira Dearborn, Keith Manning, Terje Dokland

Staphylococcus aureus pathogenicity islands (SaPIs) are one medium for virulence factor transfer among *S. aureus* bacteria to create new strains detrimental to public health. SaPI genomes within the host cell chromosome are derepressed upon infection by a specific helper phage, leading to their excision, replication, and packaging into phage-like transducing particles using structural proteins encoded by the helper. Some SaPI-encoded proteins facilitate redirection of helper phage capsid assembly mechanisms to make capsids too small to accommodate the phage genome. Thus, SaPIs perceive host cell infection and hijack capsid assembly mechanisms of helper phages to escape infected cells. This project addresses several topics in the structural characterization of how SaPIs modify viral machines for their own purposes. First, electron microscopy and 3D reconstruction were used to generate models of the 80α phage baseplate, which is used by SaPI1 in recognizing and entering new bacteria. Second, NMR spectroscopy was used to investigate the interaction between SaPI1 capsid morphogenesis protein B (CpmB) and 80α capsid protein, which is critical for assembly of capsids suitable only to the SaPI genome. Third, we compared capsids made in the presence and absence of the minor capsid protein gp44, which is essential for phage viability but dispensable to the SaPI. Elucidation of these structural features of SaPI transduction are necessary for improved understanding of the spread of SaPI-contained virulence factors.

Elucidating the Mechanism by which Gag Polyprotein Interacts with Host Plasma Membrane

R. Elliot Murphy, Jiri Vlach, Jamil Saad

During the late phase of HIV-1 infection, the Gag polyproteins are trafficked to the host cell plasma membrane for assembly. Gag binding to the membrane is mediated by the matrix (MA) domain. Subsequent Gag oligomerization is thought to occur via the capsid (CA) domain which is directly adjacent to MA in the Gag polyprotein. To understand this process, we are employing NMR and cryoEM methods to characterize the interactions between MA/MACA and nanodiscs (a sophisticated membrane mimetic). In the future, we will employ a set of NMR and other biophysical techniques to determine the mechanism by which Gag binds to the host membrane.

Investigating the effects of BMH-21 on rRNA synthesis in *Saccharomyces cerevisiae*

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Ribosome biogenesis is a process that requires the activities of three nuclear polymerases, RNA Polymerases I, II, and III, to produce rRNA, mRNAs encoding ribosomal proteins, and tRNAs, respectively. The activity of RNA Polymerase I (Pol I) is considered rate limiting for ribosome production. Cancerous cells manipulate this role for Pol I by upregulating its activity to increase protein synthesis and allow for tumorigenesis.

Because of the linkage between rRNA synthesis and cell growth, there is interest in designing inhibitors that target Pol I activity as a potential cancer therapy; one of these inhibitors is BMH-21. Remarkably, a short treatment with BMH-21 resulted in degradation of RPA194 (the largest subunit of Pol I), reduction of rRNA synthesis, and a marked decrease in cell viability. These data suggest that a pathway exists to monitor integrity of rDNA transcription. To investigate this pathway, we aim to use *Saccharomyces cerevisiae* (yeast) as a model system. Initial results reproduce effects seen in a mammalian system in the yeast system, suggesting that if such a pathway exists, it is conserved in yeast. We will take advantage of the genetic tools available in yeast to identify the genes required for this novel checkpoint of rDNA transcription.

Isolation, Characterization of *Staphylococcus aureus* Cu^{II}gtsm resistant mutants to study the mechanism of action of copper complexes.

Jyoti Jyoti, F. Wolschendorf

Copper-dependent antimicrobials are effective against highly drug resistant bacteria and are being investigated as potential compounds to refuel the dwindling drug development pipeline. A well characterized example of a copper-dependent antibiotic is the bis(thiosemicarbazone) GTSM. Previously, we demonstrated that the copper complex of GTSM, Cu^{II}GTSM, is a potent inhibitor of methicillin-resistant *Staphylococcus aureus* (MRSA). A limited structure activity relationship study conducted on 3 related bis(thiosemicarbazone) copper complexes, linked antibacterial potency to redox potential thereby suggesting that reductive deterioration of the complex and the subsequent release of copper ions into the cytoplasm may be the source of its toxicity. Corroborating this finding, a *S. aureus* transposon mutant deficient of its major copper efflux pump, CopA, displays a 4-fold higher sensitivity to Cu^{II}GTSM than the wild type strains. To identify the potential molecular targets of Cu^{II}GTSM, or alternative genetic determinants of resistance, mutants were generated by the agar plate and liquid broth dilution method. Resistant colonies occurred with a frequency of 3.5×10^{-6} Cells/ml. Three mutants with varied resistance to Cu^{II}GTSM were isolated and further characterized. Primarily, we found that resistance is specific to the copper complex of GTSM as no increase in copper resistance was noted. Sequencing of several genomic copper resistance genes showed no genetic alterations suggesting that the resistance phenotype is independent of copper resistance. Based on our data we propose that these copper complexes may act by Copper overload or by targeting specific pathways like efflux pathways or by inducing oxidative stress.

Heme oxygenase-1 protects against pulmonary tuberculosis via anti-oxidative and anti-inflammatory activities

Krishna C. Chinta[1], Vikram Saini[1], Joel N. Glasgow[1], Gene P. Siegal[2], Amie Traylor[3], Shepherd Nhamoyebonde[4], Alasdair Leslie[4], Md. A. Rahman[4], Hollis Shen[4], Ryan M. Wells[4], Rajhmun Madansein[5], Gerard R. Alexander[5], Veena B. Antony[6], Jessy Deshane[6], Anupam Agarwal[3] and Adrie J.C. Steyn[1,4,7*]

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Tissue damage resulting from sustained inflammation causes permanent pulmonary disability in approximately half of surviving patients with tuberculosis (TB). Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that regulates redox homeostasis and inflammatory responses during lung diseases; however, the role of HO-1 in TB disease progression is unknown. Here we tested the hypothesis that HO-1 is essential for effective immune and oxidative stress control of localized *Mtb* infection in mice and in human tuberculous lungs. Flow cytometry analysis of human tuberculous lung tissue revealed that neutrophils are the primary source of HO-1 in *Mtb*-infected human lungs. Intriguingly, HO-1 levels were decreased in regions of severe tissue damage compared to uninvolved tissue. Further, ROI and RNI levels were increased in these severely damaged lung regions, suggesting that HO-1 protects the host from redox-mediated tissue damage. Transcriptomic analysis of *Mtb*-infected HO-$1^{+/+}$ and HO-$1^{-/-}$ mice revealed that pathways involved in neutrophil migration are significantly regulated by HO-1 during *Mtb* infection. In *Mtb*-infected HO-$1^{-/-}$ mice, IL-6, IL-8 and TGF-β levels were significantly higher as was the accumulation of granulocytic myeloid derived suppressor cells (G-MDSC) and neutrophils, which correlated with decreased T-cell activation and increased T-reg accumulation. Further, a lack of HO-1 lead to increased bacillary load and decreased survival in *Mtb*-infected HO-$1^{-/-}$ mice. These findings establish a novel role for HO-1 mediated host protection during TB and suggests HO-1 as potential therapeutic target to improve clinical outcomes of TB.

Investigating the role of CD38 in TLR2-mediated effector functions of macrophages

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The NAD glycohydrolase CD38 is widely expressed in many cells and tissues, and is a multifunctional ecto-enzyme capable of regulating innate immunity and inflammation. We previously showed that CD38 deficiency in mice (*Cd38*$^{-/-}$) attenuated early *in vivo* resistance to virulent *Listeria monocytogenes* (*Lm*) infection and resulted in increased morbidity and mortality compared to wild-type (WT) animals. Furthermore, bone marrow chimera experiments revealed that the increased susceptibility of *Cd38*$^{-/-}$ mice was due to defective myeloid lineage cells. Given the key role that myeloid lineage macrophages play in the innate immune response against *Lm* infection, we hypothesize that CD38 regulates macrophage effector function and defense against *Lm*. In the present study, we are investigating whether TLR2-dependent macrophage effector function requires CD38. Primary splenic, peritoneal and bone marrow-derived macrophages were harvested from WT and *Cd38*$^{-/-}$ mice, and stimulated *in vitro* with different concentrations of the Toll-like receptor 2 (TLR2) agonists Heat-Killed *Listeria monocytogenes* (HKLM) and Lipoteichoic acid (LTA). Cell culture supernatants were collected for the measurement of macrophage-secreted pro-inflammatory cytokines, including TNFα, IL-6, IL-12 and IFNγ, as well as IL-10, an inhibitor of macrophage effector function. The levels of reactive oxygen/nitrogen species (ROS/RNS) will be measured in the supernatants, and cell lysates will be analyzed by qRT-PCR for differences in the expression of TLR2-specific pro/anti-inflammatory and pro/anti-oxidant genes. These experiments are crucial for identifying the CD38-dependent pathway(s) that modulate macrophage effector function and provide early protective immunity to *Lm*.

2015 Microbiology Departmental Retreat Abstract Submission

The Localization of Outer Membrane Protein CpnT is dependent on a Type VII secretion system in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis has a highly specialized quintet of paralogous Type VII secretion systems, known as ESX, which are responsible for secreting proteins related to pathogenesis, metal homeostasis and cytotoxicity. While the complete protein architecture of ESX systems remains enigmatic, core components are conserved and a secretion motif of target proteins has been identified. Thus far, bona-fide Esx substrates have been limited to the PE, PPE and Esx protein families which are frequently encoded within the *esx* genetic loci. We have recently identified the outer membrane protein CpnT that possesses an N-terminal channel domain and a C-terminal toxin. In this study, we demonstrate that CpnT localization to the outer membrane is dependent upon an N-terminal Esx motif. Further, we show that this localization is relevant during infection as it abrogates the secretion of the toxin in macrophages. This is the first demonstration in any bacteria that an integral outer membrane protein is dependent on a type VII secretion system. Our finding may also help to explain the loss of membrane stability and pronounced virulence defect observed in select Esx system mutants.

Streptococcus pneumoniae forms biofilms within the heart.

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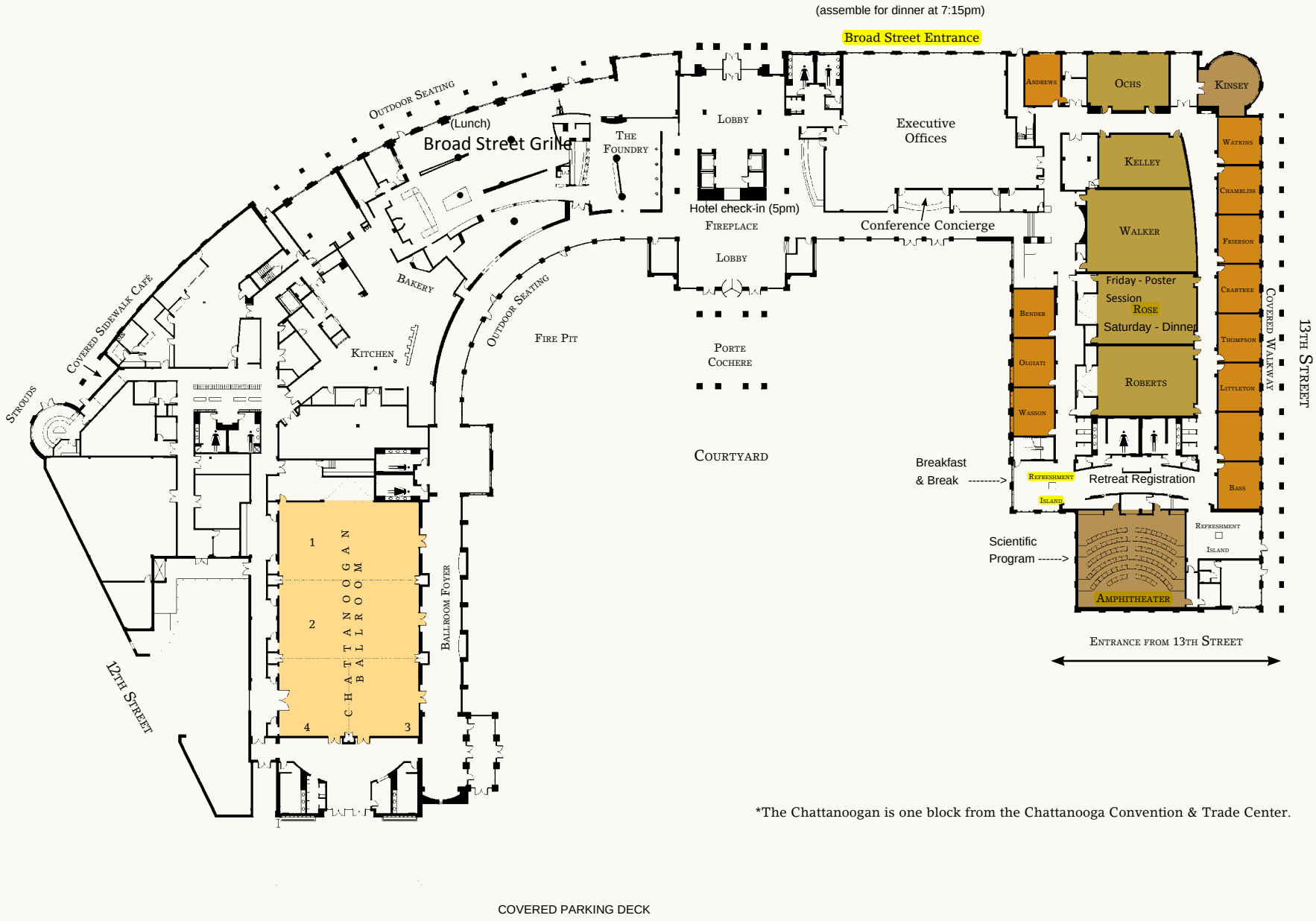
During nasopharyngeal colonization, *Streptococcus pneumoniae* (the pneumococcus), forms biofilms that are resistant to host immunity and environmental stressors. The pneumococcus also causes cardiac complications like arrhythmia and heart failure in elderly hospitalized with pneumococcal pneumonia in addition to opportunistic infections like community-acquired pneumonia, bacteremia, and meningitis. We previously reported that pneumococci invade the myocardium during invasive pneumococcal disease (IPD) forming bacteria-filled microlesions that disrupt cardiac functionality. Microscopic observations of the ventricular tissue showed increase in number and size of microlesions consistent with persistent bacteremia and opportunistic pneumococcal access to the heart. Fluorescent and electron microscopic analysis revealed equidistantly spaced non-encapsulated pneumococci with a striking absence of infiltrating immune cells within the microlesions; a finding that is suggestive of immunoquiescent biofilms as described in the nasopharynx. The latter was confirmed by the presence of extracellular matrix components poly-N-acetylglucosamine, host and bacterial eDNA within the microlesions. Furthermore, pneumococci isolated from the myocardium were more resistant to antibiotics and exhibited heightened priming for subsequent cardiac invasion when compared to circulating pneumococci. Comparative gene expression profiling of *in vitro* biofilms and planktonic pneumococci using RNA-Seq identified numerous differentially expressed genes including previously reported virulence determinants required for pathogenicity *in vivo*. Ongoing studies are examining the requirement for these virulence determinants during cardiac microlesion formation in experimentally challenged mice. These novel findings may provide a deeper insight into the host-pathogen interactions *in vivo*, identify strategies to prevent pneumococcal colonization of the myocardium and may also allow development of treatment strategies for IPD- associated cardiac complications.



*The Chattanoogan is one block from the Chattanooga Convention & Trade Center.

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