

Collaboration Roundtable

February 26, 2010

Poster Abstracts

1. Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG): Maximizing the Comparability and Value of Safety Data from HIV Vaccine Trials
Presenter: Robert Chen, CDC

Background: Various viral vectors are being explored for use in vaccines against diseases such as HIV, TB, and malaria. Although unpredicted, the “STEP” trial results highlight the importance of safety assessments of candidate vaccines. Safety parameters generally can not be measured directly, but require indirect inference from the frequency of multiple adverse events (AEs). We seek harmonization of AE assessments to maximize their comparability and value across trials of multiple vaccine candidates.

Methods: The Brighton Collaboration (BC) was created to develop high quality information about vaccine safety; case definitions on 24 AEs have been published. The BC formed the Viral Vector Vaccines Safety Working Group (V3SWG) in October, 2008. The BC secretariat organizes and supports monthly conference calls.

Results: To date, the V3SWG has 1) recruited ~30 volunteers from stakeholders representing academia, industry, and government for balance between virology and safety expertise, 2) agreed on a standard template developed with IAVI for collection of data on each vector and risk assessment framework, and 3) developed a workplan to harmonize assessment of the following issues: a) potential for vector recombination with wild type pathogenic strains, b) implications of prior infections on safety, c) genetic stability of replicating vaccine viruses in vivo, d) potential changes of vaccine viral tropism, e) tests for absence of reversion to virulence, f) absence of replication-competent virus when replication incompetent vectors are used, g) vaccine effects on innate immunity and on the induction of an immuno-suppressive window or immune-activation, h) length of time for monitoring AEs, i) inclusion of adventitious agents in cell culture, and j) possible secondary transmission of vaccine virus.

Conclusion: The BC has successfully launched the V3SWG as its first entrée into harmonization of pre-licensure safety assessments. The guidelines in development should improve our ability to prioritize vector selection and interpret safety data of viral vector vaccines.

2. Longitudinal Study of HPV16 Variants in Anal and Cervical Samples
Presenter: Martin Steinau, CDC

Background: We investigated HPV16 variants in synchronous samples from anus and cervix, as well as longitudinal samples from anus or cervix, derived from a cohort study of HIV-infected patients. Methods: We used a pyrosequencing method to determine HPV16 variants in stored extracts of all cervical and anal samples in which HPV16 was detected by the Roche linear array. Seven

polymorphic nucleotide positions within the E6 region were analyzed to determine genotype variants in 179 anal and 43 cervical swabs from 91 different subjects.

Results: Of the 222 samples sequenced, the European variants E-G350 (35%) and Ep (32%) were most frequently detected followed by Af2 (9.9%), Af1 (9%), AA (2.3%) and As (0.9%). Fourteen samples (6.3%) contained possibly undescribed HPV16 variants and 10 (4.5%) showed multiple variants. Data from both anal and cervical specimens collected on the same date were compared in 29 instances from 25 different women. Synchronous differences were found in 10 (34.5%) instances from 9 (31.0%) women. Data from samples collected from the same anatomical site on 2 or more visits at least 6 months apart were compared in 66 instances (59 anal, 9 cervical) from 59 subjects. Longitudinal changes in the variant types detected were seen in 12 (18.6%) instances from 11 (16.7%) subjects, of which 10 were anal and 2 cervical series.

Conclusions: Variant analysis indicated multiple HPV16 infections in HIV-positive subjects, both in synchronous anal and cervical samples, as well as in longitudinal samples from each anatomic site. The incidence rate of HPV16 infections is likely an underestimate since reinfections with the same variant seem likely and would not be detected by variant analysis. It remains to be determined how reflective these data are of the general population, however intra-variant changes should be considered in HPV investigations, especially if the focus is on persistence.

3. (No abstract)
4. Development and Production of Protein Reagents for Infectious Disease Research and Diagnostics
Presenter: Jason M. Goldstein, CDC

The Biologics Branch within the Division of Scientific Resources provides expertise devoted to Quality System Essentials (QSE) in development, production, and distribution services. In addition to a large variety of products (cell lines, commercial/custom buffers and growth media), the Branch provides services and products customized to infectious organisms and agents. These include biologics inventory management and distribution services, pilot-scale reagent and in-vitro diagnostic device production, and development & manufacturing of novel protein reagents and diagnostic assays. We coordinate projects between our Biosafety Laboratory (BSL-3), Antibody Development Facilities and Laboratories for Gene Expression and Protein Science. Utilizing QSE for these activities ensures biological reagents and assays that are effective and reproducible to our collaborators.

5. Research Interests and Capabilities in the Vaccinology Laboratories (Immunology) George Carlone, Sandra Steiner, Jacquelyn Sampson, Gowrisankar Rajam, Cheryl Elie, and Edwin Ades
Presenter: George Carlone, CDC

The Immunology Laboratories, (1) Integrates laboratory and epidemiologic approaches to enhance the diagnosis and surveillance of agents causing respiratory, meningococcal and other priority bacterial infections, (2) Develops, evaluates, implements, and improves serologic, immunologic and molecular biologic methods, techniques and strategies, (3) Develops and evaluates vaccines and vaccine candidates that protect against priority bacterial diseases, (4) Conducts, participates, and collaborate in vaccine clinical trials, (5) Develops, standardizes and validates correlates of protection and applies to the evaluation of new and developed priority bacterial agents, (6) Develops new diagnostic reagents and methods including the development, maintenance and evaluation of hybridomas, (7) Develops and evaluates immunizing agents and studies the role of immunologic

mechanisms in disease processes, (8) Operates and maintains a research laboratory under a Quality Management System in support of vaccine clinical trials, (9) Maintains the World Health Organization Collaborating Center for Research and Reagents for Human Immunoglobulin Subclasses, and (10) Supports global access to vaccines by providing reference materials, standardized protocols training, and consultative support to other government agencies, international collaborators including domestic and international reference laboratories, vaccine manufactures, academic institutions, The World Health Organization, and the Pan American Health Organization.

6. Public Health Administrator's Survival Kit

Presenter: Pei-Chun Tsai Wan

As a primer public health agency leader, the Centers for Disease Control and Prevention's Global AIDS Program (GAP) has been supporting multi-faceted approaches to combating the international HIV/AIDS pandemic since the 1990s. With GAP's highly-trained specialists working at headquarters in more than 25 field and 3 regional offices totaling over 1,670 staff, assist national strategies for sustainable, integrated HIV/AIDS prevention, care, and treatment programs. With GAP's daunting mission and its phenomenal growth, we described how one of it's internal Departments, the Epidemiology and Strategic Information Branch's 4 programs (surveillance, monitoring and evaluation, statistics, and health management information systems), with a budget of over \$14,647,121 and a staff of 60 tackle administrative challenges. By utilizing Situational Analysis Modeling, a cutting edge operational plan was formulated to address workforce innovation, global communication, financial management, and quality improvement.

Pre-existing tools were utilized such as e-portals (e.g. pepfar.net) and e-mail management (e.g. Outlook) to organize and streamline a multitude of information types including documents, emails, briefings, directories, partnerships, calendars, podcasts, discussion forums, and training. Moreover, we standardized the usage of international mobile telecommunication carriers, provided quarterly operational updates, created staff handbooks, and developed a hiring database which has lead to more effective branch cohesiveness, and robust collaborations with external partners. To combat a myriad of fiscal legislative mandates, rigorous financial tracking mechanisms were developed using excel spreadsheets to consolidate internal funding streams and to meet cost allocation ceilings. Periodic updating and distribution of these spreadsheets allowed a more transparent budgetary environment for the decision makers to exercise sound financial stewardship.

In conclusion, innovative use of these existing administrative tools have resulted in more sustainable operational efficiencies and provided an open environment for organizational excellence in leadership, communication and accountability. These tools can easily be incorporated synergistically from local to global public health arenas.

7. Early treatment with anti-RSV G Glycoprotein Monoclonal Antibody in the murine model of RSV infection

Presenter: Hayat Caidi, CDC

Respiratory Syncytial virus (RSV) is the most important cause of viral lower respiratory tract illness (LRI) in infants and children worldwide and the elderly and is a continuing challenge for vaccine development. The RSV G glycoprotein (RSV G protein) is of particular interest as it contains a CX3C motif in the central region (a.a. 182 to186) and has the ability to functionally mimic the CX3C motif

chemokine, fractalkine. The CX3C motif in the RSV G protein, through its ability to bind to the CX3C chemokine receptor, CX3CR1, is likely to play a role in the RSV G protein associated enhanced inflammatory response seen with RSV infection. In vitro studies have shown that an anti-RSV G protein monoclonal antibody, mAb 131-2G, which reacts with a.a.1 to 173 in both group A and group B proteins, can inhibit RSV G protein binding to CX3CR1 and RSV G protein induced chemotaxis. In a murine model of RSV infection, we recently found that early treatment with mAb 131-2G reduced several immune parameters associated with pulmonary disease and increased virus clearance. We hypothesized that another anti-RSV G monoclonal antibody that reacts proximal to the central conserved region but likely closer to the CX3C chemokine motif would also block the RSV G CX3-CX3CR1 interaction G protein induced immunomodulation. In this study, we evaluate the ability of a monoclonal antibody directed against a.a.174-215 of group A RSV strains, mAb 130-6D, and inhibits 131-2g binding for its ability to block in vivo and in vitro RSV G protein activities. We show that this anti-RSV G mAb inhibits G protein CX3C-mediated leukocyte migration. When used to treat BALB/c mice challenged with RSV, mAb 130-6D can, also, effectively reduce infection associated inflammation e.g. total bronchoalveolar lavage (BAL) cell infiltration, weight loss, interferon gamma (IFN- γ) production, lung virus titers and viral gene expression. The results from this study suggest that anti-RSV G monoclonal antibodies that bind several different regions but block CX3C-CX3CR1 related functions could be considered as potential therapeutic options for RSV disease.

8. Influenza Reagent Resource: Quality Source for Influenza Viruses and Reagents

Presenter: Joseph Miller, CDC

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The Influenza Reagent Resource (IRR) is the leading source for current high quality influenza viruses and reagents, funded by the Centers for Disease Control and Prevention and managed by ATCC[®]. The goal of the IRR is to acquire, generate, characterize, store and distribute reagents to facilitate influenza diagnostic development, drug development, and research. This resource also serves pandemic preparedness and the detection and control of seasonal influenza. This organization contains influenza viruses and reagents, including virus panels, nucleic acids, live and inactivated viruses, and antibodies. Eligible registrants include principal investigators, laboratory directors, scientists or equivalent in public, private, academic, non-profit, or for profit institutions. Our catalog gives you access to thousands of products at no cost for non-profit institutions and at cost for commercial companies. After orders are complete, most products will ship within 24-48 hours and arrive within seven business days of the processing date. Ordering is easy through our web portal www.influzareagentresource.org

9. Mismatch Amplification Mutation Assays for Genotyping *Rickettsia typhi*

Presenter: Cecilia Kato, CDC

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Background Murine typhus, caused by *Rickettsia typhi* (Rt), has a low mortality rate but may result in meningoencephalitis and interstitial pneumonia in severe cases. It manifests with many clinical symptoms like those caused by the category B bioterrorism agent *R. prowazekii*. Although these 2 agents are nearest neighbors, they are easily differentiated genetically. Genetic diversity within Rt seems limited. Mismatch amplification mutation assays (MAMA) were developed to define the genetic diversity of Rt isolates that have not been discriminated easily by other methods.

Methods Single nucleotide polymorphism (SNP) sites were identified by whole genome sequence comparisons of 3 Rt isolates. Genome sequences of Rt Wilmington (USA), B9991 (Burma), and TH1527 (Thailand) were aligned using MAVID. MAMA primers were designed for 26 SNP sites. DNAs from these 3 reference isolates were used to evaluate MAMA assays. Assays generating bands of expected sizes were tested with 15 other isolates of Rt. Genetic relationships were determined for concatenated SNPs using MEGA4.

Results MAMA assays were tested for 23 of 26 SNP sites. Ten assay sets were evaluated with all 18 Rt isolates. Genetic analysis identified 5 clades. The 3 reference isolates formed individual clades (single isolate representation for TH1527 and Wilmington; and B9991 shared a third clade with B10056). Two other Burmese isolates have 1 SNP in common with B9991 and B10056, and the largest clade included 12 isolates from the USA, Ethiopia, Pakistan, Russia, Thailand, and S. Africa. Conclusions Wilmington isolate had the greatest divergence (5 of 10 unique SNPs) which may possibly be due to the high number of yolk sac passages of this isolate. 12 isolates were identical and had the same alleles as the most prevalent type, indicating a very high level of homogeneity among low passage Rt isolates which have probably disseminated worldwide through human activities.

10. Insertion/Deletion (INDEL) Typing of Isolates of *Rickettsia rickettsii*

Presenter: Cecilia Kato, CDC

C. Y. Kato*, L. K. Robinson, F. H. White, K. Slater, S. E. Karpathy, M. E. Eremeeva, G. A. Dasch

Rickettsia rickettsii, the etiological agent of Rocky Mountain spotted fever, the most fulminant rickettsiosis, is a category B biothreat organism. Molecular typing for isolate discrimination has been accomplished by sequencing intergenic regions and characterizing variable tandem repeats by gel analysis. Both methods detected genetic markers that associated most genotypes of *R. rickettsii* jointly with geographic (Central/South America, Arizona, Montana, east of Rocky Mountains) and tick hosts (*Amblyomma*, *Rhipicephalus*, *Dermacentor variabilis* and *D. andersonii*). In order to determine if more robust differentiation of *R. rickettsii* could be achieved for use in forensics and molecular epidemiology, we evaluated 42 isolates of *R. rickettsii* and *Rickettsia* 364D for presence of

insertion/deletion (INDEL) events that differentiate the complete genome sequences of Sheila Smith and Iowa. Conserved flanking primers for amplifying 67 INDEL sites of sizes greater than 3 bp were designed and the amplicon sizes evaluated by gel electrophoresis. The INDEL sites analyzed were distributed throughout the chromosome, 25 in open reading frames and 37 in intergenic regions. Polymorphic (more than 2 types) and unexpected size bands were characterized by DNA sequencing. 62 primer sets were useful and permitted. 8 clades to be differentiated among the *R. rickettsii* isolates. Eight polymorphic INDEL sites were detected and three were highly informative. Most of the INDELS found in the attenuated Iowa vaccine strain were also found in isolates from fatal cases of RMSF from west of the Mississippi and to a lesser degree in all isolates where *D. variabilis* is the primary vector. INDEL typing confirmed that Arizona isolates associated with *Rh. sanguineus* are unique, the marked differentiation of isolates 364D and Hlp#2, and distinguished Brazilian from Central American and Colombian isolates. 364D is best regarded as a new species, *R. philipii*, while Hlp#2 is a subspecies of *R. rickettsii*.

11. Presence of *Coxiella burnetii* DNA in the environment of the United States (2006-2008)

Presenter: Gilbert Kersch, CDC

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Coxiella burnetii is an obligate intracellular bacterium that causes the human zoonotic disease Q fever. Q fever is acquired by the inhalation of aerosols containing as few as 1-10 bacteria, and most patients recover after experiencing a flu-like illness. Because *C. burnetii* is so highly infectious, can survive under a variety of environmental conditions, and has been weaponized in the past, it is classified as a select agent and is considered a potential bioweapon. The agent is present in domestic livestock and in wild animal populations, but the background levels of *C. burnetii* in the environment have not been reported.

To better understand the environmental background levels of *C. burnetii* present in the U.S., from 2006 to 2008 we collected greater than 1,600 environmental samples from 6 states. The sample types included sponge wipes, vacuum samples, and bulk soil. DNA was purified from these samples, and after it was shown to be free of PCR inhibitors, the presence of *C. burnetii* DNA was evaluated by quantitative PCR of the IS1111 repetitive element. Overall, 23.8% of the samples were positive, and the percentage of samples positive for *C. burnetii* DNA in individual states ranged from 6 to 44 percent. DNA was detected in both urban areas and rural areas near livestock. Positive sites included one or more grocery stores, post offices, banks, hospitals, and football stadiums, and a wide range of rural sites (farms, ranches, dairies, feed lots).

This study demonstrates that *C. burnetii* is fairly common in the environment in the U.S., and any analysis of *C. burnetii* after a suspected intentional release should be interpreted in light of these background levels. It also suggests that human exposure to *C. burnetii* may be more common than what is suggested by the number of reported cases of Q fever.

12. Mathematical Modeling Activities in the Chronic Viral Diseases Branch

Presenter: Brian M. Gurbaxani, CDC/Georgia Tech

While mathematical models can be intimidating to those not conversant with the techniques involved, they can also be viewed more simply as a type of accounting software for experimental results: they provide a unified, symbolic, and quantitative way to organize current knowledge¹. Of course, we are all aware of how horribly wrong accounting software can go, hence the need for validation, but we are equally aware of how indispensable it is. Once the knowledge is organized and validated, models can serve as predictive, hypothesis generating tools. Models can be at many scales and use many different techniques. In the chronic viral diseases branch (CVDB), we use a wide variety of modeling techniques, e.g. from the engineering oriented ordinary differential equation (ODE) models to the statistically oriented structural equation models (SEM), in a wide variety of applications for the analysis and control of chronic fatigue syndrome (CFS) and human papilloma virus (HPV) induced cervical cancer.

¹Gurbaxani, B.M., "Mathematical Modeling as Accounting: Predicting the Fate of Serum Proteins and Therapeutic Monoclonal Antibodies", *Clinical Immunology* (2007), 122(2):121-124

13. Next Generation of Vaccines: Oral and Transdermal Vaccines

Presenter: Martin J. D'Souza, Mercer University

Martin D'Souza¹, Syed Rizvi¹, Tuhin Bhowmik¹, Bernadette D'Souza¹, Mohammad Nasir Uddin¹, Prathap Shastri¹, Lipika Chablani¹, Archana Akalkotkar¹, Suprita Tawde¹, Lakshmi Kolluru¹, Periasamy Selvaraj², Rangaiah Murthy².

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We, at the Nanotechnology, Cancer and Vaccine Laboratory at Mercer University, Atlanta, have developed a platform technology using nanospheres and microspheres to deliver vaccines by the oral route of administration, specifically in the form of a capsule containing the bead-like encapsulated vaccine antigens. This formulation technology enables us to prepare microspheres and nanospheres containing biologically active compounds, such as vaccine antigens and protein drugs, without loss of their biological activity during the formulation process. We can also encapsulate multiple antigens, targeting agents and drugs in a single particle. Using our novel method, the vaccine prepared exists in a dry stable form. These bead-like particles containing the antigen can be administered either orally or systemically. When administered orally, these spheres have an enteric coating to protect from the acidic environment of the stomach. The enteric coating dissolves in the basic environment of the intestine and the vaccine is targeted to the Peyer's patches and the general mucosal immune system. Since these particles are particulate in nature, ranging between 0.05-2.0 microns in size, they are taken up by phagocytic antigen presenting cells (APC's), such as M cells and macrophages in the Peyer's patches of the intestines, and the antigen is presented to the lymphocytes, which are responsible for antibody production. Since these nanospheres/microspheres release the antigen (payload) intracellularly into the APC's, a higher antibody response to the encapsulated antigen is obtained. Of particular interest in this formulation is the fact that the microspheres release the antigen in a slow and sustained manner over a prolonged time period, resulting in strong mucosal and systemic immunity after oral administration, without the need for

adjuvants. Since no needles are required, this method of vaccine delivery is inexpensive and suitable for the developing world as well as for the developed world. Preliminary studies conducted in our laboratory with TB, typhoid, melanoma, and hepatitis B vaccine antigens suggest that this delivery system is highly suitable for antigens to be used for protective immunity. This method of vaccine delivery enables us to address a wide spectrum of vaccines for prophylactic and therapeutic use. Melanoma tumors are widespread in the southeastern United States. We have been working on the "Evaluation of an oral encapsulated melanoma tumor vaccine" as a collaborative research project, funded by the Georgia Research Alliance, with Dr. Periasamy Selvaraj from Emory University.

14. Whole genome annotation of bacteria – a computational genomics pipeline

Presenters: King Jordan and Lee Katz, Georgia Institute of Technology

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Next-generation sequencing technologies have accelerated the pace of genome characterization to the extent that the complete sequencing of bacterial genomes is routine. Computational analysis of the resulting data is now the rate limiting step for microbial genomics. In particular, no off-the-shelf solution exists for the integrated computational analyses necessary to make biological sense of complete genome sequence data. To address the need for such an informatics resource, we have developed a self-contained, automated, high-throughput, open-source computational genomics pipeline that can facilitate the completion of bacterial sequencing projects with minimal bioinformatics support. Because every component of the pipeline is executed on a local machine with no need to access resources over the Internet, the pipeline is suitable for projects of a sensitive nature as well as organizations wishing to customize their bioinformatics workflow. The pipeline was developed as a collaboration between the Georgia Institute of Technology (GIT) and the Meningitis Laboratory at the Centers for Disease Control and Prevention (CDC) and implemented for the analysis of *Neisseria meningitidis* genomes.

The automated computational genomics pipeline we are developing consists of three sequential analysis stages: 1) genome assembly, 2) gene prediction and 3) functional annotation. Each individual analysis step is performed via the integration of multiple distinct approaches. For each step, in addition to default options, users can employ custom settings to control how the outputs of distinct algorithms are integrated into a final result. Genome assembly is achieved through the combination of two different assembly algorithms followed by an automated scaffolding, gap

joining, and finishing procedure to further improve the assembly. Prediction of protein coding genes is based on a combination of the results of two different ab initio prokaryotic gene predictors with homology-based prediction using BLAST searches against a database of the user's choice. Non-protein coding genes, including ribosomal RNAs, transfer RNAs and insertion sequences, are predicted using specific programs for each class of genes. Functional annotation of protein coding genes is based on an integrated platform that makes use of six distinct annotation tools, four of which employ intrinsic sequence characteristics for annotation and two that use extrinsic homology-based approaches to compare sequences against databases of sequences and structures with known functions. Information on gene ontology terms, domain architecture and identity, subcellular localization, signal peptides, transmembrane helices and lipoprotein motifs is provided for each protein coding gene. Additional annotation of virulence-related features makes the pipeline particularly useful for projects working with pathogenic prokaryotes. We anticipate that our computational genomics analysis pipeline will become a viable resource for the future of microbial genome informatics.

Keywords: *Neisseria meningitidis*, computational genomics, genome informatics, genome assembly, gene prediction, functional annotation

15. Methods of purification of a microbially produced fusion protein

Presenter: George Pierce, Georgia State University

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The global demand for seasonal influenza vaccine supplies is overwhelming each year, yet production remains inefficient: it takes at least six months to make a sufficient quantity. Additionally, antigenic drift prevents accurate prediction of the season's circulating strains. In order to improve both the immunopotency of and the efficacy of production, a new approach of vaccine construction has been proposed. This approach fuses flagellin (from *S. typhimurium*), a known agonist of toll-like receptor 5), with the antigenic region of the influenza virus itself. The fusion protein (STF2.HA1-2 (SI)) is then inserted into an *E. coli* vector and cultured in a bioreactor. Once the protein has been extracted from the cell harvest, several purification steps are undergone to reduce endotoxin levels and to remove cellular materials. Another key factor in vaccine preparation is to ensure the proper folding of the final product, as this affects immune system recognition.

16. Fermentation of recombinant *E. coli* for the expression of STF2.HA1-2 fusion protein

Presenters: George Pierce and Sid Crow, Georgia State University

T-A. M. Tucker, C. Wang, K. Swensen, S. A. Crow, Jr. and G. E. Pierce.

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VaxInnate Incorporated has developed novel recombinant vaccines based upon the incorporation of a TRL-5 agonist with a specific antigen, that has potential to serve as a platform technology for the production of many different vaccines. One of the vaccine targets, is a seasonal flu vaccine based on a fusion protein comprising the Influenza HA antigen enza virus and bacterial flagellin. An example of a seasonal flu construct is [STF2.HA1 (SI)]. Phase 1 and II trials of this vaccine are extremely

promising as it is more immunopotent than traditional influenza vaccines. The fusion protein STF2.HA1 (SI)] is produced in recombinant E. Coli cells in a relatively simple multi-step fermentation process that involves (batch and fed batch phases. At the end of final phase, the OD (600 nm) values are between 58-62 with a yield 100 g/L. This work was undertaken as a technology transfer exercise prior to the initiation of a Department of Defense program to explore the potential of the VaxInnate technology to address DoD needs.

17. Rapid Detection of Pathogens with RNA Microchip

Presenter: Zhen Huang, Georgia State University

Direct, rapid, sensitive and accurate RNA detection is essential for monitoring, preventing and controlling pathogen-caused epidemics. This work demonstrates the proof of principle of a novel concept for RNA direct detection, and describes a unique RNA microchip with many advantages. We have demonstrated a novel RNA detection strategy with the enzymatic reactions on RNA microchip (spot size: 75 micron), which offers single-nucleotide specificity, rapidness (approximately 20-min detection time), and high sensitivity (at the low fmole level). We have also demonstrated simultaneous and selective detection of multi-pathogen RNAs. Furthermore, this RNA microchip allows direct detection of an individual RNA from a biological sample without the need for the time-consuming amplification and hybridization steps. Our direct and rapid RNA microchip technology will have great potentials in epidemic rapid monitoring, field detection, clinical diagnosis, and food processing monitoring.

18. Still-blinded Safety and Immunogenicity Data from a Trial of Reduced-dose, Intradermal, Influenza Vaccination by Needle-free Jet Injection

Presenter: Francisco S. Palomeque, CDC

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Introduction: Reduced-dose, intradermal (ID) influenza vaccination by disposable-syringe jet injector (DSJI) may protect greater numbers when vaccine is scarce or costly, and avoid needle-syringe (N-S) risks. **Design:** An n=450 trial administered 2 doses 4 weeks apart of Vaxigrip® (Sanofi) to children 6 to 24 months of age randomized to 3 groups: 0.1 mL ID by Biojector® 2000 DSJI; 0.1 mL intramuscularly (IM) by N-S; or full-dose 0.25 mL IM by N-S. Blinded investigators assessed adverse events (AEs) on days 0, 2, 7, and 28 after each dose; blinded parents completed diaries for days 0 to 28. Sera from days 0, 28, and 56 were assayed. **Results:** Among 390 participants, local pain was mild in 100 (26%), moderate in 8 (2%), and severe in 4 (1%). Moderate AE sizes of ≥10-<25 mm occurred for erythema (8%), swelling (2%), hematoma (2%), induration (2%), and nodules (1%). Severe sizes of ≥25-<50 mm occurred in <1% for erythema, swelling, and hematoma. Systemic AEs were diarrhea (38%), fever ≥38.0°C (32%), loss of appetite (31%), vomiting (29%), sleepiness (18%), unusual crying (17%), irritability (13%), and convulsions (2%). Serious AEs (n=25): 10 asthma-related, 4 varicella, 4 febrile convulsions, 1 death (trauma), and 6 miscellaneous others. All but one *possibly-related*

convulsion 23 days after dose 1 were deemed *unrelated* by DSMB. HAI inverse GMTs after dose 2 for multiple formulations and strains (n=207) were 193 for H1N1, 45 for H3N2, and 101 for B. Seroconversion was 72%, 25%, and 63%; seroprotection 88%, 49%, and 85%, respectively. H3N2/Brisbane/10/2007 strain responses were poor, and under investigation. **Conclusion:** Local AEs were tolerable, and immunity generally good. Definitive assessment awaits unblinded analysis by group of all participants.

19. Impact of helminth infection on response to HIV-1 vaccines

Presenter: Don Harn, University of Georgia

Eliciting optimal vaccine responses will require that vaccine recipients are relatively healthy and that their immune systems are able to properly respond to HIV-1 vaccines. Unfortunately, the majority of individuals in developing countries are infected with one or more species of parasitic helminthes that induce immune suppression and also drive systemic Th2-biasing. Helminth infection has been shown to suppress immune responses to Th1-type bacterial vaccines and impair the expansion of pathogen-specific cytotoxic CD8+ T cell responses. Recently, we demonstrated that mice infected with the helminth parasite *Schistosoma mansoni* were unable to mount significant HIV-1 vaccine-specific T cell responses to a plasmid DNA HIV-1 vaccine, even when the vaccine was enhanced. This observation, taken together with other studies examining virus-specific immune responses in helminth infected mice suggests that helminth infection will pose a significant problem for the development of HIV-1 vaccines designed to induce viral-specific Th1-type CD4+ and cytotoxic CD8+ T cell responses.

Fortunately, inexpensive, highly efficacious anthelmintic drugs are available and shortly after elimination of helminth parasites, host immune responses restore to a normal Th1-Th2 bias. We determined that drug elimination of helminth parasites restores immune responsiveness to HIV-1 vaccines. We are now looking for vectors for HIV-1 vaccines that can overcome the suppressive effect of helminth infection.

20. Chimeric Plasmodium CSP/MSP1 hybrid protein induce protection against infection and severe anemia

Presenter: Alberto Moreno, Emory Vaccine Center

We have previously reported the design of two chimeric recombinant proteins based on the circumsporozoite protein (CSP) and the Merozoite Surface Protein-1 (MSP1) derived from *Plasmodium yoelii*. Proof of principle studies using these constructs indicated that each protein used as a single immunogen induced protective immunity in mice. A distinctive feature of such chimeric proteins is the expression of promiscuous CD4+ T cell epitopes derived from the native homologous proteins. To evaluate the potential synergistic effect of combining these chimeric proteins in a single immunogen, we constructed a synthetic gene codon optimized for expression in *E. coli* that encodes both chimeric antigens associated in tandem. The hybrid chimeric protein, that contain the amino terminal chimeric CSP fused to the chimeric MSP1 protein interspaced with Gly-Pro-Gly-Pro-Gly spacers and a tag sequence, was expressed in soluble form with high yield. Antibodies produced against different segments of the recombinant protein and anti-tag sequences were used for biochemical characterization of the protein and analysis of the antigenic integrity. Groups of CAF1/J mice were used to test the immunogenicity of the hybrid construct. The immune responses elicited

by immunization with the hybrid protein were compared with that obtained using a mixture of the two chimeric proteins. A single immunization with the hybrid protein induced higher antibody titers against individual component in comparison with mice that received the mixture of the two proteins. Both vaccine formulations induced robust protection to the experimental challenge with sporozoites but only the hybrid protein was able to induce sterilizing immunity. Relevantly, efficacy against experimental challenge includes protection against hyper-parasitemia and malarial anemia. The fine specificity of the immune responses induced by immunization with the chimeric hybrid protein and the effector mechanisms involved in protection will be discussed.

21. Quantifying the impact of immune escape on transmission dynamics of influenza

Presenter: Andrew Park, University of Georgia

Influenza virus evades prevailing natural and vaccine-induced immunity by accumulating antigenic change in the haemagglutinin surface protein. Linking amino acid substitutions in haemagglutinin epitopes to epidemiology has been problematic because of the scarcity of data connecting these scales. We use experiments on equine influenza virus to address this issue, quantifying how key parameters of viral establishment and shedding increase the probability of transmission with genetic distance between previously immunizing virus and challenge virus. Qualitatively similar patterns emerge from analyses based on antigenic distance and from a published human influenza study. Combination of the equine data and epidemiological models allows us to calculate the effective reproductive number of transmission as a function of relevant genetic change in the virus, illuminating the probability of influenza epidemics as a function of immunity.

22. Cryo-electron tomography of whole viruses and cells

Presenter: Elizabeth R. Wright, Emory University

Cryo-electron tomography (cryo-ET) is a powerful technology used for the ultrastructural analysis of complex biological systems at macromolecular resolution (2 to 4 nm). Cryo-ET is uniquely suited for the visualization of pleomorphic viruses, such as HIV-1, and small whole cells. The structural analysis of HIV-1, Measles virus, virus-infected cells, and the utility of cryo-ET for rational vaccine and therapeutic development will be presented.

23. Mechanisms and Probes of the Protein Arginine Methyltransferases

Presenter: Y. George Zheng, Georgia State University

Epigenetic modifications of chromatin, which include DNA methylation and histone modifications, have been established as a fundamental mechanism for control of cell proliferation, growth and differentiation as well as organ development. Notably, many of these histone modifying enzymes are implicated in various types of cancers. We are particularly interested in investigating the biological functions of protein arginine methyltransferases (PRMTs), a type of essential chromatin modifying enzymes involved in the transcriptional regulation of gene expression in eukaryotic cells. PRMTs catalyze the transfer of a methyl group from S-adenosyl-L-methionine (AdoMet) to the guanidino group of arginines in protein substrates, resulting in mono and di-methylarginine residues in substrate proteins. A growing body of evidence in recent years has identified several PRMT proteins as new biomarkers in malignant diseases. Nevertheless, the molecular mechanisms of many HATs and PRMTs in pathogenesis are still very elusive. Meanwhile, enormous efforts are needed to

invent effective chemical probes for investigating the biochemical and cellular functions of those epigenetic factors in different cellular contexts. We will present our recent research results on the development of fluorescent chemical probes of PRMTs as well as enzymatic mechanism study.

24. *Neisseria meningitidis* Capsular Polysaccharides Induce Inflammatory Responses via TLR2 and TLR4-MD-2

Presenter: Susu Zughailer, Emory University

Susu Zughailer PhD and David Stephens MD

Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine.

Capsular polysaccharides (CPS) are a major virulence factor in meningococcal infections and form the basis for serogroup designation and protective vaccines. CPS is anchored in the outer membrane through a 1,2-diacylglycerol moiety. The immunostimulatory activity of meningococcal CPS polymers and their lipid anchors is largely unexplored. Well established human and murine macrophage cell lines and HEK/TLRs stably transfected cells were stimulated with CPS purified from the endotoxin-deficient meningococcal serogroup B NMB-*lpxA* mutant. We found that *Neisseria meningitidis* CPS induce inflammatory responses via TLR2 and TLR4-MD-2. Meningococcal CPS induced a dose-dependent release of cytokines (TNF α , IL-6, IL-8, CXCL10) and nitric oxide from human and murine macrophages respectively. CPS induced IL-8 release from HEK cells stably transfected with TLR2/6, TLR2, TLR2/CD14 and TLR4/MD2/CD14 cells. A significant reduction in TNF α and IL-8 release was seen when THP-1 and HEK-TLR4/MD2-CD14 transfected cells were stimulated with CPS in presence of Eritoran (E5564), a lipid A antagonist that binds to MD2; and similar reduction in nitric oxide and TNF α release was also seen in RAW 264.7 cells with Eritoran. In contrast, no reduction in IL-8 release from HEK-TLR2 or HEK-TLR2/6 transfected cells stimulated with CPS in presence of Eritoran. In time-course and dose-response experiments, Eritoran inhibited CPS induced responses only in TLR4-MD2 expressing cells confirming that MD2 is required for TLR4 but not for TLR2-mediated CPS bioactivity. However, cleavage of lipid anchors from purified CPS resulted in attenuation of CPS bioactivity via TLR2. Thus, meningococcal CPS polymers induce inflammatory responses via TLR4-MD2 and lipid anchors play an important role in facilitating human macrophages activation via TLR2.

25. Biomedical Informatics for Clinical Decision Support and Translational Research

Presenter: Alfredo Tirado, Emory University

In order to perform more sophisticated clinical investigations on heterogeneous data that may be integrated from a variety of high-quality data repositories and resources, or to facilitate multidisciplinary translational research, experts can greatly benefit from virtual collaborative environments that provide user-friendly spaces for building and executing complex studies and workflows. To this end, a number of relevant efforts are under way at the Emory University Center for Comprehensive Informatics (CCI), a recently created multi-disciplinary center that promotes and fosters collaborative projects between software system researchers and scientific research groups. One of such efforts is the work on distributed software architectures within the Biomedical Informatics Program (BIP), under the umbrella of the Atlanta Clinical and Translational Science Institute (ACTSI), where we are investigating complex use cases where support for clinical data management on HIV-related clinical trials can be integrated to the Atlanta-wide infrastructure of the consortium. Another effort is taking place on the design of knowledge base technologies for the

semantic integration of AIDS-defining and AIDS-associated malignancy data on underserved populations, to facilitate overall finding discovery. Another effort is under way focusing on frameworks for comparative effectiveness research in Newborn dried Bloodspot Screening (NBS) for long-term follow up. A common thread along these research efforts is the investigation of innovative ways to access and integrate complementary types of information sources and large volumes of data, in order to identify and understand the mechanisms underlying physical and biological functions and processes that may lead to new scientific knowledge discovery.

26. Thiostrepton: Biosynthesis and Biosynthetic Engineering
Presenter: Wendy Kelly, Georgia Institute of Technology

The thiopeptide antibiotics are widely distributed metabolites isolated from Gram-positive bacteria of both terrestrial and marine origin. Thiostrepton A, often considered the “prototype” of this family, was first isolated in the 1950s. The highly modified thiopeptides are characterized by a macrocycle possessing a central nitrogen-containing six-membered ring that varies in its oxidation state. Thiostrepton and other thiopeptides are potent antibacterial agents against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and have also been reported to demonstrate antimalarial activity. Development of the thiopeptides into clinically useful antibacterial agents has been limited in part by the lipophilicity of these metabolites. There is therefore great interest in the generation of analogs with improved water solubility by the means of biosynthetic engineering, synthesis, semisynthesis, or a combination thereof.

27. Simple UV-HPLC System for Sunscreen-Efficacy Analysis
Presenter: Khin Lay Maw, Georgia State University

In order to prevent the DNA damages caused by the ultraviolet radiation of sunlight, sunscreens are commonly used to protect the human skin. Current analysis of sunscreens effectiveness is done through complicated procedures, including human exposure. Thus, we sought to design a simple UV-HPLC system using thymidine-thymidine (TT) dinucleotide to analyze the effectiveness of sunscreens and the formation of TT photolesions. Efficient sunscreen has protective effects against UV irradiation damage. Our research indicates that the analytical results from UV are consistent with those of HPLC, which is used to monitor the formation of the TT photolesions. Moreover, both UV and HPLC analyses indicate that TT dinucleotide is better protected, against UV damage, using the sunscreens with higher SPF (UVB sunburn protection factor) values, and that sunscreen with higher SPF leads to lesser photolesion formation. In addition, our UV and HPLC analyses confirm the SPF grading of commercial sunscreens. In conclusion, we have established a simple strategy to analyze the effectiveness of sunscreens and the quality of these potential cancer-preventive products.

28. Haloenol Lactone-Containing Peptides: Novel Serinase Inhibitors for Leads in Drug Discovery
Presenter: Timothy Long, University of Georgia

Novel mechanism-based inhibitors of serinase enzymes (i.e. proteases, phospholipases) containing peptidyl scaffolds will be describe. Short chain oligopeptides (2-8 aa) containing haloenol lactones are being designed to target microbial-specific enzymes. Their optimization as irreversible

inactivators of enzymes required for survival by pathogenic microbes would provide opportunities for new therapeutics of different infectious disease types.

29. Mucosal delivery of PsaA subunit antigen microspheres

Presenter: Simon Paulos, Mercer University

Strong mucosal immunity is of critical importance in preventing pneumonia, which mainly invades the body via the upper respiratory tract. In this work, we have shown that a *Streptococcus pneumoniae* surface antigen PsaA has the potential to develop immunity in mice models after oral delivery when given in a microsphere formulation. The formulation is designed to help prevent the protein antigens from the stomach acidity and was optimized for size and charge and administered by oral gavage to BALB/c mice following which systemic and mucosal immune responses were evaluated. Elevated serum antibody levels of IgG2a and IgA were detected in the group which received oral antigen entrapped polycaprolactone-maltodextrin microspheres versus naked protein antigens upon challenge with a pneumococcal bacterial strain. Additionally, cytokine responses upon ex-vivo re-stimulation of cells from lymphoid organs with PsaA antigen were evaluated for IL-2, IL-4, IL-6, IFN- γ and TNF- α .

30. DEVELOPMENT, CHARACTERIZATION AND EFFICACY OF ANTI-PROSTATE CANCER DRUG LOADED NANOPARTICLES

Presenter: Bindhu M. Rayaprolu, Mercer University

Prostate Cancer still remains a significant public health problem worldwide especially in developing nations due to high incidence and associated cost of detection and treatment. Nanotechnology has a tremendous potential in making an important contribution to the treatment of Prostate Cancer. We are investigating the potential of anti prostate cancer drug loaded nanoparticles as drug delivery devices. Our studies are involved in the development, in vitro and in vivo studies of Methyl Jasmonate, Paclitaxel and Triptorelin loaded Nanoparticles. The above drug loaded Nanoparticles are developed by double emulsion and Nanoprecipitation techniques. The above formulation techniques yielded spherical nanoparticles which are of 200-300nm in size and with an average zeta potential of -30mV. Increased in vivo specificity and efficacy of nanoparticles was observed with the attachment of a prostate specific membrane antigen (PSMA) ligand to the surface of these nanoparticles. Our studies showed that under in vitro conditions, the formulation exhibited sustained release of the drugs and intratumoral injection of the formulation in animals demonstrated significant (P

31. The Contribution of Vertical Transmission with Poultry Integrators to Chicken Carcass Contamination with *Salmonella enteric*

Presenter: John Maurer, University of Georgia

The Centers for Disease Control and Prevention estimate that 76 million illnesses are caused by foodborne pathogens annually in the US. Consumption of poultry and poultry products are recognized risk factors for foodborne outbreaks of salmonellosis and campylobacteriosis. The federal government has invested significant resources in diagnosis and prevention of foodborne disease. However, these surveillance networks do not address pre-harvest food safety and therefore a major approach to *Salmonella* control is neglected. Due to the integrated nature of the poultry

industry, Salmonella can be introduced at any point within this production system. We believe that vertical transmission, as high as the pedigree flock; play a significant role in chicken carcass contamination with Salmonella enterica. Poultry farms in northeastern Georgia were surveyed for Salmonella, starting at the pullet level. Salmonella positive pullet flocks were followed to broiler-breeder farms and their progeny were followed onto broiler farms and ultimately the processing plant, sampling the farm environment, as well as chicken carcasses for Salmonella. Salmonella isolates were typed to serotype and subtype level by PCR and pulsed-field gel electrophoresis (PFGE) respectively. Phylogenetic analysis, using Dice coefficient, was used to compare Salmonella PFGE patterns and identify genetically related S. enterica strains. The same S. enterica strains were also isolated from broiler farms paired to specific broiler breeder flocks monitored in this study. These Salmonella strains were obtained broiler chick box liners and subsequently isolated from broiler farm environment and broiler chicken carcasses. The same Salmonella strains were also isolated from reproductive tract of hens supplying broiler chicks to farms monitored in this study. Several S. enterica strains identified on broiler farm or chicken carcasses matched as far back as the pullet farms, where the broiler-breeder flocks were initially raised. Of the serotypes identified, S. Kentucky appeared to be the dominant serovar transmitted all the way from the pullet level down to the broiler chickens. Vertical transmission contributes to chicken carcass contamination with Salmonella and intervention steps (ex. vaccination) introduced at the pullet or broiler-breeder level may reduce Salmonella contamination of poultry meats.

32. Prophylactic use of flagellin: A novel method to boost immune reconstitution in allogeneic HSCT recipients with limited GvHD

Presenter: Mohammad Sohrab Hossain, Winship Cancer Institute, Emory University

Background: Graft-vs-host disease (GvHD) is a major complication in allogeneic Hematopoietic Stem Cell Transplant (HSCT) recipients. Immunosuppressive drugs limit clinical GvHD but increase relapse and susceptibility to opportunistic infections and also result in drug related toxicities. To develop an alternative approach to control GvHD, we tested the immunomodulatory immune properties of flagellin, a bacterial protein that agonistically binds with TLR5 and protects mice from radiation-induced gut injury, in murine allo-BMT models.

Methods: We used established BA.B10 (H-2K) ? C57BL/6 (H-2b) MHC mismatched experimental models of allogeneic HSCT in which GvHD is a major complication. 50 µg LPS-free purified flagellin in PBS or PBS alone were administered intraperitoneally in two doses: 3 hours before fractionated irradiation (5.5Gy X 2 fractions) and 1 day post-transplant. Allografts were performed 1 day after irradiation and contained 5 million (M) T-cell depleted bone marrow (BM) cells and 5 M plastic non-adherent splenocytes from naïve BA.B10 donors. The primary end-points was survival; HSCT recipients were monitored twice a day for mortality and GvHD signs and recipients having more than 25% weight loss were sacrificed. Blood, spleen, thymus and BM were collected from surviving mice on day 132 post transplant, live cells counted, and immune phenotypes were analyzed by FACS. The numbers and phenotype of immune cells in organs from flagellin-treated HSCT recipients were compared to the similar immune cells per organs analyzed from a normal B6 mouse having similar age of HSCT recipients.

Results: Flagellin treated recipients had 15% weight-loss and 33% transplant-related death by 132 days post transplant versus severe acute GvHD and 100% early post-transplant mortality among

control HSCT recipients that received PBS. Flagellin-treated recipients had 100% donor chimerism with limited clinical signs of GvHD. While total cell numbers per spleen ($8.2 \pm 5.4M$) and thymus ($7.1 \pm 4.9M$) were very low in flagellin-treated recipients compared to normal B6 mice ($>100M/organ$), the cell numbers isolated from blood ($8.9 \pm 2.6 M/ml$) and BM ($104.5 \pm 37.4 M$) were similar to non-transplanted B6 mice ($11.4M/ml$ blood and $108 M/BM$, respectively). BM of flagellin-treated HSCT recipients contained similar numbers of CD4+ T cells ($4.6 \pm 2.7 M$) and CD8+ T cells ($2.5 \pm 1.4 M$) as normal B6 mice ($4.03M$ and $1.3M$, respectively). Numbers of naïve and memory CD4+ T-cells in the BM were similar between flagellin-treated and control mice: CD4+CD62L+ (0.7 ± 0.2 versus $0.5M$); CD4+CD62L- (3.9 ± 2.5 versus $3.5 M$); CD4+ CD44hi (2.8 ± 1.4 versus B6 $3.6M$); and CD44lo ($1.7 \pm 1.3 M$ versus $0.44M$). In contrast, flagellin-treated HSCT recipients had more naïve CD8+ T-cells but similar memory CD8+ T-cells in their BM compared with control mice: CD8+CD62L+ (2.6 ± 1.4 versus $1.0M$); CD8+CD62L- ($1.7 \pm 1.2M$ versus $0.3 M$); CD8+CD44hi (0.8 ± 1.1 versus $0.7M$); and CD8+CD44lo ($0.7 \pm 0.3M$ versus $0.6 M$). The numbers of total CD3+ T cells, NK cells, and lin-CD11b-Sca-1+Ckit+ Stem cells in the BM were also similar comparing flagellin-treated recipients with non-transplanted B6 control mice. The number of CD3-B220+ B cells in the BM were lower in flagellin-treated recipients compared to B6 mouse ($18.1 \pm 3.2M$ versus $43.1M$) as were the numbers of T-cells and B-cells per mL blood of flagellin-treated mice were found lower compared with the blood of normal B6 mouse: $0.8 \pm 0.2M$ T-cells/mL versus $2.1M/mL$; $5.5 \pm 2.5M$ B cells/mL versus $9.1M/mL$. Although the cellularity of the thymus in flagellin-treated animals was very low compared to normal B6 mice, a usual percentage ($62.5 \pm 10.5\%$) of thymocytes were of CD4/CD8 double positive, indicating functional thymopoiesis in these recipients.

Conclusion: Flagellin protected allogeneic HSCT recipients from irradiation-induced BM damage and prevented lethal GvHD in a major MHC mis-matched model of GvHD. Flagellin and other TLR5 agonists may be novel therapeutic approaches to prevent or reduce GvHD in allogeneic HSCT recipients.

33. Immunomodulating effects of vitamin D hormone during Chlamydia infection

Presenter: Qing He, Morehouse School of Medicine

Chlamydia trachomatis is a major infectious bacterial agent of sexually transmitted disease (STD) in the United States. Approximately three million non-institutionalized U.S. civilians ages 14-39 are infected with *Chlamydia*, based on reports from the Centers for Disease Prevention and Control (CDC) and the U.S. National Health and Nutrition Examination Survey [1]. There are also significant racial disparities in the prevalence of *Chlamydia* infection in the United States. The *Chlamydia* infection rate among African Americans was over 8 times that of whites [2, 3]. These studies provide initial evidence suggesting that controlling infection of high-risk African-Americans may be important from a public health standpoint. Interestingly, recent clinical studies have revealed that African-Americans have lower serum levels of 25-hydroxyvitamin D (the biomarker of vitamin D status) and weaker resistance to certain infectious diseases [4, 5, 6, 7] than other ethnic population. Therefore, to study whether low Vitamin D status play a role in the high rate of genital *Chlamydia* infection in African-American population is important for control, treatment and prevention of *Chlamydia* infection. Recent epidemiologic and experimental studies have provided evidences that 1,25-(OH)₂ D₃, a activated form of vitamin D has functions beyond the physiological regulation of calcium transport and bone mineralization. It stimulates genetic expression of antimicrobial peptides (AMPs) in human respiratory tract epithelial cells. The ability of 1,25-(OH)₂ D₃ to induce

AMPs, such as defensins and cathelicidins with direct anti-microbial action provided a possible link between vitamin D deficiency and the occurrence of respiratory infections, and tuberculosis [8, 9]. The chance that 1,25-(OH)₂ D₃ could play a role in host defense against Chlamydia disease is, however, untested. The purpose of this study was to determine the effect of 1,25-(OH)₂ D₃ on Chlamydia infection in vivo and in vitro and its possible mechanisms.

34. Morehouse School of Medicine Training in Genomics and Hemoglobinopathies Program
Presenter: Jonathan K. Stiles, Morehouse School of Medicine

Hemoglobinopathies affect disproportionately minorities in the US and significant portions of the global population, mostly residing in countries with low income. The MTGHP, funded by NIH/Fogarty, recruits and trains postdoctoral fellows from underrepresented US minority populations, particularly those affected by hemoglobinopathies over a period of two-three years. MTGHP includes international (University of Ghana; Sickle Cell Trust, Jamaica) and local collaborators (Emory and Medical College of Georgia). Members of the MTGHP group conduct laboratory, clinical and population-based studies that examine interactions between Sickle Cell Disease (SCD) and nutrition, inflammation, vascular biology, susceptibility to infection and possible therapeutic interventions. Recent studies examined the relationship of resting energy expenditure with inflammation in patients with SCD,[1] allocation of the increased metabolic demand,[2] as well effect of high protein diet on the inflammatory response in SCD mouse models.[3] Determinants of vascular injury are being elucidated, with demonstration, for the first time, of the enhanced vasculogenic potential at steady-state in patients with SCD.[4] These may serve as markers of disease severity. Population based studies examined the nature of increased susceptibility to pathogens, methods of prevention,[5] and their possible global impact. In addition to the effect of nutrition,[1-3] other therapeutic modalities explored include stem cell translation modalities,[6] and interaction between chronic transfusion iron overload toxicity, inflammation and hemolysis.[7] With the participation of fellows, the program is currently laying the groundwork for SCD population based screening to determine reliable markers of disease severity in the context of complex and diverse environments, including hitherto seldom examined populations (Tunisia) as well as in Ghana. In summary, the aim of this program is to cultivate outstanding researchers who will strive to improve care of individuals both burdened by hemoglobinopathies and resource disparities, while exploring critical, but neglected interactions of human biology that possibly affect large segments of the global population. Research Interests: Members of the MTGHP group conduct laboratory, clinical and population-based studies that examine interactions between Sickle Cell Disease (SCD) and nutrition, inflammation, vascular biology, susceptibility to infection and possible therapeutic interventions. Recent studies examined the relationship of resting energy expenditure with inflammation in patients with SCD, allocation of the increased metabolic demand, as well effect of high protein diet on the inflammatory response in SCD mouse models. Determinants of vascular injury are being elucidated, with demonstration, for the first time, of the enhanced vasculogenic potential at steady-state in patients with SCD. [4] These may serve as markers of disease severity. Population based studies examined the nature of increased susceptibility to pathogens, methods of prevention, and their possible global impact. In addition to the effect of nutrition, other therapeutic modalities explored include stem cell translation modalities, and interaction between chronic transfusion iron overload toxicity, inflammation and hemolysis.

35. GlycamWeb: Free Online Tools for Oligosaccharide Structure Prediction

Presenter: Robert Woods, University of Georgia/CCRC

A suite of modeling tools are presented which facilitate prediction of the 3D structures of glycans of relevance to carbohydrate-based vaccine design

36. Analysis of Virus Evolution Through Sequencing of Near-Full-Length Single Genomes from Subtype A Epidemiologically Linked Transmission Pairs from Rwanda

Presenter: Ling Yue, Emory University

Background: Previous studies of subtype A and C epidemiologically-linked heterosexual HIV-1 transmission pairs in Rwanda and Zambia demonstrated that a single viral variant from the donor partner established infection in 90% of transmissions. The goals of this study were to compare the near full-length viral sequences of individual viruses in the transmitting partner to that establishing infection in the newly infected recipient, and to evaluate, across the entire viral genome, the evolution of the newly transmitted variant in response to both cytotoxic T-lymphocytes (CTL) and neutralizing antibodies during the first 6 months of infection.

Methods: Amplicons produced by single genome amplification (SGA) of near full-length (>9000bp) HIV-1 genomes from patient PBMC and plasma viral RNA were sequenced directly. Full-length viral sequences from 2 transmission pairs including viruses from both the chronically infected viral quasi-species and the linked acutely infecting virus population at 3 different time points (enrollment, month 3 and month 6) were analyzed phylogenetically to establish the founder virus sequence and the appearance of potential escape mutations. Non-synonymous changes in CTL epitopes were characterized according to donor and recipient HLA types.

Results: In heterosexual transmission pairs R880 and R463, which were identified at a p24 positive time point, a single genetic variant from the chronically infected partner established infection. In pair R880, a variant carrying mutations in the ISW9, TW10 and KF11 epitopes was transmitted from the B5703+ donor to the newly infected partner. Viral load was well-controlled in the B*1503 recipient, but escape at the WF9 epitope in Nef and potential Nab induced changes in the ?2 and V5 regions of gp120 were observed at 3 months post-infection. In the newly infected partner from R463, viral load was not controlled, despite evidence for a strong CTL response to the Tat FY10 epitope during the p24+ phase of infection. Potential CTL escape mutations in Nef and Gag epitopes were also observed in this subject at 2 and 6 months of infection respectively. Changes in the Env V1, ?2 and V4 regions were observed 6 months after infection.

Conclusions: These findings provide unique insight into the evolution of the founder virus across the entire proteome in response to the adaptive immune response and will facilitate characterization of viral fitness costs associated with escape.

37. Smart Particle based Delivery of Vaccines and Bioactive Compounds via the Oral and Transdermal Routes

Presenter: Tuhin Bhowmik, Mercer University

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We are engaged in developing smart nano/microparticulate carriers to deliver vaccines and other bioactive compounds. The efficacies of these particles are being studied by administering them via the oral, transdermal and subcutaneous routes. These nanocarriers retain biological activity following a proprietary "one-step" spray drying process which yields dry stable particles. Since these smart particles are positively charged and range between 800nm-2um in size, they are readily phagocytosed by the antigen presenting cells (APC's) to generate a strong immune response for vaccine formulations.

For oral delivery of vaccines, the vaccine antigens are formulated into particles with excellent enteric properties that protect the antigen from the harsh acidic environment of the stomach. These particles are designed to either release their payload in the intestine or to be selectively targeted to the M cells in Peyer's patches of the small intestine with the use of M-cell targeting lectins as an added matrix component in the design of the particles. The unique property of the polymer matrix employed, helps to maintain the particulate nature due to its pH dependent sustained release profile. Immuno-stimulatory cytokines such as Interleukins (IL-2/12) can be added to the polymer matrix to enhance the vaccine response. We have achieved promising results for anti-cancer vaccines such as breast and melanoma, as well as for vaccines against infectious diseases like tuberculosis, typhoid, and hepatitis B. Studies are underway to test vaccines for ovarian and prostate cancer and for infectious diseases such as pneumonia and influenza. We also have facilities to image whole animals/organs/tissues using a whole body Odyssey Infra-Red Bio-Imager (LI-COR Biosystems) that is useful to carry out real time tumor imaging and measurement studies in live animals. Apart from these prophylactic and therapeutic vaccines, bioactive compounds such as insulin and NF-kB have been successfully delivered using these sustained release particulate delivery system.

In addition to the oral route of administration, we are also evaluating our particulate delivery system via the transdermal route combined with Iontophoresis enhancement techniques, These nanoparticulate delivery systems are being tested for vaccine and protein delivery using a truly innovative microneedle pen device (AdminPen[®]) and a microneedle patch device (AdminPatch[®]) in collaboration with NanoBiosciences LLC.

Our research collaborations extend to Emory University and the CDC through grants funded by the

Georgia Research Alliance (GRA), the Georgia Cancer Coalition (GCC) and private and federal funding agencies.

In summary we have demonstrated excellent results using bio-compatible/degradable and non toxic nanocarriers. We welcome collaborations on these and related research areas from academic or industrial collaborators.

38. Deciphering Structural Aspects of Mucin Glycoprotein Recognition

Presenter: David Live, University of Georgia

Mucin glycoprotein domains are components of a number of cell surface molecules and are important contributors to cellular communication, impacting a range of functional and developmental processes. Their underlying protein sequence, with its high density of S and T residues as possible sites of glycosylation, is constant for a particular glycoprotein. However, there are temporal variations of the pendant glycans on these molecules, reflecting the normal or diseased condition of the cell, and stages of cell development. This property makes them valuable biomarkers with therapeutic and diagnostic potential, and has attracted considerable attention because of the linkage of aberrant cell surface mucin glycosylation with cancer. We are therefore interested in the molecular basis for recognition of these glycoconjugate features.

Glycoarrays have emerged as an effective technology for high-throughput screening of molecular recognition, and we have exploited microarrays in determining the recognition elements of mucin structures. Because the complexity of these glycoproteins isolated from natural sources has complicated analysis, the definition of their epitopes has been limited to the minimal element of a Ser or Thr residue and attached carbohydrate, with S/T-?-GalNAc, the Tn antigen, being the simplest element. Using a glycopeptide microarray we have assembled from synthetic glycopeptides, it has been demonstrated that this is inadequate, and that the larger context of glycan and polypeptide is important in conferring specific glycoprotein recognition. These results have important implications in diagnostic applications of anti-mucin antibodies, and in development of glycoconjugate based vaccines for optimally targeting diseases such as cancer.

39. Cryo electron tomography of prokaryotic cells and viruses

Presenter: Jens Michael Holl, Emory University

Caulobacter project: Bacteriophages do not infect mammalian cells, instead targeting bacteria specifically. Emerging research on infectious diseases, biofilm formation and healthcare associated infections takes advantage of the strong bacteriophage specificity and promotes the use of phages as therapeutic strategies for the prevention and treatment of bacterial infections and surface colonization (i.e. phage therapy). Biofilm tolerance to antimicrobial agents and the ability of biofilm-detached cells to elicit disease has encouraged the initiation of research efforts leading towards the use of phage therapy. *Caulobacter crescentus* has become the main model system for examination of the cell cycle in prokaryotic organisms. Each cell division in this organism produces two, morphologically and physiologically distinct daughter cells (a sessile, stalked cell and a motile, swarming, flagellated cell: Figure 2, upper panel) allowing for their easy synchronization and almost pure isolation of each cell type. Importantly, characterization of bacteriophages active against stalk forming bacteria led to the isolation and description of 23 phages infecting the genera *Caulobacter*

and *Asticcacaulis*. One of these phages was fCb13, a large virus with a non-contractile tail (Order Caudovirales) and double stranded DNA (dsDNA).

It is our interest to shed light onto the mechanisms involved in bacterial phage interaction such as adsorption, maturation and cell lysis and liberation of phage progeny. To achieve this goal we use the *C. crescentus* model exploring the ultrastructure of bacterial phage interaction. Examination of *C. crescentus* and its phages (caulophages) will contribute to the elucidation of mechanisms responsible for phage adsorption and maturation during the bacterial cell cycle. Our study constitutes the first known attempt to observe the interaction between bacteria and their phages in a near-native state using cryo-ET.

Neisseria project: *Neisseria meningitidis* serogroup A is responsible for massive epidemics of meningococcal meningitis and septicemia in sub-Saharan Africa. Capsular polysaccharides (CPS), a major virulence factor, of serogroup A are formed from four biosynthetic genes, *sacA-D*. According to a recent study, CPS from *SacD* mutants showed increased immuno-stimulatory activity. Using Cryo-electron tomography (cryo-ET), we seek to characterize the nature of the CPS polymer in wild-type (WT) cells, *SacD*, and CPS-negative mutants, including its physical dimensions and anchorage characteristics in the outer membrane.

Measles virus project: Protein-mediated membrane fusion is essential for vesicular transport between cellular compartments and is employed by a variety of viral families to gain cell entry. However, little is known about the mechanistic principles that govern the formation of functional viral fusion complexes. We have chosen to examine viral fusion in measles virus because many members of the paramyxovirus family constitute major human respiratory pathogens, in particular for the pediatric population. The development of novel vaccines and antivirals targeted to this viral family would significantly reduce childhood illness and mortality rates. In addition, paramyxovirus glycoproteins constitute a particularly powerful model system to examine this mechanism because partial structures of the viral attachment protein, ectodomain structures of the fusion protein in both the metastable prefusion and the final postfusion conformation have been solved. With the ultimate goal of elucidating the basic principles of viral fusion, this project will address two basic questions: What is the geometry of fusogenic membrane glycoprotein complexes in the prefusion conformation? How does receptor-binding result in the triggering of irreversible conformational rearrangements? In order to address these questions, we will probe the geometry of native, hydrated hetero-oligomers in situ through cryo-electron tomography (cryo-ET) of purified MV particles. Available crystal structures of envelope glycoprotein will be superimposed onto reconstructed images to achieve high-resolution hetero-oligomer pseudoatomic structures.

40. *Salmonella* in wildbirds: a reservoir for sporadic human cases of salmonellosis?

Presenter: Susan Sanchez, University of Georgia

Between April and August of 2006, 247 wild songbirds, across 21 sites in northern Georgia, USA, were sampled for *Salmonella*. Three *S. enterica* isolates were obtained from a Brown Thrasher (*Toxostoma rufum*), Northern Cardinal (*Cardinalis cardinalis*), and Carolina Wren (*Thryothorus ludovicianus*); all were obtained from the same location and typed as serovar Muenchen. The overall prevalence of *S. enterica* was 1.2%. Two *S. enterica* Muenchen strains were identified by pulsed-field gel electrophoresis (PFGE); these songbird isolates were unrelated to *S. Muenchen* from other

animal species, but similar to patient isolates present in the CDC PulseNet database. Most human cases of *S. Muenchen* have been observed in the South Atlantic states, paralleling the geographic range of bird species testing positive for *Salmonella*. We suggest that *Salmonella* surveillance in wildlife populations can identify potential sources of infection and indicate the degree to which songbirds maintain pathogens that pose risks for human health

41. Cell Culture and Biomanufacturing Opportunities at the Bioexpression and Fermentation Facility
Presenter: Frank Sugar, University of Georgia

The Bioexpression and Fermentation Facility (BFF) at the University of Georgia provides state-of-the-art equipment and wide-ranging expertise in biotechnological applications to academic and industry clients. In addition to a microbial fermentation suite consisting of fermentors ranging from 1-800 liter scale, our newly-furnished high containment cell culture laboratory is a unique resource offering BSL2 and BSL3 level projects and monoclonal antibody and vaccine production.

42. (No abstract)

43. Cells expressing Indoleamine 2,3 Dioxygenase (IDO) attenuate T cell responses to prophylactic & therapeutic vaccine adjuvants
Presenter: Lei Huang, Medical College of Georgia

Therapeutic vaccines to treat cancer and established (chronic, persistent) infections must overcome innate immunologic resistance ("immune privilege"). Long before clinical presentation chronic inflammation associated with malignancies or infections activates immune regulatory processes that protect developing malignancies and local infections from natural and vaccine induced immunity. Recent reports show that even prophylactic vaccines can stimulate local host regulatory responses that attenuate immune responses and compromise vaccine efficacy.

Cells expressing the tryptophan catabolizing enzyme indoleamine 2,3 dioxygenase (IDO) help create immune privilege at the maternal-fetal interface during pregnancy, at pre-malignant lesions and at sites of microbial infections. IDO may have evolved to protect inflamed tissues from destructive pathology caused by excessive (host) T cell immunity. However IDO also impedes immune responses to therapeutic vaccines. Here we summarize our ongoing studies to elucidate immunologic processes involving IDO that create local immune privilege in inflamed tissues, and the effects of IDO inhibitors on responses to vaccines and vaccine adjuvants.

44. Applications of bacterial ghosts (BGs) in Biomedicine
Presenter: Francis Eko, Morehouse School of Medicine

The bacterial ghost platform is a novel drug/vaccine delivery system endowed with intrinsic adjuvant properties as well as carrier and targeting functions. Bacterial ghosts (BGs) are non-living cells devoid of cytoplasmic contents that retain the morphological characteristics and structural integrity of their living counterparts. They are produced by the controlled expression of a phage lysis gene (PhiX174 gene E) and have a high capacity to simultaneously carry and present multiple antigens to the immune system. BGs are environmentally stable, cold-chain-free lyophilized preparations that provide a simple method for packaging drugs/vaccine antigens for effective delivery. Similar to liposomes in structural organization, the BG platform represents a novel

approach in drug delivery and vaccine development and offers an exceptional opportunity to develop multisubunit or combination vaccines.

45. Structural Investigation of HBsAg: A Progress Report

Presenter: Quentin Florence, University of Georgia

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Hepatitis B virus (HBV) is a blood-born-associated virus that has infected over 2 billion people worldwide. It is also one of the major causes of liver cancer. The overall goal of this study is to provide structural insight of the HBsAg protein, the antigenic component of the currently licensed vaccine for HBV. Despite its worldwide usage and its application as a carrier for epitope presentation, the tertiary structure of this important protein is still unknown. In addition, the lack of a HBsAg structure has (1) prevented us from understanding the effects of mutations on polymerase drug-resistance and on the antigenic properties of the HBsAg major neutralizing antigenic epitope and (2) impeded the development of bivalent vaccines where HBsAg is used as a carrier for foreign antigenic epitopes.

In summary, the results of the proposed studies will provide the basis for understanding how various mutations and fusion of foreign epitopes may affect the tertiary structure of the HBsAg neutralizing epitope. In addition, the studies should provide sufficient understanding of molecular recognition between the immunogenic site ('a'-determinant region) of HBsAg and antibodies. Such knowledge is necessary for the development of improved HBV vaccines, bivalent vaccines and understanding the role of drug-resistant mutations in HBV immune escape.

Our structural studies have been focused on conducting Small Angle X-ray Scattering (SAXS) experiments and the production of diffraction quality crystals for X-ray diffraction experiments. In conjunction with the structural studies, surface plasmon resonance experiments were conducted with HBsAg and a monoclonal antibody to investigate the production of antibody-antigen crystal complexes. Results from SAXS experiments, initial crystallization screens, and surface plasmon resonance experiments will be presented.

46. Sulfoglucuronosyl paragloboside expression under inflammatory conditions is regulated via NFκB signaling in human cerebrovascular endothelial cells

Presenter: Robert Yu, Medical College of Georgia

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Inflammatory cytokines such as TNF α and IL-1 β stimulate glucuronosyltransferase genes (GlcAT-S and GlcAT-P) and elevate sulfoglucuronosyl paragloboside (SGPG) synthesis in a human cerebrovascular endothelial (SV-HCEC) cell line; such stimulation also promotes T cell adhesion [Dasgupta et al. J. Neurosci. Res. 85:1086, 2007]. Transfecting SV-HCECs with a SGPG-specific inhibitor, such as HNK-1 sulfotransferase (HNK-1ST) siRNA, downregulates the SGPG expression by suppressing three genes, HNK-1ST, GlcAT-S and GlcAT-P and this suppression mechanism proceeds

via NF κ B signaling. HNK-1ST siRNA- transfection not only down regulates NF κ B signaling in SV-HCECs, but also reduces the TNF α /IL-1 β -stimulated NF κ B signaling [Dasgupta et al. J. Neurosci. Res. 2009, in press]. To delineate the molecular mechanism of NF κ B signaling with SGPG regulation, we have investigated additional signaling pathways, including I κ B, ERK, and Akt phosphorylation. HNK-1ST siRNA transfection inhibited I κ B and Akt phosphorylation, but not ERK activation. Although I κ B was immediately activated (within 30 minutes) with addition of both cytokines, the optimal time of activation varied. For example, IL-1 β -mediated I κ B activation peaked between 2-4 h while TNF α stimulation required 8 h for peak activation. Understanding the precise regulation of SGPG expression via NF κ B signaling and its participation as a T-cell ligand should lead to the development of specific anti-inflammatory agents in the nervous system (Supported by NIH NS11853, NS26994).

47. Inhibition of sodium ion channel activity by anti-lipid A antibody of a chicken with campylobacteriosis

Presenter: Robert Yu, Medical College of Georgia

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A microbiologically confirmed strain VLA2/18 of *Campylobacter jejuni* (non-serotyped) was isolated from a patient with campylobacteriosis. The serum of chicken infected by VLA2/18 contained a high-titer anti-Lipid A antibody (anti-Lipid A Ab) against Lipid A from VLA2/18. The innate immune systems of humans and other animals are activated by lipopolysaccharides (LPSs) of Gram-negative bacteria. Kdo2-Lipid A is a minimal structure of Toll-Like Receptor 4 ligand required for activation of human macrophages. Our chicken serum also reacted with Kdo2-Lipid A as a cross-reacting antigen. We studied the effect of the chicken anti-Lipid A Ab on voltage-gated Na currents in whole-cell patch-clamped NSC-34 cells. A decrease of Na current was observed, which was dependent only on the anti-Kdo2-Lipid A Ab activity in the chicken serum. Based on experiments using tunicamycin-treated cells, anti-Kdo2-Lipid A Ab was shown to induce a functional inhibition of Na channels (Nav) at the protein portion instead of the N-glycan portion. We hypothesized that certain epitopes of the extracellular domains of Nav channels might cross-react with the anti-Lipid A AB. Using a molecular overlapping method; we showed that certain dipeptides of the extracellular Nav channel domains are homologous to the Lipid A structure. Since the lipooligosaccharides (LOSs) of *C. jejuni* are thought to play an important role in the pathogenesis of Guillain-Barré syndrome (GBS), our finding suggests that the anti-Lipid A Ab, generated in the infected chicken, may cross-react with the dipeptides, resulting in depression of the Nav function to account for some of the neurophysiological changes in GBS. (Supported by USPHS RO1 NS26994-20 to RKY)