2012
International Symposium
on Molecular Medicine
and Infectious Disease
2012 International Symposium on Molecular Medicine and Infectious Disease

Drexel University College of Medicine, Philadelphia, PA
June 19-21, 2012

The Annual Symposium of the Institute for Molecular Medicine & Infectious Disease

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Director’s Message

I would like to welcome everyone to the 2012 International Symposium on Molecular Medicine and Infectious Disease at Drexel University College of Medicine. This is an exciting time at Drexel University and the College of Medicine as we embark on the implementation of our new strategic plan and continue to expand and improve the quality of everything we do in meaningful and engaging ways with our colleagues, collaborators, and the surrounding community. This Symposium will be the first in what will become an Annual Symposium Series of the College’s Institute for Molecular Medicine and Infectious Disease. The Symposium will serve to highlight the scientific achievements of the Institute’s faculty, postdoctoral fellows, graduate students, and research staff relating to molecular mechanisms, diagnosis, treatment, and prevention of infectious, inflammatory, oncogenic, and metabolic disease. In what will become an annual tradition, the Symposium will also recognize seminal scientific achievements in the form of Drexel Prizes in Infectious Disease, Immunology, and Translational Medicine. The quality of the 2012 selections makes a very strong statement in this regard. At the heart of the Institute for Molecular Medicine and Infectious Disease is the performance and promotion of basic scientific discovery and the advancement of these discoveries by utilizing innovative collaborations within academia and with the pharmaceutical industry to translate today’s discoveries into tomorrow’s products to diagnose, prevent and treat human disease. This approach is at the cultural heart of the Drexel University scientific community. In conclusion, I want to thank the more than 60 Drexel scientists that were involved in speaker selection and acknowledge the sponsorship of the Dean’s Office, the Office of the Vice Dean for Research, and several industrial sponsors, including TherimuneX Pharmaceuticals, Inc., and Chemalytics.

Brian Wigdahl, Ph.D., Professor and Chair
Department of Microbiology and Immunology
Director, Institute for Molecular Medicine and Infectious Disease
2012 International Symposium
on Molecular Medicine and Infectious Disease

Drexel University College of Medicine, Philadelphia, PA

June 19-21, 2012

The Annual Symposium of the Institute for Molecular Medicine & Infectious Disease

About the Symposium
Speakers selected by the Organizing Committee represent Drexel University, including ten basic and clinical science departments in the College of Medicine, College of Arts and Sciences, School of Public Health, and School of Biomedical Engineering, Science and Health Systems; Thomas Jefferson Medical College; the University of Pennsylvania School of Medicine; Temple University School of Medicine; Johns Hopkins University; and 20 other academic research centers across the country, as well as the National Institutes of Health.

About Drexel University College of Medicine
Drexel University College of Medicine has established some of the most highly innovative and rigorous academic programs available today, incorporating the University’s expertise in engineering and technology into traditional medical training. The College of Medicine is home to one of the nation’s leading centers for spinal cord research; one of the foremost centers for malaria study; and an outstanding HIV/AIDS program with extensive NIH-funded research in prevention and therapeutic interventions. Drexel University College of Medicine has been designated a Vanguard National Center of Excellence in Women’s Health by the U.S. Department of Health & Human Services, and is highly respected in numerous other specialties including cardiology and pain management. Visit www.drexelmed.edu for more information.
About the Institute for Molecular Medicine and Infectious Disease

Director, Brian Wigdahl, Ph.D.

Research Centers of Excellence
Cancer Biology
Molecular Parasitology
Virology & Therapeutics
Immunology & Vaccine Science
Clinical & Translational Research
Molecular & Functional Genomics
International Research & Medicine
Bacterial Pathogenesis & Biodefense
Neuroimmunology & CNS Therapeutics
Immunogenetics & Inflammatory Disease
Molecular Virology & Translational Neuroscience
Scientific Administration & Training
Scientific Communication & Outreach

Mission Statement
The Drexel University College of Medicine Institute for Molecular Medicine and Infectious Disease is a collaborative inter-campus enterprise focused on research, diagnosis, treatment, prevention, and clinical management of infectious, inflammatory, oncogenic, and metabolic disease.

For more information on the Institute for Molecular Medicine and Infectious Disease, visit www.drexelmed.edu/immid
2012 International Symposium on Molecular Medicine and Infectious Disease Committees

Executive Committee
Brian Wigdahl (Chair), Elizabeth Blankenhorn, Timothy Block, Irwin Chaiken, Thomas Edlind, Garth Erhlich, Jeffrey Jacobson, Peter Katsikis, Fred Krebs, Raphael Lukov, Julio Martin-Garcia, Anand Mehta, Olimpia Meucci, Michael Nonnemacher, Richard Rest, Aydin Tozeren, Akhil Vaidya, and Barry Waterhouse

Organizing Committee
Brian Wigdahl (Chair), Carol Artlett, Peter Bass, Lawrence Bergman, Elizabeth Blankenhorn, Timothy Block, Michael Bouchard, James Burns, Irwin Chaiken, Thomas Edlind, Garth Erhlich, Haitao Guo, Fred Krebs, Uri Hershberg, Jeffrey Jacobson, Pooja Jain, Suresh Joshi, Peter Katsikis, Sandhya Kortagere, Michele Kutzler, Patrick Loll, Julio Martin-Garcia, Anand Mehta, Olimpia Meucci, Sonia Navas-Martin, Shira Ninio, Michael Nonnemacher, Vanessa Pirrone, Patrick Romano, Christian Sell, Ying-Hsiu Su, Claudio Torres, Aydin Tozeren, Jeffrey Twiss, Richard Rest, Sandra Urdaneta-Hartmann, Akhil Vaidya, Barry Waterhouse

Session 1 selection committee:
Julio Martin-Garcia, Barry Waterhouse, Olimpia Meucci, Michael Nonnemacher, Jeff Twiss, and Peter Bass

Session 2 selection committee:
Garth Ehrlich, Jane Clifford, Lawrence Bergman, James Barrett

Session 3 selection committee:
Lawrence Bergman, Akhil Vaidya, James Burns, Sandhya Kortagere, and Jeffrey Jacobson

Session 4 selection committee:
Richard Rest, Shira Ninio, Patrick Loll, and Suresh Joshi

Session 5 selection committee:
Akhil Vaidya, Patrick Loll, Lawrence Bergman, Sandhya Kortagere, and Michele Kutzler

Session 6 selection committee:
Olimpia Meucci, Jeffrey Jacobson, Michele Kutzler, Michael Nonnemacher, and Brian Wigdahl

Session 7 selection committee:
Jeffrey Jacobson, Timothy Block, Thomas Edlind, and Akhil Vaidya

Session 8 selection committee:
Elizabeth Blankenhorn, Peter Katsikis, Carol Artlett, Uri Hershberg, and Jeffrey Jacobson

Session 9 selection committee:
Timothy Block, Michael Bouchard, Haitao Guo, Anand Mehta, and Sonia Navas-Martin

Session 10 selection committee:
Anand Mehta, Michael Bouchard, Peter Katsikis, and Pooja Jain

Session 11 selection committee:
Anand Mehta, Vanessa Pirrone, Pamela Norton, Ying-Hsiu Su, and Aydin Tozeren

Session 12 selection committee:
Peter Katsikis, Elizabeth Blankenhorn, Carol Artlett, and Michele Kutzler

Session 13 selection committee:
Irwin Chaiken, Peter Katsikis, Jeffrey Jacobson, Fred Krebs, and Sandra Urdaneta-Hartmann

Session 14 selection committee:
Jeffrey Jacobson, Barry Waterhouse, Claudio Torres, Christian Sell, and Vanessa Pirrone

Session 15 selection committee:
Garth Ehrlich, Lawrence Bergman, Elizabeth Blankenhorn, Michael Nonnemacher, and Aydin Tozeren
Symposium Agenda

Tuesday, June 19, 2012

7:30-9:00 am  Registration and Continental Breakfast

9:00-9:10 am  Introductory Remarks

Brian Wigdahl, Ph.D.
Director, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA

Daniel Schidlow, M.D.
Interim Dean, Drexel University College of Medicine, Philadelphia, PA

Kenny Simansky, Ph.D.
Vice Dean for Research
Drexel University College of Medicine, Philadelphia, PA

9:10-11:00 am  Session 1: HIV/AIDS, brain impairment, and neuroprotection

Co-Chairs: Julio Martin-Garcia, Barry Waterhouse

9:10-9:40 am  Avindra Nath, M.D., Senior Investigator and Director, Section of Infections of the Nervous System, National Institutes of Health, Bethesda, MD

HIV reservoir in the brain: Can it be cured?

9:45-10:05 am  Dennis Kolson, M.D., Ph.D., Professor, Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA

Human immunodeficiency virus-mediated neurodegeneration: Therapeutic targeting of the macrophage anti-oxidant response

10:10-10:30 am  Timothy J. Cunningham, Ph.D., Professor, Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA

Inflammation and neuron death: Therapeutic studies with secreted phospholipase A2 inhibitors

10:35-10:55 am  Olimpia Meucci, M.D., Ph.D., Professor, Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA

Neuroprotective function of the chemokine CXCL12 and its potential role in HIV-associated neurocognitive disorders

11:00-12:00 noon  Session 2: Drexel Prize in Translational Medicine

Introduction: Kenny Simansky, Noshin Kathuria

Paul Offit, M.D., Chief, Division of Infectious Diseases, Professor of Pediatrics, and Director, Vaccine Education Center, Children's Hospital of Philadelphia, Philadelphia, PA

The rotavirus vaccine: From bench to bedside

12:00-1:30 pm  Lunch and Poster Session 1

18th floor, New College Building
<table>
<thead>
<tr>
<th>Time</th>
<th>Session 3: Molecular mechanisms of malarial disease</th>
<th>Session 4: Pathogenic mechanisms of bacterial disease</th>
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<tbody>
<tr>
<td>1:30-3:00 pm</td>
<td>Co-Chairs: Heather Painter, Akhil Vaidya</td>
<td>Co-Chairs: Garth Erhlich, Richard Rest</td>
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<td>1:30-1:55 pm</td>
<td>Kiaran Kirk, Ph.D., Professor, Research School of Biology, The Australian National University, Canberra, Australia</td>
<td>David Mosser, Ph.D., Professor, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD</td>
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<td>A sodium pump as an antimalarial drug target</td>
<td>The role of macrophages in promoting and preventing inflammatory diseases</td>
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<td>2:00-2:20 pm</td>
<td>Lawrence W. Bergman, Ph.D., Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA</td>
<td>Jacob Russell, Ph.D., Assistant Professor, Department of Biology, College of Arts and Sciences, Drexel University, Philadelphia, PA</td>
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<td>A new myosin motor in malaria parasites</td>
<td>Exploring the microbiomes of insect guts: Effects of diet and relatedness on the composition of symbiotic communities</td>
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<td>2:25-2:40 pm</td>
<td>Michael Mather, Ph.D., Research Assistant Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA</td>
<td>Shira Ninio, Ph.D., Assistant Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA</td>
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<td>The unusual ATP synthase complexes of a ciliate and a malaria parasite: Evolution of a central player in bioenergetics</td>
<td>Novel virulence determinants of Legionella pneumophila identified using an injected bacterial protein</td>
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<td>2:45-2:55 pm</td>
<td>Elamaran Meibalan, M.S., Graduate Student, Microbiology and Immunology Graduate Program, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA</td>
<td>Jeffrey P. Holt, Graduate Student in Biological Sciences, College of Arts and Sciences, Drexel University, Philadelphia, PA</td>
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<td>Trafficking in Plasmodium yoelii: Export of proteins from the intracellular malarial parasite to the host reticulocyte membrane</td>
<td>Identification of a phase-variable restriction barrier of Campylobacter jejuni</td>
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5:30-5:50 pm  Purnima Bhanot, Ph.D., Assistant Professor, Department of Microbiology and Molecular Genetics, UMDNJ – New Jersey Medical School, Newark, NJ  
Preventing malaria infection by targeting plasmodium pre-erythrocytic stages

5:55-6:15 pm  Akhil Vaidya, Ph.D., Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA  
Molecular pathways targeted by novel antimalarial drugs

6:20-6:30 pm  Scott Baliban, Graduate Student, Microbiology and Immunology Graduate Program, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA  
Toxin-based DNA vaccination for the prevention of Clostridium difficile-associated disease

Wednesday, June 20, 2012

8:00-9:00 am  Continental Breakfast

9:00-11:00 am  Session 6: HIV/AIDS and substance abuse  
Co-Chairs: Olimpia Meucci, Jay Rappaport

9:00-9:25 am  Sabita Roy, Ph.D., Professor, Department of Surgery, and Director, Division of Infection, Inflammation and Vascular Biology, University of Minnesota, Minneapolis, MN  
Morphine induction of toll-like receptors on microglial cells potentiates HIV-1 TAT induced neuropathogenesis in a pneumococcal pneumoniae co-infection model

9:35-10:00 am  Ellen Unterwald, Ph.D., Professor, Department of Pharmacology, and Director, Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA  
Modulation of cocaine-induced behaviors and neurotransmission by the chemokine CXCL12

10:05-10:25 am  Tracy Fischer-Smith, Ph.D., Assistant Professor, Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA  
Morphine and SIV-mediated neuroimmune modulation in rhesus macaques

10:30-10:40 am  Nirzari Parikh, M.S., Graduate Student, Molecular and Cellular Biology and Genetics Program, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA  
Cocaine alters the immunomodulatory profile in HIV-1-infected patients

10:45-10:55 am  Jonathan Pitcher, MD/PhD Student, Microbiology and Immunology Graduate Program, Department of Pharmacology, Drexel University College of Medicine, Philadelphia, PA  
Morphine-induced alteration of ferritin heavy chain and disruption of the chemokine receptor CXCR4 in neurons

11:00-12:00 noon  Session 7: Drexel Prize in Infectious Disease  
Introduction: Richard Rest, Jennifer Kress-Bennett

John Mekalanos, Ph.D., Professor and Chair, Department of Microbiology and Immunology, Harvard Medical School, Boston, MA  
A view to a kill: Molecular and cellular interactions in pathogenesis
12:00-1:30 pm  Lunch and Poster Session 2  
18th floor, New College Building

1:30-3:00 pm  Session 8: Molecular mechanisms of inflammation  
Co-Chair: Stephen Jennings, Peter Katsikis,

1:30-1:55 pm  Thirumala-Devi Kanneganti, Ph.D., Associate Member, Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN  
Regulators of inflammatory responses

2:00-2:20 pm  Carol M. Arlett, Ph.D., Associate Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA  
Inflammasome activation in fibrotic disease

2:25-2:40 pm  Uri Hershberg, Ph.D., Assistant Professor, School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA  
Detecting inflammatory diversification of the B cell repertoire at the level of a single clone and as a whole

2:45-2:55 pm  Alina Boesteanu, Ph.D., Research Assistant Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA  
The role of PI3K p110delta isoform in reducing morbidity and mortality in Influenza virus-infected mice

3:00-4:30 pm  Session 9: Pathogenic mechanisms of hepatitis B/C  
Co-Chairs: Michael Bouchard, Laura Steel

3:00-3:25 pm  David B. Weiner, Ph.D., Professor, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA  
Synthetic DNA vaccine approaches for difficult pathogens

3:30-3:50 pm  Anand Mehta, D. Phil., Associate Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Doylestown, PA  
Human hepatitis B virus utilizes a genetic approach to limit antigen presentation

3:55-4:10 pm  Sonia Navas-Martin, Ph.D., Assistant Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA  
Hepatitis C virus and macrophages

4:15-4:25 pm  Siddhartha Rawat, Graduate Student, Molecular and Cell Biology and Genetics Program, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA  
Akt and NF-κB pathways differentially regulate HBV replication

4:30-5:00 pm  Break

5:00-6:40 pm  Session 10: Viral oncogenic disease  
Co-Chairs: Jennifer Gordon, Brian Wigdahl

5:00-5:25 pm  Kamel Khalili, Ph.D., Laura H. Carnell Professor of Neuroscience, Chair, Department of Neuroscience, and Director, Center for Neurovirology, Temple University School of Medicine, Philadelphia, PA  
Viral oncogenesis in brain: Lesson from human neurotrophic virus, JCV
5:30-5:55 pm  James C. Alwine, Ph.D., Professor, Department of Cancer Biology, University of Pennsylvania School of Medicine, Philadelphia, PA
Dealing with stress: The maintenance of mTOR kinase activity during human cytomegalovirus infection

6:00-6:15 pm  Michael Bouchard, Ph.D., Associate Professor, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA
Hepatitis B virus regulation of calcium signaling: a potential factor in the development of hepatitis B virus associated liver cancer

6:20-6:35 pm  Pooja Jain, Ph.D., Associate Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Doylestown, PA
The dynamic interaction of Tax, RNAi, and chromatin remodelers determines the fate of HTLV-1 infection

Thursday, June 21, 2012

8:00-9:00 am  Continental Breakfast

9:00-11:00 am  Session 11: Biomarkers of human infectious disease and cancer
Co-Chairs: Anand Mehta, Ying-Hsiu Su

9:00-9:25 am  Richard G. Pestell, M.D., Ph.D., Professor and Chairman, Department of Cancer Biology, Jefferson Medical College, and Director, Kimmel Cancer Center at Jefferson, Thomas Jefferson University, Philadelphia, PA
Novel roles of cyclins in cancer

9:30-9:50 am  Lynn Pulliam, Ph.D., Professor of Laboratory Medicine and Medicine, University of California, San Francisco; Chief, Microbiology, Veterans Affairs Medical Center, San Francisco, CA
Search for predictive biomarkers of neurocognitive impairment in HIV and/or HCV infections

9:55-10:15 am  Avi Ma’ayan, Ph.D., Assistant Professor, Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, Icahn Medicine Institute, New York, NY
Data mining strategies in systems biology and systems pharmacology

10:20-10:35 am  Peter Clark, Ph.D., Postdoctoral Fellow, Computational Medicine Center, Thomas Jefferson University, Philadelphia, PA
Comprehensive transcriptome profiling of colorectal cancer Dukes staging reveals miRNA interactions and phenotype markers

10:40-10:55 am  Surbhi Jain, Ph.D., Postdoctoral Fellow, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA
Epigenetic modifications of the viral and host DNA in HCC: A biomarker perspective
11:00-12:00 noon  
**Session 12: Drexel Prize in Immunology**  
Introduction: Peter Katsikis, Donald Gracias

**Bruce Beutler, M.D., 2011 Nobel Prize in Physiology or Medicine Recipient**, Professor and Director, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX

*How mammals sense infection*

12:00-1:30 pm  
**Lunch and Poster Session 3**  
18th floor, New College Building

1:30-3:00 pm  
**Session 13: Treatment and prevention of HIV disease**  
Co-Chairs: Irwin Chaiken, Fred Krebs

1:30-1:55 pm  
**Phalguni Gupta, Ph.D.,** Professor and Assistant Chair, Department of Infectious Disease and Microbiology, University of Pittsburgh, Pittsburgh, PA

*Development of a ring formulated microbicide against HIV*

2:00-2:20 pm  
**Peter Katsikis, M.D., Ph.D.,** Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA

*Targeting HIV-1 and TLR7/9 as a microbicide strategy*

2:25-2:40 pm  
**Michele Kutzler, Ph.D.,** Assistant Professor, Department of Medicine, Division of Infectious Diseases and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA

*Improving prophylactic HIV-1 DNA vaccines through optimization, delivery and immunoadjuvants: From bench to bedside*

2:45-2:55 pm  
**Rosemary Bastian,** Graduate Student, Biomedical Engineering Program, School of Biomedical Engineering, Science and Health Systems, Drexel University, and Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA

*HIV-1 inactivation and virolysis by gp120 targeting before host cell exposure*

3:00-4:35 pm  
**Session 14: Impact of aging on HIV disease**  
Co-Chairs: Andres Kriete, Vanessa Pirrone

3:00-3:30 pm  
**Rita Effros, Ph.D.,** Professor, Department of Pathology and Laboratory Medicine, University of California, Los Angeles, David Geffen School of Medicine at UCLA, Los Angeles, CA

*HIV/AIDS: accelerated aging of the adaptive immune system*

3:35-4:00 pm  
**Donna Murasko, Ph.D.,** Professor, Department of Biology, and Dean, College of Arts and Sciences, Drexel University, Philadelphia, PA

*Changes in immune response with age: Nature or nurture?*

4:05-4:30 pm  
**Claudio Torres, Ph.D.,** Research Assistant Professor, Department of Pathology and Laboratory Medicine, Drexel University College of Medicine, Philadelphia, PA

*Astrocyte senescence and neurodegenerative disease: Implications for HIV-associated neurocognitive disorder*

4:35-5:00 pm  
Break
5:00-6:30 pm  Session 15: Molecular genetics and infectious disease
Co-Chairs: Jane Clifford, Brian Moldover

5:00-5:25 pm  Garth D. Ehrlich, Ph.D., Executive Director, Center for Genomic Sciences, Allegheny-Singer Research Institute, Pittsburgh, PA
Development of global unbiased rational approaches for identifying bacterial virulence genes

5:30-5:50 pm  Aydin Tozeren, Ph.D., Distinguished Professor and Director, Center for Integrated Bioinformatics, School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA
Bioinformatics as a tool for deciphering the grammar of crosstalk between host and pathogen

5:55-6:10 pm  Michael Nonnemacher, Ph.D., Assistant Professor, Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA
HIV-1 LTR polymorphisms associated with alterations in clinical course and neurologic disease

6:15-6:25 pm  Jennifer Kress-Bennett, Graduate Student, Microbiology and Immunology Graduate Program, Center for Genomic Sciences, Allegheny Singer Research Institute, Pittsburgh, PA; and Drexel University College of Medicine, Philadelphia, PA
Large-scale comparative genomics analysis provided for the characterization of a novel gene family in Haemophilus influenzae associated with invasiveness
Drexel Prize in Translational Medicine

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<tr>
<th>Paul Offit, M.D.</th>
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<tr>
<td>Chief, Division of Infectious Diseases</td>
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<tr>
<td>Director, Vaccine Education Center</td>
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<td>Children's Hospital of Philadelphia</td>
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<td>Philadelphia, PA, USA</td>
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Maurice R. Hilleman Professor of Vaccinology  
Professor of Pediatrics  
University of Pennsylvania School of Medicine  
Philadelphia, PA, USA

The rotavirus vaccine: From bench to bedside

Paul A. Offit, M.D., is the Chief of the Division of Infectious Diseases and the Director of the Vaccine Education Center at the Children's Hospital of Philadelphia. In addition, Dr. Offit is the Maurice R. Hilleman Professor of Vaccinology and a Professor of Pediatrics at the University of Pennsylvania School of Medicine. He is a recipient of many awards, including the J. Edmund Bradley Prize for Excellence in Pediatrics from the University of Maryland Medical School, the Young Investigator Award in Vaccine Development from the Infectious Diseases Society of America, and a Research Career Development Award from the National Institutes of Health. He is also the co-inventor of the rotavirus vaccine, RotaTeq, recommended for universal use in infants by the Centers for Disease Control and Prevention (CDC). For this achievement Dr. Offit received the Luigi Mastroianni and William Osler awards from the University of Pennsylvania School of Medicine, and the Charles Mérieux Award from the National Foundation for Infectious Diseases, and was honored by Bill and Melinda Gates during the launch of their foundation’s Living Proof Project for global health. In 2009, Dr. Offit received the President’s Certificate for Outstanding Service from the American Academy of Pediatrics. In 2011, Dr. Offit received the Humanitarian of the Year Award from the Biologics Industry Organization (BIO), the Distinguished Medical Achievement Award from the College of Physicians of Philadelphia, the David E. Rogers Award from the Association of American Medical Colleges, and the Odyssey Award from the Center for Medicine in the Public Interest, and was elected to the Institute of Medicine of the National Academy of Sciences. Dr. Offit was a member of the Advisory Committee on Immunization Practices to the CDC and is a founding advisory board member of the Autism Science Foundation and the Foundation for Vaccine Research. He is also the author of five medical narratives, including Vaccinated: One Man's Quest to Defeat the World's Deadliest Diseases (HarperCollins, 2007), for which he won an award from the American Medical Writers Association.
John Mekalanos, Ph.D., is the Adele H. Lehman Professor of Microbiology and Molecular Genetics at Harvard Medical School and has served as chair of the Department of Microbiology and Immunobiology since 1996. His research has involved multiple facets of bacterial pathogenesis with an emphasis on using genetic and functional genomic approaches to explore virulence gene regulation and host-pathogen interactions. Dr. Mekalanos has been at the forefront of cholera research from his graduate studies at UCLA in 1974-78 to the present. The Mekalanos group has provided classic insights such as the identification of the regulatory factors that control production of both cholera toxin and the intestinal colonization factor TCP, identification of the filamentous bacteriophage that carries the genes for cholera toxin, development of reporters for virulence gene expression in vivo, and identification of small molecules that inhibit virulence in expression during infection. They have developed prototype vaccines effective against cholera, typhoid, anthrax and other encapsulated microorganisms, as well as providing evidence that bacteriophages control cholera epidemics in natural endemic settings. Recent work in his laboratory led to the identification of type VI secretion systems and dynamic phage-related organelles found in numerous bacteria that inject proteins into target eukaryotic and bacterial cells. Dr. Mekalanos has received many honors, including election to the National Academy of Sciences and the American Society for Microbiology; the American Society for Microbiology Eli Lilly Award, the American Association for the Advancement of Science Newcomb Cleveland Prize, and the City of Medicine Award. He has been a member of the FDA Advisory Committee on Vaccines and Related Biologics, and has consulted for numerous governmental and private agencies, including the National Institutes of Health, the World Health Organization, the International Vaccine Institute, the National Academy of Sciences, Massachusetts Public Health Biological Laboratories, and the US-Japan Cooperative Medical Science Program.
Bruce Beutler, M.D., Regental Professor and Director
Center for the Genetics of Host Defense
Raymond and Ellen Willie Distinguished Chair in Cancer Research
UT Southwestern Medical Center, Dallas, TX, USA

Recipient of The Nobel Prize in Physiology or Medicine 2011

How mammals sense infection

Bruce Beutler, M.D., was born in 1957, in Chicago, and grew up in southern California. He received his undergraduate degree from UCSD in 1976 and his medical degree from the University of Chicago in 1981. After medical school, he completed two years of residency at UT Southwestern Medical Center in Dallas, studying internal medicine and neurology. He was a postdoctoral fellow and an assistant professor at the Rockefeller University from 1983 to 1986. During those years, he isolated mouse tumor necrosis factor and established that TNF acts as a key executor of the inflammatory response. Returning to UT Southwestern in 1986 as an Howard Hughes Medical Institute investigator, he designed a recombinant inhibitor of TNF that became widely used in clinical practice as Enbrel, or Etanercept. Between 1993 and 1998, he used a classical genetic approach to identify the mammalian LPS receptor. His discovery was recognized 13 years later by the 2011 Nobel Prize in Physiology or Medicine. Moving to the Scripps Research Institute in 2000, Beutler developed the largest mouse mutagenesis program in the world, and applied a forward genetic approach to decipher the signaling pathways activated by toll-like receptors (TLRs). He also identified many other molecules with non-redundant function in the immune response. Beutler returned to UT Southwestern once again in 2011, and is currently a Regental Professor and Director of the Center for Genetics of Host Defense. He also holds the Raymond and Ellen Willie Distinguished Chair in Cancer Research in Honor of Laverne and Raymond Willie, Sr. Prior to the Nobel Prize, Beutler received the Shaw Prize (2011), the Albany Medical Center Prize (2009), the Frederik B. Bang Award (2008), the Balzan Prize (2007), the Gran Prix Charles-Leopold Mayer (2006), the William B. Coley Award (2005), the Robert Koch Prize (2004), and other honors recognizing his many contributions to the field of innate immunity. In 2008 he was elected to both the National Academy of Sciences and the Institute of Medicine.
A1

Comprehensive NeuroAIDS Center

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The Comprehensive NeuroAIDS Center (CNAC) at Temple University is one of nine Centers of its kind in the United States funded by the National Institute of Mental Health. It was founded in 2011. CNAC's mission is to improve and extend public health impact of bench-to-clinic research associated with HIV-induced neurological diseases and cognitive disorders. Neurological complications and cognitive disorders are among the most devastating clinical manifestations of HIV-1 infection in AIDS patients today – including patients receiving treatment with antiretroviral therapy. In the Philadelphia area, the rate of HIV-1 infection is five times the national average, with more than 19,000 individuals currently infected with the virus. Temple University Hospital's Comprehensive HIV Program treats more than 10% of AIDS patients in Philadelphia, and 10% of its inpatient neurology discharges are HIV-1 positive individuals with neurological disorders. CNAC's goal is to improve and extend the public health benefits of bench-to-clinic research related to NeuroAIDS in the Philadelphia region and beyond. To this end, CNAC brings together a large group of AIDS and neuroscience investigators based at Temple and other leading academic medical centers. CNAC offers expert research services and technologies, an established clinical HIV program including neuropsychiatric and neurocognitive assessment, mentorship/training programs, and seed funding for new translational research projects addressing NeuroAIDS and HIV-1 associated neurocognitive and behavioral disorders. CNAC offers a multidisciplinary research approach by providing scientific services in cell culture and neurotropic viruses, animal model development and behavioral testing, proteomic biomarker discovery, as well as expertise and consultation in neuroscience and neuropathology. Overall, CNAC’s strategy is to achieve success in advancing HIV and AIDS research by providing services to the scientific community and fostering translational collaborative efforts for worldwide NeuroAIDS research. CNAC is integrated with the Temple/Drexel Interdisciplinary and Translational Research Training Program in NeuroAIDS and efforts are under way to partner with other related Centers and Training programs nationally.

For more information visit: www.temple.edu/medicine/cnac

A2

Interdisciplinary and Translational Research Training Program in NeuroAIDS

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The Interdisciplinary and Translational Research Training Program (ITRTP) for predoctoral students in NeuroAIDS and related areas of research is a joint program from two institutions, Temple University and Drexel University, located in close proximity in Philadelphia, Pennsylvania. The program creates a city-wide interdisciplinary and translational research training program in NeuroAIDS through shared resources, joint research seminar series, journal club, symposia, invited speakers, thesis mentoring and educational opportunities at both institutions. The graduate curriculum at both institutions is designed to provide a broad based scientific foundation in biomedical science including Neuroscience, Immunology,
Microbiology, Pharmacology and Physiology. This curriculum including Scientific Communication, Scientific Integrity and Bioethics, and Statistics, as well as courses in Molecular and Cellular Neurobiology and Pathogenesis of Neurobiological Diseases prepares graduate students for thesis research in NeuroAIDS. Within Temple University School of Medicine, research opportunities in NeuroAIDS and related areas are available within the Department of Neuroscience, Department of Microbiology and Immunology, Department of Pharmacology, and Department of Physiology with additional opportunities in the School of Engineering. At Drexel University College of Medicine, research opportunities in NeuroAIDS and related areas are available in the Department of Microbiology and Immunology, Department of Neurobiology and Anatomy, Department of Pharmacology and Physiology and Department of Biochemistry and Molecular Biology. Additional research opportunities are available in the School of Biomedical Engineering and Health Science Systems. This program brings together multiple biomedical basic science departments at two institutions, and integrates joint training activities with nearby University of Pennsylvania. With the inclusion of clinical AIDS investigators, the program is not only interdisciplinary, but exposes students to training in neuroAIDS basic sciences, AIDS, and NeuroAIDS related clinical perspectives. This training program interdigitates closely with the NIMH supported Temple/Drexel Comprehensive NeuroAIDS Center (CNAC). Overall, this program provides formal comprehensive training in neuroAIDS in areas ranging from molecular studies, in vitro systems, and pathogenesis studies in human and non-human systems. Future efforts will expand the training activities of this T32 program in the development of a comprehensive neuroAIDS training course in collaboration with CNAC and other related T32 programs nationally.

A3

Immunogenicity of an engineered HIV-1 Clade A consensus-based envelope DNA vaccine

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Sub-Saharan Africa remains the most heavily affected region accounting for 71% of all new HIV infections in 2008. The majority of infections in the region (56%) were estimated to be caused by subtype C, with smaller proportions caused by other subtypes, including 14% by subtype A. These high prevalence rates underscore the need for an effective vaccine that could impact the spread of HIV-1. DNA vaccines require engineering in order to generate strong cellular responses in both non-human primates and humans. In this study, we designed a HIV-1 clade A envelope construct (EY1E1-A) to decrease the genetic distances of virus isolates within the clade through the use of consensus sequences that were optimized for increased expression. When studied as a DNA vaccine delivered intramuscularly followed by in vivo electroporation in Balb/c mice, we observed a significant antigen-specific cellular immune response as measured by IFN-γ ELISpot supporting immunogenicity in mice. Importantly this construct not only induces stronger cross-reactive cellular responses within clade A, it also induces immune responses against clades B and C envelope peptide pools. Epitope mapping demonstrated that EY1E1-A was able to induce clade A envelope-specific immune responses against 12 out of 28 peptide pools, indicating breadth of induced immune responses. In addition, frequencies of antigen-specific CD3⁺/CD4⁺ and CD3⁺CD8⁺ T-cells that secreted HIV-1 peptide induced IFN-γ, IL-2 and TNF-α were detected in the spleen of immunized mice when compared to control vaccinated supporting a functional antigen specific response. Anti-envA antibody responses were measured and show that the engineered consensus A gene is capable of eliciting antigen specific IgG. These findings suggest that synthetic HIV-1 Env clade A immunogens should be examined for their potential as part of an efficient HIV DNA vaccine in humans. This work is supported by the following NIH/NIAIDS grants: PO1AI071739 HIVRAD and U19AI078675-01 (MK/DBW).
**A4**

Toll-like receptor 2 is required for complete protection against lethal Herpes simplex virus type 1 (HSV-1) encephalitis in a murine lip scarification model

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HSV can lead to a lethal encephalitis in rare instances. The immediate recognition of HSV-1 by pattern recognition receptors is crucial for establishing an antiviral state and limiting nervous system involvement. The importance of TLR3 and TLR9 in the type I interferon-dependent protection against HSV disease has been well documented, however, the role of TLR2 is unclear. This study aims to define the function of TLR2 in a natural infection model of HSV-1. Here we demonstrate that Tlr2−/− mice are significantly more susceptible to HSV-1 disease than WT controls. Whereas Tlr2−/− mice have indistinguishable levels of virus at the inoculation site, they display faster nervous system invasion and elevated viral loads in the trigeminal ganglia, brainstem, and brain, indicating that TLR2-expressing cells in the nervous system aid in controlling HSV-1 replication and spread. Mortality from HSV-1 CNS infection is due to virus-mediated cytotoxicity in combination with the ensuing neuroinflammatory response. Analysis of inflammatory gene expression revealed the susceptible Tlr2−/− mice display exaggerated nitric oxide production relative to controls. Thus, TLR2 is required for limiting the amount of HSV-1 that gains access to the CNS; mice lacking this receptor succumb to virus-mediated neurotoxicity in addition to inflammatory reactions generated independently of TLR2. Current work is focused on tracking the migration of inflammatory cells to the HSV-infected CNS, with the aim of elucidating the cellular mechanisms responsible for lethality. Understanding the factors that cause an exacerbated inflammation in models of viral encephalitis will aid in the design of therapeutics for afflicted patients.

**A5**

TLR3 activation on dendritic cells induces the up-regulation of miR-155 that correlates with maturation and a complete suppression of HIV-1 infection.

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Human Immunodeficiency Virus type-1 (HIV-1) is transmitted primarily through mucosal routes and dendritic cells (DCs) are among the first cells to interact with HIV-1. In addition to supporting viral replication, DCs facilitate viral dissemination and contribute to HIV-1 pathogenesis by migrating to the lymph nodes and promoting the infection of CD4+T cells. Upon maturation, DCs seem to be less susceptible to HIV-1 infection. This maturation process appears to be regulated by different cellular factors such as microRNA-155 (miR-155). However, little is known about how miR-155 is involved in DC maturation and whether it plays a role in DCs susceptibility to HIV-1 infection. Our goal is to determine if miR-155 mediates maturation of DCs and its potential effect in the modulation of DCs susceptibility to HIV-1 infection. To achieve this goal, we activated immature DCs with different Toll-like receptor (TLR) ligands to obtain a mature phenotype and determined the expression levels of miR-155 and their susceptibility to HIV-1 infection. We observed that the expression of miR-155 was mainly up-regulated upon TLR3L stimulation in comparison to non-stimulated cells (p=0.003). This up-regulation in TLR3L-stimulated cells correlates with increased expression maturation markers CD80, CD86, HLA-DR and CD83 (p=0.03) and a complete suppression of HIV-1 infection (p=0.031). These results suggest that miR-155 may be involved in the development of DC maturation and in the decrease of HIV-1 infection in mature DCs. Future studies will attempt to define the specific mechanism(s) involved in these processes.
A6

Induction of BST-2 in primary neurons: a potential antiviral role against measles virus

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The last step in the viral life cycle, viral release, is crucial for productive infection of a virus. Several viruses, including HIV, influenza and filoviruses, assemble and release viral particles from areas in the host cell membrane enriched with lipid rafts utilizing the host cell’s cytoskeleton for budding. Measles virus also typically buds from lipid rafts except, apparently, during neuronal infection, such as Subacute Sclerosing Panencephalitis (SSPE). In SSPE, no extracellular virus is detectable, despite widespread antigen-positive neurons in brain biopsies, and this is replicated in vitro with measles virus unable release from neurons. Similarly, vesicular stomatitis virus (VSV) release from neuroblastoma cells is virtually inhibited upon treatment with interferon-. Interferons (IFN) are well known for their potent ability to induce several antiviral proteins. Measles virus has the ability to induce both Type I and Type II IFN production with Type II IFN being essential for measles virus clearance from the CNS. One of the more newly discovered interferon-induced antiviral proteins, BST-2 has been shown to limit free extracellular virus of many enveloped viruses including HIV, lassa virus, and VSV from non-neuronal cells. BST-2, which is induced by both Type I and Type II IFN, is localized to lipid rafts and involved in cytoskeletal structure, a location ideal for viral egress. Our data demonstrates that BST-2 can be induced in primary neurons following stimulation with either Type I or Type II IFN. More importantly BST-2 is also induced during measles virus infection of neurons indicating a potential role for this anti-viral protein in the neuronal innate response to viral infection.

A7

Neuronal and non-neuronal cells produce distinct gene expression patterns in the face of viral infection

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The immune system can limit viral spread in the brain, although the mechanisms by which infected neurons respond to the inflammatory environment created by immune infiltration into the brain remain largely undefined. Interferon-gamma (IFN-γ) and interferon-beta (IFN-β) are potent anti-viral cytokines that classically signal through activation of STAT-1 (IFN-γ) or STAT-1 and STAT-2 (IFN-β). Recently, we showed that basal STAT-1 levels in primary mouse hippocampal neurons are reduced as compared to control mouse embryonic fibroblasts (MEFs). Consequently, phosphorylation and nuclear localization of STAT-1, is muted and delayed in hippocampal neurons treated with IFN-γ. Nevertheless, IFN-γ treatment protects primary neurons from measles virus (MV) infection. Here, we show that neuronal STAT-1 and STAT-2 activation in response to IFN-β do not appreciably differ from MEFs. However, while IFN-β has a modest inhibitory effect on MV replication in neurons, the pattern of interferon-stimulated gene (ISG) expression in neurons differs significantly from MEFs. These data support the hypothesis that neurons utilize distinct signaling responses to anti-viral cytokines, both at the levels of signal transduction factors and gene expression profiles. We propose that these cell type-specific differences in type I interferon responsiveness play a key role in the ability of neurons to survive viral infection.
A8

PFF1320c, a putative myosin light chain of Plasmodium

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Little is known regarding the nature and function of the myosin motors of Plasmodium. Analysis of the genome reveals that there are six myosin heavy chains but only the role of Myosin A, along with its cognate light chain partner, myosin A tail interacting protein (MTIP), has been well characterized in the process of parasite invasion. To date, only MTIP has been characterized as a Plasmodium myosin light chain. We have begun characterizing an annotated putative myosin light chain, however there is no biochemical evidence suggesting that the molecule functions in this manner. PFF1320c has been conserved within the primate infecting plasmodia, P. vivax and P. knowlesi, in addition to P. falciparum, yet this protein has been evolutionarily lost in the rodent parasites, P. berghei, P. yoelii and P. chabaudi. Interestingly, in P. falciparum this protein appears essential due to our inability to disrupt the gene. However, upon expression of an HA tagged version of the gene at an ectopic site, we were able to knock out the gene. Preliminary studies have shown that this presumptive myosin light chain assembles in a high molecular weight complex indicative of formation of a myosin motor. Further studies are currently underway to identify the myosin heavy chain partner, determine the subcellular localization of this myosin motor and to characterize the biological role of this complex.

A9

Development of a urine test for the early detection of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC), the 5th most frequent cancer worldwide, has a 5-year survival rate of 14% because it is difficult to diagnose early. The only available biomarker for screening, serum α-fetoprotein (AFP), has a low sensitivity of detection (40%-60%), so the need for a better detection method is urgent. The goal of this project is to construct a panel of circulation-derived DNA markers to use in a urine test for the early detection of HCC to improve its prognosis. In order to detect cell-free circulating DNA markers in urine (<300 bp), PCR based assays customized for short DNA templates were developed for three known HCC DNA markers, including both the genetic mutation, the HCC-specific p53 codon 249T mutation, and epigenetic modifications, methylated GSTP1 (mGSTP1), and methylated RASSF1 (mRASSF1). Urine samples from HCC (n=41), hepatitis (n=44), and cirrhosis (n=49) patients were tested in each of the three assays and analyzed. The ROC curves were generated for each DNA marker alone or in combination with serum AFP levels. The sensitivity and specificity for these markers is: p53 249T mutation (55.4%, 89.8%), mGSTP1 (43.9%, 94.6%), mRASSF1 (31.7%, 98.9%), and the combination of these three markers with serum AFP levels (81.1%, 75.5%). This work is in progress to include additional DNA markers to obtain high sensitivity for the early diagnosis of HCC. The potentials of how this urine test could lead to a paradigm shift for screening and effective management of HCC is discussed.
**A10**

**PyMAIR, a putative parasite-encoded adhesin expressed on the surface of Plasmodium yoelii 17X infected reticulocytes**

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Adherence of Plasmodium falciparum infected erythrocytes to vascular endothelial cells contributes to pathogenesis and disease severity during malaria infection. This adherence is primarily mediated by var gene-encoded P. falciparum erythrocyte membrane protein-1 (PfEMP-1), which is unique to P. falciparum. Red blood cells infected with other malarial parasites, like Plasmodium vivax, are reported to adhere to vascular endothelium, although the parasite-encoded RBC surface proteins mediating this adherence are less well studied. Using the reticulocyte-restricted parasite Plasmodium yoelii 17X, we developed an adherence assay that allows for isolation of parasites from adherent and non-adherent reticulocytes. Using P. yoelii DNA microarrays, we identified six genes encoding putative adhesins whose expression was up-regulated in parasites from adherent versus non-adherent reticulocytes. We focused on one of these, which we have named P. yoelii Mediator of Adherence in Infected Reticulocytes (PyMAIR). PyMAIR encodes a ~193 kDa protein that is conserved across malarial species. We have expressed a 26 kDa fragment of the protein in E. coli and have generated rabbit antisera. PyMAIR protein is present in a P. yoelii-infected reticulocyte membrane protein (PyRMP) preparation and immunofluorescence using live, unfixed P. yoelii 17X infected RBCs (iRBCs) indicates that PyMAIR is expressed on the surface of a subset of schizont-stage infected reticulocytes. Sera raised against PyMAIR can also partially block adherence of P. yoelii 17X iRBCs to the mouse endothelial cell line bEnd.3. These results indicate that the interaction of PyMAIR with endothelial receptors may contribute to adherence of iRBCs in host tissues. Studies to characterize a PyMAIR gene knock out in P. yoelii 17X are in progress.

**A11**

**A differential echinocandin phenotype in Candida glabrata: sphingolipids modulate echinocandin-Fks interaction**

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Infections with the azole-refractory yeast Candida glabrata are now commonly treated with the echinocandin antifungal drugs caspofungin (CSP) or micafungin (MCF). True resistance (>32-fold decreased susceptibility) to these lipopeptide inhibitors of cell wall synthesis is rare and strictly associated with mutations in integral membrane proteins Fks1 or Fks2. In contrast, mutants exhibiting 4 to 32-fold CSP reduced susceptibility (CRS) were readily selected in vitro, and surprisingly demonstrated 4 to 32-fold MCF increased susceptibility (MIS). Sequencing and gene deletion demonstrated that formation of CRS-MIS is Fks-independent. To explore alternative mechanisms, we initially employed Saccharomyces cerevisiae as a genetic model, and observed that CRS was conferred by multiple mutations (fen1δ, sur4δ, cka2δ, and tsc10-ts) disrupting sphingolipid biosynthesis. Following this lead, C. glabrata fen1δ and cka2δ deletants were constructed, and shown to exhibit CRS-MIS. Sphingolipid analysis of CRS-MIS laboratory mutants and clinical isolates demonstrated elevated dihydrosphingosine (DHS) and phytosphingosine (PHS) levels. Consistent with this, sequencing revealed fen1, sur4, ifa38 and sur2 mutations. Moreover, exogenous DHS or PHS conferred a CRS-MIS phenotype on wild-type C. glabrata. Exogenous PHS failed, however, to suppress CRS-MIS in a sur2 mutant blocked in conversion of DHS to PHS, implying that accumulation of these intermediates confers CRS-MIS. Importantly, we have also identified three C. glabrata clinical isolates that demonstrate a CRS-MIS phenotype, increased levels of DHS and/or PHS, and fen1 or ifa38 mutations. The relevance of a Fen1 point mutation found in one of
these clinical isolates was confirmed upon expression in the fen1δ strain. Together these data suggest that the sphingolipid environment of the plasma membrane is a key contributor to the specificity of echinocandin-Fks interaction. Clinically, echinocandin activity versus C. glabrata may be modulated by the lipid environment, and potentially enhanced by addition of sphingolipid pathway inhibitors.

A12
Protective Role of Toll-like Receptor 3-Induced Type I Interferon in Murine Coronavirus Infection of Macrophages
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Toll-like Receptors (TLRs) sense viral infections and induce production of type I interferons (IFNs), other cytokines, and chemokines. Viral recognition by TLRs and other pattern recognition receptors (PRRs) has been proven to be cell-type specific. Triggering of TLRs with selected ligands can be beneficial against some viral infections. Macrophages are antigen-presenting cells that express TLRs and have a key role in the innate and adaptive immunity against viruses. Coronaviruses (CoVs) are single-stranded, positive-sense RNA viruses that cause acute and chronic infections and can productively infect macrophages. Investigation of the interplay between CoVs and PRRs is in its infancy. We assessed the effect of triggering TLR2, TLR3, TLR4, and TLR7 with selected ligands on the susceptibility of the J774A.1 macrophage cell line to infection with murine coronavirus (mouse hepatitis virus, [MHV]). Stimulation of TLR2, TLR4, or TLR7 did not affect MHV production. In contrast, pre-stimulation of TLR3 with polyinosinic-polycytidylic acid (poly I:C) hindered MHV infection through induction of IFN-β in macrophages. We demonstrate that activation of TLR3 with the synthetic ligand poly I:C mediates antiviral immunity that diminishes (MHV-A59) or suppresses (MHV-JHM, MHV-3) virus production in macrophages.

A13
The heme biosynthesis pathway is not essential for malaria parasites during blood-stage growth
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Malaria parasites appear to have a functional heme biosynthesis pathway, although they potentially have access to the large amount of heme resulting from hemoglobin digestion in the digestive vacuole. They are believed not to salvage heme since almost all digestive heme is crystallized to form hemozoin. The generally accepted view in the field has been that the de novo heme synthesis pathway is essential for malaria parasites, and thus a good prospective drug target. Thus it came as a surprise that we were able to knock out genes for the first and the last enzymes individually or in combination, viz. 5-aminolevulinate synthetase (ALAS) and ferrochelatase (FC), of the malarial heme biosynthesis pathway. ALAS synthesizes 5-aminolevulinic acid from glycine and succinyl-CoA while FC inserts a ferrous iron into protoporphyrin IX to generate the final product, heme. Parasites of the three knockout lines exhibited fully normal growth, suggesting the heme biosynthesis pathway is not essential for malaria parasites during the asexual blood stages. Since heme is the prosthetic group of several cytochromes (a-, b-, & c-types), which are components of the essential mitochondrial electron transport chain (mtETC), malaria parasites must apparently salvage heme, most likely from hemoglobin digestion. These results are remarkable because 1) they challenge the dogma that heme biosynthesis is essential in malaria parasites; 2) they support the idea that malaria parasites can also salvage heme; and 3) they may inform investigations involving antimalarial drug discovery. Further characterizations of these knockout parasites are underway to fully realize the implications of these unexpected findings.
A14

Delayed CD8+ T cell Response in Neonatal Mice to Influenza Infection

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To examine the influenza virus-specific primary CD8+ T cell response in neonatal mice, three day old neonatal mice were infected intranasally with PR8 influenza virus; adult mice served as controls. The animals were harvested at Day 6, 10, and 14 post-infection and lungs and spleens were analyzed for the number of nucleoprotein (NP366)-specific CD8+ T cells by flow cytometry. When adult C57Bl/6 mice have a primary infection with PR8 influenza virus, they mount an immunodominant NP366-specific CD8+ T cell response in the lungs that peaks on days 10-11. We have found that when day 3 neonatal C57Bl/6 mice are infected with influenza virus they mount a greatly reduced and delayed lung NP366-specific CD8+ T cell response on day 10 compared to adult mice (p<0.003). The neonatal response continues to increase at day 14, while the adult response is already contracting. However, despite the altered kinetics of the viral specific CD8+ T cell response, the neonatal mice are able to clear the virus, as shown by similar viral loads in the lungs, compared to adults. The exact mechanism of viral clearance is unknown. There is greatly increased activation of Natural Killer (NK) cells and T cells on day 6 post-infection (p<0.05), which is an indication of a general pro-inflammatory state. The reduced and delayed viral specific response in neonates indicates that there must be an alternate mechanism for viral clearance. Naïve CD8+ T cells could exhibit differences in their response to cues that direct the CD8+ T cell response during viral infection. These could involve responsiveness to TCR activation, costimulation, cytokines, or defective antigen presentation. Further studies are needed to investigate the different mechanisms of viral clearance, which may allow us to develop novel therapeutic strategies against viral respiratory infections that reduce morbidity and mortality in neonates.

A15

Differential regulation of glycolytic pathway by HIV-1 infection of its target cells.

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Regulation of Glucose metabolism has been shown to play an important role in pathogenesis of many diseases. Primarily because deregulation of this metabolic pathway can lead to either apoptosis or extended life span of the cells involved. Human Immunodeficiency Virus Type-1 (HIV-1) infects both activated CD4+T cells as well as terminally differentiated macrophages during the course of HIV-1 pathogenesis. Hence we focused on the possible regulation of glycolytic pathways by HIV-1 infection in two different cell types; by monitoring the effect of HIV-1 infection on the activities of important glycolytic enzymes: Hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD) and Pyruvate kinase (Muscle) 2. We used human CD4+T cell line Jurkat latently infected with HIV-1 J.Lat 6.3 cell line and a monocyte cell line U1 latently infected with HIV-1.Jurkat and U937 cell lines served as controls for T-cell lines and monocyte derived macrophages respectively. We observed that the activity of HK, G6PD and PKM2 enzymes were markedly different under HIV-1 replication in the two cell lines. The T-Cell line showed 5 times increase in HK activity whereas the U1 cell line showed 6 times decrease in HK activity. However in the case of G6PD activity the trend was reversed such that in U1 cells G6PD activity was increased about 3 fold. The G6PD activity of infected T-cell line decreased about 1.3fold. PKM2 enzyme also showed cell line based difference in its activity; in U1 cell the activity decreased about 2 fold with viral replication but in J.Lat cell line PKM2 activity did not show any steady trend of variation. These observations suggest that the glycolytic pathway in different HIV-1 target cells is differently...
affected by viral replication. It can further unveil any role of metabolic pathway in HIV-1 pathogenesis and survival of infected cells. The study was possible by grant awarded by NIH to SA.

**A16**

**Genetic and Phenotypic characterization of the envelope glycoprotein of two highly neurotoxic CSF derived HIV-1 primary isolates**

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HIV-1 enters the CNS early after systemic infection, leading to a variety of neurocognitive and motor impairments referred as HIV-1 associated neurocognitive diseases (HAND). Our goal is to elucidate the role of the HIV-1 envelope glycoprotein during the HIV-1 infection in the CNS. Here, we characterized the genotypes and the phenotypes of the envelope glycoproteins (env) of two highly-neurotoxic CSF-derived primary HIV-1 isolates (Jago and Doge) obtained from patients with HIV-1 associated dementia (HAD). First we amplified HIV-1 env using conventional PCR and single genome amplification, which avoid the resampling and recombination problems associated with conventional PCR procedures, to study genuine envelope glycoproteins derived from these isolates. Both methods generated functional envelopes in the context of linear expression cassettes (LEC). These LEC-encoding env were subjected to phenotypic studies such as macrophage tropism, ability to use low levels of CD4 for infection, CD4 avidity, sensitivity to entry and fusion inhibitors, and high fusogenicity. We identified that some, but not all the env from Jago and Doge, present phenotypic characteristics that have been previously reported in CNS isolates. Altogether, these genetic and phenotypic characteristics may allow the virus to have a better adaptation to infect a CNS-like microenvironment.

**A17**

**Titer and specificity of antibodies elicited by immunization with PfMSP1/8, a chimeric blood stage malaria vaccine**

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In 2010, the World Health Organization reported 216 million cases and 655,000 deaths due to malaria, largely attributed to Plasmodium falciparum and Plasmodium vivax. Despite considerable efforts, there is no vaccine available. Poor immunogenicity and antigen polymorphism are ongoing challenges. We developed a chimeric P. falciparum blood-stage vaccine candidate by fusing the dimorphic, C-terminal epidermal growth factor-like domains of merozoite surface protein 1 (PfMSP119) to nearly full length, highly conserved merozoite surface protein 8 (PfMSP8). This recombinant PfMSP1/8 vaccine induces strong T and B cell responses to PfMSP8 and high titers of antibodies to the protective B cell epitopes of PfMSP119. Our previous data showed that PfMSP1/8-immunized rabbits produce high titers of antibodies that inhibited the in vitro growth of P. falciparum. To further evaluate the immunogenicity of PfMSP1/8, we immunized CD1 outbred mice with PfMSP1/8 formulated with two distinct adjuvants: Quil A or Montanide + CpG ODN. By ELISA, we determined IgG levels in individual sera that bound to PfMSP1/8, the PfMSP8 domain, and the two major alleles of PfMSP119 (FVO and 3D7). Irrespective of adjuvant, immunization with PfMSP1/8 elicited highest antibody titers in all mice, with a large proportion of the response directed against PfMSP8-specific epitopes. However, all animals also mounted a significant antibody response to MSP119. Remarkably, these PfMSP119-specific antibodies recognized conserved epitopes common to the two PfMSP119 allelic variants. Since poor immunogenicity and
induction of strain specific responses are not a concern, these results are encouraging for the further development of this chimeric PfMSP1/8 vaccine.

A18

A Role for miR-155 Modulation in the Anti-HIV-1 Effects of TLR3 Stimulation in Macrophages

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Toll-like receptor (TLR) stimulation modulates the microRNA (miRNA) profiles in immune cells, and some miRNAs are known to play a role in HIV-1 infection by targeting viral or cellular mRNAs. We aimed to define whether miRNA modulation contributes to the differential effect in susceptibility to infection observed in monocyte-derived macrophages (MDM) upon stimulation with several TLR ligands. We found that stimulation of MDM with poly(I:C) or LPS (TLR3 and 4 ligands, respectively), but not with Imiquimod or CpG (TLR7 and 9), abrogated infection by both BaL- and VSV-G-pseudotyped viruses. miR-155 was significantly up-regulated in both TLR3 and 4-stimulated MDM, but not in TLR7-stimulated cells, and poly(I:C) induced significantly higher miR-155 levels in MDM than LPS. A miR-155 antagonist prevented miR-155 induction by poly(I:C) and led to significantly higher infection, while miR-155 over-expression in MDM significantly reduced susceptibility to infection. Both poly(I:C) stimulation and miR-155 over-expression resulted in unaltered relative amount of early RT product, but increased relative levels of late RT product, and greatly reduced or suppressed detection of integrated provirus, and these changes were abrogated by the miR-155 antagonist. Finally, reduced mRNA levels of ADAM 10, TNPO3, NUP153 and LEDGF have been observed in miR-155-expressing MDM. Increased miR-155 levels induced by poly(I:C) or ectopic expression in MDM suppresses/reduces their susceptibility to HIV-1 infection by inducing a post-entry, pre-nuclear import restriction mechanism. Interestingly, miR-155 over-expression leads to reduction in mRNA levels of several host proteins known or suggested to play a role in trafficking and/or nuclear import of the viral pre-integration complex, which warrants further investigation.

A19

Investigating the Putative b Subunit of the ATP Synthase Complex in Blood Stage Plasmodium falciparum

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The central player in the bioenergetics of most organisms is the rotary nanomotor, ATP synthase. The F-type ATP synthase consists of two domains - the catalytic domain, F1, and the membrane/stator domain, FO, which conducts the protons and provides a stator against the rotary action of the ATP synthase complex. Components of both domains are conserved across prokaryotes and eukaryotes. Surprisingly, several genes encoding components of the FO domain were not detected in the sequenced genomes of a variety of apicomplexan parasites, such as Plasmodium falciparum, and other unicellular organisms of the superphylum Alveolata. Recently calculated updates to the Pfam (protein families) database, however, indicated that PF3D7_1125100, a conserved Plasmodium protein, has similarity to mitochondrial ATP synthase B chain precursor family. We initiated an investigation of this protein in the asexual blood stages of the parasite to determine if it is indeed the b subunit of the Plasmodium ATP synthase complex. We integrated an HA-tagged version of the putative b gene in an ectopic site of the Plasmodium genome. However, we were not able to detect expression of HA tagged protein via western blots or immunofluorescence assays (IFA). We next determined by PCR analysis that not only was the HA-tagged mRNA not being expressed, but mRNA of the endogenous “b” gene was not detected, as well.
Previously published proteomics data suggested that the expression of the putative b protein increased in the sexual gametocyte stages of the parasite life cycle. We generated gametocytes in culture, but initial attempts to detect the protein were not successful. Isolation of mRNA and generation of cDNA from gametocytes will be performed next. It is important to determine expression and localization of the putative subunit to determine whether it may be the missing b subunit.

A20

Arrest of nuclear division in Plasmodium through blockage of erythrocyte surface exposed ribosomal protein P2

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Malaria parasites reside inside erythrocytes and the disease manifestations are linked to the growth inside infected erythrocytes (IE). The growth of the parasite is mostly confined to the trophozoite stage during which nuclear division occurs followed by the formation of cell bodies (schizogony). The mechanism and regulation of schizogony are poorly understood. Here we show a novel role for a Plasmodium falciparum 60S stalk ribosomal acidic protein P2 (PfP2) (PFC0400w), which gets exported on the IE surface for 6-8 hrs during early schizogony, starting around 26-28 hrs post-merozoite invasion. The surface exposure is demonstrated using multiple PfP2-specific monoclonal antibodies, and is confirmed through transfection using PfP2-GFP. The IE surface-exposed PfP2-protein occurs mainly as SDS-resistant P2-homotetramers. Treatment with anti-PfP2 monoclonals causes arrest of IEs at the first nuclear division. Upon removal of the antibodies, about 80-85% of synchronized parasites can be released even after 24 hrs of antibody treatment. It has been reported that a tubovesicular network (TVN) is set up in early trophozoites which is used for nutrient import. Anti-P2 monoclonal antibody causes a complete fragmentation of TVN by 36 hrs, and impairs lipid import in IEs. These may be downstream causes for the cell-cycle arrest. Upon antibody removal, the TVN is reconstituted, and the cell division progresses. Each of the above properties is observed in the rodent malaria parasite species P. yoelii and P. berghei. The translocation of the P2 protein at the IE surface is therefore likely to be of fundamental importance in Plasmodium cell division.

A21

Identification and characterization of a Plasmodium falciparum orthologue of the yeast ubiquinone-binding protein, Coq10p

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Coenzyme Q (CoQ, ubiquinone) is a central electron carrier in mitochondrial respiration. CoQ is synthesized through multiple steps involving a number of different proteins. The prevailing view that the CoQ used in respiration exists as a free pool that diffuses throughout the mitochondrial inner membrane bilayer has recently been challenged. In the yeast Saccharomyces cerevisiae, deletion of the gene encoding Coq10p results in respiration deficiency without altering total size of the available CoQ pool, suggesting that the Coq10p is critical for the delivery of CoQ to the site(s) of respiration. The precise mechanism by which this is achieved remains unknown at present. Because mitochondrial respiration is a validated target for antimalarial drugs such as atovaquone, we are interested in examining its regulation in malaria parasites. We have identified an orthologue of Coq10p, PfCoq10, in P. falciparum, the most virulent species of malaria parasite, and demonstrated that a GFP-tagged version of PfCoq10 localized to the parasite mitochondrion. Expression of PfCoq10 in the S. cerevisiae coq10 deletion strain restored the capability of the yeast to grow on respiratory substrates, suggesting a remarkable functional conservation
of this protein over a vast evolutionary distance, and despite a relatively low level of amino acid sequence identity. We are currently assessing effects of PfCoq10 overexpression on the atovaquone sensitivity of P. falciparum. We are also examining the possibility of altered response to atovaquone in yeast mitochondria expressing the parasite Coq10. These studies may provide insights into respiration regulation in general, as well as in malaria parasites.

A22
Co-regulation of Hepatitis B Virus Replication and the Oncoprotein Lin28B
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The oncogenic protein Lin28B plays an integral role in development and cellular differentiation. Lin28B can negatively regulate, and be regulated by, the let-7 family of miRNAs, which can target factors such as MYC and RAS to modulate cell proliferation. Expression of Lin28B is also stimulated by proliferation- and survival-related factors such as MYC and NF-κB. Interestingly, the hepatitis B virus (HBV) X protein (HBx) can also affect many of these Lin28B-regulated pathways. HBx is a small HBV protein that can modulate many cellular signaling pathways. HBx plays a critical role in HBV replication, and considerable evidence exists to support its oncogenic potential. Because of the distinct overlap between Lin28B and HBx activities, we are investigating the role of Lin28B in HBx-mediated changes in cellular signaling pathways and the relationship between levels of Lin28B and HBV replication. We hypothesize that HBV, and specifically HBx, elevates Lin28B levels to decrease expression of tumor suppressors such as let-7, and activates cellular proliferation and survival pathways, creating a more tumorigenic environment. In support of our hypothesis, we have shown that the expression pattern of Lin28B directly correlates with the expression level of the HBV core protein in cell lines expressing integrated HBV genome. These results are important because they imply that HBV alters the levels of Lin28B, a known oncoprotein. Additional data also suggest increasing levels of Lin28B by transient transfection may increase the levels of HBV replication in primary rat hepatocytes, implying a potential feedback loop in which levels of Lin28B and HBV replication may be co-regulated. Globally, chronic HBV infections are the leading cause of hepatocellular carcinoma, and future studies that continue to interrogate the effects of HBV and HBx on Lin28B levels and functions could contribute to our understanding of mechanisms that are associated with HBV-related tumorigenesis.

A23
miRNA-155 is critical for the generation of primary and memory CD8+ T cells during microbial infections
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Micro-RNAs (miRNAs) have been described as important regulators of gene expression, mediating their effect either by inhibiting protein translation or by degrading the mRNA transcript. miRNAs such as miRNA-155 (bic/miR-155) may greatly influence immune responses, by regulating the function of macrophages, dendritic cells, B and CD4+ T cells. However, their role in CD8+ T cells responses has been largely unexplored. In our study, we have found miR-155 to be up-regulated by activated CD8+ T cells in vitro as well as by effector and effector memory CD8+ T cells from in vivo infection. To test the in vivo role of miR-155 in CD8+ T cell responses, we adoptively transferred bic'/OT-I and OT-I transgenic CD8+ T cells into C57BL/6 hosts, which were then infected with influenza virus strain WSN-OVA expressing the SIINFEKL peptide. At day 10 post-infection with WSN-OVA, the number of bic'/OT-I cells in the lungs was reduced approximately 60-fold compared to control OT-I cells (p<0.001). Interestingly,
generation of memory bic^−α5D4−OT-I cells was significantly reduced after day 60 of WSN-OVA infection. Gene expression analysis revealed bic^−α5CD8^− T cells to have an increased enrichment of genes induced by Type I Interferon (IFN) signaling. In vitro stimulation assays with OT-I and bic^−α5OT-I cells revealed bic^−α5CD8^− T cells to be increasingly susceptible to type I IFNs, showing reduced proliferation and survival. Our study thus implicates miR-155 as being critical for in vivo CD8^− T cell responses against pathogens.

**A24**

T-cell receptor alpha and beta chain skewing in the islets of pre-diabetic LEW.1WR1 rats: Germline-encoded TCR repertoire as a determinant of Type 1 diabetes susceptibility.

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Autoimmune type-1 diabetes (T1D) is common among inbred rat strains with a high-risk MHC-II (RT1.B/Du). Prior genetic studies have mapped a robust diabetes susceptibility locus (Iddm14) on chromosome 4 using five T1D susceptible (including LEW.1WR1) and three resistant rat strains (e.g. WF and LEW.1W). We then identified a T-cell receptor (TCR) beta chain variable region gene, Tcrb^VL3, as the probable Iddm14 gene. We have shown that deletion of Vβ13+ T cells prevents T1D (Liu et al., Diabetes, 2012), suggesting that TCR-Vβ13+ T-cells recognize a critical islet auto-antigen. If true, we should detect clonal expansion of Vβ13+ T cells in pre-diabetic LEW.1WR1 islets. We sequenced Vβ13+ CDR3 regions from T cells isolated from spleen, islets, and T cells cultured ex vivo from islets of pre-diabetic LEW.1WR1 rats, as well as from T1D resistant LEW.1W islets. We observed significant skewing of the TCRβ repertoire, with pauciclonal expansion of Vβ13-CDR3 sequences from islet T-cells compared to a high diversity of Vβ13-CDR3 regions in spleen. T-cells were also present in the T1D resistant LEW.1W islet but are not antigen focused. These data indicate that an oligoclonal Vβ13 response to pancreatic beta cells exists early in progression to autoimmune diabetes. In NOD/LtJ mice, TCR-Vα5D4 is frequently used in the T-cell response to islet antigen, specifically recognizing insulin B:9-23, in combination with multiple TCRβ chains. We analyzed rat Vα5D4+ CDR3 sequences from pre-diabetic islets, cultured islet T cell exfiltrates, and Vβ13+ and Vβ13- sorted exfiltrates. We observed pauciclonal expansion of islet homing Vα5D4+ T-cells in LEW.1WR1 but not the T1D resistant LEW.1W, suggesting an autoimmune response to insulin occurs early in rat T1D pathogenesis. Additionally we show the presence of TCR-Vα5D4 repertoire skewing among islet homing TCR-Vβ13+ cells. We conclude that the TCR variable region genotype is a critical genetic determinant of T1D susceptibility.

**A25**

Two Distinct Mechanisms of Host Vacuole Remodeling by the Pathogen Legionella pneumophila

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Legionella pneumophila is an opportunistic intracellular pathogen, and the causative agent of a severe respiratory infection known as Legionnaires’ disease. To cause disease, intracellular pathogens, like the bacterium L. pneumophila, must modulate the processes within the host cell that inhibit the growth and replication of the pathogens. L. pneumophila achieves this by injecting effector proteins directly into the cytoplasm of the host cell via the Dot/Icm Type IV secretion system. Injected effectors then act to modify the surface of the host vacuole containing L. pneumophila (LCV), affecting the trafficking of this vacuole, and facilitating the survival and replication of the residing bacteria. To study the mechanism controlling effector binding to the vacuole, we analyzed the binding requirements of two different effectors. One of these, PieA, has been shown to be recruited to LCV during infection. The second
effector, SidC, was previously shown to interact with phosphatidylinositol-4-phosphate in the membrane of the LCV. We found that the c-terminal portions of PieA and SidC are sufficient for binding and, both PieA(511-699) and SidC(609-917) have the capacity to bind to the LCV in-vitro. The binding of PieA to the vacuole is shown to be mediated by protein-protein interaction, and is abolished if the vacuoles are pre-treated with protease. However, SidC was found to bind to the vacuole by a different mechanism, independent of protein-protein interactions, most likely involving lipids. The two effectors therefore represent two distinct mechanisms contributing to host vacuole remodeling by the intracellular pathogen L. pneumophila.

A26
Mapping of the mutation in the Tight Skin 2 model of systemic sclerosis
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The Tight Skin 2 (Tsk2/+) mouse is a genetic mouse model of systematic sclerosis. This model has several similarities to human disease, including tight skin, increases in collagen, and alterations in the extracellular matrix. The Tsk2/+ mutation first occurred in a 101/H mouse due to exposure to a mutagenic agent, which likely caused a point mutation. Because the mutation is homozygous lethal the mice are bred as heterozygotes. While the mutation has not been identified it is known to be located on chromosome 1 between 42.5 and 52.5 megabases (Mb). We backcrossed the hybrid Tsk2/+ mice to a B6 background and mapped the location of the mutation by microsatellite and single nucleotide polymorphism (SNP) typing. We bred the B6.Tsk2/+ mice to a consomic B6.chr 1-A/J background to distinguish Tsk2/+ from its parentals. At Dartmouth our collaborators used RNA-Seq to sequence the exons using skin samples from Tsk2/+ mice to identify novel SNPs within the interval. We designed SNP primers to compare the SNPs identified by RNA-Seq to 101/H DNA to eliminate SNPs not specific to the Tsk2/+ mutation. Microsatellite typing narrowed the interval to 9.5 Mb, while SNP typing narrowed the interval further to only 3 Mb. RNA-Seq identified 13 genes with SNPs found exclusively in Tsk2/+ samples, 8 of which have been previously observed in other mouse strains. SNP primers designed for the remaining 5 SNPs found 3 were specific to Tsk2/+ and 2 were present in the parental 101/H strain. Two of the novel SNPs were within a Gulp1 intron and one was within the second exon of Col3a1. In conclusion, we have narrowed the probable location of the Tsk2/+ mutation to one of three locations. Due to abnormalities in collagen in the Tsk2/+ mouse we believe the Col3a1 mutation to be the most likely candidate.

A27
Role of HIV Envelope Glycoprotein gp120 and Inflammatory Cytokines in Altering Neuronal Ferritin Heavy Chain Expression
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Ferritin is a ubiquitous protein involved in iron sequestration and storage, composed of two different subunits, Heavy chain (FHC) and Light chain (FLC). Recent evidence has identified a novel role for FHC as a negative regulator of the chemokine receptor CXCR4. Our lab has previously demonstrated that µ-opioid receptor agonists, including morphine, upregulate FHC protein levels in neurons, both in vitro and in vivo, which results in inhibition of prosurvival signaling mediated by CXCR4. Furthermore, greater expression of FHC and inhibition of CXCR4 (Ser 339) phosphorylation, an index of CXCR4 activation, was found in brain tissue of HIV-positive drug users (HIV/DU) as well as in morphine-treated SIV infected macaques. Interestingly, a similar trend in FHC/CXCR4 changes was also observed in HIV (or SIV) subjects with no history of drug abuse - though these changes were less pronounced. Overall these data suggest that HIV infection and opiate abuse could exert a synergistic action on FHC and deprive...
neurons of important neuroprotective mechanisms driven by CXCR4. To test this hypothesis we studied the effect of viral proteins (i.e. gp120) and the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) on FHC protein expression in neurons. Our initial data suggest that different and potentially additive molecular mechanisms may be responsible for FHC changes caused by opiates and HIV infection.

A28

**Human seminal fluid contains factors that modulate cervical epithelial monolayer integrity and may impact male-to-female HIV-1 transmission**

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The role of semen during male-to-female human immunodeficiency virus type 1 (HIV-1) transmission likely extends beyond a passive carrier of virions and HIV-1-infected cells. Factors in seminal fluid (SF) that have integral roles in reproductive biology may also alter the ability of HIV-1 to penetrate the cervicovaginal epithelium and infect target immune cells. We hypothesized that one consequence of SF exposure may be alterations in cervicovaginal epithelial tissue tight junction integrity, resulting in changes in epithelial barrier function with respect to HIV-1. To address this possibility, human cell lines derived from three different regions of the female reproductive tract - vagina, ectocervix, and endocervix - were cultured in a transwell cell culture system to form polarized epithelial monolayers. Changes in barrier integrity were measured over time by monitoring transepithelial electrical resistance (TEER). Apical exposure to SF caused a rapid increase in epithelial integrity across all cell lines by 30 min post-exposure and a sustained peak in TEER by 3-4 h post-exposure. The elevated TEER persisted for approximately 48 h post-exposure. Although all three cell lines were responsive to SF, the endocervical cells responded with the greatest change in TEER and the highest absolute TEER value, while the vaginal cells were the least responsive to SF. These results suggest that factors present in SF may initially protect the female reproductive tract from HIV-1 infection during sexual intercourse. Ongoing studies are investigating changes in epithelial permeability to HIV-1 following SF exposure and the mechanism(s) by which SF affects epithelial cell tight junction integrity.
**B29**

Nonthermal dielectric-barrier discharge plasma-induced inactivation involves oxidative DNA damage and membrane lipid peroxidation in Escherichia coli

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Nonthermal plasma at room air generates many reactive species when applied to biological samples using the floating-electrode dielectric-barrier discharge (FE-DBD) technique. Some of the species being characterized in our laboratories are hydrogen peroxide, singlet oxygen, superoxide, hydroxyl radical, and nitric oxide. All or most of these species are capable of causing oxidative damage to bacteria, and eventually death. Recently, we reported a successful application FE-DBD technique for rapid inactivation of virulent bacterial contaminants (S. G. Joshi et al., Am. J. Infect. Control 38:293-301, 2010). There are many speculative reports supporting this mechanism, and although the exact changes occurring are not yet known, the involvement of reactive oxygen species (ROS) and oxidative damage is more likely to be involved. In the present report, we demonstrate that FE-DBD plasma-mediated inactivation involves membrane lipid peroxidation in E. coli. In-house built plasma probe and samples holders, with commercial plasma generator, and E. coli ATCC 25922 used in this study. The samples were exposed to plasma for 0 (0), 3 (0.39), 6 (0.78), 12 (1.56), 15 (1.95), 24 (3.12), 30 (3.9), 60 (7.8), 90 (11.7), 120 (15.6), and 180 (23.4) seconds (the plasma energy in J/cm2 is shown in parentheses), depending upon the type of experiment. Standard techniques such as colony assay, XTT assay, bright-field and fluorescence microscopies, reactive species-specific fluorescent probe assays, MDA assay, ELISA used. Dose-dependent ROS, such as singlet oxygen and hydrogen peroxide-like species generated during plasma-induced oxidative stress, were responsible for membrane lipid peroxidation, and ROS scavengers, such as α-tocopherol (vitamin E), were able to significantly inhibit the extent of lipid peroxidation and oxidative DNA damage. These findings indicate that this is a major mechanism involved in FE-DBD plasma-mediated inactivation of bacteria and that ROS are the key regulators in this process, induce lipid peroxidation in E. coli.

**B30**

The NLRP3 Inflammasome is a Drugable Target for a New Class of Anti-Infective Agents

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We describe the molecular mode of action and pharmacodynamics of a new molecular entity (NME) that induces the NLRP3 inflammasome-mediated innate immune response. This innate response reduces the pathogen load in an experimentally induced MRSA infection, enhances survival in an experimentally induced Gram-negative bacteremia, and overrides the escape mechanism of an obligate intracellular pathogen, viz. Chlamydia pneumoniae. Furthermore, the NME is more effective than standard-of-care antibiotic therapy in a clinically established multi-factorial bacterial infection. Analysis of transcriptional regulation of inflammasome signaling genes and innate/adaptive immune genes revealed consistent and significant host changes responsible for the improved outcomes in these infections. These studies pave the way for the development of first-in-class drugs that enhance inflammasome-mediated pathogen clearance and identify the NLRP3 inflammasome as a druggable target to address the global problem of emerging new infectious diseases and the re-emergence of old diseases in an antibiotic resistant form.
**B31**

The tug-of-war between dendritic cells and HTLV-1: lessons from in vivo studies

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The human immune system is under constant challenge from many viruses, some of which the body is successfully able to clear. Other viruses have evolved to escape the host immune responses and thus persist, leading to the development of chronic diseases. Dendritic cells (DCs) are professional antigen presenting cells that play a major role in both innate and adaptive immunity against different pathogens. For the past few years our efforts have been focused on exploring the participation of DCs in the pathogenesis of HTLV-1. We observed previously that depletion of DCs in CD11c-DTR transgenic mice enhanced the susceptibility to cell-free HTLV-1 infection. We further performed the host-pathogen interaction studies utilizing Flt3 ligand derived murine bone marrow DCs (FL-DCs). First, the kinetics of viral entry, proviral integration, and expression of the viral protein Tax was established and then effects of cell-free HTLV-1 was examined on these cells. Phenotypically, FL-DCs demonstrated activation and produced an array of proinflammatory cytokines as well as IFN-α. Virus-matured FL-DCs also stimulated proliferation of autologous CD3⁺ T cells and IFN-γ production. Gene expression studies revealed upregulation of interferon-stimulated genes, most cytokines, and transcription factors but a distinct downregulation of many chemokines. Overall, these results highlight the critical interaction of DCs with a human chronic virus important for the early immune responses.

**B32**

In vivo imaging and mechanistic studies of CCL2-mediated transmigration of dendritic cells into the central nervous system

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Although it has been established that dendritic cells (DCs) play critical roles in the development of CNS autoimmune inflammation, the molecular mechanisms of how circulating DCs traffick across the blood-brain barrier (BBB) is not understood completely, a process that is highly influenced by chemokines. The chemokine ligand 2 (CCL2), also known as monocyte chemotactic protein 1 (MCP-1), is one such potent chemottractant that has been implicated in the recruitment of leukocytes at the BBB. To study the influence of this chemokine on DC trafficking, we have undertaken both in vivo and in vitro imaging studies in conjunction with transmigration assays. Near Infra-Red imaging of DC transmigration into the CNS correlated with the severity of inflammation during experimental autoimmune encephalomyelitis. Histology confirmed the presence of CCL2 in EAE lesions in concurrence with DCs emerging from perivascular spaces. Accordingly, DCs exhibited an efficient transmigration pattern during steady-state as well as under the influence of CCL2 as compared to T cells in an in vitro BBB model consisting of human brain microvascular endothelial cells. Transwell imaging studies indicated a paracellular versus transcellular pattern of migration by DCs and T cells, respectively suggesting differential mechanisms of action utilized by these cells. At the molecular level, CCL2 seems to facilitate DC transmigration in an ERK1/2-dependent manner linked with an increased surface expression of integrins on DCs. In summary, these comprehensive studies provide state-of-the-art view of DCs within the CNS, elucidate their path across the BBB and highlight potential mechanisms involved in CCL2-mediated DC trafficking.
**B33**

**Potentiating dendritic cells to target hypoxic environment of brain tumor**

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Glioblastoma multiforme (GBM) is the most common type of brain tumor, as well as the most malignant with mean survival time of 1 year. The most difficult problem to treat tumors is their hypoxic microenvironment that changes the phenotypic characteristic of immune cells. In hypoxic conditions, HIF-1α, a transcription factor, stimulates various genes that promote angiogenesis and tumor growth, is accumulated in large amounts. Our hypothesis is to equip dendritic cells (DCs) with anti-HIF-1α through antisense RNA technology in order to improve their antigen presenting capacity under hypoxic conditions. We first confirmed that hypoxic environment (created by the use of Cobalt chloride, CoCl2) downregulates the expression of HLA-DR, CD86 and HLA-ABC on DCs and affects their antigen processing and presentation capability. We then injected DCs containing antisense HIF-1α plasmid into mice with GBM. In vivo imaging reveals 125I radiolabeled DCs (co-labeled with IRDye680LT) migrating away from the site of injection into the spleen and mediastinal lymph nodes by 54h post infection indicating chemotraction of DCs to areas of ongoing inflammation. Mice injected with plasmid alone showed GFP signal in its axillary and brachial lymph nodes by 48h indicating antisense HIF-1α being expressed upon likely encounter with metastasized tumor cells. Currently, our efforts are focused on screening plasmid transfected into DCs in vitro for its anti-sense capability and introducing them into mice with high grade GBM expressing HIF-1α to measure its attenuation. This translational approach has applicability in eradicating tumors by DC-based vaccination or being adjunct to existing radio- and chemotherapy.

**B34**

**Generation of a new hybrid mouse colony containing HLA-A2/DTR transgenes to assess dendritic cells involvement in human class I-restricted immune responses**

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Individually, HLA-A2 and CD11c-DTR transgenic mouse strains have been used to investigate the pathogenesis of different viruses as well as other pathogens and provided important insights into our current understanding of host-pathogen interactions in vivo. A hybrid strain of the two will further provide a powerful tool to investigate dendritic cell (DC)s’ involvement in human class-I-restricted immune responses. Therefore, employing a cross breeding strategy we successfully generated a HLA-A2+/DTR+ transgenic mouse hybrid strain that carries a HLA-A2 gene for human class I-specific antigen presentation, and diphtheria toxin receptor gene for transient depletion of CD11c+ DCs. This new hybrid strain enabled us to study CD8-immune response against a known A2-specific viral epitope (HTLV-1 Tax 11-19) delivered along with tetanus helper peptide (THP) without or with adjuvant (IFA-Incomplete Freund’s adjuvant) in the absence and presence of DCs. We first confirmed depletion of CD11c+ DCs and presentation of Tax11-19 viral epitope. Further, upon in vitro stimulation of splenocytes with autologous bone marrow-derived DCs pulsed with Tax11-19 antigen, DC-depleted mice showed marked attenuation in proliferation of CD8+ T cells when compared with the non DC-depleted mice. Also, mice immunized with both antigen and adjuvant demonstrated a much higher antigen (Tax11-19)-specific response compared to the non-adjuvant group. Each experiment was conducted within 5-day time period to avoid repopulation of DCs. This study therefore illustrates a broader application of the new HLA-A2/DTR transgenic hybrid mouse for studying the importance of DCs in priming and sensitization of an antigen-specific immune response in the context of human MHC Class I molecule.
**B35**

A novel high-throughput screening assay to identify inhibitors of HIV-1 gp120 protein interaction with DC-SIGN

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The 2010 UNAIDS report states that approximately 34 million people are living with human immunodeficiency virus type 1 (HIV-1), despite highly active antiretroviral therapy (HAART). Despite being effective, ARV therapy has many disadvantages including a cost trajectory unsustainable for economically challenged countries, serious side effects, and the development of drug-resistant strains. Several measures are under way to develop alternatives for ARV therapy, particularly for the control of early HIV-1 infection, but lack of efficient drug targets and assays hinders the search of potential ARV molecules. The dendritic cells present in the mucosal tissue, together with CD4+ T lymphocytes and macrophages, are among the first cells to encounter HIV-1. The dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) molecule plays a crucial role by establishing a high affinity interaction with viral envelope glycoprotein gp120. DC-SIGN, a mannose-binding C-type lectin expressed on cells in the mucosal tissue of the rectum, uterus and cervix, facilitates early HIV-1 infection after sexual transmission. In this study we report a novel target-specific high-throughput screening (HTS) assay capable of quantifying the binding as well as the inhibition of DC-SIGN/gp120 interaction. First, the assay was miniaturized from a 96 to a 384 well format. The specificity of the assay was determined through competitive inhibition while optimization occurred for DMSO tolerance (0.5%), Z’ factor (0.51), signal-to-noise ratio (3.26), and coefficient of variation (5.1%). For assay validation known antagonists of DC-SIGN/gp120 binding were tested demonstrating the suitability of the assay for future HTS screen of potential HIV-1 inhibitors.

**B36**

Myocyte Enhancer Factor-2 (MEF-2) is involved in HTLV-1 Tax-mediated chromatin remodeling and plays critical role in viral infection

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The transactivator protein of human T cell leukemia virus type 1 (HTLV-1), Tax, is required for the activity of viral promoter LTR (long terminal repeat) and is capable of regulating both virus and host transcription. However, the clear molecular mechanism of Tax-mediated viral gene expression is not known. In the studies proposed herein, we have performed the extensive Protein/DNA transcription factor array analyses on the HTLV-1 infected cell lines (MT-2, and SLB-1), a latent cell line (MT-1) and on a Tax-expressing cell line (C8166) in comparison to normal CD4+ T cell line (Jurkat), the primary target infected cell population. Interestingly, both MT-2 and SLB-1 cells demonstrated upregulated transcriptome profile while MT-1 and C8166 cells showed either no expression or downregulation of a majority of the factors analyzed. Significant changes were observed in several new nuclear and cytoplasmic factors in the case of HTLV-1 infected cells whose LTR-binding activity was confirmed by the promoter binding assays. Of these factors, Myocyte Enhancer Factor-2 (MEF-2), a chromatin-remodeling factor, was found to be of great interest, and therefore, its recruitment on HTLV-1 LTR was confirmed by the CHIP assay in both MT-2 and HTLV-1-infected primary CD4+ T cells. Furthermore, an increase in MEF-2 expression was observed upon HTLV-1 infection. In order to understand the
mechanism of MEF-2 activity, we confirmed its binding with Tax by co-immunoprecipitation, investigated its role in multiple signaling pathways and observed its direct effect on HTLV-1 infection. Overall, these studies are first to elucidate the involvement of a novel chromatin remodeling factor, MEF-2, in HTLV-1 pathogenesis.

**B37**

**Pharmacologic inducers of the macrophage antioxidant response inhibit HIV infection and neurotoxin production**

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HIV CNS infection causes a syndrome of cognitive, motor, and behavioral abnormalities collectively known as HIV-associated neurocognitive disorders (HAND). HIV-infected monocyte-derived-macrophages (HIV/MDM), a primary CNS reservoir, promote neurodegeneration through induction of neuroinflammation and neurotoxin release. These neurotoxins include small (<3kDa), heat-stable, protease-resistant molecules which excite N-methyl-D-aspartate (NMDA) receptors. The excitotoxins glutamate and quinolinic acid (QUIN) both correlate with degree of neurologic impairments in HIV-infected individuals. Although antiretroviral therapy (ART) improves clinical outcomes, the prevalence and associated morbidity of HAND remain high (~50%). Thus adjunctive neuroprotective therapies that address the pathological processes persisting in ART-treated individuals are needed. We have shown that HIV infection alters the macrophage antioxidant response (AR) and that pharmacological induction of the AR attenuates HIV replication and neurotoxicity. We hypothesized that HIV infection of MDM alters components of glutamate and QUIN biosynthesis and that inducers of the AR attenuate HIV neurotoxicity by modulating these neurotoxin pathways. Monocytes and MDM were obtained from PBMCs of human volunteers. HIV/MDM were infected with cell-free HIV-1. RT activity assays determined viral replication in supernatants. AR activation was determined by western blot. Cell-based MAP2 ELISAs determined neuronal survival. Significance was defined as p <0.05 using ANOVA or student’s t-test. We have demonstrated that HIV replication reduces expression of heme oxygenase 1 (HO-1), a key component of the AR, and also alters components of the glutamate and QUIN biosynthesis pathways. Inducers of the AR decrease neurotoxin production, even without altering HIV replication, in HIV/MDM. Our results suggest that inducers of the antioxidant response have therapeutic potential for HAND through suppression of HIV replication and neurotoxin production in MDM. This reduction of neurotoxin production may work through alteration of the biosynthetic pathways of glutamate and QUIN. Studies are ongoing to define the mechanism by which HO-1 modulates neurotoxin production.

**B38**

**Neurofibromatosis Type 2 tumor suppressor protein, NF2, induces proteasome-mediated degradation of JC virus T-antigen in human glial cells**

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The human polyomavirus, JC virus (JCV), is the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML) and displays a strong tropism toward glial cells. JCV typically replicates under immunosuppressive conditions, making this virus a threat to AIDS patients and those undergoing immunomodulatory therapies. Understanding the molecular mechanisms responsible for viral replication can have a pivotal affect on deterring viral resurgence in these patients. Expression of the viral early promoter is the initiating step in the virus life cycle and leads to the production of the major viral regulatory protein, T-antigen. To promote viral replication, T-antigen must hijack the cell cycle machinery of the host to enhance expression of itself and other JCV proteins. Studies on JCV T-antigen transgenic mice, which develop tumors resembling the malignant peripheral nerve sheath tumors (mpnst),
have led to the identification of neurofibromatosis type 2, NF2, as a binding partner for T-antigen. NF2 is a cytoplasmic scaffolding protein affecting cell motility and morphology which has tumor suppressor properties and has also shown to be present in the nucleus. We have found that NF2 downregulated expression of both large and small T-antigen proteins and activity of the JCV bidirectional promoter. Upon further characterization of this interaction, we determined that NF2 utilizes a novel approach to suppress T-antigen expression, whereby it promotes the accumulation of mature T-antigen mRNA and the proteasomal degradation of T-antigen protein. Collectively, these results show that NF2 is a negative regulator of JCV T-antigen expression, and suggests a novel role of NF2 as an inhibitor of JCV reactivation. The utility of NF2 as a potential strategy to block JCV replication in glial cells is discussed.

**B39**

Rapamycin improves mitochondrial homeostasis and delays the onset of senescence by altering p62/SQSTM1 dynamics.

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The Target of Rapamycin (TOR) is an evolutionarily conserved protein kinase that integrates signals from growth factors, cellular nutrients, oxygen and energy availability in order to regulate cell growth and proliferation. Inhibition of the TOR pathway extends lifespan in several model organisms, and represents a potential strategy for anti-aging intervention in humans. We explored the effects of chronic mammalian TOR (mTOR) inhibition in primary human fibroblasts treated with rapamycin, a highly specific TOR inhibitor. Prolonged exposure to rapamycin increased autophagy, reduced the half-life of mitochondrial proteins, and increased the turnover of p62/SQSTM1 and its localization with cytochrome c, suggesting increased mitophagy of damaged mitochondria in human fibroblasts. Consequently, cultures exposed to rapamycin had lower levels of reactive oxygen species and their mitochondria had increased membrane potential and higher resistance to oxidative stress. In the long term, inhibition of mTOR prevented the progressive accumulation of depolarized mitochondria, typical of cells approaching senescence, and decreased the activation of p38MAPK, a protein kinase involved in the establishment of senescence. Rapamycin treatment extended the maximum lifespan of cells (measured as cumulative population doubling, CPDL) by 14% and reduced the presence of senescence-associated markers at high CPDL. Interestingly, lowering p62/SQSTM1 expression increased mitochondrial depolarization to levels comparable to control and promoted the expression of the senescence marker p16INK4a in cells exposed to rapamycin. In conclusion, we find that inhibition of mTOR with rapamycin delays the onset of senescence by improving mitochondrial homeostasis and lowering oxidative stress. Interestingly, we find that rapamycin alters p62/SQSTM1 expression and localization and that p62/SQSTM1 is required to reduce the accumulation of depolarized mitochondria (and lower stress levels). Our results identify p62/SQSTM1 as a novel factor involved in the biology of aging and suggest that interventions on its expression and activity could lead to new strategies to slow the aging process.

**B40**

Differential regulation of miR-146a and EAAT2 by IL-1β and glutamate induced NF-κB activation in astrocytes

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The astroglial EAAT2 glutamate transporter is essential for clearing the critical neurotransmitter, glutamate in the central nervous system and protecting against excitotoxicity. The transcription factor NF-κB is a positive regulator of microRNA 146a and a negative regulator of EAAT2 expression. Glutamate is known to induce the transcription factor NF-κB. In the present study, we investigated the effect of
glutamate-induced activation of NF-κB in glial cells of the CNS, including primary astrocytes on the regulation of mir-146a and EAAT2 promoter regulation. Our studies indicated that glutamate-mediated NF-κB activation induced EAAT2 promoter but had no effect on mir-146a promoter. We also demonstrated that glutamate induced p65-IκBα dissociation occurred in the absence of IκBα phosphorylation or degradation. Furthermore, glutamate induced the rapid phosphorylation of p65 at Ser536. In contrast, HIV-induced pro-inflammatory cytokine IL-1β induced NF-κB activation which in turn, activated mir-146a promoter but inhibited EAAT2 promoter activity. Also, pro-inflammatory cytokine IL-1β induced rapid phosphorylation of p65 at Ser536 and IκBα phosphorylation or degradation. Our results provide insight into a glutamate-induced regulatory pathway that is distinct from cytokine-induced NF-κB activation in regulation of mir-146a and EAAT2 in astrocytes.

**B41**

**JCV-NCCR is targeted by negative transcription factors.**

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Patients undergoing immune modulatory therapies for the treatment of autoimmune diseases such as multiple sclerosis, and individuals with an impaired-immune system, most notably AIDS patients, are in the high risk group of developing progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the white matter caused by human neurotropic polyomavirus, JCV. It reactsivate under immunosuppressive conditions and replicates in oligodendrocytes leading to multifocal demyelinated lesions in the brain. We have previously demonstrated that the cellular alternative splicing factor SF2/ASF is a negative regulator of JCV in glial cells. SF2/ASF only inhibited splicing of JCV genes but also showed a strong suppression of basal transcription of the viral early and late promoters. This negative impact of SF2/ASF was dependent on its ability to bind a specific region within viral promoter mapped to the 98bp repeated region of the virus. In order to investigate the importance of the second 98bp repeated region in regulation of JCV gene expression and replication, we created a mutant JCV strain with only one 98bp repeated domain which also served only one binding site for SF2/ASF [JCV-Mad1-(1X98)]. Surprisingly, this large deletion within viral NCCR increased the rate of viral early transcription assessed by reporter gene constructs. However, the replication efficiency of this mutant virus was similar to the wild type virus. We also created a mutant strain with no SF2/ASF binding site [JCV-Mad1-δCR3 (1X73)]. Transcription and replication efficiency of the mutant virus was analyzed in PHFA cells. Interestingly, JCV-Mad1-δCR3 (1X73) showed three and two fold higher early promoter activity than JCV-Mad1-WT and JCV-Mad1-(1X98), respectively. However, this mutant virus was unable to propagate in PHFA cells. Further analyses of the transcription mediated by mutant promoter sequences revealed that JCV-Mad1-δCR3 (1X73) was defective in late gene transcription.

**B42**

**Cocaine-induced loss of white matter proteins in the adult mouse nucleus accumbens is inhibited by administration of a β-lactam antibiotic during withdrawal**

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Cocaine remains among the most commonly abused drugs worldwide and poses a substantial health and economic burden. Imaging studies and post-mortem microarray data from human cocaine abusers reveal significant deficits in white matter (WM) integrity and myelin-related genes in discrete brain regions,
including the nucleus accumbens (NAc). While underlying mechanisms remain largely unknown, WM abnormalities likely contribute to the cognitive, motor, and psychological deficits commonly afflicting cocaine abusers. Furthermore, human studies indicate that abstinence from cocaine allows for recovery of some cognitive function and gray matter density. Interestingly, WM density does not appear to recover in these patients, indicating long-term and potentially irreversible damage as a result of repeated cocaine exposure. As human studies are often confounded by poly-drug abuse and co-morbid conditions, in vivo animal models are needed to delineate pathways contributing to WM loss and highlight potential treatment avenues. In this context, we have uncovered a new paradigm by which chronic cocaine treatment significantly decreases the expression of WM proteins in the NAc of adult mice. Furthermore, we demonstrate that this WM loss can be inhibited and/or reversed by administration of a β-lactam antibiotic, ceftriaxone, during a period of cocaine withdrawal. Mice exposed to a chronic cocaine paradigm (15 mg/kg daily for 14 days) followed by 30 days of cocaine withdrawal and a challenge dose displayed significant decreases in myelin basic protein, proteolipid protein, myelin-oligodendrocyte glycoprotein, and myelin-associated glycoprotein in the NAc. Moreover, these changes were inhibited by administration of ceftriaxone (200 mg/kg daily) during withdrawal. No effect on WM loss was observed in mice treated with ceftriaxone during cocaine treatment but vehicle only during the 30 day withdrawal prior to challenge. Our observations identify cocaine-mediated myelin loss in an adult mouse model and highlight a potential pharmacological intervention to reverse and/or prevent cocaine-induced WM loss.

**B43**

**CD135 (flt3) up-regulation in the CNS in SIV infection and encephalitis (SIVE) and by M-CSF in vitro: Support for CD135 and M-CSF as potential therapeutic targets for HIV infection and CNS disease**

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Macrophage-colony stimulating factor (M-CSF) is believed to play a prominent role in AIDS progression and the development of HIV-associated neurocognitive disorders (HAND). In addition to increasing HIV infection and virus production in monocytes, M-CSF increases CD16+ monocyte frequency in circulation and CD163 expression. These cells are phenotypically similar to macrophages that accumulate in the CNS in HIV encephalitis (HIVE) and are believed to contribute to the pathogenesis of HAND. Therefore, M-CSF may be a viable therapeutic target for treating or preventing HAND. Here, we report several tyrosine kinase inhibitors (TKI) with M-CSF receptor (CD115) activity, reduce the frequency and/or expression of CD16, CD163, CCR5, M-CSF and CD115 by monocytes in M-CSF treated peripheral blood mononuclear cells (PBMC). Interestingly, these data suggest that a proprietary TKI reported to be exclusive to CD115, decreases the frequency of CD135 [fms-like tyrosine kinase 3 (flt3)], a receptor tyrosine kinase expressed by monocytes and believed to interact exclusively with flt3 ligand (FL). M-CSF-treated PBMC showed increased frequency of CD135 by monocytes without changing CD135 expression level. Because M-CSF is elevated in cerebral spinal fluid (CSF) of patients with HIVE, it may alter expression of CD135 in the CNS. We examined CNS tissue from a relevant animal model for HIV-related CNS disease, SIV infected rhesus macaques, for CD135 expression. These studies showed greater expression of CD135 in CNS of SIV+ macaques with even greater expression seen in those with encephalitis (SIVE), as compared to seronegative animals. This was observed as an increase in the number of CD135+ cells, as well as the degree of expression. These findings suggest that M-CSF/CD115 signaling may be involved in the regulation of CD135 and/or its ligand. Modulation of CD135 may be a potential adjunctive therapy, in addition to targeting CD115, as a therapeutic strategy for treating or preventing HAND.
**B44**

**Increased levels of tetra-antennary N-linked glycan but not core fucosylation are associated with hepatocellular carcinoma tissue**

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Alterations in glycosylation have long been associated with the development of cancer. In the case of primary hepatocellular carcinoma (HCC), one alteration that has often been associated is increased amounts of fucose attached to the N-glycans of serum proteins secreted by the liver. In an effort to determine the nature of this increased fucosylation, we have performed N-linked glycan analysis of HCC tissue, the surrounding non tumor tissue, and compared this to tissue from a non diseased adult liver. Surprisingly, no difference in the level of fucosylation was observed from the three donor groups, suggesting that the increased levels of fucosylation observed in serum of those with HCC is not the result of increased synthesis of fucosylated proteins. On the other hand, increased levels of a tetra-antennary glycan were observed in the HCC tissue as compared to the surrounding tissue and the non diseased tissue from an independent liver. This represents the first report associating increased levels of branching with the development of HCC.

**B45**

**HIV viral protein Tat impairs early differentiation of neural stem cells through inhibiting NF-κB signaling**

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In the era of highly active antiretroviral therapy, a milder form of HIV-associated neurocognitive disorders (HAND) has become prevalent among the HIV-infected population. Both clinical and animal studies have demonstrated the impairment of neurogenesis induced by HIV infection or viral proteins. Neurogenesis is initiated from the self-renewing neural stem cells (NSCs) that produce committed daughter neural progenitor cells. Previous studies demonstrated the inhibition of neuronal differentiation and promotion of astrogliosis by HIV viral proteins. However, the underlying molecular mechanisms remain poorly understood. Our recent studies demonstrated that NF-κB signaling is required for NSC initial differentiation. To gain insight into the potential role of NF-κB in mediating HIV viral protein-induced inhibition of neuronogenesis, we examined the effects of Tat on the NF-κB activity, self-renewal and tripotential differentiation of NSCs. Primary neurospheres were cultured from neonatal mouse brain. Dissociated single cells were cultured as monolayer in matrigel-coated plate under proliferation or differentiation condition and treated with or without viral protein Tat for 24 hours. The activity of NF-κB signaling was determined using adenovirus-mediated NF-κB -luciferase reporter assay. The stemness frequency was determined by assessing the self-renewing and multipotent capability of NSCs using modified NeuroCult® neural colony-forming cell assay. We found that Tat treatment for 24 h had no effect on the constitutive NF-κB activity and the stemness frequency of NSCs under proliferation conditions. However, the activation of NF-κB signaling under differentiation condition (withdrawal of EGF/FGF2) was significantly inhibited by Tat exposure. Differentiation-induced reduction of the stemness frequency was significantly reversed by Tat treatment. These data suggest that Tat inhibited the initial differentiation of NSCs and such inhibition might result from the blockade of NF-κB signaling. Impediment in the first rate-limiting step of neuronogenesis by HIV infection may lead to aberrant neuronal lineage differentiation and consequent neurocognitive disorders in the milder HAND.
Type I interferon increases apoptosis and proapoptotic Bak expression in T cells from chronically HIV-infected individuals

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The role of Type I IFN in priming T cells for apoptosis, a characteristic of T cells in HIV infection, is unclear. We examined the effect of type I IFN on pro- and antiapoptotic molecule expression and apoptosis sensitivity in T cells from HIV infected and healthy individuals. Although proapoptotic Bak, Bax and Bim levels were significantly increased in T cells from HIV-1-infected patients, only Bak expression in CD4⁺ T cells correlated directly with CD95/Fas-mediated apoptosis and inversely with CD4⁺ T cell counts. IFNa/β increased Bak expression and the sensitivity of healthy donor T cells to CD95/Fas-mediated death. Type I IFN further primed HIV-specific CD8⁺ T cells from HIV-1-infected patients to CD95/Fas-mediated apoptosis. Exposure of PBMC to infectious and non-infectious HIV-1 sensitized CD4⁺ T cells and CD8⁺ T cells to CD95/Fas-induced death and this apoptosis induction was mediated by TLR7/9 and Type I IFN. Ex vivo IFNa-induced IF16-16 gene expression in PBMC from HIV-1-infected patients was increased and positively associated with Bak expression and CD95/Fas mediated T cell apoptosis, while it correlated inversely with CD4⁺ T cell counts. This inverse correlation was also seen between plasma IFNa and CD4⁺ T cell counts during chronic SIV infection. Our findings indicate that Type I IFN produced during chronic HIV-1 infection may contribute to the loss of CD4⁺ T cells and the failure of HIV-specific CD8⁺ T cells to control viral replication by upregulating Bak and sensitizing T cells to CD95/Fas-induced apoptosis.

TNF-α stimulates outgrowth of neuronal processes upon injury via stimulation of NF-κB1-induced EPHB2 signaling.

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HIV-1 infection of central nervous system (CNS) causes neuronal injury and synaptic loss, both of which correlate with cognitive decline in HIV-1/AIDS patients. Neuronal damage results from CNS viral replication and neurotoxic effects of inflammatory chemokines and cytokines, including TNF-α. It was previously suggested that TNF-α signaling is involved in regulation of glutamatergic transmission. To determine whether IGF-1 influences TNF-α-mediated effects on synaptic plasticity, the expression of genes central to synaptic alterations during learning and memory were analyzed in human neurosphere cultures using quantitative RT-PCR. Our results demonstrate that TNF-α significantly altered expression of immediate-early response genes ARC, EGR1, EGR4, and HOMER1. TNF-α treatment induced expression of Ephrin type-B receptor 2, which is involved in the NMDA signaling pathway; glutamate receptor subunits ζ1 and ε-3; PICK1, which interacts with glutamate receptors; and neuronal pentraxin-2 (NPTX2), which is involved in excitatory synapse formation. TNF-α also induced expression of genes of proteins involved in extracellular matrix breakdown and proteolytic processing: Reelin, MMP9, and PLAT. These proteins are key effectors of ECM remodeling. In addition, Reelin is involved in the maintenance of long-term potentiation and dendritic spine development. Observed effects of TNF-α were opposed by IGF-1. We utilized a scratch assay to demonstrate the response of primary human neurons to injury upon treatment with TNF-α, IGF-1, and TNF-α combined with IGF-1. Our results show that TNF-α induces more neurite outgrowth, both in length and complexity. It has been shown that TNF-α can induce neuroprotective effects through the NF-κB pathway, therefore, we propose that upon TNF-α treatment
following induced injury, neurite outgrowth occurs primarily through EPHB2 signaling via stimulation of NF-κB1. Our observations provide a new avenue for the investigation on the impact of HIV-1 in neuronal cell damage and the involvement of TNF-α and IGF-1, along with other cytokines in this event.

**B48**

**PINCH in the cellular stress response to Tau-hyper phosphorylation**

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Hyperphosphorylated Tau (hp-Tau) detaches from microtubules and can form paired helical filaments and tangles leading to neuronal dysfunction. In healthy ageing, aberrant proteins such as hp-Tau are cleared from the cell by the heat shock response (HSR) machinery. In the case of hp-Tau, the HSR complex sorts aberrant Tau for either repair or degradation. However, if the cellular machinery fails to clear abnormal proteins, accumulation of aberrant proteins can occur. Accumulation of hp-Tau accounts for more than 20 neuropathological diseases including AD and HIV-1. In this context, we have discovered that a protein called PINCH binds to hp-Tau and may function in its clearance from neurons. Likewise, PINCH is detectable in the CSF of HIV and AD patients, but is nearly undetectable in healthy controls. Briefly, PINCH is composed of 5 double zinc finger domains and has no catalytic activity. PINCH is highly conserved, plays a key role in multi-protein complex formation, and facilitates cell spreading, migration and survival. Growing evidence points to significant overlap between mechanisms involved in HIV-associated neurocognitive disorders (HAND) and age-related neurodegenerative diseases. HIV+ individuals diagnosed decades ago are beginning to face age-associated CNS changes. Combined with infection and long-term exposure to cART, age-related neurodegeneration may be exacerbated. During neuronal stress, PINCH protein is required to maintain neurite extensions. Additionally, studies in cancer suggest that PINCH promotes cell survival, but no direct mechanism has been identified. We show that during the cellular stress response, PINCH binds to hp-Tau and may contribute to changes in intracellular levels of hpTau. These studies address a new mechanism by which aging and HAND may interact. We hypothesize that in diseases with a tauopathy component, PINCH is expressed by neurons to promote cell survival through its interactions with Tau and the heat shock protein response machinery.

**B49**

**Pushing the limits of expression: highly optimized next generation molecular adjuvants for mucosa targeting DNA vaccines**

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Scientists who are working to develop DNA-vaccine platforms against a wide range of diseases and conditions have begun to employ multiple technical advancements such as gene optimization strategies, usage of immune adjuvants, and more effective delivery methods that lead to enhanced gene expression and subsequent immunogenicity. In an effort to augment both systemic and mucosal immune responses to our laboratory's HIV-1gag/pol DNA-vaccine, we carried out a series of gene modifications to the molecular adjuvants, CCL25, CCL27 and CCL28, members of the mucosal chemokine family. We hypothesized that by adding an efficient secretory leader and KOZAK context, optimizing codons for G-C-content, as well as removal of cis-acting sequence motifs and RNA secondary structures that impede translation, protein expression of the mucosal-targeting molecules would be further augmented. Following synthesis and cloning of the highly optimized genes into expression vectors, we tested the specific chemokine protein levels in comparison to nonoptimized chemokine controls. Following
transient transfection of HELa cells, cell lysates or supernatants were harvested 48 hours after transfection. Using western blotting and ELISA techniques, we observed a modest increase in CCL27 expression, a 7-fold increase in CCL28 expression (673 μg/ml +/-112 SEM to 4843 μg/ml +/-450 SEM) and a 5-fold increase in CCL25 expression (496 μg/ml +/-87 SEM to 2439 μg/ml +/-485 SEM). Supernatants from transfected cell cultures contained enhanced chemokine bioactivity as up to a 10-fold increase in migration of primary splenocytes and mesenteric lymph node cells were observed using transwell assays. Moreover, pCCL25, -27 and -28 in vivo co-immunization of balb/c mice as adjuvants in combination with HIV-1 DNA antigens resulted in a 1.5-, 1.2- and 3.9-fold increase in HIV-1 gag specific IgG and a 1.6-, 1.2- and 9.3-fold increase in HIV-1 gag specific IgA, respectively, as measured by B-cell ELISpot. These data support the use of highly optimized molecular adjuvants in our HIV-1 gag/pol vaccine platform for targeting HIV-1 specific T and B cells to mucosal sites.

B50
An Increasing Incidence of ESBL-producing Multidrug-Resistant Acinetobacter baumannii from a Tertiary-Care Teaching Hospital

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Extended-spectrum β-lactamases (ESBLs) imperil novel β-lactams, and their presence has a huge impact on success of antimicrobial chemotherapy against important nosocomials such as Acinetobacter (Aci), causing life threatening infections. These pathogens survive for months in hospital environment. This study analyzes the occurrence of ESBL and ampC from the clinical isolates of Aci. During Jan 2003-Dec 2007, 60964 suspected infectious samples were processed, and Aci identified using standard microbiological methods, including API and VITEK-2 systems. The genomic assay and phenotypic tests were carried out for speciation. Other characteristics were determined by disk diffusion for antibiogram, broth microdilution for MICs, nitrocefin test for β-lactamase, phenotypic confirmatory disk diffusion test (PCDDT) for phenotypic ESBL and ampC detection, PCR assay and sequencing for 16S rRNA identification and bla-ESBL genes, and the safranin microtiter plate assay for biofilm detection. From 17097 infective samples, 632 non-repetitive isolates were identified as Aci, of which 582 (92%) were A. baumannii (Ab). Over 70% showed multidrug resistance. A β-lactam/β-lactamase inhibitor combination had negligible effect on Aci. Tigecycline, colistin and polymyxin-B were the most effective antimicrobials, showed sensitivity of 91.77%, 91.45% and 89.87% respectively. MIC90 value for most of the tested agents was >265 μg/ml, and colistin, <16 μg/ml and tigecycline, <64 μg/ml, demonstrates their increasing resistance levels. Total 237 (37.5%) and 11 (1.74%) of Aci were ESBL-positive and ampC-positive, respectively, and all were Ab, and strong biofilm-producers. The major genes were bla-TEM T1-T2, followed by Bla-ADC-7 from representative pan-drug resistant Ab. Multidrug resistance in Ab is rapidly rising, and the empiric β-lactam/β-lactamase inhibitor therapy is no longer beneficial in infections due to such phenotypes, and colistin and tigecycline MICs are steadily rising, a serious concern. To our knowledge, for the first time we report bla-TEM-1, 2 and bla-ADC-7 genes in MDR Aci from Indian hospital.
Alginate gels serve as an ideal wound dressing by virtue of their ability to absorb exudates, maintain a moist wound healing environment, and serve as a vehicle for drug delivery, but lack antimicrobial capabilities. Currently, several alginate gel based antimicrobial dressings available which control wound contamination, but have appreciable cytotoxicity. Non-thermal dielectric barrier discharge (DBD) plasma is known to generate reactive oxygen / nitrogen species that can react with and inactivate bacteria. Here we proposed a plasma-activated antimicrobial dressing, which does not show cell toxicity, and promotes cell proliferation and migration. Sodium alginate gels (d = 2.3 cm) either untreated or treated for 1 minute on each side under plasma conditions, with a frequency setting of 1.5 kHz, voltage of 31.6 kV, (6.1 watts/cm2 power) using microsecond pulse. For antimicrobial studies, these gels were then inoculated with starting concentrations around 10^7 of multiple pathogenic strains. For endothelial cell (EC) studies, the gels were submerged in serum free cell media over time. Multiple samples were taken over a 48 hour range, and compared to samples of untreated gels in serum free media, serum free media with no gel (negative control) and a chlorhexidine gluconate gel (positive control). The Oris Cell Migration Assay was used to confirm the proliferation and migration benefits of the plasma treated alginate gels. Total inactivation of all pathogens observed after 15 seconds of exposure to the plasma treated alginate gel. Indirect exposure of ECs with treated alginate gels produced a greater proliferative effect as compared to untreated alginate gels. Commercially available chlorhexidine gel had relatively significantly more cytotoxicity, compared to plasma-treated gels. The ECs exposed to the plasma treated gels were able to migrate and occlude the simulated wound faster than the non-treated gels. In summary, plasma-activated alginate gels have better in-vitro wound decontamination and wound healing capabilities.

**B52**

**Antimicrobial Properties of Liquids Treated by Non-Thermal DBD Plasma**

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Disinfection of contaminated surfaces and control of biofilm-embedded pathogens is a major problem in hospital environment. Many of the commercially available biocidal agents are either not sufficient to inactivate bacterial pathogens (especially embedded in biofilms) or detrimental to delicate surfaces. Thus, there is an unmet need of efficient broad-spectrum antimicrobial and antibiofilm agents. By applying floating electrode-dielectric barrier discharge nonthermal plasma technique, we have treated fluids [deionized water or a phosphate-buffered saline or N-acetyl-cysteine (NAC) solution], and generated antimicrobial solutions, tested for properties such as changes in pH, temperature, delay time, contact (holding) time, fluid-aging, and detection and comparison of acid and hydrogen peroxide. The pathogens were tested in their planktonic and biofilm forms, and colony assays and XTT [2,3-Bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide)] assays were used to detect bacterial viability. All plasma-treated solutions showed strong biocidal activity, and among them, N-acetyl-cysteine solution was the most powerful, which inactivated biofilms of Acinetobacter baumannii, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis, Candida albicans, and C. glabrata in 15 min of holding/contact time. During accelerated aging experiments,
plasma-treated N-acetyl-cysteine solution was found extremely stable and exhibited the equivalent of 2 years of shelf life at temperatures of 50ºC. These results indicate that the plasma-activated solutions retain their antimicrobial properties for an extended period against a wide range of multidrug-resistant pathogens, making then an excellent candidate for further testing in vivo. The effect may not be totally due to a change in the H₂O₂ or nitric acid or pH, but a combination of all the species or their product.

B53

A close association of biofilm-producing Staphylococci and Atopic Dermatitis: Is the association etiologically important?

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Atopic dermatitis (AD) is an inflammatory, pruritic skin disorder, has multiple clinical presentations, and predominantly coexists with other atopic diseases such as asthma and hay fever, and thought to be associated with autoimmunity and familial trait. Biologically, a coexistence of Staphylococcus aureus and AD has been reported over two decades but very less is known about their etiologic involvement. Study involves skin swabs from 40 patients attending clinic for AD treatment, collected aseptically, and processed for microbial identification using traditional microbiological methods and API. The identified isolates assayed for MICs using Gram +ve sensitiitre plate, extracellular biomass formation by Congo-Red Agar (CRA) test, biofilm formation by XTT assay, staphylococcal biofilm in skin tissues by Gram staining, and confocal microscopy, and detection of biofilm mediating genes, icaD and aap using PCR assay. All the samples (irrespective of the site of AD lesion) were positive for grow of multidrug resistant (MDR) staphylococci, including methicillin-resistance. API staph identification showed that 37/40 (90%) of the isolates were staphylococci, and speciation revealed S. aureus (42%) and S. epidermidis (20%) as the predominant species. CRA test demonstrated all the isolates as strongly positive for extracellular polysaccharide (biomass) producing staphylococci. XTT assay showed 85% of the isolates strong biofilm producers and 15% moderately positive. PCR detection revealed the biofilm formation mediating aap gene in 12.5% and icaD gene in 20% of the isolates. Other biofilm mediating genes are being studied. Biofilm formation by AD-associated staphylococci such as S. aureus and S. epidermidis, probably play a major role in occlusion of eccrine ducts, and leading in part to setting inflammation and pruritus. The MDR status of the isolates didn’t allow them to disappear during antibiotics-based therapy, and the icaD and aap genes in part are involved in mediating such biofilms. Further molecular studies are in progress.

B54

Presence of blaOXA-51 like genes in carbapenem-resistant Acinetobacter baumannii in hospitalized patients in Philadelphia, PA

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Acinetobacter baumannii is third commonest gram-negative coccobacilli involved in hospital-acquired infections, and multidrug resistant (MDR) phenotypes are major concern. Carbapenems are often the last resort to treat MDR A. baumannii infections, but recently carbapenem-resistance reportedly emerging worldwide, including the United States. The metallo-β-lactamases (MBLs) and OXA-like carbapenemases are major resistance mechanisms. In this study we report the analysis of ISAba1 insertion sequence preceded blaOXA-like carbapenemase & MBL genes in virulent carbapenem-resistant A. baumannii (CRAB). During Jun 2010 to Jan 2011, 104 A. baumannii were isolated from hospitalized patients. All the isolates were analyzed for antibiotic susceptibility pattern, and also screened by the Hodge test and the double disc synergy (DDS) test. The isolates were analyzed by PCR amplification for the presence commonest genes such as, blaOXA-23, blaOXA-40, blaOXA51, blaOXA58, blalMP, blalVIP, blalSIM, blalGIM, blalSPM, blalDIM, and blalNDM-like genes. All carbapenem-susceptible
isolates and 16 CRAB isolates were also analyzed for the presence of insertion sequence ISAba1. An appropriate ATCC reference strains were included as controls. Out of 104 isolates, 84 (80.8%) isolates were resistant to meropenem, and showed multidrug resistance. For Hodge test, 16 isolates were positive; but negative for double disc synergy test. No blaMBL genes were detected. All CRAB, and 4 carbapenem-susceptible isolates were positive for blaOXA-51 like genes. All isolates had ISAba1 preceded blaOXA-51 genes in their genome whereas only 6 carbapenem-susceptible isolates were detected having ISAba1, not preceding bla-OXA-51 sequences. The presence of blaOXA-51 gene has some role in carbapenem resistance as the resistant isolates are negative for DDS tests and MBL genes, which confirms the absence of metallo β-lactamases. We believe that the expression of blaOXA-51 is regulated by ISAba1 in our locally isolated CRABs, and that differential expression of OXA-51 carbapenemase would be an important characteristic. Further molecular characterization is in process.

B55
Characterization of Acinetobacter infection at tertiary care teaching hospital
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Acinetobacter baumannii (Ab) is a gram-negative nosocomial pathogen, involved in hospital-acquired infections; and multidrug resistant (MDR) phenotypes are continuously on rise worldwide. The last resort antibiotics used for treatment are carbapenems. In this study we report on the characteristics of carbapenem-resistant Ab through clinical data analysis. Risk factors for the development of such resistance are also evaluated. During June 2010-Dec 2011, 100 Ab cases were analyzed retrospectively, involving 64 patients and 66 distinct incidences of hospital admission. The isolates were subjected for API identification and antibiogram for the first and repeat (second) isolates. The risk factors and demographic features were analyzed to derive relative risk factor and odds ratio. Of the 100 samples, 49% were sputum samples, collected from the ventilated patients. Total 57% of the cases had an associated coinfection. MRSA, Pseudomonas aeruginosa and Klebsiella pneumoniae were the significant co-infecting agents. Prior hospitalization, progressive care units and nursing units, respiratory ventilation, polymicrobial infections involving Ab, were the significant risk factors. Antibiogram demonstrated a multidrug resistance in majority of the isolates. Relative risk associated with ventilation, diabetes and surgery was higher in repeat isolates compared to first isolates. Multi drug resistance was observed in majority of the isolates. The repeat isolates displayed an increased antibiotic resistance. The resistance against all carbapenems and colistin is increasing slowly; and the resistance became double in repeat (second) isolates compared to initial (first) isolates, against colistin, the drug of choice for such infections.

B56
Mechanism of Action of a p53-Derived Peptide that Targets a Novel Death Pathway Inducing Selective Necrosis in Cancer Cells
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Our laboratory identified specific domains from the p53 protein that possess anti-cancer activity. In a series of preliminary in vitro and in vivo studies we have shown that p53-derived peptides consisting of residues 12-26 and 17-26 from the murine double minute binding domain (MDM-2) possess anti-cancer activity. These peptides are linked to a trans-membrane penetrating sequence (penetratin) derived from the antennapedia homeodomain of Drosophila and are designated PNC-27 and PNC-28, respectively. We
also found that our previously determined 3D structure of the p53 residues of PNC-27 is directly superimposable on the same residues bound to MDM-2, suggesting that these peptides may target MDM-2 in the membrane of cancer cells. These peptide constructs appear to promote cancer cell death through a novel pathway that leads to necrosis rather than apoptosis while sparing their untransformed counterparts. In our initial experiments, the critical role of the MDM-2 oncoprotein as a potential target molecule for these PNC peptides was elucidated when a sequence specific MDM-2 antibody blocked PNC-27 activity on cancer cells. Our current studies suggest that these PNC peptides promote selected cancer cell cytotoxicity by a novel p53-independent mechanism in which the peptide induces pore forming cancer cell membranolysis dependent on binding of the peptide to HDM-2 in the cancer cell membrane.
C57

Comprehensive NeuroAIDS Center

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The Comprehensive NeuroAIDS Center (CNAC) at Temple University is one of nine Centers of its kind in the United States funded by the National Institute of Mental Health. It was founded in 2011. CNACs mission is to improve and extend public health impact of bench-to-clinic research associated with HIV-induced neurological diseases and cognitive disorders. Neurological complications and cognitive disorders are among the most devastating clinical manifestations of HIV-1 infection in AIDS patients today – including patients receiving treatment with antiretroviral therapy. In the Philadelphia area, the rate of HIV-1 infection is five times the national average, with more than 19,000 individuals currently infected with the virus. Temple University Hospital’s Comprehensive HIV Program treats more than 10% of AIDS patients in Philadelphia, and 10% of its inpatient neurology discharges are HIV-1 positive individuals with neurological disorders. CNAC’s goal is to improve and extend the public health benefits of bench-to-clinic research related to NeuroAIDS in the Philadelphia region and beyond. To this end, CNAC brings together a large group of AIDS and neuroscience investigators based at Temple and other leading academic medical centers. CNAC offers expert research services and technologies, an established clinical HIV program including neuropsychiatric and neurocognitive assessment, mentorship/training programs, and seed funding for new translational research projects addressing NeuroAIDS and HIV-1 associated neurocognitive and behavioral disorders. CNAC offers a multidisciplinary research approach by providing scientific services in cell culture and neurotropic viruses, animal model development and behavioral testing, proteomic biomarker discovery, as well as expertise and consultation in neuroscience and neuropathology. Overall, CNAC’s strategy is to achieve success in advancing HIV and AIDS research by providing services to the scientific community and fostering translational collaborative efforts for worldwide NeuroAIDS research. CNAC is integrated with the Temple/Drexel Interdisciplinary and Translational Research Training Program in NeuroAIDS and efforts are under way to partner with other related Centers and Training programs nationally.

For more information visit: www.temple.edu/medicine/cnac

C58

Interdisciplinary and Translational Research Training Program in NeuroAIDS

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The Interdisciplinary and Translational Research Training Program (ITRTP) for predoctoral students in NeuroAIDS and related areas of research is a joint program from two institutions, Temple University and Drexel University, located in close proximity in Philadelphia, Pennsylvania. The program creates a city-wide interdisciplinary and translational research training program in NeuroAIDS through shared resources, joint research seminar series, journal club, symposia, invited speakers, thesis mentoring and educational opportunities at both institutions. The graduate curriculum at both institutions is designed to provide a broad based scientific foundation in biomedical science including Neuroscience, Immunology,
Microbiology, Pharmacology and Physiology. This curriculum including Scientific Communication, Scientific Integrity and Bioethics, and Statistics, as well as courses in Molecular and Cellular Neurobiology and Pathogenesis of Neurobiological Diseases prepares graduate students for thesis research in NeuroAIDS. Within Temple University School of Medicine, research opportunities in NeuroAIDS and related areas are available within the Department of Neuroscience, Department of Microbiology and Immunology, Department of Pharmacology, and Department of Physiology with additional opportunities in the School of Engineering. At Drexel University College of Medicine, research opportunities in NeuroAIDS and related areas are available in the Department of Microbiology and Immunology, Department of Neurobiology and Anatomy, Department of Pharmacology and Physiology and Department of Biochemistry and Molecular Biology. Additional research opportunities are available in the School of Biomedical Engineering and Health Science Systems. This program brings together multiple biomedical basic science departments at two institutions, and integrates joint training activities with nearby University of Pennsylvania. With the inclusion of clinical AIDS investigators, the program is not only interdisciplinary, but exposes students to training in neuroAIDS basic sciences, AIDS, and NeuroAIDS related clinical perspectives. This training program interdigitates closely with the NIMH supported Temple/Drexel Comprehensive NeuroAIDS Center (CNAC). Overall, this program provides formal comprehensive training in NeuroAIDS in areas ranging from molecular studies, in vitro systems, and pathogenesis studies in human and non-human systems. Future efforts will expand the training activities of this T32 program in the development of a comprehensive neuroAIDS training course in collaboration with CNAC and other related T32 programs nationally.

C59
An Animal Model To Study The Effects Of Primary Tumor Excision On Metastatic Potential And Organ-Tropism Of Residual Breast Cancer Cells

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Despite the high 5-year survival rate following surgical excision of a locally invasive breast tumor, the risk of dying from metastatic disease fifteen years after surgery is currently between 30% and 36%. As only 37% of breasts are found tumor-free following initial lumpectomy, between 22 and 59% of patients currently need re-intervention because positive or close margins are detected. Local factors produced at the site of the surgical wound may affect the residual cancer cells by selecting for cancer stem phenotypes, increase metastatic potential and promote the dissemination to specific secondary organs. This study aims to: 1) establish whether surgery on primary breast tumors leads to the emergence of cellular phenotypes with higher malignancy, associated with an increase in local growth, invasiveness and metastatic potential; 2) determine whether breast surgery alters the organ-tropism of residual cancer cells. For our experiment, BALB/c mice inoculated in the mammary fat pad with 4T1 murine breast cancer cells develop tumors that are either left to progress or surgically excised. Approximately 50% of animals in the surgery group presented with local recurrence, although all the excised tumors had positive margins. Local recurrences showed higher Ki-67 staining compared to their respective primary tumors and these data were in agreement with human samples of primary and recurrent breast tumors. Interestingly, the overall survival of mice in the control and surgery with recurrence groups were similar. Furthermore, macroscopic lung metastases were detected in the control and local recurrence groups, whereas mice without post-surgery recurrence failed to develop lung metastatic lesions. Finally, differences in the dissemination and growth of 4T1 cells to brain and bone between control, surgery plus recurrence and surgery without recurrence groups were investigated.
**C60**

**Molecular determinants for the bone-metastatic potential of prostate cancer cells.**

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Skeletal metastasis is the major cause of morbidity and death in patients with advanced prostate cancer (PCa). The propensity of cancer cells to progress into macroscopic lesions at secondary sites relies on the successful adaptation of Disseminated Tumor Cells (DTCs) to the new microenvironment. This requirement explains the predilection of most tumors to produce clinical metastases in selected organs although they can seed malignant cells also in several other tissues. It seems plausible that the metastatic fate of DTCs is dictated by the pleiotropic effects of specific genes and their encoded products establishing functional interactions with the surrounding stroma. The main objective of this study was to identify these molecular mediators of cell survival and growth in the bone with the ultimate goal of identifying effective therapeutic targets to counteract PCa metastases. Using a pre-clinical animal model of experimental metastasis, we have recently shown that exogenous over-expression of PDGFRα in non-metastatic prostate phenotypes conferred bone-metastatic potential and a monoclonal antibody targeting the receptor reduced bone metastatic tumors by more than 70%. Here we report that comparative gene-expression analyses conducted with PCa cells showing different intrinsic metastatic potential, as well as prostate phenotypes that fail to increase their metastatic behavior upon PDGFRα expression, resulted in a bone-metastatic gene signature including three genes encoding for soluble, secreted proteins. This suggests that DTCs with aggressive metastatic behavior modify the bone microenvironment in their favor, not only coercing the stroma to support their own survival, but also allowing other malignant phenotypes, that would otherwise fail to thrive, to grow into macroscopic tumors. We are currently testing this cell-cooperation in metastasis as well as functionally validating our three-gene signature by stably silencing or over-expressing each of the three newly identified genes in bone-metastatic and non-metastatic PCa cells, respectively.

**C61**

**Effects of Morphine on Ferritin Subunits in Neurons, and a Resulting Inhibition of CXCR4 Signaling**

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CXCR4 is a chemokine receptor constitutively expressed in the central nervous system, well characterized for its homeostatic pro-survival effects on developing, as well as mature neurons. Recent studies have identified morphine as a negative regulator of neuronal CXCR4 function, an effect relying on an increase in levels of the heavy chain subunit of ferritin (FHC). Although FHC is best known for its role in the iron-sequestering functions of ferritin, a protein complex formed with its partner subunit ferritin light chain (FLC), FHC has been shown to interact specifically with chemokine receptors, including CXCR4, and to inhibit endogenous CXCR4 signaling. This study analyzes the relationship between iron-binding and CXCR4-regulatory functions of FHC, and suggests a pathological relevance of elevated FHC levels in opiate abuse. Here we demonstrate a preferential effect of morphine on increasing FHC levels, as compared to FLC levels, suggesting a functional outcome specific to activity of the heavy chain subunit. We then investigate the role of FHC’s iron-binding activity on CXCR4 signaling, and demonstrate an ability of mutant non-iron-binding, as well as wild-type FHC, to inhibit CXCR4 signaling. Together these data suggest that CXCR4 regulation is a distinct function of FHC, independent from its primary role in storing iron. A specific physiological consequence of altered FHC levels is investigated by measuring changes in dendritic spine density. CXCR4 activation is shown to increase, and morphine to decrease spine density, therefore future and ongoing experiments aim to analyze the involvement of FHC in the harmful effects of morphine on dendritic spines. Together these data suggest a neuropathological outcome
of increased FHC, which may disrupt multiple cellular processes, and may mediate neuronal dysfunction as a consequence of opiate abuse.

**C62**

**Mechanistic aspects of DC:T cell interaction during HTLV-1 associated oncogenesis and neuroinflammation**

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Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of two immunologically distinct diseases: adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the HTLV-1-specific CD8+ cytotoxic T cell response is seen in both pathogenic states, its actual significance in preventing viral load and controlling disease progression remains questionable. Utilizing a newly standardized dendritic cell and a T cell polychromatic antibody cocktail, we investigated the immune activation of these cells in a patients cohort from Jamaica including HTLV-1 seronegative controls, asymptomatic carriers (ACs), ATL, and HAM/TSP. Extensive immune profiling revealed that CD8+ T cells from both HAM/TSP and ATL patient samples demonstrated some functional responses, albeit to a much lesser extent than those responses seen in ACs. Furthermore, DCs from HTLV-1-diseased individuals exhibited an altered maturation and adhesion phenotype as compared to ACs. The expression of an inhibitory molecule PD-1 and its ligand, PD-L1 was upregulated in CTLs and DCs, respectively in both diseased groups. While comparing the matched proviral loads to the flow cytometry results, we identified unique immune signatures distinguishing ACs from ATL and HAM/TSP patients. Collectively, these results uniquely demonstrate the critical role of DCs in their ability to prime immune responses during initial cell-free HTLV-1 infection and highlight a significant aspect of viral immunopathogenesis related to the development of ATL and HAM/TSP. In addition, these results suggest that modulation of both DCs and CD8+ T cells and/or blockade of the PD-1/PD-L1 pathway may be useful in therapeutic interventions of ATL and/or HAM/TSP.

**C63**

**A polychromatic antibody cocktail to investigate phenotypic and functional markers on human dendritic cells and its applicability in a cohort of HIV-1/HCV patients**

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Antigen presentation is a critical feature of adaptive immunity and essential in self versus nonself discrimination as well as in stimulating immune responses against pathogens. Dendritic cells (DCs) are considered to be the most potent antigen-presenting cells and have long been recognized as key regulators of the immune system. Because DCs are such crucial cells of the immune system, an extensive assessment of their quality in infected individuals is critical. Therefore, we have standardized a unique DC cocktail containing 12 different functional DC markers using an LSR II polychromatic flow cytometer. To this point, we have tested our newly standardized cocktail on the fresh, frozen, and cultured peripheral blood mononuclear cell (PBMCs) as well as on monocyte-derived DCs (MDDCs), a widely used surrogate system for primary blood DCs. This study emphasizes the practicality of utilizing frozen versus fresh cells, especially in rare disease states, wherein obtaining fresh material is virtually impossible. Furthermore, we focus on the feasibility of PBMCs compared to whole blood as starting material. To our knowledge this is the first comprehensive DC phenotyping protocol that uses an extensive list of functional markers (>8). Collectively, these investigations possess great potential to
enable investigators to perform immune cell monitoring and develop diagnostic and therapeutic strategies. This newly standardized cocktail has already been utilized successfully in a patient cohort of HTLV-1-infected individuals from Jamaica and currently being investigated for a HIV-1/HCV patient cohort from North America.

**C64**

**HTLV-1 Tax mediated downregulation of miRNAs associated with chromatin remodeling factors in T cells with stably integrated viral promoter**

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RNA interference (RNAi) is a natural cellular mechanism to silence gene expression and is predominantly mediated by microRNAs (miRNAs) that target messenger RNA. Viruses can manipulate the cellular processes necessary for their replication by targeting the host RNAi machinery. This study explores the effect of human T-cell leukemia virus type 1 (HTLV-1) transactivating protein Tax on the RNAi pathway in the context of a chromosomally integrated viral long terminal repeat (LTR) using a CD4+ T-cell line, Jurkat. Transcription factor profiling of the HTLV-1 LTR stably integrated T-cell clone transfected with Tax demonstrates increased activation of substrates and factors associated with chromatin remodeling complexes. Using a miRNA microarray and bioinformatics experimental approach, Tax was also shown to downregulate the expression of miRNAs associated with the translational regulation of factors required for chromatin remodeling. These observations were validated with selected miRNAs and an HTLV-1 infected T cells line, MT-2. miR-149 and miR-873 were found to be capable of directly targeting p300 and p/CAF, chromatin remodeling factors known to play critical role in HTLV-1 pathogenesis. Overall, these results are first in line establishing HTLV-1/Tax-miRNA-chromatin concept and open new avenues toward understanding retroviral latency and/or replication in a given cell type.

**C65**

**Upregulation of flt3 receptor (CD135) expression in SIV and SIV encephalitis: Neuroprotective or neurotoxic?**

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HIV-associated neurocognitive dysfunction (HAND) is often observed in individuals with HIV. While the pathogenesis of HAND is not completely understood, infected and non-infected activated macrophages (Mφ) and microglia are believed to contribute significantly to neuronal injury through a number of factors that are directly or indirectly neurotoxic. Accordingly, significant Mφ accumulation is seen in HIV encephalitis (HIVE), the neuropathological correlate of the most severe form of HAND, and is involved in its pathological features. Previously, we reported expansion of a circulating monocyte subset (CD14+/CD163+/CD16+) in HIV infected patients that is phenotypically similar to Mφs that accumulate in the CNS in HIVE. We hypothesize that the cytokine, macrophage colony stimulating factor (M-CSF), is driving the expansion of this monocyte/ Mφ subset, as M-CSF is elevated in HIV and upregulates CD16 and CD163 expression. Interestingly, our in vitro studies of M-CSF treated peripheral blood mononuclear cells (PBMC) show it also increases the frequency of CD135+ monocytes. Because M-CSF is elevated in the CSF of patients with HIVE and may contribute to HIVE pathogenesis, we examined CNS tissue from SIV infected rhesus macaques for possible alterations in CD135 expression. Here, we report elevated CD135 expression in the CNS of SIV+ macaques with even greater expression seen in those with encephalitis (SIVE), as compared to seronegative animals. In grey matter, cells with neuronal morphology are CD135+, irrespective of the presence or absence of SIV infection. In contrast, CD135 positivity is only seen in white matter of animals with SIV and SIVE. Colocalization of CD135
with CD163 by cells in the white matter suggests these are Mϕ s/microglia. The CD135 ligand, Flt3 ligand, reportedly acts as an adjunct trophic factor in the CNS. The role of CD135 upregulation in SIV, however, remains unclear and may be, at the same time, neuroprotective and contributory to the disease process.

**C66**

**Phosphoinositide-3 kinase p110δ isoform and pathogenicity of influenza virus infection**

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We have found that mice deficient in a tissue specific isoform of the signaling enzyme phosphoinositide-3 kinase (PI3K), p110δ, are protected from influenza virus infection and have reduced lung viral loads. We tested whether p110δ deficient macrophages can control murine PR8 influenza virus infection in vitro. Supernatants from p110δ−/− macrophages exposed to gamma radiation inactivated X31 influenza virus can successfully inhibit viral production if added to mouse embryonic fibroblasts (MEFs) before PR8 infection. Supernatant from p110δ−/− macrophages inhibit viral replication in a 48 hr assay. These studies suggest that p110δ−/− macrophages secrete anti-viral factors when stimulated by virus. These findings raise the issue that differences in protection seen with p110δ deficient mice may be due to alveolar macrophages that exhibit enhanced antiviral immunity. These data suggest the tissue specific PI3K signaling cascade is important for influenza virus protection.

**C67**

**Sensitivity of Peptide Triazole Function to HIV-1 Envelope Mutagenesis**

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Human Immunodeficiency Virus Type 1 (HIV-1), the causative agent for Acquired Immunodeficiency Syndrome (AIDS), still remains an epidemic worldwide with over 33 million people infected and roughly 2.7 million new infections annually (1). Fortunately with the advent of highly active antiretroviral therapy (HAART), the death toll attributed to HIV-1 has leveled off (1). However, there is still a strong need for drug intervention to prevent HIV-1 infection. Without the availability of an effective HIV-1 vaccine, another method of prevention is needed to control acquisition of the virus. Topical microbicides represent a class of drugs that can be applied to tissue to prevent infection. Agents that can act prior to HIV-1 integration are ideal candidates for this approach. HIV-1 entry is an optimal target because it occurs as the pivotal first step of the viral infection process. Entry is mediated by binding of HIV-1 envelope, consisting of a trimeric protein complex of gp120 and gp41, to host cell CD4 receptor and a chemokine co-receptor CCR5 or CXCR4. The peptide triazole class of entry inhibitors that our lab works with appears to conformationally entrap HIV-1 gp120 to prevent both receptor and co-receptor recognition and therefore stop entry of the viruses across a broad range of subtypes (2). Recently, our lab has found a sulfhydryl-containing peptide triazole capable of causing cell-free virolysis of HIV-1 (3). In the current work, we have initiated a study to address the mutagenic affects of gp120 on peptide triazole function. Mutations of varying degrees of conservation have been shown to have different affects on both binding and virolysis. The most profound affects observed so far are mutants within the peptide triazole functional footprint. These mutations provide insight on the mechanism of differing classes of peptide triazoles and potential resistance that can arise with treatment.
**C68**

**Dimethyl Fumarate Strongly Induces Heme Oxygenase-1 (HO-1) Expression In Human Neuronal Cells and Rat Astrocytes: Implications for HIV Mediated Neurotoxicity**

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Recent work from our lab has demonstrated that HIV-1 infection in primary monocyte-derived macrophages (MDM) leads to a significant decrease in the levels of the anti-oxidant protein heme oxygenase-1 (HO-1), and that pharmacological induction of HO-1 in infected MDMs strongly reduces HIV-mediated neurotoxicity. A potential therapeutic inducer of HO-1 (and other antioxidant response genes) is dimethyl fumarate (DMF), which is quickly converted to its primary metabolite monomethyl fumarate (MMF). Treating infected MDMs with DMF or MMF restores HO-1 levels and strongly suppresses HIV-mediated neurotoxicity, and can do so despite continued viral replication (Cross, et al. 2011, J. Immunol.). As DMF/MMF can cross the blood-brain-barrier, we hypothesized that DMF/MMF may induce HO-1 expression in neurons and astrocytes, which may be further protective to HIV-induced toxicity. We report that DMF/MMF does induce HO-1 expression in both human differentiated neuronal lines (NT2.N), and in primary rat astrocytes as measured by qPCR. However, the kinetics of expression differ widely among cell types. In MDM, MMF induces a modest increase in HO-1 expression (1.8X) by 7h post-treatment, followed by a large decrease at 24h-48h (0.20X). However, in differentiated NT2.N neurons, HO-1 levels peak at 24h (3.3X), remain high at 48h (2.2X), and drop further but remain higher than controls after 6 days (1.5X). In astrocytes, MMF is more potent in inducing HO-1 expression, increasing expression nearly 10X at 24h, followed by lower, but consistent elevations (3.3X) at 48h. These results suggest DMF/MMF may have neuroprotective effects against HIV neurotoxicity not only through effects in infected macrophages, but also through effects in neurons and astrocytes. These studies provide further support for the potential role for DMF as a therapeutic drug for HIV associated cognitive disorder (HAND).

**C69**

**Exosomes from HIV-1 infected cells: the Trojan horses of neurodegeneration.**

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Despite antiretroviral therapy (ART), HIV infection in the brain promotes HIV-1-associated neurocognitive disorders (HAND) through persistent inflammation and release of neurotoxin from latently infected and/or activated macrophages/microglia and astrocytes. Furthermore, the lack of productive infection of neurons by HIV suggests for the involvement of viral and cellular proteins with neurotoxic activities to play significant role in neuronal degeneration. However, the exact mechanism of these processes remains elusive. We hypothesize that exosomes released from HIV infected cells may play a role in neuronal degeneration. Our studies demonstrate that exosomes can be isolated from PMA activated latently infected U1 cells and also from sera of HIV infected individuals with high viral load. These exosomes when exposed to human fetal neurons are capable of inducing neuronal beading and disintegration of neuronal processes. Furthermore, characterization of the miRNA cargo of these exosomes demonstrates the presence of numerous micro RNAs (miRNAs) that are up regulated by at least 2-fold in comparison to exosomes derived from un-induced cells. The target genes of some of these over expressed miRNAs were identified using the miRNA database. Interestingly, we identified numerous gene targets that are involved in neuronal growth, regeneration and synaptic functions.
Systemic Immunization with Mucosal Chemokine Molecular Adjuvant TECK/CCL25 and CCL28/MEC Targets Antigen Specific Immune Responses in the Mucosa-Associated Lymphoid Tissue

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The development of a systemically-delivered vaccine approach driving mucosal T cell immune responses will be critical for protection against pathogens that use mucosal surfaces as portal of entry and replication including HIV-1. We hypothesized that immunization with chemokine adjuvant pCCL25 or pCCL28 and HIV-1 antigens encoding plasmids will augment anti-HIV-1 cell mediated and humoral immune responses at mucosal surfaces. To understand the mechanism behind enhanced mucosal immune responses we immunized chimeric C57Bl/6 mice with LCMV p14 specific CD8 T cells with pLCMVgp33 in combination with pTECK in Tabia Anterialis muscle followed by electroporation. Co-delivery of plasmid chemokine TECK mediated an increase in the frequency of antigen specific CD8 T cells with a memory phenotype in various compartments including the intra-epithelial (IEL) compartment of gastrointestinal mucosa and lungs when compared with antigen alone group. On the other hand co-immunization with MEC results in increased IgG responses in sera as well as in secretory IgA in mucosal secretions in mouse and rhesus macaques models. Antibody responses generated in response to immunization with MEC correlated with long term decrease in viral load and elevated peripheral CD4 T cell count in HIV-1 infected rhesus macaques. Co-immunization with TECK results in 1.5 fold increase in the infiltration of CCR9+ dendritic cells in draining lymph nodes when compared to pVax immunized mice at day 7 post immunization. We have shown that CCR9+ DCs generated from bone-marrow cultures and in vivo sorted CCR9+ DCs have higher ability to convert Vitamin A to retinoic acid, required for upregulation of mucosal homing markers CCR9, and αβ7 on CD8 T cells. These data support the use of highly optimized molecular adjuvant TECK and MEC in DNA vaccine platform for targeting antigen specific T and B cells to mucosal sites and support the role of retinoic acid signaling in this approach.

A novel in vivo and in vitro inflammatory breast cancer model to study the involvement of CXCR4 and CXCR7 in early stage metastases

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Human Inflammatory Breast Cancer (IBC) is an aggressive form of invasive tumor, accounting for 2.5% of breast cancer cases. It is characterized by rapid progression, regional and distant metastases - including brain metastasis - younger age of onset, and lower overall survival (less than a 5% survival rate beyond 5 years when treated with surgery or radiation therapy). Presently, there are no targeted therapies against IBC. Efficacious treatments depend on preclinical models that predict patient response, increase understanding of the metastatic process, and enable the identification of biomarkers for earlier and accurate detection of metastasis. This study aims to develop a novel in vivo/in vitro IBC model using primary cultures of tumor cells isolated from pleural exudates of IBC patients and sorted for stem cell features (CD44+/CD24+). We developed a human xenograft model where primary IBC-cells stably transduced with a EGFP-expressing lentiviral vector are inoculated in the left cardiac ventricle in mice.
Using this approach, we are evaluating the involvement of chemokine receptors CXCR4 and CXCR7 in the metastatic process. Our preliminary in vivo data show the capacity of these cells to spread to the brain and lungs. RT-PCR and IF show expression of CXCR4/CXCR7 in primary IBC-cells. Moreover, these receptors were co-expressed in individual cells and co-localized with the proliferative marker Ki67. Western blot and migration assays were performed to evaluate the activity/function of CXCL12 in vitro and the involvement of extracellular signal-regulated kinase 1/2 (ERK1/2). CXCL12 induced significant ERK1/2 activation that was strongly reduced by inhibition of CXCR4+CXCR7. In conclusion, we found that primary IBC-cells express CXCR4 and CXCR7 and the activation of ERK1/2 after CXCL12 stimulus is mediated by both receptors. Taken together, these data validate our experimental model and suggest an involvement of CXCR4 and CXCR7 in IBC disease.

C72

HIV-1 envelope glycoprotein gp120 triggers a senescence phenotype in cultured human astrocytes

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Although the molecular mechanisms underlying the pathophysiology of HIV-associated neurocognitive disorders (HAND) are not completely understood, both viral products and the cellular inflammatory response are thought to play significant roles. HIV-1 envelope glycoprotein gp120 mediates viral entry into the host cell, can be found in the plasma and cerebrospinal fluid of HIV-1 infected individuals, and additionally plays a role in HAND pathogenesis. Our laboratory is currently exploring the hypothesis that cellular senescence in astrocytes is a causative event in age-related neuropathology, including HAND. We previously demonstrated that 1) astrocytes undergo senescence in response to stress and 2) this response could be physiologically-relevant given that we are able to detect senescent astrocytes in aged and brain tissue and in neurodegenerative disease. HIV-1 infection of astrocytes or acute exposure to HIV-1 gp120 results in profound changes in astrocyte gene expression and the release of inflammatory mediators; however, the role of gp120 as an inducer of astrocyte senescence is still undefined. In order to examine the cellular response to gp120, human astrocytes derived from fetal cortex were challenged with a range of concentrations of recombinant gp120 with different tropisms (IIIB and Bal). We investigated the effect of gp120 on the induction of the senescence phenotype through senescence-associated beta-galactosidase (SA β-gal) staining. We also profiled the secretion pattern of senescent astrocytes in vitro and found that senescent astrocytes produce a number of inflammatory cytokines including interleukin-6 (IL-6) indicative of a senescence-associated secretory phenotype (SASP), which seems to be regulated by p38MAPK. Astrocyte senescence may be an important component of the development and progression of HAND through the generation of a SASP and/or the perturbation of astrocyte functions that are critical for the maintenance of neuronal homeostasis.

C73

Transcriptional Regulators Rsp and CrgA Repress lst Expression in Neisseria gonorrhoeae

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There is no vaccine to prevent the ~62 million global Neisseria gonorrhoeae (Ng) infections acquired every year, a fact made more troubling by the recent isolation of strains resistant to all antibiotic classes. Lipooligosaccharide (LOS) sialyltransferase (Lst) is a critical surface-expressed virulence determinant that catalyzes LOS sialylation and promotes Ng survival in vivo. Whereas LOS sialylation increases virulence by enhancing Ng resistance to complement and neutrophil killing, it decreases Ng association with urogenital epithelial cells, which may be necessary for invasive disease. This suggests that Lst expression is, in part, controlled by niche-dependent mechanisms. To address this hypothesis, we analyzed lst transcript levels in the presence and absence of cells. As expected, cell-association promoted
lst down-regulation. We next sought to identify regulators of lst by utilizing a Himar1 mariner transposon library to screen for increased resistance to serum killing. Through this screen, we identified regulator-sensor protein (rsp) which, when deleted from wild type strains, promoted enhanced lst transcription and translation. We also examined lst expression by Ng deleted for crgA (contact-regulated gene A), since CrgA directly represses expression of surface proteins by cell-associated N. meningitidis. Like δrsp, δcrgA gonococci exhibited increased lst transcription and translation, and resistance to serum killing. These data suggest that rsp and crgA contribute to lst regulation during contact-dependent and -independent growth. Ongoing studies will determine if repression is a result of direct binding of CrgA and Rsp to the lst or crgA promoters and if other genes are involved in Lst control.

C74

Extracellular HIV-1 Viral Protein R (Vpr) Influences Astrocytic GAPDH Activity and Metabolism Affecting Neuronal Glutathione Synthesis and Survival

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Studies have demonstrated the accumulation of extracellular human immunodeficiency virus type 1 (HIV-1) Vpr in the serum and cerebrospinal fluid of HIV-1-infected patients in direct correlation with viral loads and disease progression. Late stage HIV-1-infected patients also suffer from different inflammatory conditions and manifest excessive intracellular oxidation, a condition that may potentially escalate and lead to irreversible fatal effects. While several other HIV-1 proteins (gp120, Tat and Nef) were found in different bodily fluids, very little is known about the downstream effects of Vpr. As one approach to examine the role of extracellular Vpr in HIV-1 disease progression, we have demonstrated that recombinant Vpr decreased the levels of glutathione (GSH) and ATP pools in cultured astrocytes, causing oxidative stress. Based on these results, and the aforementioned clinical manifestations, we hypothesized a correlation between enhanced extracellular Vpr in the CNS and a decline in astrocytic levels of GSH and ATP with disease progression. Results demonstrated declines in the levels of ATP and GSH were unrelated and due to a Vpr-induced decrease in GAPDH activity, thereby affecting the glycolytic pathway. In addition, exposure to extracellular Vpr promoted caspase-dependent apoptosis in astrocytes along with secretion of pro-inflammatory cytokines (IL-6 and IL-8) and chemoattractants (MCP-1 and MIF). Furthermore, excessive astrocytic GSH oxidation lowered extracellular levels of GSH and the supply of cysteine for neurons, which affected GSH synthesis within the neuronal compartment. This cascade of events within the astrocytic compartment thus led to oxidative stress within the neuronal population, impairing survival. Partial rescue of these effects was obtained upon supplementation with the anti-oxidant compound N-acetyl-cysteine. These results support a role for HIV-1 extracellular Vpr in deregulating the neuronal-astrocytic network, which possibly accelerates disease progression, but also offers a therapeutic approach aimed at targeting one of the causative agents of the astrocytic-neuronal network disruption.

C75

Single nucleotide polymorphisms in the human immunodeficiency virus type 1 viral promoter precede the onset of neurocognitive impairment

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The advent of HAART therapy has led to a decrease in the incidence of HAND, but its prevalence remains unchanged with milder forms predominating. In addition, HAART therapy as well as immune selective pressure placed on HIV-1 can cause genotypic and/or phenotypic viral alterations which can alter the ability of the virus to infect and/or persist in particular cell types. Cross-sectional studies in the pre-HAART era demonstrated that single nucleotide polymorphisms (SNPs) in peripheral blood-derived LTRs [a C-to-T change at position 3 of C/EBP site I (3T) and/or at position 5 of Sp site III (5T)] were identified, and were often encountered together in the LTR of the integrated provirus in late stage HIV disease. Additionally, the 3T variant correlated with HIV-1-associated dementia. LTR sequences derived from longitudinal sampling of peripheral blood from a single patient in the DrexelMed HIV/AIDS Genetic Analysis Cohort resulted in the detection of the 3T and 5T co-selected SNPs before the onset of neurologic impairment, suggesting that these SNPs may be useful in predicting HIV-associated neurological complications. The relative fitness of the LTRs containing the 3T and/or 5T co-selected SNPs as they evolved in their native patient-derived LTR backbone structure demonstrated a spectrum of basal, activated, and Tat-mediated transcriptional activities. In silico predictions utilizing co-linear envelope sequence suggested that the patient’s peripheral blood virus evolved from an X4 to an R5 swarm prior to the development of neurological complications and more advanced HIV disease. These results suggest that the viral swarm evolves during the course of disease in response to selective pressures, from both the host immune response as well as HAART therapy, and can lead to changes in prevalence of specific polymorphisms in the LTR and/or the envelope gene that could predict the onset of neurological disease and result in alterations in viral function.

C76

Single nucleotide polymorphisms within the HIV-1 LTR correlate with use of drugs of abuse in the DrexelMed HIV/AIDS Genetic Analysis Cohort

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HIV infection is prevalent among substance abusers. However, the effects of illicit drugs on HIV-1 disease progression are not well established. We evaluated the relationship between illicit drug use and HIV-1 disease progression in patients enrolled in the DrexelMed HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T cell count, and viral load were performed approximately every 6 months. Drug abuse is common in the cohort, with 87.6% of patients admitting past use; 29.7%, currently abusing drugs; and 36.2% testing positive for drug use at the time of visit. Cocaine and marijuana use are heavily favored, with 80.5% of drug-using patients admitting to past or current cocaine use and 72% admitting to marijuana use. Most patients use multiple drugs simultaneously. The cohort can be categorized into non-users (PN), cocaine only (preferential, PC) users, cannabinoid only (preferential, PM) users, and multidrug users. Nonusers are more likely to remain on HAART (94.4%), whereas PC and PM are less likely (83.4% and 78%, respectively). Drug
users exhibited lower current CD4+ T cell count, lower nadir CD4+ T cell, higher current viral loads, and higher peak viral loads than non-users. In addition, single nucleotide polymorphisms (SNPs) that are unique to cocaine, marijuana, or non-users were identified. In conclusion, illicit drug use appears to facilitate HIV-1 disease progression and selects for genetic variations unique to mono- and multi-using HIV/AIDS patient cohorts.

**C77**

**Exposure of an in vitro model of the blood-brain barrier to chronic morphine: Impact on structure and function**

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About one-third of human immunodeficiency virus type 1 (HIV-1) cases leading to acquired immunodeficiency syndrome (AIDS) in the United States have been attributed to injection drug use, frequently involving the abuse of opioids. Opioid abuse by HIV-1-infected individuals leads to more rapid disease progression, increased viral replication and peripheral viral load, and increased incidence and severity of neurocognitive abnormalities compared to non-drug abusers. The blood-brain barrier (BBB) is an obstacle that must be overcome during neuroinvasion and development of HIV-associated neurocognitive disorders (HAND). HIV-1 proteins can directly impact BBB permeability, and drugs of abuse, including cocaine and methamphetamine, have been shown to increase BBB leakiness and cellular transmigration. Previous in vitro and in vivo studies addressing the role of mu-opioids in altering BBB permeability suggest that exposure increases cellular transmigration and overall barrier leakiness. Currently, in this study, a human brain microvascular endothelial cell (hBMEC) line, hCMEC/D3, was used to establish an in vitro transwell model of the BBB to investigate the effects of chronic (24, 48, or 72 h) morphine treatment on barrier structure and function. We observed that hCMEC/D3 cells form a confluent monolayer with a basal rate of passage of a 70 kDa tracer molecule comparable to primary hBMECs. While chronic morphine treatment does not induce overall barrier leakiness, changes in mRNA levels of tight junction proteins were observed throughout the course of chronic treatment. Future experiments will investigate the impact of chronic morphine treatment on transcellular migration of mononuclear cells, tight junction protein expression, adhesion molecule surface expression, and cytokine/chemokine secretion.

**C78**

**C/EBP and NFAT act via a novel downstream element in the LTR to regulate HIV-1 transcription**

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Cellular and viral factors regulate HIV-1 transcription by binding to DNA elements present both upstream and downstream of the transcriptional start site in the long terminal repeat (LTR) in cells of the monocyte-macrophage lineage. TRANSFAC analysis of the HIV-1 subtype B LAI LTR revealed a potential downstream CCAAT enhancer binding protein (C/EBP) binding site (DS3). This element is present immediately downstream of nucleosome 1 in the HIV-1 LTR, which suggested that it may have a functional role in regulating production of HIV-1 transcripts. Analysis across multiple HIV-1 subtypes indicated that the DS3 element has a high degree of conservation both in terms of nucleotide sequence and physical location in the LTR. Interestingly, this element overlaps with a previously identified AP3-like element, which has been shown to bind members of the nuclear factor of activated T-cells (NFAT)
family of proteins. Results indicated that NFATc2 has a higher relative affinity for this element as compared to members of the C/EBP family (C/EBPα and C/EBPβ). It was also observed that this element was able to compete efficiently with a low affinity upstream C/EBP binding site I (US1) with respect to C/EBP binding, suggesting utilization of both NFAT and C/EBP. Moreover, cyclosporine-A treatment, which prevents dephosphorylation and nuclear translocation of NFAT isoforms, showed enhanced binding for C/EBPα. Also, a binding knockout mutant for DS3 (T to C change at position 9) demonstrated reduced transcriptional activity suggesting that DS3 serves as a positive regulator of LTR-directed transcription. We are currently investigating the relative importance of NFAT and C/EBP isoforms with respect to DS3-mediated transcription and how extracellular signals like cytokines impact DS3-mediated, HIV-1 LTR-directed transcription in cells of the monocyte-macrophage lineage.

HIV-1 LTR single nucleotide polymorphisms (SNPs) correlate with clinical disease parameters

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The long terminal repeat (LTR) regulates HIV-1 gene expression by interacting with multiple host and viral factors. Cross-sectional studies in the pre-HAART era demonstrated that single nucleotide polymorphisms (SNPs) in C/EBP site I and Sp site III from peripheral blood-derivedLTRs increased in frequency as disease severity increased and correlated with HIV-1-associated dementia. Current studies focus on the identification of LTR signatures derived from peripheral blood virus that can be used as molecular markers to identify HIV-1-infected individuals more prone to developing advanced stage disease and/or neurologic disease. A prospective, longitudinal study was conducted on 458 HIV-1-seropositive patients currently enrolled in the DrexelMed HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T-cell count, and viral load were collected approximately every 6 months. SNP density within the entire HIV-1 LTR was determined by comparison to the conB reference sequence and showed areas of increased variability. The collection of extensive clinical parameters on these patients have allowed for cross-population and longitudinal analyses of the impact of these parameters on the development of SNPs during the course of disease. To date, multiple SNPs have been detected to be significantly associated with CD4 T-cell count and viral load, as well as with change in CD4 T-cell count and change in viral load. These SNPs were identified in areas of the LTR that have been previously reported to be important to viral promoter function and some in areas that have been less well functionally characterized. These results suggest that the HIV-1 genomic swarm may evolve during the course of disease in response to selective pressures that lead to changes in prevalence of LTR SNPs and that LTR SNPs may produce alterations in viral function and can be predictive of more advanced stage HIV disease.
The effect of naturally occurring SNPs on the functionality of the HIV-1 LTR within model cell lines

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Human immunodeficiency virus type 1 (HIV-1) gene expression is driven by the long terminal repeat (LTR), which contains binding sites that interact with multiple host and viral factors. Selective pressures within the host as well as the low efficiency of reverse transcriptase lead to genetic alterations within the viral genome resulting in viral quasispecies that can be differentially regulated and can potentially form niches within specific cell types and tissues. Previous studies identified a single nucleotide polymorphism (SNP) within C/EBP site I (3T, C-to-T change at position 3 of the site) that correlated with HIV-1-associated dementia. In addition, our current patient cohort shows a SNP within Sp site III (5T, C-to-T change at position 5 of the site) that occurs as frequently as the consensus subtype B sequence. Stably transfected cell lines were developed using bone marrow progenitor, T, and monocytic cell lines (TF-1, Jurkat, and U-937, respectively) to explore the LTR phenotype associated with these genotypic changes from an integrated microenvironment. The LAI LTR was coupled to the green fluorescent protein (GFP), and polyclonal HIV-1 LTR-GFP stable cell lines were developed. To examine the mechanism of LTR driven gene expression as well as epigenetic modifications that may control it, clones were derived from each population of cells. The clones were examined with respect to basal transcription, cytokine treatment, and Tat transactivation. Results suggest that non-expressing (NE) clones containing the 3T5T LTR within TF-1 cell lines can be induced to express. Additionally, the NE LAI 3T and LAI 5T Jurkat clone genotypes could be induced into expression. Results demonstrate that genetic signature, epigenetic modifications to viral and host DNA, and cellular phenotype may determine the overall level of LTR activity and the potential of the LTR to be activated from a quiescent state.

Cocaine as an immunomodulator within patients in the DrexelMed HIV/AIDS Genetic Analysis Cohort

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HIV infection is prevalent among substance abusers. We evaluated the relationship between illicit drug use and HIV-1 disease progression in HIV-1-infected patients enrolled in the DrexelMed HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T cell count, and viral load were assessed approximately every 6 months. Drug abuse is common in the cohort, with 87.6% of patients admitting past use; 29.7%, currently abusing drugs; and 36.2% testing positive for drug use at the time of visit. Cocaine use is heavily favored, with 80.5% of drug-using patients admitting to past or current cocaine use. Most patients use multiple drugs simultaneously. The cohort can be categorized into non-users (PN), cocaine only (preferential, PC) users,
cannabinoid only (preferential, PM) users, and multidrug users. Nonusers are more likely to remain on HAART (94.4%), whereas PC are less likely (83.4%). The overall health of the PN subcohort is better than that of the PC subcohort. Patients in PN are less likely to suffer from opportunistic infections and have higher current and nadir CD4 counts. Additionally, the peak and the current viral loads in PN are substantially lower than those in PC patients. Since, cocaine is known to have immunomodulatory effects, the cytokine profiles of PN and PC individuals were analyzed to understand the effects of cocaine on cytokine modulation and HIV-1 disease progression. Among the 30 cytokines investigated, interestingly, differential levels of the HIV-1 suppressive factors, MIP-1β, and RANTES, were established within the PC subcohort. The cytokine profiles have also been analyzed with respect to clinical parameters such as age, gender, viral load, CD4+ T cell count and neurocognitive impairment status. In conclusion, illicit drug use appears to facilitate HIV-1 disease progression based on these assessments.

C82
Human beta-Defensins 2 and 3 inhibit HIV in macrophages: implications for HIV infection in the CNS.

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Human beta-Defensins (hBD) are secreted, broad-spectrum antimicrobial peptides that we and others have shown to inhibit HIV in primary CD4+ T cells. The inhibitory activity is mediated in T cells by CCR6, a receptor that binds both the chemokine MIP-3a and hBD2 and -3, and eventuates in transcriptional induction of the antiretroviral cellular factor APOBEC3G. Intriguingly, our data show that hBD2 is expressed in the CNS, but very little is known about the role played by defensins in the brain. We hypothesized that, besides T cells, hBDs could protect microglial cells and perivascular macrophages from productive HIV infection. Our data in primary monocyte-derived macrophages (MDM), which we used as an in vitro model for microglial cells, show that hBD2 and -3 inhibit HIV at an early stage of infection in association with increased levels of APOBEC3G. Flow cytometry analyses show that MDM express CCR6 and CCR2, another shared chemokine-defensin receptor, although with high variability among donors. Since agonist and antagonists of chemokine receptors are becoming available, our data could lead to the development of new therapeutic approaches to treat HIV infection systemically and in the CNS. In addition, decreased defensin production that we observed in HIV-infected subjects could be associated with increased occurrence of HIV-associated symptoms.

C83
M-CSF receptor: An important oncogene in AML and potential therapeutic target?

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The oncogenic potential of M-CSF receptor (cFMS) has been noted for over thirty years, however, few current studies have focused on the role of the receptor in AML. In a clinical trial for AML, Sunitinib was found to hold some efficacy for treating the disease. The authors hypothesized that the primary therapeutic target of Sunitinib in AML is FLT3 kinase. However, FLT3 inhibition alone has not been shown to recapitulate all the effects of Sunitinib in vitro and the drug is known to have cross reactivity to other potential oncogenic receptors as well. In this study, we treated three myeloid cell lines, Mono-Mac 1, THP-1, and U937 with Sunitinib or a small molecule (cFMS-I) optimized for cFMS inhibitory activity to test the anti-cancer effect in of such treatment. We observed that only Mono-Mac 1 cells had diminished proliferation in vitro. Mono-Mac 1 cells had inhibited ERK as a result of cFMS inhibition and showed a dose dependent increase in cFMS expression with both Sunitinib and cFMS-I. Our results suggest potential for cFMS as an important target of Sunitinib or other similar drugs AML, either
independently or in combination with other therapeutics. Alternatively, cFMS may be a marker for differentiation of AML and may be linked with responsiveness to certain therapeutics. In both cases, the future study of cFMS may produce more targeted therapeutic approaches and may be a suitable tool for the development of personalized medicine for AML.

**C84**

**Vpr as a regulator of NF-κB in HIV-1: Selective inhibition of the TNF-α pathway**

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Numerous studies have reported that Vpr alters NF-κB signaling in various cells, however, the findings have been largely conflicting with reports of both stimulatory and inhibitory effects of Vpr. Our aim was to investigate the role of Vpr signaling in myeloid cells and address controversies that have developed in the field. Our results show that Vpr expressed intracellularly is inhibitory to NF-κB, while extracellular Vpr may have opposite stimulatory effects. Consistent with this notion, we report that Vpr has inhibitory effects that are specific to the TNF-α pathway, but not affecting the LPS pathway, suggesting that differential targets of Vpr may exist for NF-κB regulation. Further, we identify VprBP as one possible cellular component of Vpr’s regulation of IκBα in response to TNF-α stimulation. We did not identify such a role for HSP27, which instead seems to inhibit Vpr functions. Finally, our findings suggest that NF-κB regulation by Vpr is further influenced by the presence of other HIV-1 components within infected cells, as chronically HIV-1 infected U1 cells with knockdown constructs for Vpr were unexpectedly less responsive to TNF-α. This data suggests that Vpr may serve an important role in vivo by selectively inhibiting immune activation while stimulating NF-κB mediated viral production in HIV-1 infected T-cells and myeloid cells.
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Drexel University College of Medicine, Philadelphia, PA, USA • June 19-21, 2012