Welcome
12th Annual International Meeting of the Institute of Human Virology of the University of Maryland School of Medicine

By
Robert C. Gallo, Founder and Director, Institute of Human Virology
and
Giovanni Latorre, President, University of Calabria

Dear colleagues and friends,

University of Calabria President Giovanni Latorre and I welcome you to the 12th Annual Meeting of the Institute of Human Virology (IHV). For the first time since the meeting began in the mid-1970s at the National Cancer Institute, it is taking place outside the Baltimore/Washington, DC area. The setting for this year’s meeting, Tropea, Italy, is one of extraordinary beauty and ancient historical contributions. The region is among the oldest civilization in Europe and includes the location of Pythagoras, Herodotus, and other greats of the ancient world, as well as the land of origin of many famous Greek legends, some told by Homer. Today, Tropea attracts visitors to the Costa degli Dei (Coast of the Gods), one of the most beautiful regions in Italy and food aficionados seeking the famous local red onion that was hailed by the Roman Pliny the Elder for its gastronomic and healing potential. Nearby are the ancient Aeolian islands. Together with Calabria and Sicily, their cultures dating back thousands of years before Christ, also with their many legends.

It is important that we acknowledge Professor Arnaldo Caruso, of the University of Brescia but who originally hails from Calabria, chair of the Italian Organizing Committee; without his vision and support, this meeting in Calabria would not be possible. Dr. Caruso currently holds an Adjunct Professorship at IHV and the University of Maryland School of Medicine. This collaboration planted the idea for changing the venue of this 12th Annual Meeting. Our meeting has always been a venue for science of AIDS and cancer; this year we bring the meeting to Italy, in part, as a recognition of our many international colleagues and collaborators.

We would like to thank several organizations who have committed their support while we are still in the early stages of planning this event: the National Cancer Institute and National Institute of Allergy and Infectious Disease of the National Institutes of Health, the Office of AIDS Research, Italian Society for Medical Virology (SIVIM), Medestea, Sanofi-Pasteur, and Sanyo, Inc.. We will be pleased to recognize other supporting partners as the list grows.

The Annual Meeting has a traditional emphasis on the biology of HIV/AIDS, including the perspectives of basic science, clinical science and new treatment and prevention approaches. In 2010 we are expanding the emphasis on vaccines to recognize the importance of this area and learn how recent successes may guide new directions in HIV vaccine research and development. International vaccine experts will describe vaccine immunogens that are moving through human clinical trials, efforts to define immune correlates of protection, possible applications for therapeutic...

Continued on page 4
vaccination and a range of vaccine delivery strategies that are bringing us closer, everyday, to effective protection from HIV disease. Recognizing the important parallels of HIV and cancer, we include discussions of cancer-causing viruses, new cancer vaccines and fundamental research in cancer. In developed countries, cancer is emerging as the leading cause of death for persons with HIV, and these fields are inexorably linked in terms of both science and medicine. We also emphasize the problems of HIV/AIDS in the developing world by inviting experts on the specific impacts of HIV/AIDS on sub-Saharan Africa.

This year, in keeping with the meeting’s focus on vaccines, we are honored to present the IHV Lifetime Achievement Award for Scientific Contributions to Dr. Rino Rappuoli of Novartis Vaccines in Sienna, Italy. Rappuoli, currently the Global Head of Vaccines Research for Novartis Vaccines & Diagnostics (Sienna, Italy), was elected as a foreign associate of the National Academy of Sciences in 2005. He spent his career developing vaccines for pertussis, meningitis, and Helicobacter pylori and is jointly responsible for engineering the carrier protein used in many conjugate vaccines. He is credited with launching the field of reverse vaccinology, the first fruits of which are revealed in the special Inaugural Article of the Proceedings of the US National Academy of Sciences, where he describes a universal vaccine against serogroup B meningococcus. Previous recipients of the IHV Lifetime Achievement Award for Scientific Contributions are: Isaac Witz, Maxine Singer, Manfred Eigen, Paul Zamecnik, Jan Svoboda, Alexander Rich, Hilary Koprowski, Maurice Hilleman, and George Klein.

We will also be presenting the IHV Lifetime Achievement Award for Public Service to Harry Huge, Esq. Harry is a friend of the Institute and was with us at the beginning of the Institute of Human Virology, where he describes a universal vaccine against serogroup B meningococcus. Previous recipients of the IHV Lifetime Achievement Award for Scientific Contributions are: Isaac Witz, Maxine Singer, Manfred Eigen, Paul Zamecnik, Jan Svoboda, Alexander Rich, Hilary Koprowski, Maurice Hilleman, and George Klein.

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The Institute of Human Virology
www.ihv.org

Mission Statement

The Institute of Human Virology (IHV) is a world-class center of excellence focusing on chronic viral diseases and virally linked cancers. IHV is dedicated to biomedical research leading to improved treatment and prevention of these diseases.

Our unique structure connects cohesive, multidisciplinary research and clinical programs so that new treatments are streamlined from discovery to patients. IHV is forging local and international programs for research and treatment of human disease.

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Co-Director, Division of Basic Science and Vaccine Research

William A. Blattner
Associate Director
Director, Division of Epidemiology and Prevention

Robert R. Redfield
Associate Director
Director, Division of Clinical Care and Research

C. David Pauza
Associate Director

George Lewis
Co-Director, Division of Basic Science and Vaccine Research

Joseph L. Bryant
Director, Division of Animal Models

Dave Wilkins
Chief Operating Officer
Tropea, Calabria, Italy

We are pleased to announce the IHV Meeting, for the first time in its history, will be held outside the Baltimore/Washington, DC area. The location for the meeting is Tropea, Italy. Tropea, specifically, and its region, Calabria, are a well-kept secret by American standards, and those who join us are in for a special treat.

Located on the western tip of the Italian peninsula on the Tyrrhenian Sea, the coastline is affectionately known as the “Costa degli Dei,” or the “Coast of the Gods.” Tropea is a very popular vacation destination among Italians and other Europeans. It is best known for stunning beaches, medieval architecture, and the Tropea Red Onion. However, the entire region of Calabria is of great historical interest, a region which was among the first to develop what we call civilization in the Western world.

IHV is very fortunate for the opportunity to share hosting honors with the University of Calabria and the Italian Society for Medical Virology. We all hope that you will join us for what will surely be a special experience.

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Communications and Press Policy
12th Annual International Meeting of the Institute of Human Virology

To enhance the exchange of information and communication among attendees of the Institute of Human Virology Annual International Meeting, the following must be adhered to by all participants:

- All comments at sessions are off-the-record and are not for attribution.
- No coverage, reporting or publication of scientific data or presentations at the Institute of Human Virology Annual Meeting is permitted without the consent of the presenter(s).
- One-on-one interviews with scientists and media may be arranged by contacting Nora Grannell, Director of Public Relations and Marketing, Institute of Human Virology, ngrannell@ihv.umaryland.edu or (410) 706-1954.
International Organizing Committee

The Institute of Human Virology of the University of Maryland School of Medicine is very grateful for the assistance provided by our International Organizing Committee.

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Global HIV Vaccine Enterprise  
New York, New York, USA

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Special Acknowledgements

The Institute of Human Virology of the University of Maryland and the University of Calabria would like to thank the following organizations. Without their continued and generous support, this meeting would not be possible.

**Benefactor**
- Italian Society for Medical Virology – SIVIM
- Medestea
- National Institutes of Health
- National Institute of Allergy and Infectious Diseases
- Office of AIDS Research

**Underwriter**
- US Military HIV Research Program (MHRP)
- The Fondazione Bonino Pulejo

**Supporters**
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- Sanyo, Inc.
- Sysmex GmbH
- University of Calabria
2010 IHV Lifetime Achievement Award for Scientific Contributions

Rino Rappuoli, Ph.D.

This year, in keeping with the meeting’s focus on vaccines, we are honored to present the IHV Lifetime Achievement Award for Scientific Contributions to Dr. Rino Rappuoli of Novartis Vaccines in Sienna, Italy. Rappuoli, currently the Global Head of Vaccines Research for Novartis Vaccines & Diagnostics (Siena, Italy), was elected as a foreign associate of the National Academy of Sciences in 2005. He is considered one of the major world experts in R&D of vaccines. In fact, Rino Rappuoli has developed the first recombinant bacterial vaccine (against pertussis) and a conjugate vaccine against meningococcus C. Both products have been approved for human use. Currently, he is involved in the development of a vaccine against group B meningococcus using a genome-based approach, which has been termed reverse vaccinology, in the development of influenza vaccines produced in cell culture, and the development of vaccines against avian influenza. He is co-founder of the field of cellular microbiology, a discipline that has merged cell biology and microbiology.
2010 IHV Lifetime Achievement Award for Public Service

Harry Huge

We will also be presenting the IHV Lifetime Achievement Award for Public Service to Harry Huge, Esq. Harry is a friend of the Institute and was with us at the beginning when the State of Maryland recruited Bob Redfield, Bill Blattner, and me. He is a practicing attorney in Charleston, South Carolina, and Washington, DC, and has enjoyed a long and distinguished law career, serving as an arbitrator on the National Tobacco Arbitration Panel, a Special Master for the U.S. District Court for the District of Columbia; and chairman of the United Mine Workers Health and Retirement Fund. He is currently co-counsel representing family members of the victims of the September 11, 2001 attacks and in late 2002, he was appointed co-prosecutor with the Chief German Federal Prosecutor by the German Federal Criminal Court in the trials of Hamburg cell terrorists. Harry was also awarded the Order of the Cross of Terra Mariana from the country of Estonia in 2006, for work by him on behalf of Estonia in the late 1980's and early 1990's when Estonia regained its independence from the Soviet Union in 1991 in a bloodless transition. Huge worked on behalf of Estonia with the U.S. Administration and Congress during this period as well as in Estonia. Harry and his wife, Reba, founded and operate two foundations which award college scholarships to Nebraska high school seniors. Over the past five years, they have awarded 30 scholarships to students who attend colleges and universities in Nebraska, Iowa, Kansas and South Dakota.
2010 IHV Lifetime Achievement Award for Public Service

Michele LaPlaca, M.D.

For the first time, we are presenting an award for teaching excellence. Michele La Placa, MD has been educating medical students and researchers in the field of microbiology since 1954. Dr. La Placa’s academic career began as a Lecturer in Microbiology at the Medical School of the University of Bologna, November 1, 1954. He is the author of the manual “Principles of Medical Microbiology,” (Esclapio, Bologna, Italy), the most widely used textbook of microbiology in the medical schools of Italian universities, which is currently in its 12th edition. Dr. LaPlaca’s students, many of whom are now leaders in their own right, say “…he was an excellent teacher: able to involve and captivate students and colleagues in an enraptured way, since he always believed in the importance of microbiology, spending his days studying before teaching.”

His colorful career was characterized by intensive laboratory research in all fields of microbiology and virology, and he spent many years working on HIV pathogenesis with great success. The link between progenitor cells and Tat/gp120 drew his attention and prompted him to create a group for intensive study of this topic. We have not enough time to describe his value. We can only say - Thank you, Michele.
Previous Recipients of
Lifetime Achievement Awards

Scientific Contributions

1999  George Klein, Karolinska Institute, Stockholm, Sweden
2000  Maurice Hilleman, Merck Research Laboratories, Sumneytown, Pennsylvania
2001  Hilary Koprowski, Thomas Jefferson University, Philadelphia, Pennsylvania
2002  Alexander Rich, Massachusetts Institute of Technology, Cambridge, Massachusetts
2003  Jan Svoboda, Institute of Molecular Genetics, Prague, Czech Republic
2004  Paul Zamecnik, Massachusetts General Hospital, Boston, Massachusetts
2005  Manfred Eigen, Max Planck Institute, Göttingen, Germany
2006  Maxine Singer, National Institutes of Health, Bethesda, Maryland
2008  Isaac P. Witz, Tel Aviv University, Tel Aviv, Israel

Public Service

2004  Stewart Greenebaum, Greenebaum and Rose Associates, Inc., Baltimore, Maryland
2006  Martin Delaney, Project Inform, San Francisco, California
Mark Your Calendar!

13th Annual International Meeting of the Institute of Human Virology

Marriott Waterfront Hotel
Baltimore, MD

October 30 - November 2, 2011
Evening Events Schedule

Monday, October 4, 2010

6 p.m. Welcome Reception and Dinner
Location: Sunshine Club Hotel Ballroom

Tuesday, October 5, 2010

6 - 7:30 p.m. Aperitivo
8:30 p.m. Lifetime Achievement Gala Banquet and Awards Ceremony
Location: Sunshine Club Hotel Ballroom

Wednesday, October 6, 2010

6 - 7:30 p.m. Aperitivo
8:30 p.m. Historical Lecture with Regional Dance Performances
Location: Sunshine Club Hotel Ballroom

Thursday, October 7, 2010

Scintille Jewelry Fashion Event - By Invitation Only
No other events scheduled

Friday, October 8, 2010

7:30 p.m. Evening Boat Ride to Stromboli
At participants’ expense
Opening Ceremonies

Monday, October 4, 2010

6:00 p.m.  Reception

Welcome Remarks  Robert C. Gallo, Director – Institute of Human Virology
Kathleen Kennedy Townsend – Chair – IHV Board of Advisors
Ferruccio Fazio, Minister of Health
Giuseppe Scopelliti, Governor of Calabria
Giovanni Latorre, Rector – University of Calabria

Keynote Speaker  Robert C. Gallo, Director – Institute of Human Virology
Key Problems in HIV Research: A Brief Outline

Dinner

Awards Reception and Banquet

Tuesday, October 5, 2010

8:30 p.m.  Presentation of Lifetime Achievement Awards

Award for Teaching Excellence and Leadership
Michele La Placa - Remarks by Robert Gallo, Maria C. Re, and Luigi Buonaguro

Award for Public Service
Harry Huge - Remarks by Robert Gallo, Reba Huge, and Robert Redfield

Award for Scientific Contributions
Rino Rappuoli - Remarks by Robert Gallo, Gary Nabel, and Alan Bernstein
Schedule of Events

Monday, October 4, 2010

10:30 a.m. Satellite Symposium – Fogarty Scholars Forum
1:00 p.m. Introduction Satellite Symposium – Italian Society for Medical Virology
2:18 p.m. Special Symposium on HIV and Malignant Diseases In Africa
3:48 p.m. Roundtable: Research Needs and Challenges in Africa and the Developing World
4:00 p.m. Adjourn
6:00 p.m. Formal Opening Ceremony, Welcome and Reception

Tuesday, October 5, 2010

9:00 a.m. Insights from Basic Science 1
11:10 a.m. Insights from Basic Science 2
12:58 p.m. Lunch Break
2:30 p.m. Institute of Human Virology Lifetime Scientific Achievement Award 2010
3:00 p.m. Selected Abstracts
4:00 p.m. Adjourn
6:00 p.m. Aperitivo
8:30 p.m. Lifetime Achievement Gala Banquet and Awards Ceremony

Wednesday, October 6, 2010

9:00 a.m. HIV Vaccine Part 1: Challenges to HIV Vaccine Development
10:12 a.m. HIV Vaccine Part 2: Antibody Response to HIV Env
11:44 a.m. Special Lecture
12:02 p.m. Roundtable: What Has Been Learned from Clinical Trials
12:30 p.m. HIV Vaccine Part 3: Alternatives for Vaccine Delivery
1:30 p.m. Lunch Break
3:00 p.m. HIV Vaccine Part 3: Alternatives for Vaccine Delivery
3:54 p.m. Selected Abstracts
4:40 p.m. Adjourn
6:00 p.m. Aperitivo
8:30 p.m. Historical Lecture with Regional Dance Performance
Thursday, October 7, 2010

9:00 a.m.  HIV Vaccine Part 4: Therapeutic Vaccines for HIV and Cancer
10:12 a.m. HIV Vaccine Part 5: Defining Targets for Protective Immunity
11:44 a.m. HIV Vaccine Part 6: Lessons from Animal Models
12:56 p.m. HIV Clinical Science and Therapy
1:30 p.m.  Lunch Break
3:00 p.m.  HIV Clinical Science and Therapy
3:45 p.m.  New Treatment Approaches to HIV Disease
4:45 p.m.  Adjourn

Friday, October 8, 2010

9:00 a.m.  Some Infectious Causes or Possible Causes of Cancer
11:10 a.m. HIV-Associated Cancers
12:22 p.m. Tumor Growth and Inhibition
1:00 p.m.  Lunch Break
2:30 p.m.  Tumor Growth and Inhibition
3:06 p.m.  Selected Abstracts
4:00 p.m.  Adjourn
Monday, October 4, 2010

10:30 a.m.  Satellite Symposium

Fogarty Scholars Forum
Chair: William A. Blattner, M.D. – Institute of Human Virology

Italian Society for Medical Virology (SIVIM) Symposium

HIV in Italy
Chairs: Maria C. Re, Ph.D. – University of Bologna and Adriano Lazzarin, M.D. – Università Vita-Salute San Raffaele

1:00 p.m.  Introduction: Giuseppe Ippolito, M.D., Scientific Director – National Institute for Infectious Diseases
           HIV/AIDS in Italy: Divers and Dynamics

1:05 p.m.  Paola Cinque, M.D. – San Raffaele Scientific Institute
           Immunoactivation and HIV

1:23 p.m.  Gabriella D’Ettorre, M.D. – La Sapienza University
           HIV-DNA and Therapy

1:41 p.m.  Filippo Canducci, M.D. – Vita-Salute San Raffaele University
           Viral Fitness and Therapy of HIV Infection

2:00 p.m.  Mauro Pistello, Ph.D. – University of Pisa
           Lessons Learned from the Feline Immunodeficiency Virus/AIDS Model

Special Symposium on HIV and Malignant Disease in Africa
Chairs: Mauro Moroni, M.D. – Ospedale Niguarda Ca’ Granda, Umberto Tirelli, M.D. – National Cancer Institute and Joe O’Neill – University of Maryland, Baltimore

2:18 p.m.  William Blattner, M.D. – Institute of Human Virology
           HIV and Viral Associated Malignancies

*Abstracts appear as provided by authors
2:36 p.m. Clement Adebamowo, D.Sc., M.D., FWACS, FACS – Institute of Human Virology
Cancer Research in Africa

2:54 p.m. Annie Sasco, M.D., Ph.D. – Victor Ségalen Bordeaux 2 University
Cancer and HIV in Sub-Saharan Africa: Not Exactly as in the North

3:12 p.m. Max Essex, DVM, Ph.D. – Harvard University School of Public Health
HIV-1C of Southern Africa: Why is the Virus More Fit?

3:30 p.m. Sam Mbulaiteye, M.D. – National Cancer Institute
Epidemiology of AIDS-Related Malignancies: Current Knowledge and Opportunities for Etiological Studies in the Setting of HIV Treatment Programs

Roundtable: Research Needs and Challenges in Africa and the Developing World
Moderator: Joe O’Neill, M.D – University of Maryland, Baltimore

William Blattner, M.D. – Institute of Human Virology, Clement Adebamowo, D.Sc., M.D., FWACS, FACS – Institute of Human Virology, Annie Sasco, M.D., Ph.D. – Victor Ségalen Bordeaux 2 University, Max Essex, DVM, Ph.D. – Harvard University School of Public Health, Sam Mbulaiteye, M.D. – National Cancer Institute

4:00 p.m. ADJOURN

Formal Opening Ceremony, Welcome and Reception

6:00 p.m. Refreshments

6:30 p.m. Welcome Remarks by Robert C. Gallo
Kathleen Kennedy Townsend, Chair IHV Board of Advisors
Ferruccio Fazio, Minister of Health
Giuseppe Scopelliti, Governor of Calabria
Giovanni Latorre, Ph.D, Rector – University of Calabria

7:00 p.m. Robert C. Gallo, M.D. – Institute of Human Virology
Key Problems in HIV Research

7:15 p.m. Light Dinner: Speakers, participants, and guests are invited to attend.
Tuesday, October 5, 2010

Insights from Basic Science 1
Chairs: Giuseppe Teti, M.D. – University of Messina Medical School and Roberto Accolla, M.D. – University of Insubria

9:00 a.m. Andrea Cerutti, M.D. – Weill Cornell Medical College
Nef-Trafficking Intercellular Highways for HIV Evasion of Antibody Production

9:18 a.m. Suzanne Gartner, Ph.D. – Institute of Human Virology
Generation of Cells Within Human Nurse Macrophages and Consequences Following HIV Infection

9:36 a.m. Guido Poli, M.D. – San Raffaele Scientific Institute
Multifaceted Infection and Replication of HIV-1 in Human Macrophages

9:54 a.m. Warner Greene, M.D., Ph.D. – Gladstone Institute of Virology and Immunology
Murder on the HIV Express: Insights into How CD4 T Cells are Killed in Lymphoid Tissues

10:12 a.m. Leonid Margolis, Ph.D. – National Institutes of Health
HIV Interactions With Other Viruses in Human Tissues as Determinants of HIV Infection

10:30 a.m. William Hall, M.D., Ph.D. – University College Dublin
HIV-1 Rev and Regulation of Rev Nucleo-Cytoplasmic Shuttling

20 minutes BREAK

Special Lecture
10:47 a.m. Zeng Yi, Ph.D. – Institute for Viral Disease Prevention and Control Study on Therapeutic Vaccine for AIDS Treatment

Insights from Basic Science 2
Chairs: Umberto Bertazzoni, Ph.D. – University of Verona and Vittorio Colizzi, M.D., Ph.D. – University of Rome, Tor Vergata

11:10 a.m. John Moore, Ph.D. – Weill Cornell Medical College
Inhibiting CCR5 in vitro and in vivo

11:28 a.m. Mario Stevenson, Ph.D. – University of Massachusetts Medical Center
Viral Attack and Cellular Defense: The Role of Cellular Restrictions in the Biology of Primate Lentiviruses
11:46 a.m. Alain Lafeuillade, M.D. – Chalucet Hospital
*HIV Persistence Challenging Therapeutic Success*

12:04 p.m. Marie-Lise Gougeon, Ph.D. – Institute Pasteur
*Contributions of Innate Immunity to the Establishment of HIV Reservoir in DC: Role for DC:NK Cross-Talk*

12:22 p.m. Monsef Benkirane, Ph.D. – Institute of Human Genetics
*Interplay Between HIV Replication and MicroRNAs*

12:40 p.m. Eric Verdin, M.D. – Gladstone Institute of Virology and Immunology
*Molecular Mechanisms of HIV Latency*

12:58 p.m. LUNCH

2:30 p.m. **Institute of Human Virology Lifetime Scientific Achievement Award 2010**
Robert C. Gallo, M.D. – Institute of Human Virology
*Introduction of Rino Rappuoli, Ph.D., 2010 Award Recipient*

**Special Seminar**
Rino Rappuoli, Ph.D.
*Novartis Vaccines and Diagnostics*

3:00 p.m. **Selected Abstracts for Oral Presentation**
*Chairs: Fernando Aiuti, M.D. – University of Rome and Nancy Miller, M.D. – National Institute of Allergy and Infectious Diseases*

3:05 p.m. Alfredo Garzino-Demo, Ph.D. – Institute of Human Virology
*CCR6 Ligands Inhibit HIV in Highly Susceptible CD4+CCR6+ T Cells by Inducing APOBEC3G Expression*

3:12 p.m. Giulia Della Chiara, Ph.D. – San Raffaele Scientific Institute
*Naturally c-Terminally Truncated STAT5 (STAT5Δ): A Negative Controller of HIV-1 Transcription and Expression*

3:19 p.m. Marina Lusic, Ph.D. – San Raffaele Scientific Institute
*PML Nuclear Bodies Determine the Repressive Environment and Restrict Viral Gene Expression in Primary Human Lymphocytes*
3:26 p.m. Elisa Vicenzi – San Raffaele Scientific Institute
Characterization of Tripartite-Motif (TRIM) 22-Mediated Inhibition of HIV-1 Transcription

3:33 p.m. Olga Latinovic, Ph.D. – Institute of Human Virology
Suppression of CCR5 Density as a Novel Way to Enhance the Anti-HIV Activity of Fusion Inhibitors and CCR5 Antagonists

3:40 p.m. Apostolos Rizos, Ph.D. – University of Crete
HIV and Immune System Deregulation

3:47 p.m. Ming Zeng, Ph.D. – University of Minnesota
Lymphoid Tissue Niche Damage in SIV Infection Depletes Naïve T Cells

3:54 p.m. A.J. Smith, Ph.D. – University of Minnesota
The Role of Chitinase 3-Like-1 in the Pathological Deposition of Collagen in Lymphatic Tissue During HIV-1 Infection

4:01 p.m. Michael Thomson, Ph.D. – Institute de Salud Carlos III
Identification of New Patterns of Splice Site Usage by Transcripts of HIV-1 Primary Isolates of Diverse Subtypes

4:08 p.m. Ramesh Akkina, Ph.D. – Colorado State University
Aptamer-siRNA Chimera Therapy Suppresses HIV-1 Viral Loads and Protects From CD4 T Cell Loss in Humanized (RAG-hu) Mice

4:15 p.m. Cristiana Cairo, Ph.D. – Institute of Human Virology
Population Level Variations in the Baseline Value for Vδ2 T Cells and Implications for the Study of Infectious Diseases

4:22 p.m. Natalia Freund, Ph.D. – Tel Aviv University
Reconstitution of Conformational B-Cell Epitopes Using Combinatorial Conformer Libraries

4:29 p.m. Chiara Orlandi, Ph.D. – University of Insubria
HTLV-2 Tax-2 Transactivator Increases the Expression and the Function of its Inhibitor CIITA, the Master Regulator of HLA-II Gene Transcription

4:36 p.m. ADJOURN
Wednesday, October 6, 2010

**HIV Vaccine Part 1: Challenges to HIV Vaccine Development**

**Chairs:** Robert C. Gallo, M.D – Institute of Human Virology and Franco Buonaguro, M.D – National Cancer Institute, Fond Pascale

- **9:00 a.m.** Robert C. Gallo, M.D. – Institute of Human Virology  
  *Introduction – The Key Basic Science Issues in Developing an HIV Vaccine*

- **9:18 a.m.** Nelson L. Michael, M.D., Ph.D. – Walter Reed Army Institute of Research  
  *Building on the Results of the RV144 Prime Boost HIV Vaccine Efficacy Study*

- **9:36 a.m.** Jay Berzofsky, M.D., Ph.D. – National Cancer Institute  
  *A Novel Nanoparticle Approach to Induce Colorectal and Vaginal Mucosal Immunity*

- **9:54 a.m.** Mark J. Newman, Ph.D. – GeoVax Inc.  
  *Induction of Efficacious Immune Responses Using Heterologous Prime Boost Regimens of Recombinant DNA and MVA HIV Vaccines and GM-CSF as the Adjuvant*

**HIV Vaccine Part 2: Antibody Response to HIV Env**

**Chairs:** Alan Bernstein, OC, Ph.D., FRSC – Global HIV Vaccine Enterprise and Massimo Clementi, M.D. – Università Vita-Salute San Raffaele

- **10:12 a.m.** Leonidas Stamatatos, Ph.D. – Seattle Biomedical Research Institute  
  *Characteristics of the Earliest Cross-Neutralizing Antibody Response to HIV-1*

- **20 minutes** BREAK
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**Abstracts**

*OCTOBER 4-8, 2010 International Meeting of the Institute of Human Virology*

**Special Lecture**

11:44 a.m. Hans Wigzell, M.D., Director of the Center for Medical Innovations  
- Karolinska Institute

**Roundtable: What Has Been Learned from Clinical Trials**

**Moderator:** Alan Bernstein, OC, Ph.D., FRSC – Global HIV Vaccine Enterprise  
Gary Nabel, M.D., Ph.D. – National Institutes of Health; Nelson L. Michael, M.D., Ph.D. – Walter Reed Army Institute of Research; Robert C. Gallo, M.D. – Institute of Human Virology; Jim Tartaglia, Ph.D. – Sanofi Pasteur, Ltd.; Hans Wigzell, M.D., Director of the Center for Medical Innovations – Karolinska Institute; and David Montefiori, Ph.D. - Duke University

**HIV Vaccine Part 3: Alternatives for Vaccine Delivery**

**Chairs:** Giorgio Palù, M.D. – University of Padua and Carlo F. Perno, M.D. – University of Rome Tor Vergata

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<td>Gunnel Biberfeld, M.D., Ph.D. – Karolinska Institutet</td>
<td>Strong and Broad Immunogenicity of a Multigene, Multiclade HIV-1 DNA Prime MVA Boost Vaccine Regimen Among Healthy Tanzanian Volunteers</td>
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<td>12:48 p.m.</td>
<td>Gary Nabel, M.D., Ph.D. – National Institutes of Health</td>
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<td>Jim Tartaglia, Ph.D. – Sanofi Pasteur, Ltd.</td>
<td>Moving Forward From RV144: An Industrial Perspective</td>
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<td>3:00 p.m.</td>
<td>Dan Barouch, M.D., Ph.D. – Beth Israel Deaconess Medical Center</td>
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Novel Vectors and Antigens for a Next Generation HIV-1 Vaccine

3:18 p.m.  Genoveffa Franchini, Ph.D. – National Cancer Institute
Retooling the Macaque Model as a Necessary Step to Evaluate the Relative Efficacy of Vaccines for HIV

3:36 p.m.  Giuseppe Pantaleo, M.D. – Centre Hospitalier Universitaire Vaudois
Role of Poxvirus Vectors in the Development of an HIV Vaccine

3:54 p.m.  Selected Abstracts for Oral Presentation
Chairs: Michele La Placa, M.D. – University of Bologna and David Pauza – Institute of Human Virology

4:00 p.m.  Roberto F Speck, Ph.D. – University Hospital of Zurich
TLR8 Triggering Induces HIV from Latently Infected Cells of Myeloid Monocytic Origin and Indirectly from Latently Infected CD4+ T Cells Via the MAPK Pathway and TNF-α Respectively

4:07 p.m.  Fabio Romerio, Ph.D. – Institute of Human Virology
Modeling HIV-1 Latency in vitro

4:14 p.m.  Massimiliano Secchi, Ph.D. – San Raffaele Scientific Institute
Anti-HIV-1 RANTES Derivatives Acting as CCR5 Antagonists Present Full Additivity or Synergy in Combination With Different Entry/Fusion Inhibitors

4:21 p.m.  Elisa Saba, Ph.D. – San Raffaele Scientific Institute
HIV-1 Infection and Replication of Cervico-Vaginal Histocultures

4:28 p.m.  Laurel Lagenaur, Ph.D. – National Cancer Institute
A Live Microbicide Shows Efficacy in a Repeated Low Dose Challenge Model

4:35 p.m.  Donato Zipeto, Ph.D. – University of Verona
Fusion Complexes and CD-4 Independent Env for the Induction of Broad Spectrum Neutralizing Antibodies Against HIV-1

4:40 p.m.  ADJOURN

6:00 p.m.  Aperitivo

8:30 p.m.  Historical Lecture with Regional Dance Performance
(Coordinated by Arnaldo Caruso and Sebastiano Ando)
Location: Sunshine Club Hotel Ballroom
Thursday, October 7, 2010

HIV Vaccine Part 4: Therapeutic Vaccines for HIV and Cancer

**Chairs:** Cornel Fraefel, Ph.D. – ATH University of Zurich and Giampiero Carosi, M.D. – University of Brescia

9:00 a.m. Arnaldo Caruso, M.D. – University of Brescia

_HIV-1 Matrix Protein p17: A Candidate Antigen for Therapeutic Vaccines Against AIDS_

9:18 a.m. Luigi Buonaguro, M.D. – Istituto Nazionale Tumori

_Immune Signatures and Systems Biology of Vaccines_

9:36 a.m. Jeffrey Schlom, Ph.D. – National Cancer Institute

_Recombinant Vector-based Vaccines for Cancer Therapy_

Zwi Berneman, MD, Ph.D. – Antwerp University Hospital

_Dendritic Cell Vaccination in Acute Myeloid Leukemia_

9:54 a.m. Daniel Zagury, M.D. – NEOVACS

_Active Anti-IFN α Immnotherapy Applied to Chronic Viral and Autoimmune Diseases_

HIV Vaccine Part 5: Defining Targets for Protective Immunity

**Chairs:** Nelson L. Michael, M.D., Ph.D. – Walter Reed Army Institute of Research and Maria C. Re, Ph.D. – University of Bologna

10:12 a.m. Lucia Lopalco, Ph.D. – Fondazione San Raffaele del Monte Tabor

_New Immune Strategies to Reduce CCR5 Expression and Block HIV Infection_

10:30 a.m. Susan B. Zolla-Pazner, Ph.D. – NYU School of Medicine, Veterans Affairs Medical Center

_Using Epitopes Recognized by Monoclonal Antibodies as Vaccine Templates_

20 minutes BREAK

10:50 a.m. Ben Berkhout, Ph.D. – University of Amsterdam

_Reconsidering a Live-Attenuated HIV Vaccine?_
11:08 a.m. George Lewis, Ph.D. – Institute of Human Virology
   *Fc-Mediated Anti-HIV Effector Function Mediated by New Monoclonal Antibodies That Recognize Epitopes Selectively Exposed During Viral Entry*

11:26 a.m. David Montefiori, Ph.D. – Duke University Medical Center
   *Magnitude and Breadth of the Neutralizing Antibody Response in RV144*

### HIV Vaccine Part 6: Lessons from Animal Models

**Chairs:** Maria Salvato, Ph.D. – Institute of Human Virology and Massimo Galli, M.D. – University of Milan

11:44 a.m. Anna Aldovini, M.D. – Children’s Hospital Boston
   *SIV DNA/rMVA Nasal Vaccination Provides Better Control of Viremia and Disease Progression in Female than Male Macaques*

12:02 p.m. Guido Silvestri, M.D. – University of Pennsylvania
   *SIV Infection of Natural Hosts*

12:20 p.m. Ruth Ruprecht, M.D., Ph.D. – Dana-Farber Cancer Institute
   *Recombinant Protein Immunogens: Protection Against R5 Clade C SHIV Transmission and Correlates of Protection*

12:38 p.m. Christopher Miller, DVM, Ph.D. – California National Primate Research Center
   *A Protective Live-attenuated AIDS Vaccine Suppresses Innate Immunity and Inflammation in Immunized Rhesus Macaques*

### HIV Clinical Science and Therapy

**Chairs:** Gioacchino Angarano, M.D. – University of Bari and Robert Redfield, M.D. – Institute of Human Virology

12:56 p.m. **Introduction** - Stefano Vella, M.D. – Istituto Superiore di Sanità

1:00 p.m. Mark Wainberg, Ph.D. – McGill AIDS Centre
   *Monitoring HIV Drug Resistance as a Guide to Understanding Viral Pathogenesis*

1:15 p.m. Giuseppe Tambussi, M.D. – San Raffaele Scientific Institute
   *Cytokine-based Therapy in HIV Infection: from IL-2 to IL-7*

1:30 p.m. **LUNCH**
3:00 p.m.  Antonella Castagna, M.D. – San Raffaele Hospital  
*Salvage Therapy in HIV-1 Infected Patients*

3:15 p.m.  James Mullins, Ph.D. – University of Washington  
*HIV-1 Evolution in Primary Infection is Affected by Stochastic Followed by Selective Processes*

3:30 p.m.  Carl June, M.D. – University of Pennsylvania School of Medicine  
*Exploring the Potential of CCR5 and CXCR4 Modified CD4 T Cells to Target the HIV-1 Reservoir*

3:45 p.m.  BREAK

**New Treatment Approaches to HIV Disease**

**Chairs:** Roger Pomerantz, M.D. – Merck and Anita De Rossi, Ph.D. – University of Padua

4:00 p.m.  **Introduction:** Roger Pomerantz, M.D. – Merck  
*Is a Cure Possible for HIV Disease?*

4:15 p.m.  Anders Vahlne, M.D., Ph.D. – Karolinska Institutet  
*Peptide Linkers Between Natural Antibodies and HIV Targets*

4:30 p.m.  Robert Redfield, M.D. – Institute of Human Virology  
*Targeting Cell Cycle to Enhance the Potency of Antiretroviral Therapeutic Agents*

4:45 p.m.  Saverio Parisi, M.D. – University of Padua  
*Residual Viremia and HIV Compartmentalization in HAART Treated Patients*

5:00 p.m.  ADJOURN

*Scintille Fashion Show – by invitation only*

**Friday, October 8, 2010**

**Some Infectious Causes or Possible Causes of Cancer**

**Chairs:** Robert C. Gallo, M.D. – Institute of Human Virology and Kiyoshi Takatsuki, M.D., Ph.D. – Kumamoto University School of Medicine

9:00 a.m.  Kiyoshi Takatsuki, M.D., Ph.D. – Kumamoto University School of Medicine  
*Adult T-Cell Leukemia/Lymphoma in Japan: Reminiscences and Perspectives*
9:18 a.m.  Kuan-Teh Jeang, M.D., Ph.D. – National Institutes of Health
HTLV-1 Research After Three Decades: Insights into Cellular Transformation
Mechanisms for ATL (Adult T-Cell Leukemia)

9:36 a.m.  Toshiki Watanabe, M.D., Ph.D. – University of Tokyo
Risk Indicators for Disease Progression in Asymptomatic HTLV-1 Carriers:
A Nationwide Prospective Study in Japan and Expression Profile Analysis
Based on the Material Bank

9:54 a.m.  Mark Kaplan, M.D. – University of Michigan Medical Center
HIV Exposes Major “ Trafficking” in HERV-K HML-2 Viruses Which are Critical
to the Pathogenesis of HIV Infection and Lymphoma

10:12 a.m.  Reinhard Kurth, Ph.D. – Robert Koch Institute
Beneficial and Detrimental Effects of Human Endogenous Retroviruses (HERVs)

10:30 a.m.  David Markovitz, M.D. – University of Michigan
Do Modern Endogenous Retroviruses Still Replicate?

20 minutes  BREAK

HIV-Associated Cancers
Chairs: Marvin Reitz, Ph.D. – Institute of Human Virology and Eva Klein, M.D. – Karolinska Institutet

11:10 a.m.  George Klein, M.D., Ph.D. – Karolinska Institutet
Awakening of Dormant Tumor Cells by Inflammation

11:28 a.m.  Dario Di Luca, M.D. – University of Ferrara
KSHV, Angionesis and Kaposi Sarcoma

11:46 a.m.  Parkash Gill, M.D. – University of Southern California
VEGF-Notch-EphrinB2 Pathways in AIDS-Related Kaposi Sarcoma

12:04 p.m.  James Goedert, M.D. – National Cancer Institute
HIV Tropism and Decreased Risk of Breast Cancer

Tumor Growth and Inhibition
Chairs: Luigi Chieco-Bianchi, M.D. – University of Padua and Sebastiano Andò, M.D – University of Calabria

12:22 p.m.  Wuyuan Lu, Ph.D. – Institute of Human Virology
Novel Classes of p53 Activators for Cancer Therapy
12:40 p.m. Eitan Yefenof, Ph.D. – The Hebrew University, Hadassah Medical School
Sensitizing Hemopoietic Malignant Cells to Glucocorticoid Induced Apoptosis by PK Inhibitors
1:00 p.m. LUNCH
2:30 p.m. Isaac Witz, Ph.D. – Tel Aviv University
Cross-Talk with the Microenvironment: The Case of Melanoma Brain Metastasis
2:48 p.m. Andrei Kozlov, Ph.D. – St. Petersburg University
The Possible Evolutionary Role of Tumors in the Origin of New Cell Types

Selected Abstracts for Oral Presentation
Chairs: Guido Poli, M.D. – San Raffaele Scientific Institute and Mark Kaplan, M.D. – University of Michigan Medical Center

3:06 p.m. Franco Buonaguro, M.D. – National Cancer Institute, Fond Pascale
HPV-Related Cancers in HIV-Infected Subjects
3:13 p.m. Sayed Abdelwahab, Ph.D. – Minia University
Hepatitis C Virus-Specific Immune Response Among Egyptian Healthcare Workers at High Risk of Infection Without Viremia or Seroconversion
3:20 p.m. Jean Carr, Ph.D. – Institute of Human Virology
Newly Identified Circulating Recombinant Form Coming in Nigeria
3:27 p.m. Judith Torimiro, Ph.D. – Chantal Biya International Reference Centre
Population Level Drug Resistance in HIV Type 1 Protease and Reverse Transcriptase in Cameroon: 1995-2010 Review
3:39 p.m. Badran Bassam, Ph.D. – Institut Jules Bordet, Universite Libre de Bruxelles
Identification of a Human Natureal Regulatory T-Cell Micro-RNA Signature and Demonstration of the Major Role Played by miR-31, miR-21 and Valproate in Foxp3 Expression
3:46 p.m. Marco Rusnati, Ph.D. – University of Brescia
Role of Heparan Sulfate Proteoglycans in HIV-l Tat-Induced Transendothelial Migration of Lymphoid Cells
4:00 p.m. ADJOURN
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HIV-DNA and HIV Therapy

d’Ettorre Gabriella, Lorenzo Zaffiri, Giancarlo Ceccarelli, Claudio M. Mastroianni and Vincenzo Vullo
Department of Hygiene, Public Health and Infectious Diseases

The introduction of the HAART has not only dramatically reduced the mortality and morbidity of AIDS but has also transformed HIV infection in a manageable, chronic condition. HIV infection requires frequent virologic and immunologic monitoring to detect early virologic failure to the therapy and to monitoring the evolution of viral variants. To date the standard markers are: CD4+ T cells and HIV-RNA. The study of HIV-DNA could be useful to improve the life long treatment of HIV infection. HIV-DNA plays an important role in the pathogenesis of AIDS, in the establishment and persistence of the reservoir and for the monitoring of therapeutic strategies. The persistence of the virus depends on the long term survival of a pool of infected cells called “resting CD4+ lymphocytes” which represent the HIV reservoir. The frequency of latently infected cells is approximately 1 in 106 resting CD4+ T cells. This reservoir is generated early during an acute infection by reversion of the infected CD4+ into quiescent memory lymphocytes. These resting CD4+ T cells are transcriptionally silent but they can produce new infectious virions when activated again. The stability of this set of cells can depend on several factors: cellular, host, and by limits of HAART.

New therapeutic approaches are needed to purge these latent provirus, otherwise the goal to eradicate HIV infection will be out of reach. The hypothetical introduction of regimens active on the reservoir requires new markers for the monitoring of the size of reservoir. HIV-DNA load may give an estimate on the number of infected cells, including CD4+ T lymphocytes, and on the size of the viral reservoir which infectious virions can potentially be released. Secondly, it can be useful in clinical management in predicting very early failures and in sequencing the combined antiretroviral therapy.

105
HIV and Viral Associated Malignancy

William A. Blattner, M.D.
Institute of Human Virology, School of Medicine, University of Maryland, Baltimore, MD

HIV has been linked to malignancy since the first reports of Kaposi Sarcoma were a harbinger of the pandemic to come. Two herpes viruses, EBV (certain lymphomas) and HHV-8 (KSHV) (Kaposi Sarcoma) and pathogenic strains of HPV (cervical cancer) have been linked to the majority of AIDS-defining malignancies. These viruses are also linked to a number of cancers elevated in patients with HIV including some lymphomas (EBV and HHV8), Primary Effusion lymphoma (HHV-8), hepatocellular cancers (HBV, HCV) and penile and anal cancers (HPV). Through AIDS cancer matching studies and HIV cohort and clinical analyses a broader spectrum of HIV-associated malignancies have been reported and in European and US cohorts cancer is the leading cause of death in the HAART era as patients survive longer. Some such as Hodgkins disease are EBV associate while other such as lung cancer have yet to have an etiologic association with viruses. In sub-Saharan Africa, information on HIV-associated malignancies is sparse and with the exception of an AIDS Cancer Match study from Uganda are descriptive. With the scale up of antiretroviral therapy, the PEPFAR program provides an important platform for expansion of cancer research since it represents the largest cohort of HIV infected patients ever assembled. There are significant opportunities to define the spectrum of HIV-associated malignancies through improved integration of HIV and oncology services. Additionally, studies of these malignancies may provide new insights concerning viral etiology of cancer in HIV-infected cohorts. For example in West Africa, new strains of HTLV, HTLV-3 and HTLV-4 have been described that have yet to be linked to human disease, and emerging data link HPV to certain forms of head and neck cancer. Linking epidemiological cohorts to cancer virology basic study provides a path for expanding insights about the role of viruses in malignancy.
Cancer Research in Africa  
Clement Adebamowo, Sally Akarolo-Anthony and Hadiza Rasheed  
Institute of Human Virology, Nigeria

Risk of cancer in sub-Saharan Africa is linked to demographic shifts, urbanization, changing lifestyle, preventable oncogenic infections including the HIV/AIDS pandemic. Of the 12.4 million incident cancer cases for 2008, more than half will occur in developing countries accounting for two-thirds of the estimated global cancer deaths. In Africa, low levels of awareness, poverty, limited budgets and poor health care systems complicate cancer prevention, and treatment. Coordinated response by international agencies, NGOs and national governments targeting education, training, cancer control and management are a priority along with good quality clinical and laboratory practice, accessible and affordable chemotherapy, and treatment programs designed for low resource environments. Effective utilization of existing treatment and prevention strategies will decrease cancer mortality in Africa by more than 50% and implementation science research to evaluate challenges in deploying these strategies is urgently needed. There are unique opportunities for gaining novel insights about cancer etiology and biology by conducting studies in Africa to exploit the diversity of genomic and environmental factors and their interaction, an opportunity not replicable in other environments and populations. The African genome is the most heterogeneous with high frequency of mutations and short Haplotype lengths; its geography includes most of the world's flora and fauna; it is home to diverse culture with different social, economic and cultural characteristics and its biodiversity is yet to be adequately systematically exploited for phyto-pharmaceuticals. Heightened international collaborative such as the Human Heredity and Health in Africa project (H3Africa) co-sponsored by NIH and the Wellcome Trust explore role of genomic factors and their interaction with a range of environmental factors and are model for other collaborative ventures to unlock new therapies based on natural products drug discovery and new etiologic agents.

Cancer and HIV in Sub-Saharan Africa: Not Exactly as in the North  
Annie J. Sasco, Antoine Jaquet, François Dabis and Denis Malvy  
Inserm U 897, Victor Segalen Bordeaux 2 University, 146 rue Leo Saignat, 33 076 Bordeaux , France

Infectious diseases have long been regarded as diseases of the South and chronic diseases as those of the North. With the globalization of exposures, climate change, and improvement in the control of transmissible diseases, these frontiers are becoming somewhat blurred. With the lengthening of life-expectancy of HIV positive patients, linked to the use of effective anti-retroviral therapy, and the accompanying aging of the HIV positive populations, cancer became a noticeable cause of serious morbidity and mortality in the North. Studies in the South are still limited despite the fact that two-thirds of the world HIV positive population lives in sub-Saharan Africa.

Comparing results of studies on HIV and cancer from the South and the North, a pattern emerges. For Kaposi's sarcoma, non-Hodgkin lymphoma and cervical cancer, the association with HIV positivity, albeit remaining strong is weaker than the one found in the North. For non-AIDS classifying cancers, the directions of the associations are the same as in the North but detailed comparison of their magnitude is prevented by the paucity of data.

Potential explanations for these results include different background rates of the cancers in the South and the North (weaker association with higher background rates), as well as more frequent competing causes of death and later or missed diagnosis of cancer in the South. We propose a common physiopathological mechanism based on the role of immunity or lack thereof in cancer occurrence. The most likely candidate for this impaired immune background is the role in the South of infectious and tropical diseases. We thus suggest to investigate the association between a wide panel of biological agents and cancers, with evaluation of interaction with other carcinogenic behavioral and environmental exposures. This can only be done through a concerted effort of South and North epidemiologists, clinicians and biologists with complementary expertises.
108
HIV-1C of Southern Africa: Why Is the Virus More Fit?
M. Essex
Harvard School of Public Health, Boston MA

Over the last 3–5 years, the pandemic of HIV/AIDS appears to have reached a plateau for prevalence rates in most areas of the world. In all major regions except sub-Saharan Africa, saturation levels for HIV prevalence are below 1%. In sub-Saharan Africa, the WHO estimates are about 5% for adults aged 15–49. However, within sub-Saharan Africa, southern Africa has a much higher rate, about 18%, while the rest of sub-Saharan Africa is 3–4%.

Why are “saturation-level” HIV prevalence rates about 5-fold higher in southern Africa? Behavioral practices, differences in human genetics, and different viruses are all theoretical possibilities. The HIV-1C which causes the southern Africa epidemic has several features that appear to make it different. These include different rates of genomic variation and different patterns of drug-resistant mutations.

About one quarter of HIV-1C-infected adults show a prolonged pattern of high viral load (VL) following infection. Because high VL is associated with transmissibility, this suggests that such individuals may be “hypertransmitters” who are more highly infectious to their sexual contacts. Antiretroviral drug treatment (ART) dramatically decreases VL and HIV transmission. It then follows that to decrease transmission among adults, targeting those with high VL may reap the greatest benefit. Our current project in Mochudi, Botswana, utilizes this approach while incorporating HIV envelope sequence fingerprinting as a tag to monitor transmission.

109
Epidemiology of AIDS-related Malignancies in Africa: Current Knowledge and Opportunities for Etiological Studies in the Setting of HIV Treatment Programs
Sam M. Mbulaiteye, M.D.
Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS

The human immunodeficiency virus (HIV) infection epidemic was heralded by eruption of Kaposi sarcoma (KS) and aggressive non-Hodgkin lymphoma (NHL), including Burkitt lymphoma, in young homosexual men in the West. Risks for KS and for aggressive NHL were shown to be dramatically elevated (>10,000-fold and 50-600-fold, respectively) prompting these tumors to be designated AIDS-defining. Risks were elevated, albeit modestly, for cervical cancer (10-fold), Hodgkin lymphoma (10-fold), anal cancer (15-30-fold), but not for the most common malignancies in the general population: lung, breast, colon/rectum, stomach, liver, and prostate cancer. Data are scanty for Africa, where the 75% of the epidemic is concentrated. Incomplete case ascertainment and inaccurate diagnosis limit the value of quantitative estimates from Africa. Risk for KS and conjunctival squamous cell carcinoma are 20-90-fold and 10-fold increased with HIV infection. The associations of HIV infection with NHL and cervical cancer are controversial. No association has been shown for many virally-associated cancers, including cancers of the liver, nasopharynx, and penis. The introduction of immune restoring highly active anti-retroviral therapy (HAART) in Western countries in 1996 has decreased acute mortality from HIV and is changing the profile of cancers in people with chronic HIV infection. Access to life-extending HAART has been rapidly expanding since 2002 in Africa and it is dramatically improving survival of patients. The impact of increased survival on cancer burden or relative risk in Africa cannot be predicted from current data, but the HIV treatment cohorts across the continent provide a rare opportunity to study the relationships between cancer, HIV subtype infection, immunosuppression, and host-genotype. Such studies may reveal new associations of HIV with some virally or not-virally associated cancers. New associations or manifestations of cancer with HIV may become apparent as HAART becomes widely available and HIV survival in developing countries improves.
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Nef-Trafficking Intercellular Highways for HIV Evasion of Antibody Production

Weifeng Xu, Paul A. Santini, John S. Sullivan, Bing He, Meimei Shan, Susan C. Ball, Wayne B. Dyer, Thomas J. Ketas, Amy Chadburn, Leona Cohen-Gould, Daniel M. Knowles, April Chiu, Rogier W. Sanders, Kang Chen and Andrea Cerutti*

Mount Sinai School of Medicine, Immunology Institute, Catalan Institute for Research and Advanced Studies, Barcelona Biomedical Research Park, IMIM-Hospital del Mar

Contact-dependent communication between immune cells generates protection, but also facilitates viral spread. We found that macrophages formed long-range actin-propelled conduits in response to negative factor (Nef), a human immunodeficiency virus type-1 (HIV-1) protein with immunosuppressive functions. Conduits attenuated immunoglobulin G2 (IgG2) and IgA class switching in systemic and intestinal lymphoid follicles by shuttling Nef from infected macrophages to B cells through a guanine exchange factor-dependent pathway involving the amino-terminal anchor, central core and carboxy-terminal flexible loop of Nef. By showing stronger virus-specific IgG2 and IgA responses in patients harboring Nef-deficient virions, our data suggest that HIV-1 exploits intercellular highways as a “Trojan horse” to deliver Nef to B cells and evade humoral immunity systemically and at mucosal sites of entry.

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Generation of Cells Within Human Nurse Macrophages and Consequences Following HIV Infection

Suzanne Gartner, Senthilkumar Natesan and Yiling Liu

Institute of Human Virology

We have observed that a subset of cultured human monocyte-derived macrophages can behave as “nurse” cells with functional capabilities that include the de novo generation, within themselves, of CD4+ T-lymphocytes and a previously unknown small cell with monocyte-macrophage characteristics. We named these novel cells “self-renewing monocytoid cells” (SRMC) because they could develop into nurse macrophages (NM) that produced another generation of SRMC. The NM/SRMC production cycle could continue in vitro for several generations. SRMC expressed CCR5, the coreceptor for macrophage-tropic HIV isolates, and unlike monocytes and mature macrophages, were highly susceptible to HIV entry leading to productive infection. Moreover, in the presence of HIV expression, infected SRMC differentiated into macrophages, including SRMC-producing NM, and the NM/SRMC cycle was maintained. We hypothesize that perpetual cycles of NM/SRMC production could maintain HIV within the body indefinitely. Because this mode of persistence does not require new rounds of infection, it would escape the effects of most antiretrovirals. HIV infection of NM decreased production of CD4+ T-lymphocytes in macrophage cultures. This was attributable to a severe, preferential loss of the CCR5+ CD4+ subpopulation. Rather than being released, the developing T-lymphocytes accumulated within infected NM, resulting in cells with the appearance of classic HIV+ multinucleated giant cells. Confocal microscopy revealed individual HIV-expressing NM simultaneously producing both virus-expressing SRMC and non-expressing T-cells, suggesting that NM might be a source of latently infected CD4+ T-lymphocytes. Real-time PCR experiments further supported this contention by showing ~10-fold more HIV genome-positive than virus-expressing T-cells. We extend these observations to infected individuals and propose that NM play a major role in maintenance of HIV persistence via the NM/SRMC production cycle, that they are a source of latently infected T-cells, and that their infection contributes to the CD4 T-cell decline that characterizes HIV/AIDS.
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**Multifaceted Infection and Replication of HIV-1 in Human Macrophages**  
Luca Cassetta, Edana Cassol, Anna Kajaste-Rudnitski, Elisa Vicenzi, Massimo Alfano and Guido Poli  
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After CD4+ T lymphocytes, mononuclear phagocytes were demonstrated to be infected in vivo and infectable in vitro by HIV-1. Unlike T cells, they are relatively resistant to the cytopathic effect of the virus therefore resulting in a persistent state of infection oscillating from latency to active virion production. In addition, macrophages can bud and accumulated ex novo produced virions in intracellular vacuoles of debated nature, a feature that has generated the so-called “Trojan horse” hypothesis on the role of these cells in HIV infection. In this scenario, we have recently observed that polarization of monocyte-derived macrophages (MDM) into classically activated (M1) or alternatively activated (M2a) cells by their “pulsed” stimulation with either TNF-α plus IFN-γ or IL-4, respectively, results in the inhibition of virus replication likely by different mechanisms. While M1-polarization acts at an early, pre-integration level in the virus life cycle, M2a-MDM appear restricted by post-integration mechanisms under intense investigation (E. Cassol, J Immunol. 182: 6237, 2009). Furthermore, M2a cells also express high levels of DC-SIGN that, conversely, facilitate infection and cell-mediated transmission of HIV-1 to CD4+ T cells. Since tissue macrophages, unlike dendritic cells, are mostly residential, M2a-driven DC-SIGN expression may play an important role in the local spreading of infection in mucosal rather than lymphatic tissues. In conclusion, macrophage polarization in vivo is likely to span a spectrum of activated phenotypes that may change their state of permissiveness to infection and profoundly influence their capacity to propagate HIV-1 to neighboring CD4+ cells.

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**Murder on the HIV Express: Insights into How CD4 T Cells Are Killed in Lymphoid Tissues**  
Gilad Doitsh, Marielle Cavrois, Kara Lassen, Orlando Zepeda, Zhiyuan Yang, Mario Santiago, Andrew M. Hebbeler and Warner C. Greene  
Gladstone Institute of Virology and Immunology

The mechanism underlying CD4 T-cell depletion in HIV-infected hosts remains poorly understood. Loss of these cells underlies subsequent clinical development of AIDS. Both direct and indirect mechanisms of CD4 T cell killing have been proposed. In ex vivo cultures of primary human tonsil tissue, CD4 T cells undergo a dramatic cytopathic response following HIV infection. Strikingly, we find that 95% of these dying lymphoid cells are not productively infected with HIV but instead correspond to bystander cells that are abortively infected. Abortive infection reflects the non-permissive state of most resting CD4 T cells where the viral life cycle is arrested prior to the completion of reverse transcription. CD4 T cell killing is prevented by HIV entry and fusion inhibitors and by inhibitors that block the initiation of reverse transcription (NNRTI’s including efavirenz and nevirapine). Conversely, RT inhibitors that act later in the reverse transcription process as chain terminators (NRTI’s including AZT and 3TC) or inhibitors of viral integration (raltegravir) do not rescue CD4 T cells from this form of death. The accumulation of cytoplasmic viral DNA is sensed in these cells activating an innate immune response that ultimately culminates in death of the host cell. The nature of this innate response will be discussed. Together, these findings highlight how a protective host response centrally contributes to the demise of CD4 T cells during HIV infection ultimately culminating in AIDS.
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HIV Interactions With Other Viruses in Human Tissues as Determinants of HIV Infection

Leonid Margolis
National Institute of Child Health and Human Development

Human tissues, where critical events of HIV disease occur, are not sterile. Unlike cell cultures used in the laboratories, human tissues are coinfected with other microbes, in particular viruses which interact with HIV-1 and affect HIV infection. Exploiting them may reveal new anti-HIV strategies. Towards this goal, we study HIV interactions with human herpesvirus (HHV) in coinfected human lymphoid, rectal, and cervico-vaginal tissues ex vivo. We infected tissues ex vivo with HIV and various HHVs, including highly pathogenic HSV-2 and low pathogenic HHV-6 and HHV-7, and found that in coinfected tissues these viruses interact with each other.

Moreover, it is possible to establish a new mode of HIV-1 interaction with HHVs by administrating acyclovir, a common antitherpetic drug. This drug, upon phosphorylation, inhibits both HHV and HIV-1, suppressing HIV reverse transcriptase (RT). We developed monophosphorylated acyclovir derivatives that do not require activation by HHVs and inhibit HIV-1 in HHV-free systems. Activated acyclovir and its monophosphorylated derivatives suppress replication of both R5 and X4 HIV-1 variants, including variants that are resistant to other NTRIs. Although an ACV-resistant HIV variant with a dominant V75I RT mutation has been identified in ex vivo tissues, there is no evidence that this mutation evolves in HIV-infected patients treated with acyclovir.

Interactions between HIV and other viruses, particularly HHVs, largely determine the course of HIV disease and can be exploited and mimicked to develop new anti-HIV-1 strategies.

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HIV-1 Rev and Regulation of Rev Nucleo-Cytoplasmic Shuttling

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The HIV-1 regulatory protein Rev is essential for viral replication and mediates the nuclear export of unspliced viral transcripts, a process that requires active nucleocytoplasmic shuttling. Rev nuclear import is facilitated by the recognition of its nuclear localisation signal (NLS) by a number of import factors, which include importin β and transportin. However it is unclear which nuclear import pathway(s) predominate in vivo, and whether other specific cellular factors can modulate Rev shuttling. In this study we have demonstrated an interaction of the cellular protein HIC (Human I-mfα domain-Containing protein) with Rev. It could be shown that HIC interferes with the Rev NLS - importin β interaction and inhibits nuclear import. In contrast HIC does not affect transportin mediated Rev nuclear import, suggesting that Rev-NLS recognition by the various import factors may involve distinct mechanisms. Our findings suggest that the cytoplasmic sequestration of Rev by HIC is one mechanism for the regulation of Rev function, and that the employment of different import factors by Rev enables the protein to change its nuclear trafficking strategy depending on the cellular environment.
Inhibiting CCR5 in vitro and in vivo
John Moore
Cornell

I will describe collaborative research on CCR5 small molecule inhibitors of HIV-1 entry. In particular I will discuss how HIV-1 develops resistance to this drug class in vitro. I will also show how these compounds can be used to prevent HIV-1 transmission in the macaque model when applied as a vaginal microbicide, both as a gel and as a vaginal ring. I will also address how such approaches can be combined with vaccination to make a more effective prevention science strategy.

Viral Attack and Cellular Defense: The Role of Cellular Restrictions in the Biology of Primate Lentiviruses
Mario Stevenson
University of Massachusetts Medical School

The replication of retroviruses is dependent upon their ability to commandeer cellular factors at various stages in the replication cycle. Research over the past several years however, has revealed the presence of “cellular restrictions” that potently antagonize the replication of viruses such as HIV-1. For example, the Apobec 3 proteins are cytidine deaminases that compromise the formation and integrity of viral cDNA while Bst2/tetherin prevents detachment of budding viruses from the surface of the infected cell. In order to counteract these restrictions, primate lentiviruses have evolved accessory proteins: the Vif protein targets Apobec 3 for proteasomal destruction while Vpu mislocalizes Bst2/tetherin away from sites of virus budding. We have recently obtained evidence for novel restrictions that are expressed in cells of macrophage lineage. One restriction potently antagonizes retrovirus replication including the lentiviruses, HIV-1/2 and SIV as well as the gamma retrovirus, MLV. We have evidence that this restriction is counteracted by the viral accessory proteins Vpx/Vpr. These proteins commandeer a damaged DNA response protein (DDB1) to target the restriction to the proteasome. We have further obtained evidence that this restriction dictates the cell cycle dependence of retrovirus infection and is the obstacle to monocyte infection by retroviruses. Therefore when the restriction is neutralized, macrophages, which are normally refractory to MLV infection, are rendered permissive to MLV transduction. The second restriction antagonizes virus egress and is counteracted by the Vpu proteins of HIV-1/SIV. Given our increasing understanding of the function of the viral accessory proteins, they remain highly attractive targets for therapeutic intervention in HIV/AIDS.
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HIV Persistence Challenging Therapeutic Success
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HIV integrates in host cells, in particular in a pool of latently resting CD4 cells with a half life of several decades. Despite the availability of 30 different ARVs to block viral replication at different steps of its life cycle, this reservoir rekindles infection each time therapy is interrupted. In fact, HIV RNA persists at a few copies/ml in the plasma of patients under effective combination therapy. Whether these low levels are the result of cryptic replication or the release from previously infected cells, remains debated. Intensification trials have failed to show any effect on these residual RNA levels. However, a recent study found an increase in 2 LTR circles and a decrease in activation markers in about 30% of raltegravir intensified cases. In another study changes, although not significant, were only found in ileum biopsies, raising the possibility that events in plasma doesn’t correlate with those in tissues. The origin of this persistent viremia is not clear, and the contribution of different cell types, including haematopoietic progenitors, has been proposed. It is also possible that some anatomic reservoirs are involved. However, sites like the central nervous system more probably act as sanctuaries, with the observation of a high prevalence of neurocognitive disorders, even in ‘successfully’ treated patients.

Another negative aspect of HIV persistence despite effective viral control is the presence of abnormal inflammation in most patients and its consequences in terms of increased atherosclerosis and premature aging.

Finally, even if ARVs are currently less toxic, long term side effects remain frequent like lipid, bone density and renal function alterations.

Consequently, as patients suffer from the added penalties of progressive infection in some compartments, collateral effects of inflammation and drug toxicities, the search for a cure remains a realistic goal even

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Functional Implications γδ T Cell Depletion in HIV Disease
Cristiana Cairo, Elizabeth Urban, Haishan Li, Bhawna Poonia, Cheryl L. Armstrong, David Riedel, Robert Redfield and C. David Pauza
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Acute HIV infection is characterized by uncontrolled viremia and rapid depletion of CD4+ and CD4- cell subsets. Cell depletion is a cascade of direct virus cytopathicity, immune activation and FasL-dependent cell death that remodels the immune system and allows the establishment of persistent infection. Among the cell subsets that are impacted during acute infection are the γδ T and NK cells. These subsets have overlapping functions for secreting Type 1 cytokines (TNFα and IFNγ), direct cytolysis of infected cells and indirect cell killing through antibody-dependent cellular cytotoxicity (ADCC). Effector cells from both subsets are characterized by expression of CD56 and NKG2D. The acute loss of CD56+ γδ T and NK cells may be linked through cell regulatory networks controlled by costimulatory molecules including 4-1BB, and it is possible that a single event leads to changes in both lymphocyte populations. We also noted a strong correlation between HIV acquisition and high activity alleles of the FCGR3A genes (Fc receptor γIIa), suggesting that natural antibody may have a role in signaling these innate immune cells and triggering the events of cell depletion. The loss of potent ADCC effector subsets during acute HIV disease, will prevent nascent, HIV-specific antibodies from attacking infected cells and slowing virus spread. Decreased levels of pro-inflammatory cytokines TNFα and IFNγ, will lower the effectiveness of cellular immunity. The impact of HIV infection to debilitate innate immune responses and blunt the effectiveness of virus-specific acquired immunity, reveals a complex mechanism for immune evasion and the establishment of persistent infection. Persons with natural control of viremia do not have γδ T or NK cell defects and this difference accounts in part, for their ability to avoid progressing disease.
The outcome of HIV-1 infection results from complex interactions between viral compounds and host cell factors. In most cases, HIV-1 successfully hijacks cellular pathways and bypasses restriction factors for optimal replication leading to continuous rounds of infection, replication and cell death. Continuous viral replication causes the loss of CD4+ T cells and progression to immunodeficiency in infected individuals. However, situations where successful control of virus replication was achieved have been reported. First, HAART treatment revealed the existence of a pool of resting memory CD4+ T cells harbouring integrated but silent HIV-1 provirus. Although this situation occurs in a small number of cells, it suggests that intracellular defence mechanisms can be effective against HIV. Second, HIV-infected individuals who are able to control their plasma viremia to undetectable levels for many years in absence of any treatment have been identified and referred to as Elite HIV controllers. Again, this is a rare situation observed in 0.5% of infected patients. Still, it demonstrates that it is possible to effectively control HIV replication and disease progression. We will discuss how HIV-1 uses microRNA pathway and cellular miRNAs to overcome restriction factors activity and their implication in viral latency.

The presence of latent reservoirs has prevented the eradication of Human Immunodeficiency Virus (HIV) from infected patients. The mechanism of postintegration latency is poorly understood, partly because of the lack of an in vitro model. We previously reported that the chromatin environment at the site of integration of HIV into the cell genome plays an important role in its transcriptional activity. We also reported on the use of an HIV molecular clone expressing green fluorescent protein (GFP) to highly enrich for latently infected cells after infection. HIV latency occurred reproducibly, albeit with low frequency (1.5%), during infection. Clonal cell lines derived from this latent population showed no detectable basal expression, but could be induced fully after treatment with a variety of biological and pharmacological agents. I will discuss our study of these clonal cell lines and the mechanisms responsible for the establishment, the maintenance and the reactivation of latent HIV in this system.
CCR6 Ligands Inhibit HIV in Highly Susceptible CD4+CCR6+ T Cells by Inducing APOBEC3G Expression

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CD4+CCR6+ T cells are highly susceptible to infection by both R5 and X4 isolates of HIV, as compared to CD4+CCR6- cells. We had shown that two CCR6 ligands (hBD2 and hBD3) inhibit HIV infection. We hypothesized that CCR6 mediates a novel type of antiretroviral activity.

We observed that treatment with CCR6 ligands hBD2, -3, and MIP3α resulted in HIV inhibition and decreased abundance of early products of reverse transcription, which are abrogated by treating cells with PTx, an inhibitor of Gi proteins that are associated to signal transduction mediated by chemokine receptors. We found that expression of the cellular protein APOBEC3G is increased concomitantly with treatment with CCR6 ligands and with decrement of early reverse transcription products. Treatment of cells with a specific siRNA targeting APOBEC3G abrogates HIV inhibition, demonstrating the central role of APOBEC3G in the effects of CCR6 ligands. Induction of APOBEC3G by CCR6 ligands is also abrogated by treatment with pertussis toxin, indicating the requirement of intracellular signaling in this effect. We found that expression of CCR6 ligands is lower in HIV infected subjects than in controls.

Our findings show that CCR6-induced antiviral activity can be a target for novel approaches to treatment and prevention of HIV infection, since CCR6 is expressed on cells most relevant to HIV infection, including CD4+CCR5+ memory T cells, Th17 cells, DC, and activated macrophages.

Naturally C-Terminally Truncated STAT5 (STAT5Δ): A Negative Controller of HIV-1 Transcription and Expression

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Signal transducers and activator of transcription (STAT) proteins, namely STAT1 and STAT5, are often constitutively activated in the PBMC of most of HIV-1+ individuals; furthermore, most patients are characterized by the dominant expression of a C-terminally truncated isoform of STAT5 (STAT5Δ). STAT5Δ is also the prevalent isoform of STAT5 found in the chronically HIV-1 infected promonocytic cell line U1, characterized by a constitutive state of viral latency and inducibility of virus expression by PMA or several cytokines. GM-CSF-mediated STAT5Δ activation induced a delayed expression of integrated HIV-1 in U1 cells. Selective inhibition of STAT5Δ expression or activation enhanced viral expression in GM-CSF stimulated U1 cells. Moreover, activated STAT5Δ can directly in vivo bind to STAT consensus sequences in the HIV-LTR promoter with an impaired recruitment of RNAPol II in U1 cells stimulated with GM-CSF. STAT5Δ can act as a negative regulator of HIV-1 expression in GM-CSF stimulated U1 cells. We are currently investigating whether the reduced recruitment of RNA Pol II and the consequent decreased viral transcription and delayed kinetics of HIV expression that follow GM-CSF stimulation could be entirely attributed to the negative role of STAT5Δ alone or whether other proteins participate to the negative control of HIV transcription in U1 cells.
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PML Nuclear Bodies Determine the Repressive Environment and Restrict Viral Gene Expression in Primary Human Lymphocytes
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Various members of tripartite motif (TRIM) protein family display antiviral properties, targeting retroviruses in particular. The activity of TRIM19, better known as promyelocytic leukemia protein (PML) against several viruses has been well documented, and yet its role in HIV-1 infection remains elusive.

P-TEFb kinase complex, composed of CDK9 and CyclinT1, is one of the most important cellular partners playing a key role in HIV-1 transcription. We have previously demonstrated that both members of P-TEFb localize inside the PML Nuclear Bodies (PML NBs) and that the acetylated and enzymatically inactive form of CDK9 interacts with PML. Since acetylated CDK9 was found to bind the integrated and transcriptionally silent viral genome, we hypothesized that the latter might reside in the proximity of PML NBs.

To test this hypothesis, we developed 3D Immuno DNA FISH on latently infected Jurkat cells; we observed close proximity between silent HIV-1 provirus and PML NBs, whereas transcriptional activation induced by TNF-α or TPA led to a significant displacement of PML NBs. This result was confirmed by ChIP, showing PML occupancy at the HIV LTR in resting conditions and its dynamic release after induction. To establish the functional role for PML in HIV-1 repression, we disrupted PML NBs by arsenic trioxide treatment or by stably knocking-down PML protein with a lentiviral vector; both treatments induced a very significant increase in the levels of viral transcription. Finally, we confirmed the association of HIV with PML and the role of these bodies in a primary model of HIV latency, using CD4+ cells purified from human blood and successively infected in vitro.

Thus, provirus resides in the close proximity to PML NBs, nuclear neighborhood that represses viral gene expression and contributes to viral latency.

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Characterization of Tripartite-Motif (TRIM) 22-Mediated Inhibition of HIV-1 Transcription
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The family of TRIM proteins, including TRIM5α, has been previously described as capable of interfering with HIV-1 or SIV at different steps of the retroviral life cycle. In a model of permissive (P) and non-permissive (NP) clones of the promonocytic U937 cell line, we have recently observed that endogenous TRIM22 is responsible for impairing HIV-1 long terminal repeat (LTR)-driven gene expression in NP cells, whereas its expression is absent in P cells (AKR et al., submitted). HIV transcription is regulated by a variety of cis-acting DNA sequence elements within the proviral LTR, responsive to specific host transcription factors. With the aim of identifying which of these factors could be involved in the TRIM22-mediated inhibition of HIV-1 transcription, an HIV-1 LTR Luciferase (Luc) reporter construct was transfected in 293T cells along with increasing amounts of TRIM22-expressing plasmid followed by cell stimulation with phorbol myristate acetate (PMA) plus ionomycin (I). TRIM22 expression inhibited both basal and PMA+I induced HIV-1 LTR activity whereas Tat-mediated transactivation of HIV-1 LTR was not affected. Furthermore, increasing amounts of TRIM22-expressing plasmid inhibited NFAT-1, NF-kB or AP-1-driven Luc expression to basal level in 293T cells along with, followed by stimulation with PMA+I. These results highlight a broad inhibitory effect of TRIM22 on Tat-independent HIV-1 LTR driven transcription. Since no DNA interacting domains are known to be present in TRIM22, our results suggest that this host factor might either interact, directly or indirectly, with the above mentioned cellular transcription factors or that it may act via an epigenetic mechanism regulating HIV-1 transcription.
Suppression of CCR5 Density as a Novel Way to Enhance the Anti-HIV Activity of Fusion Inhibitors and CCR5 Antagonists

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The CCR5 chemokine receptor plays a crucial role in HIV-1 infection. We have established methods to quantify CCR5 density and to evaluate its impact on virus infectivity in spreading/single cycle infection and direct virus-cell fusion assays. In our preliminary studies, we use a stoichiometry model (Kuhmann et al, 2006) to calculate a number of CCR5 molecules needed for HIV-1 pseudovirus infection. In addition, using low doses of the drug Rapamycin, a CCR5 suppressor, we demonstrate decreased R5 HIV-1 infectivity and enhanced potency of entry inhibitors. Our data show that Rapamycin reduction of CCR5 density in lymphocytes increased sensitivity to Vicriviroc (VCV) in VCV-resistant strains, inhibiting production by ~90%. Novel Beta-lactamase (BlaM) entry assay revealed the differences in the activity between VCV sensitive and VCV resistant virus. In the case of the resistant virus, there is no inhibition in higher CCR5 expressive cells, but in cells with physiological expression of CCR5, we do observe susceptibility to VCV resistant virus.

As an alternative anti-HIV therapy approach, we successfully employ the CCR5 antibodies in order to determine the affinity of resistant virus Envelope in cases when it is free versus occupied CCR5 site by the CCR5 antagonist, Maraviroc. Identifying the most effective drug combinations of Rapamycin or CCR5 antibodies with the CCR5 antagonists (with the lowest possible side effects) has relevant clinical implications in anti-viral therapy.

HIV and Immune System Deregulation

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Tremendous advances have been made over the last decade in our understanding of the complex interaction between the human immunodeficiency virus (HIV) and the host immune system that has laid ground for the development of new potent antiviral drugs. However, many central questions that bear on the feasibility of developing an HIV vaccine still remain unanswered, including identification of protective immune mechanisms, addressing the high variability of the virus and its ability to evade immune responses. In this context the most important unanswered question is the mechanism by which HIV inactivates or kills T cells. It is now well established that the third hypervariable domain (V3) of the human immunodeficiency virus-1 (HIV-1) glycoprotein gp120 plays a crucial role in the cell attachment mechanism of the virus. Using synthetic peptides we have shown that during the process of specific antigen presentation, the presence of the cationic crown of V3 induces enhanced and accelerated activation of the responding CD4+ T cells, followed by a sharp apoptosis. The degree of activation was related to the cationic potential of the V3 peptide. The interacting molecule on the T cell membrane was the chemokine receptor CCR5 which also acts as co receptor to the virus. This interaction is central to immune dysfunction caused by the presence of the virus and is a major contributor to the HIV-1 pathogenesis. A careful appraisal of the structure and dynamics between gp120 and CCR5 using physicochemical methods gave new insights of the underlying interactions at a molecular level and will contribute to our ability to intervene and develop novel therapeutic approaches against this infection.
The Role of Chitinase 3-Like-1 in the Pathological Deposition of Collagen in Lymphatic Tissue During HIV-1 Infection
University of Minnesota

Background: One pathological hallmark of HIV-1 infection is the aberrant deposition of collagen and consequent fibrotic damage in both gut and lymphatic tissue, a process which adversely affects the maintenance and preservation of CD4+ T cells and overall immune function in this anatomical niche. To date, mechanistic information is still needed to account for this fibrotic process during HIV-1 infection. We recently identified a gene encoding chitinase 3-like-1 (CHI3L1) in microarray studies of HIV-1 infection in lymphatic tissues and show here that CHI3L1 may play an important role in accelerating collagen deposition during HIV-1 infection.

Methods: In vivo expression of CHI3L1 was observed using CHI3L1-specific antibodies in immunofluorescent analyses of inguinal lymph node (LN) biopsies (n = 24) from HIV-1-infected individuals at various stages of disease. The ability of CHI3L1 to participate in collagen deposition was assayed ex vivo by culturing human stromal cells in the presence or absence of recombinant human CHI3L1 and then measuring collagen type I (COL1) production via immunofluorescence and quantitative ELISA. In vivo correlation analyses were performed using Pearson's correlation coefficient (r).

Results: CHI3L1 is upregulated in the LN~4-fold in acute HIV-1 infection, ~3-fold in the asymptomatic stage, and ~6-fold in AIDS compared to uninfected controls (n=4). CHI3L1 is expressed as an extracellular membrane protein predominantly in vimentin+ stromal cells/fibroblasts, and its expression mirrored COL1 deposition in vivo (r=0.8). Recombinant CHI3L1 increased COL1 synthesis ~2-fold in primary human stromal cells/fibroblasts, leading to acellular deposition of COL1; CHI3L1-specific antibodies blocked this effect.

Conclusion: Collectively, this data provides mechanistic insight surrounding aberrant collagen deposition during HIV-1 infection. CHI3L1’s expression in the LN may serve to accelerate the fibrotic process during viral infection, advocating continued research in this area to develop novel therapeutic targets to attenuate pathological collagen deposition in HIV-1 infection.

Identification of New Patterns of Splice Site Usage by Transcripts of HIV-1 Primary Isolates of Diverse Subtypes
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HIV-1 expresses all its RNAs from a single primary transcript which undergoes a complex alternative splicing process, by which more than 40 different spliced transcripts can be generated. Knowledge on HIV-1 splice site usage by primary isolates of non-B subtypes is scarce. Here we analyze HIV-1 splice site usage in acute in vitro infection of peripheral blood mononuclear cells (PBMCs) by 15 primary isolates of subtypes A, B, C, and G. HIV-1 spliced transcripts were amplified by RT-PCR coupled with nested PCR using primers recognizing sequences common to all doubly spliced (DS) or singly spliced (SS) transcripts. Fluorescent labelling of one of the primers in the nested PCR and electrophoresis in an automated sequencer allowed PCR product size determination and quantitation by using the GeneMapper program. PCR products with unexpected sizes, according to known HIV-1 splice site usage, were identified by cloning and sequencing. Wide fluctuations in the relative expression of spliced transcripts within the DS and SS classes following acute infection of PBMCs were frequently observed. In one subtype A virus high levels of expression of tat RNAs were observed, which surpassed rev RNA expression and approached nef transcript levels. All 3 analyzed subtype C viruses utilized a previously unreported splice site for generation of rev transcripts located 7 nucleotides upstream of SA4a, designated SA4d, which was the major splice site used by rev transcripts in two isolates. In one subtype B virus, rev transcripts used predominantly a newly identified splice site (SA4e) located 5 nt upstream of SA4a. In one subtype C virus, a majority of rev transcripts (but not of nef or tat RNAs) incorporated noncoding exon 3. These results show a high diversity of spliced RNA expression in primary HIV-1 isolates of different subtypes.
Aptamer-siRNA Chimera Therapy Suppresses HIV-1 Viral Loads and Protects from CD4 T-Cell Loss in Humanized (RAG-hu) Mice

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Therapeutic strategies designed to combat HIV/AIDS by siRNAs show considerable promise, but targeted delivery of these synthetic molecules into virus infected cells in vivo has been a formidable challenge. In addressing this need, using a humanized mouse model, we sought to evaluate the in vivo efficacy of a chimeric construct consisting of an HIV-1 gp120 directed aptamer with viral neutralization capacity fused to an siRNA with proven efficacy against tat/rev viral transcripts. Humanized Rag2-/- γc-/- (RAG-hu) mice with multilineage hematopoiesis were prepared by engrafting with human CD34+ hematopoietic progenitor cells. These animals were infected with HIV-1 NL4-3 virus to generate viremic mice. Subsequently, the viremic mice were injected intravenously with the aptamer-siRNA chimeras or control RNAs. The viral loads and CD4+ T-cell levels were monitored weekly. Our results show that both the aptamer and aptamer-siRNA chimera treatments markedly suppressed HIV-1 replication and prevented viral induced CD4 T-cell depletion. The addition of the siRNA to the aptamer added to the antiviral effect by triggering target specific knockdown of the HIV-1 encoded tat/rev transcripts, thus validating the dual inhibitory function of the chimeras. Moreover, the presence of siRNAs and target specific knockdown of HIV-1 tat/rev transcripts were detected in gp120 aptamer-siRNA chimera treated mice, but not in the aptamer alone-, mutated aptamer-siRNA chimeras- or naked siRNA-treated animal groups, thus validating systemic, HIV-1 infected cell specific aptamer mediated siRNA delivery. Using RACE PCR we verified the in vivo RNAi mediated cleavage of the HIV-1 tat/rev target transcripts. Collectively, this approach resulted in suppression of viral loads in vivo and most importantly resulted in protection of T-cell depletion, making the aptamer-siRNA chimeras attractive therapeutic candidates for the treatment of HIV-1 infection.

Population Level Variations in the Baseline Value for Vδ2 T Cells and Implications for the Study of Infection Diseases

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The major subset of human peripheral blood γδ T cells expresses the Vγ2Vδ2 T cell receptor and responds to infectious diseases and malignancies. We observed a significant difference in the frequency of Vδ2 cells in blood from healthy Caucasian (CA) and African American (AA) age matched donors, with CA samples having 3.71%±4.37% Vδ2 cells (as a percentage of total lymphocytes) compared to 1.18%±2.14% Vδ2 cells for AA donors (p<0.0001). Levels of Granzyme B and CD56 expression on Vδ2 cells were lower for AA donors, and Vδ2 cells from AA donors displayed a less skewed Vγ2 chain repertoire (p<0.05). However, proliferative responses to phosphoantigens for AA donors and CA donors were similar. Thus, Vγ2Vδ2 cells in both groups were selected for phosphoantigen recognition, suggesting that differences in homeostatic regulation may account for an expanded population in CA individuals.

Differences in Vδ2 cell baseline frequencies across populations should be taken into account when studying diseases affecting the Vδ2 subset. A group of African-American HIV-infected patients who suppress HIV replication without antiretroviral therapy (natural viral suppressors, NVS) had a Vδ2 T cell frequency significantly lower than an age matched CA control group (1.28%±1.21% versus 3.71%±4.37%), but showed no decrease in the proportion of circulating Vδ2 T cells when compared with a control group appropriately matched for age and ethnicity (1.28%±1.21 versus 1.18%±2.14%). In contrast, a group of AA HIV+ viremic patients had significantly lower values of circulating Vδ2 T cells than the same age and ethnicity matched control group (0.34%±0.37% versus 1.18%±2.14%). Using appropriately matched controls revealed a significant association between natural viral suppression and normal levels of Vδ2 lymphocytes.
Reconstitution of Conformational B-Cell Epitopes Using Combinatorial Conformer Libraries

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Background: A major challenge is the induction of neutralizing antibodies (nAbs) in the vaccinee. We propose to meet this challenge by developing recombinant vaccines based on the corresponding epitopes recognized by known and well defined nAbs. These “neutralizing epitopes” are concealed within the pathogen’s envelope proteins and by means of negative selection have evolved to be inaccessible or highly conformational, and therefore of suppressed immunogenicity in the context of intact pathogen.

Hypothesis: Reconstituted neutralizing epitopes used as isolated immunogens are able to elicit nAbs. To test this hypothesis we have to overcome two major obstacles: (i) mapping the neutralizing epitope at the atomic level, and (ii) reconstitution of the epitope as an independent subunit-immunogen.

Model system and results: 80R is an extremely potent nAb directed against the receptor binding domain (RBD) of the SARS virus spike protein. The 80R neutralizing epitope was initially mapped using novel bioinformatic tools, and subsequently confirmed by co-crystallization. The epitope is comprised of 32 contacts that are located on a large and extended surface of approximately 1000 angstrom2, encompassing two beta strands, one alpha-helix and two random structures. The isolated 80R epitope is predicted to be efficient as an immunogen able to elicit an 80R-like humoral response and serves as a model for epitope-based vaccine construction against other viruses such as HIV. In order to reconstitute the epitope into a functional and short recombinant peptide, a novel approach has been developed: “conformer libraries”. Such libraries use the bona fide peptide-segments of the RBD interconnected by a variety of linkers that stabilize the epitope segments in a vast collection of different conformations. Four different epitope-libraries were built using the phage display technique and screened against 80R that was used as a monitor for the desired native conformation. A number of epitope-conformers were isolated that bind 80R well. These epitope-conformers are vaccine candidates currently being evaluated as immunogens.

HTLV-2 Tax-2 Transactivator Increases the Expression and the Function of its Inhibitor CIITA, the Master Regulator of HLA-II Gene Transcription

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We previously showed that CIITA, the HLA Class-II transactivator, inhibits the transcriptional function of Tax-2 and, consequently, the replication of HTLV-2 virus in human susceptible B and T cells.

Here, we show for the first time that CIITA and Tax-2 interact in vivo and, that this interaction involves two independent regions of CIITA molecule. The physical interaction between the two transactivators may be at the basis of the observed functional effect on HTLV-2 viral replication. On the other side, Tax-2 might also influence the functional activity of CIITA. Thus we asked whether Tax-2 could affect the CIITA-dependent HLA-II expression. Expressing vectors of CIITA and Tax-2 were co-transfected in 293T cells and the cell surface expression of HLA molecules was evaluated by cytofluorometry. We observed that Tax-2 alone does not affect HLA-I or HLA-II-DR expression, whereas it increases CIITA-mediated expression of HLA-II-DR. Interestingly, in the presence of Tax-2, the expression of exogenous CIITA is significantly increased in a dose-dependent manner. Tax-2 affects also CIITA ability of interacting with NF-YB, component of the HLA-II enhanceosome, important for the recruitment of CIITA on HLA-II promoters. We ruled out a possible transcriptional effect of Tax-2 on CMV promoter driving the expression of CIITA, because Tax-2 does not increase the accumulation of CIITA internal fragment and other proteins transcribed from the same CMV promoter.

Preliminary data suggest that the increasing accumulation of CIITA molecule is the result of a slight extension of CIITA protein half-life induced by Tax-2.

Together with our previous observation that CIITA inhibits Tax-2 function, these findings might indicate that HTLV-2 virus exploits CIITA to negatively control its genes transcription and to decrease the production of new virions. This could be a new mechanism for the virus to remain latent in infected cells.
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Building on the Results of the RV 144 Prime Boost HIV Vaccine Efficacy Study
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An effective HIV vaccine is a global health priority. The Merck Ad5 phase IIb T-cell vaccine failed to show efficacy and might have increased the risk of HIV acquisition in MSM. While VaxGen gp120 alone was not efficacious in groups at high risk for HIV-1 infection, the RV144 ALVAC-HIV prime and AIDSVAX gp120 B/E boost regimen showed 31% efficacy in low incidence heterosexuals in Thailand. All trials demonstrated the limitations of available laboratory and animal models to both assess relevant vaccine-induced immune responses and to predict clinical trial outcome. We have begun an intensive, broad based attempt to discover a correlate of protection from RV 144 involving over 30 investigators at 20 institutions examining the innate and adaptive responses induced in RV 144, viral sieve and host genetic studies, and non-human primate studies. Results of some of these studies will be described. A roadmap for future HIV vaccine trials will be described designed to better define RV 144 correlates of protection, improve the durability and level of protection observed, and assess protective vaccine efficacy in diverse risk groups. New strategies examining heterologous vector prime boost, universal inserts, replicating vectors, and novel protein/adjuvant immunogens should be explored to induce both T-cell and antibody responses. These new approaches will be described within the context of the roadmap for future HIV vaccine efficacy studies. HIV vaccine development requires innovative ideas and a sustained long-term commitment of scientists, governments, and the community.

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A Novel Nanoparticle Approach to Induce Colorectal and Vaginal Mucosal Immunity
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Many infections, including HIV, are transmitted through mucosal surfaces. Where T cell immunity is needed to protect, we have previously found that local mucosal immunity is most effective. Moreover, intrarectal immunization was the most effective route of several we compared to induce such immunity in the GI mucosa. However, the intrarectal route may not be the most acceptable for human vaccination. We hypothesized that we might mimic intrarectal immunization if we could deliver the vaccine to the colorectal mucosa, and might be able to do so by a more practical oral route of delivery if we could bypass destruction in the stomach and induction of oral tolerance in the small intestine. We have now accomplished this in mice by using a novel construct of pH-dependent microparticles encapsulating nanoparticles that contain the vaccine. The particles are designed to avoid uptake of the microparticles or release of the nanoparticles until they reach the large intestine. This oral vaccine induced a level of T cell immunity in the large intestinal mucosa that rivaled that induced by direct intrarectal delivery, and resulted in comparable protection against intrarectal or intravaginal challenge with a recombinant virus. In contrast, vaccine particles targeted to the small intestine resulted in greater T cell immunity in the small intestine but not in the large intestine, and failed to protect against intrarectal or intravaginal viral challenge. Thus, with these directed particles, we have been able to demonstrate functional compartmentalization of the gut mucosal immune system for T cell immunity, and to develop an orally delivered vaccine capable of providing protection against rectal or vaginal mucosal challenge.
**Induction of Efficacious Immune Responses Using Heterologous Prime: Boost Regimens of Recombinant DNA and MVA Vectored HIV Vaccines and GM-CSF as the Adjuvant**


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The GeoVax DNA and MVA-vectored HIV vaccines are single recombinant moiety designed to express non-infectious, immature virus-like-particles bearing “native” Env protein. The DNA vaccines encode Gag, Pol and Env using HIV subgenomic splicing, with or without GM-CSF in the nef region, while the MVA vectored vaccines encode Gag, Pol and Env under the control of the mH5 early/late vaccinia promoter. This vaccine design was tested using the rhesus macaque model and SIV-239 prototype vaccines encoding the analogous SIV structural proteins, with and without GM-CSF. A heterologous (DNA + MVA) vaccination regimen was employed with standard immune response measurements and efficacy testing using weekly intra-rectal heterologous infectious challenges with SIV-E660 (MID40). Comparable cellular immune responses were induced. However, the GM-CSF-supplemented vaccine induced antibodies specific to SIV gp160 with higher binding avidity to the “native” protein and higher neutralization titers specific for a tier 1 variant of E660 (E660.11). The GM-CSF supplemented vaccine also induced a higher level of efficacy with 70% (5/7) of the animals protected against 12 challenges, compared to a 25% (2/8) level of efficacy obtained using the unadjuvanted vaccine. The use of the MVA vectored SIV vaccine alone, without GM-CSF, induced higher titers of antibodies specific to SIV gp160 and reduced cellular responses. The level of protection was comparable, if not better, to that obtained using the unadjuvanted DNA + MVA products.

In a completed Phase 1 (HVTN-065) clinical trial, similar immune response patterns were observed with the administration of the MVA vectored vaccine alone resulting in higher titers of antibodies while the use of the heterologous prime-boost regimen DNA + MVA induced higher CD4+ T cell responses and lower antibody titers. The unadjuvanted HIV vaccines are currently being tested in a Phase 2 trial (HVTN-205) while the GM-CSF supplemented vaccine represents the next generation product in the GeoVax pipeline.

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**Characteristics of the Earliest Cross-Neutralizing Antibody Response to HIV-1**

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Recent cross-sectional analyses of HIV-1+ plasmas indicated that broadly cross-reactive neutralizing antibody responses are developed by 10% - 30% of anti-retroviral naïve HIV-1+ subjects. The timing of the development of such anti-viral responses is unknown. It is also unknown whether the emergence of these responses coincides with the appearance of antibody specificities to a single or multiple regions of the viral envelope glycoprotein (Env). Here we report that anti-HIV-1 cross-neutralizing antibody responses first become evident, on average, at 2.5 years, and as early as 1 year, following infection. Broad cross-neutralizing antibody responses do not appear to develop later in infection. Although plasma neutralizing antibody responses of narrow breadth, target epitopes on the monomeric gp120 Env subunit that are located outside the conserved CD4-binding site (CD4-BS), the earliest cross-neutralizing antibody responses primarily target epitopes that are located within the CD4-BS or epitopes that are not present on monomeric gp120 but present on virion-associated trimeric form of Env. The relative contribution of these two epitope specificities to the plasma’s overall cross-neutralizing activities is patient depended. Once cross-neutralizing antibody responses emerge, they do not wane for extended periods of time and their epitope-specificities remain unchanged.
Steps Towards Epitope-Based Vaccines

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Successful AIDS vaccines will ultimately need to stimulate both cellular and antibody responses against neutralizing epitopes of HIV. The evidence that such effective epitopes exist stems from the fact that potent broadly cross-neutralizing monoclonal antibodies have been isolated in a number of labs and have proven able to protect monkeys from SHIV challenge in model systems. Hence, a paradigm for rational vaccine design could consist of a number of well defined steps: (i) isolation and characterization of broadly cross-neutralizing antibodies, (ii) physical mapping of their corresponding epitopes, (iii) reconstitution of these epitopes, (iv) development of immunogens containing the reconstituted epitopes. Steps one and two have been accomplished to various degrees. Although only a few leading monoclonals have been isolated, they amply prove that neutralizing B-cell epitopes exist in both HIV gp120 and gp41. These have been mapped via a number of methodologies; the most defining is antibody:antigen co-crystallization. Little progress, however, has been made in meeting the challenge of “Step three”, namely reconstitution of functional B-cell epitopes. This is mainly due to the highly complex, discontinuous, conformational nature of such epitopes. How does one extract the critical components of a proven epitope and display them in their proper spatial orientation? Here we describe a practical approach towards epitope reconstitution. For this, bona fide segments of a given epitope are expressed in combinatorial phage display libraries. The segments are connected via a vast collection of random linkers thus creating a library of epitope conformers. The library is then screened against its cognate antibody in order to isolate conformers whose structures are recognizable. Once this is accomplished, the phage displayed reconstituted epitopes can be used to develop immunogens which will be tested for their ability to stimulate the production of neutralizing antibodies in an animal model.

HIV Transmission: The Role of Neutralizing Antibodies

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At present, it is unknown whether the presence of HIV-specific neutralizing antibodies (NAbS) provides any protection from infection in individuals naturally exposed to HIV. Although here is evidence that select NAbS can protect in the SHIV/macaque model, these studies were limited to analysis of a few select neutralization sensitive challenge strains that are not representative of circulating HIV. We have examined whether NAbS, when present at the levels found in natural HIV-infection, can reduce the risk of HIV acquisition using two cohorts: 1) HIV positive high-risk women who continue to be exposed to new source partners and 2) breastfeeding infants of HIV positive mothers, who may have HIV NAbS through passive transfer. Both of these settings provide a situation in which the individual has pre-existing HIV-specific antibodies at the time of exposure to HIV. Among high-risk women, those who became superinfected by a second partner did not show evidence of notably weak or narrow NAb responses at the time of superinfection compared to women who did not become superinfected. In infants, the presence of broad potent neutralizing antibodies did not impact of their risk of HIV acquisition after birth. Together, these studies suggest that neutralizing antibodies that are elicited by a natural HIV infection are not of sufficient breadth or potency to protect against diverse circulating strains of HIV-1.
Protection Against Heterologous SHIV Challenge Afforded by Immunization of Rhesus Macaques With a Subunit Immunogen that Mimics a Transition State HIV Envelope Structure

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The coreceptor binding site of HIV-1 gp120 comprises some of the most conserved and functionally important residues on the viral envelope. Therefore, antibody responses to these epitopes should be highly cross-reactive and potentially useful for HIV vaccine development. To address this possibility, we have vaccinated rhesus macaques with various formulations of a subunit immunogen designed to raise humoral responses against conserved gp120 epitopes (rhesus full-length single-chain; rhFLSC). In heterologous high dose rectal challenge models, rhesus macaques were immunized with rhFLSC formulated in QS-21 or GPl-0100 adjuvants. Immunized animals challenged with heterologous SHIV162P3 exhibited vaccine dose-dependent nonsterilizing immunity; defined as accelerated clearance of plasma viremia and decreased tissue viral load compared with unvaccinated control animals. Such control correlated with stronger responses to a variety of conserved epitopes (including CD4-induced or CD4i) in rhFLSC-vaccinated animals. The control of infection was not associated with anti-CD4 responses or neutralizing activity measured in conventional assays. In a different study, macaques were immunized with rhFLSC formulated in either Iscomatrix or RC-529 adjuvants and subjected to repeat dose challenges with SHIV162P3. Evidence of temporary sterilizing protection was seen in the RC-529/rhFLSC formulation, which was distinguished by high titer antibody responses to conserved CD4i epitopes in the absence of cellular responses. Protection was not associated with conventional neutralizing activity. Reminiscent of the RV144 clinical trial in humans, macaques that became infected upon challenge did not control viremia. Collectively, these data indicate that a single subunit vaccine has the capacity to prevent or control heterologous mucosal infection as a function of humoral immunity.

Strong and Broad Immunogenicity of a Multigene, Multiclade HIV-1 DNA Prime MVA Boost Vaccine Regimen Among Healthy Tanzanian Volunteers

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A phase I trial (HIVIS01/02) of a multigene, multiclade HIV-1 DNA prime heterologous MVA boost vaccine regimen among healthy volunteers in Sweden showed that the vaccines were safe and highly immunogenic (J Inf Dis 2008;198:1482-90). A phase I/II trial (HIVIS03) using the same vaccine constructs has subsequently been conducted in Dar es Salaam, Tanzania. Sixty HIV-uninfected volunteers randomised to three groups of 20 received HIV-DNA vaccine at 1 mg intradermally (i.d) or 3.8 mg intramuscularly (i.m.) or placebo using a needle-free device. The DNA plasmids containing HIV-1 env, gag, pol of CRF01A_E or placebo at months 9 and 21. Two to four weeks after the second HIV-MVA boost, 28 (97%) of 29 vaccinees had positive IFN-gamma ELISpot responses, 27 (93%) to Gag and 23 (79%) to Env peptides. The i.d. primed vaccinees showed higher immune responses to Env compared to the i.m. primed vaccinees. Intracellular cytokine staining for Gag-specific IFN-gamma/IL-2 production 4 weeks after the second HIV-MVA boost showed both CD8 and CD4 T-cell responses. All of 25 vaccinees had lymphoproliferative responses of a similar high magnitude to AT-2-treated HIV antigens from 3 different subtypes (donated by J Lifson, NCI, USA). After the second HIV-MVA boost, 26/29 (90 %) of the vaccinees developed binding antibodies to gp160. In conclusion, this HIV-DNA prime MVA boost vaccine regimen induced strong and broad immune responses in Tanzanian volunteers.
Rational Design of Envelope Surface Identifies Broadly Neutralizing Human Monoclonal Antibodies to HIV-1

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Advances in our basic scientific understanding of the immune system and mechanisms of HIV pathogenesis have provided the tools to rationally design an effective vaccine for AIDS. Cross-reactive neutralizing antibodies (NAb) are found in the sera of many HIV-1 infected subjects, but the virologic basis of their neutralization remains poorly understood. We have used knowledge of HIV-1 Envelope (Env) structure to developed antigenically resurfaced glycoproteins specific for the structurally conserved site of CD4 receptor binding. These probes identified sera with such NAb's from infected donors and enabled the isolation of B cells that recognized the CD4-binding site (CD4bs). By expressing immunoglobulin genes from individual B cells, we identified three monoclonal antibodies, including a pair of somatic variants that neutralized over 90% of circulating HIV-1 isolates. Exceptionally broad HIV-1 neutralization can be achieved with individual antibodies targeted to the functionally conserved CD4bs of gp120, an insight critical to the development of an AIDS vaccine.

In other studies, we have continued to study basic mechanisms of pathogenesis of HIV and have recently made progress in defining the molecular basis for CD4 cell killing/depletion by HIV. The status of the rational immunogen design and novel therapeutic interventions that prevent HIV infection will be discussed.

Moving Forward from RV144: An Industrial Perspective

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Moving forward from RV144, the sanofi pasteur strategy for HIV vaccine development is to ‘substantiate and extend’ upon the viral vector prime/protein subunit boost regimen. With this, we will continue to engage in public-private partnerships to drive the scientific agenda and build upon the very important milestone from the Thai trial results. As an introduction to the vaccine session, an update will be provided on this strategy and development plans, as well as further industrial perspectives on HIV vaccine development, in general. Key data from RV 144 with relevant post-hoc analysis will be presented as a catalyst to inform the discussion around this session.
Novel Vectors and Antigens for a Next Generation HIV-1 Vaccine
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Alternative serotype Ad vectors such as rAd26 and rAd35 are biologically substantially different than rAd5 vectors. We have evaluated rAd26 and rAd35 vectors expressing SIV antigens in immunogenicity and challenge studies in rhesus monkeys, and we have recently advanced a prototype rAd26 vector expressing HIV-1 Env into a phase 1 clinical trial. Importantly, this vector has proven safe and immunogenic in humans at doses of 10^9 vp, 10^10 vp, and 10^11 vp. We have also assessed the capacity of vector-specific CD4+ T lymphocytes to traffic to mucosal surfaces following rAd vaccination in rhesus monkeys, and we have observed that trafficking of vector-specific CD4+ T lymphocytes to colorectal mucosa does not occur more readily in monkeys with baseline vector immunity as compared with monkeys without baseline vector immunity. In addition, we have demonstrated that computationally optimized “mosaic” HIV-1 Gag/Pol/Env antigens substantially expand cellular immune breadth and depth as compared with consensus or natural sequence antigens in rhesus monkeys. Taken together, these data suggest that a rAd35/rAd26 prime-boost vector regimen expressing mosaic HIV-1 antigens should be evaluated in clinical studies.

Retooling the Macaque Model as a Necessary Step to Evaluate the Relative Efficacy of Vaccines for HIV
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Vaccination with ALVAC-HIV and gp120 protein demonstrated some degree of protection against HIV acquisition (31.2%), in the RV144 Trial. We have investigated how the dose of challenge exposure to SIVmac251 affects the evaluation of relative vaccine efficacy in macaques. We have chosen vaccine regimens that closely mimic the RV144 trial, since this is the only vaccine regimen that, so far, has given some degree of protection in humans. Vaccine regimens that included DNA/ALVAC-SIV/gp120 or ALVAC-SIV/gp120 or gp120 alone have been re-evaluated using a single high dose or repeated lower doses of SIV mac 251. These vaccines induced CD4+, CD8+ T-cell responses, and antibodies levels as observed in humans. Minimal protection was observed in macaques exposed to a single high virus dose. In contrast, exposure to the lower virus doses resulted in protection from acquisition in three of the twelve vaccinated macaques. None of the naive controls were protected. The nine vaccinated animals that became infected had significantly lower virus in plasma and at the mucosal site than control macaques and, strikingly, they were also protected from the systemic CD4+ T-cells loss and experienced a significantly better reconstitution of CD4+ T-cells at mucosal sites. Analysis of immune correlates was doable only in animals that became infected because of the small number of those that were protected from acquisition (3 of 12). Nevertheless, the immune responses that correlated inversely with short and/or long term control of virus level were mainly CD4+ T-cell responses (ICC and Lymph proliferative responses for envelope) and ADCC. Our results suggest that a properly calibrated macaque model may become a useful tool to understand immune correlates of protection and promote a rapid progress in the development of more effective vaccines for HIV.
Role of Poxvirus Vectors in the Development of an HIV Vaccine
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Virus vectors together with DNA plasmid vectors have been instrumental for the development of the so-called HIV T-cell vaccines. The development of T-cell vaccines has been based predominantly on two virus vector platforms: a) adenovirus vectors and b) poxvirus vectors. Adenovirus and poxvirus vectors used alone and particularly in combination with plasmid DNA-based vaccines have shown to induce vigorous T-cell responses. Appropriate HIV-specific cellular immunity certainly plays a major direct role in the development of protective immunity and adequate CD4 T-cell help is critical for the development of optimal B-cell/antibody response. The overall concept of T-cell vaccines has been harshly questioned following the failure of a phase IIb study investigating an adenovirus serotype 5 (Ad5)-based HIV vaccine candidate expressing gag-pol-nef HIV-1 proteins from clade B (known as Step trial). The promising protective effect observed in the RV-144 phase III efficacy clinical trial in Thailand which has tested a poxvirus/gp120 combination has indicated the importance of developing immunization strategies capable of stimulating both humoral and cellular arms of the immune response. Although the protective effect was modest, the RV-144 trial has shown that a poxvirus plus protein HIV vaccine combination is able to prevent HIV infection. These results also indicate that both components of the vaccine, i.e. the priming component (ALVAC) and the boosting component (the gp120 protein) need to be improved. With regard to the improvement of the cellular component of the vaccine-induced immune response, a number of novel poxvirus vectors have been developed which have an immunogenicity profile substantially improved compared to ALVAC. These novel vectors in combination with a gp120 protein component are ready to move into large clinical trials. The limited sets of immunological results of the RV-144 trial do not allow to rule out or to favor any specific cellular or humoral mechanism of protection. Additional mechanisms of protection, i.e. innate immunity mechanisms, beyond the conventional CD4 and cytotoxic CD8 T-cell and neutralizing antibody responses may have played an important role. Hopefully, the improved vaccine combinations should aim at eliciting an integrated immune response, i.e. innate, humoral and cellular immunity. Furthermore, highly attenuated replication competent poxvirus vectors with promising immunogenicity and safety profiles are in advanced stage of development. Therefore, there is a large and interesting portfolio of novel poxvirus vectors that will be critical in sustaining the iterative process needed for the development of an effective HIV vaccine.

TLR8 Triggering Induces HIV from Latently Infected Cells of Myeloid Monocytic Origin and Indirectly from Latently Infected CD4+ T Cells Via the MAPK Pathway and TNF-α Respectively
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We have previously shown that triggering toll-like receptor (TLR) 7/8 is able to activate HIV from cells of myeloid-monocytic origin. In this work, we identified that TLR8 triggering activated latently infected cells mainly via the MAPK pathway, in particular Erk1/2 and p38α. NFκB, its activation was partially controlled by p38α, played a minor role. TNF-α, which was secreted subsequently to TLR8 triggering, contributed to the activation of those cells in an autocrine manner, pointing to a bimodal mechanism how TLR8 triggering sustains its effect for a longer time period. Notably, we found that TNF-α secreted by myeloid dendritic cells also acts in a paracrine mode in the activation of neighboring latently infected CD4+ T cells, thus reinforcing the purging effects even on cells devoid of TLR8 expression. Thus, triggering TLR8 represent a very promising strategy for attacking the silent HIV reservoir by acting on various cell types.

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Modeling HIV-1 Latency in vitro
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The human immunodeficiency virus type-1 (HIV-1) establishes latency primarily by infecting activated CD4+ T cells that later return to quiescence as memory cells. Latency allows HIV-1 to evade immune responses and to persist during anti-retroviral therapy. The lack of a valid cellular model to study HIV-1 latency has hindered advances in the understanding of its biology. We developed an in vitro model suitable to investigate the induction, maintenance and reactivation of HIV-1 latency. Our model recapitulates the events of primary and secondary antigen-driven immune responses in which CD4+ T cells are activated with dendritic cells and antigen, infected in vitro with HIV-1, and then brought back to quiescence through a resting phase in the presence of interleukin-7. During the resting phase, the latently infected cells generated in vitro with our system lack expression of activation markers; do not undergo cellular proliferation and do not sustain viral replication. All these activities resume promptly following secondary antigen stimulation. This system is suitable to study the biology of HIV-1 latency. Indeed, we have performed microarray analyses with RNA isolated from FACs-sorted, quiescent, latently infected vs. uninfected cells. The results suggest that HIV-1 achieves latency in CD4+ T cells not as a consequence of the host cell’s ability to survive clonal contraction and to establish immunological memory. Rather, HIV-1 appears to “re-program” the host cell’s gene expression profile in a way that promotes cell quiescence, supports cell survival and thus induces viral latency. In addition, a panel of genes encoding for cell surface molecules is differentially expressed in latently infected vs. uninfected cells, which may have diagnostic and therapeutic implications. The results of these analyses point to important new concepts regarding the establishment and maintenance of latency in CD4+ T cells, and thus suggest new mechanisms of viral persistence in HIV-1 patients.

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Anti-HIV-1 RANTES Derivatives Acting as CCR5 Antagonists Present Full Additivity or Synergy in Combination With Different Entry/Fusion Inhibitors
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Inhibition of HIV-1 entry by CCR5 targeting is a strategy that requires antagonism towards CCR5 to prevent pro-inflammatory conditions. RANTES-based HIV-1 blockers and maraviroc represent the state of the art in this field, and could be envisaged both as systemic drugs and as topical microbicides. In both options, the combination of different drugs represents an advantage in terms of higher efficacy, lower administration dosage and decreased risk of resistant virus strains’ emergence. Two anti-HIV-1 CCR5 antagonists have been developed in our laboratory: i) Rmax, the most potent peptide derived from the N-loop/β1-strand region of RANTES; and ii) C1C5 RANTES, a full-length RANTES mutant in which serine residues in positions 1 and 5 were replaced by cysteine. Fully additive or synergistic HIV-1 inhibition is obtained in vitro when RANTES derivatives are tested in combination with maraviroc, cyanovirin-N or T20. Rmax was chemically synthesized while C1C5 RANTES was secreted by engineered lactobacilli, and both have been used after purification. Cell-cell fusion and p24-based assays were performed to determine anti-HIV-1 activity of individual or combined compounds. Results were analyzed using the CalcuSyn software to determine inhibitory concentrations (IC) and combination indexes (CI). Rmax and C1C5 RANTES exert their activity against different laboratory and primary HIV-1 R5 strains of clade B and C. Rmax inhibits HIV-1BaL infection with an IC50 similar to that of T20. When tested in combination, full additivity was observed with maraviroc and cyanovirin-N and synergy with a CI50<0.8 was obtained with T20. C1C5 RANTES combination with maraviroc or cyanovirin-N resulted also in a significant synergistic effect on the inhibition of HIV-1BaL infection, with a CI50 of about ~0.8 for both combinations. No cellular toxicity was observed under all conditions. Our data suggest that RANTES derivatives may be included in systemic or microbicidal anti-HIV-1 cocktails.
HIV-1 Infection and Replication in Cervico-Vaginal Histocultures
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The mucosal immune system of the lower female genital tract plays a key role in HIV-1 transmission and pathogenesis. While in other tissues HIV-1 infection of CD4 T cells is known to depend on cellular activation, differentiation and expression of HIV-1 co-receptors for cervico-vaginal tissue (CVT) these key characteristics remain unknown. To address this problem we developed an ex vivo system of cervical explants that efficiently support HIV replication. We investigated by FACS analysis the differentiation and activation state of CD4 T cells extracted from CVT blocks obtained from HIV-1 negative (HIV-1neg) volunteers. CD4 T cells isolated from CVT showed an effector memory phenotype with a peculiar pattern of expression of activation markers. CVT blocks were infected with either R5-HIV-1BaL or X4-HIV-1LAI strains; productive infection of HIV-1 was documented by ELISA for p24Gag, and by RNA quantification by real-time PCR. Also, we identified p24gag expressing cells by FACS analysis. CVT inoculated with R5-HIV-1BaL efficiently supported virus replication, whereas X4-HIV-1LAI replicated only in the few tissues enriched in CD27+CD28+CD4 TEM cells. Ex novo HIV replication rather than virus absorption was demonstrated to occur and was abolished by either inhibitors of HIV reverse transcriptase (3TC) or of viral entry (PSC-RANTES). For R5-HIV-1BaL, p24Gagpos cells were mostly activated (CD38+) CD4 T cells although a similar state of activation of p24Gagneg (bystander) CD4 T cells was also observed. We can conclude that CD4 T cells present in CVT of HIV-1neg individuals are in a predominantly activated state and express CCR5. These features are likely responsible for the susceptibility of CVT to support productive infection of R5-HIV-1. In contrast, X4-HIV-1 replication was negligible except in a few tissues implying that co-expression of CD4 and CXCR4 in lymphocytes of CVT is not sufficient to support productive infection of X4 HIV-1.
157 Fusion Complexes and CD4-Independent Env for the Induction of Broad Spectrum Neutralizing Antibodies Against HIV-1

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Introduction: Broad spectrum neutralizing antibodies against HIV-1 are essential for the development of a humoral anti-AIDS vaccine. We used fusion complexes and CD4-independent gp120 as new immunogens to induce neutralizing antibodies blocking the infectivity of different primary isolates of HIV-1.

Methods: Spleen cells from mice immunized with fusion complexes were used to prepare murine hybridomas. Secreted antibodies were screened for their neutralizing activity using the pseudovirus standard neutralization assay. In parallel, the immunogenicity of CD4-independent Env, on which conserved epitopes might be exposed, has been tested.

Results: Among antibodies secreted by hybridoma clones, 8% showed a neutralizing activity higher than 40% (1 µg/ml), and the best ones showed neutralization levels as high as 80% against the pseudovirus B panel, reaching neutralization levels similar or higher than the Tri-mAb control. 10 hybridoma clones showing 80% or higher neutralization levels were selected and re-cloned by limiting dilutions. Panel C evaluation is ongoing. Preliminary results using a 1:1000 sera dilution from mice immunized with CD4-independent Env showed a neutralizing activity of 40-60% and, as expected, a 2-3 folds neutralization increase in the presence of sCD4.

Conclusions: Monoclonal antibodies obtained by immunizing with fusion complexes showed a broad spectrum neutralizing activity against all panel B pseudoviruses, as well as against a group of selected laboratory isolates. Sera from mice immunized with CD4-independent Env showed neutralizing activity against heterologous EnvVs that increases in the presence of sCD4, suggesting the elicitation of antibodies against the conserved coreceptor binding site. In conclusion, fusion complexes and CD4-independent Env represent potential new immunogens that can induce neutralizing antibodies with activity against a wide panel of HIV-1 isolates.

157a HLA-C Associates with Env and Increases HIV-1 Infectivity

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Introduction: Host cell proteins are specifically incorporated into HIV-1 envelope during budding. Virionic HLA-C reduces HIV-1 susceptibility to neutralizing antibodies (Cosma 1999). A polymorphism in the 5’ region of the HLA-C gene has been associated with individual variations in set point viral loads (Fellay 2007), suggesting a role of HLA-C expression levels in modulating HIV-1 infectivity. We have reported (Matucci 2008; Baroni 2010) that HLA-C in the HIV-1 envelope associates to Env increasing viral infectivity of both R5 and X4 tropic viruses. The purpose of this study is to elucidate this interaction and exploit it for generating new immunogens capable of conferring protective immunity.

Methods: Using recombinant HLA-C and Env molecules fused to fluorescent tags, we are studying their association using the bimolecular fluorescence complementation (BiFC) technique. BiFC allows the analysis of the interaction between associated proteins in living cells and to study their co-localization into cellular compartments. Env-deletion mutants and Env swapped domain recombinants are being tested to identify protein domains involved in their association. HLA-C coded by different alleles will be analyzed for their association with Env to study the influence of HLA-C polymorphisms in increasing HIV-1 infectivity.

Results: Preliminary results on Env-HLA-C association and sub-cellular compartmentalization, using a specific marker for ER, reveal a direct proximity between these proteins, suggesting an early association at the ER level. Similarly, analysis of co-localization between Golgi apparatus and Env-HLA-C reveal the presence of the complementation signal between the two proteins in Golgi vesicles.

Conclusions: BiFC assays allows an efficient visualization of HLA-C and HIV-1 Env association in living cells. Preliminary results obtained suggest an association between the two proteins at the ER and Golgi level. Understanding the interaction between HLA-C and HIV-1 Env might give valuable information for the design of new immunogens and/or compounds that, by reducing viral infectivity, may help controlling HIV-1 infection.
HIV-1 Matrix Protein p17: A Candidate Antigen for Therapeutic Vaccines Against AIDS
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The success in the development of anti-retroviral therapies (HAART) that contain Human Immunodeficiency Virus type 1 (HIV-1) infection are challenged by the cost of this lifelong therapy and by its toxicity. Immune-based therapeutic strategies that boost the immune response against HIV-1 proteins or protein subunits have been recently proposed to control virus replication in order to provide protection from disease development, reduce virus transmission, and help limit the use of anti-retroviral treatments. HIV-1 matrix protein p17 is a structural protein that is critically involved in most stages of the life cycle of the retrovirus. Besides its well established role in the virus life cycle, increasing evidence suggests that p17 may also be active extracellularly in deregulating biological activities of many different immune cells that are directly or indirectly involved in AIDS pathogenesis. Thus, p17 might represent a promising target for developing a therapeutic vaccine as a contribution to combating AIDS. Here we discuss the biological characteristics of HIV-1 matrix protein p17 and describe why a synthetic peptide representative of the p17 functional epitope may work as a vaccine molecule capable of inducing anti-p17 neutralizing response against p17 derived from divergent HIV-1 strains.

Immune Signatures and Systems Biology of Vaccines
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Vaccines represent a strategic successful tool to prevent or contain diseases with high morbidity as well as mortality. However, despite the extensive and wide use, we still have a limited knowledge on mechanisms underlying the effective elicitation of protective immune responses by vaccines, which represents the final outcome of a effective cooperation between the innate and adaptive arms of the immunity. Immunity is made of a multifaceted set of integrated responses involving a dynamic interaction of thousands of molecules, whose list is constantly updated to fill the several empty spaces of this puzzle. The recent development of new technologies and computational tools allows to perform a comprehensive and quantitative analysis of the interactions between all of the components of immunity over time.

The global transcriptional profile of PBMCs stimulated with HIV candidate vaccine (Virus-Like Particles, VLPs) has been evaluated in HIV-infected patients with low/high viral load compared to healthy volunteers. Baseline activation of chemokine production was observed in PBMC from HIV infected patients and innate immune stimulation with HIV-VLPs was not blunted. The immune profile among HIV-infected patients was found to be qualitatively similar but quantitatively extremely variable. This diversity was independent of viral load and it might be dependent on individual immunogenetic traits or concurrent immunological status.

This ex-vivo screening strategy represent an efficient tool for guiding modifications/optimizations of vaccination strategies and understanding failures in individuals enrolled in clinical trials.

The potential of systems biology in general in providing relevant and novel insights in the mechanisms of action of vaccines in order to improve their design and effectiveness, will be discussed.
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Recombinant Vector-Based Vaccines for Cancer Therapy  
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We have developed a recombinant viral vector-based vaccine platform consisting of a recombinant vaccinia (rV-) prime followed by multiple booster vaccinations with a recombinant fowlpox (rF-). Each vector contains the transgenes for one or more tumor-associated antigens, and transgenes for 3 human T-cell costimulatory molecules (designated TRICOM). A prostate cancer vaccine (PSA-TRICOM) has been designated PROSTVAC. Patients (n=125) with metastatic castrate-resistant prostate cancer were accrued in a 43-center, randomized placebo-controlled Phase II study. At 3 years post-study, PROSTVAC patients had a better overall survival with 30% alive vs. 17% of controls, and longer median survival by 8.5 months (25.1 vs. 16.6 months for controls; p=0.006).

A concurrent single-arm Phase II study with the same vaccine and similar patient population has also recently been completed with similar results. Regulatory T-cell suppressive function was shown to decrease following vaccine in patients surviving longer than predicted and increase in patients surviving less than predicted.

We envision these TRICOM vaccines as part of an immune-oncology platform. Several hypothesis-generating Phase II clinical studies in patients with breast and prostate cancer employing TRICOM vaccines in combination with radiation, hormone therapy or chemotherapy have recently been completed or are in progress with promising results. Several of these trials have now provided evidence for a paradigm shift in the design and evaluation of vaccine clinical trials either as monotherapy or in combination therapy.

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Dendritic Cell Vaccination in Acute Myeloid Leukemia  
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Active immunization using tumor antigen-loaded dendritic cells (DC) holds promise for the adjuvant treatment of cancer to control residual disease, but so far most DC trials have been performed in end-stage cancer patients with high tumor load. Here, in a phase I/II trial, we investigated the effect of autologous DC vaccination in 17 patients with acute myeloid leukemia (AML) in remission but at high risk of full relapse. The Wilms’ tumor 1 protein (WT1), a nearly universal tumor antigen, was chosen as an immunotherapeutic target because of its established role in leukemogenesis and superior immunogenic characteristics. Two out of 3 patients who were in partial remission with morphologically demonstrable disease after chemotherapy were brought into complete remission following intradermal administration of WT1 mRNA-electroporated DC. In those 2 patients as well as in 7 other patients who were in complete remission but who had molecularly demonstrable residual disease, there was a return to normal of the AML-associated tumor marker following DC vaccination, compatible with the induction of molecular remission in 9/17 patients vaccinated thus far. Survival in responders is significantly longer than in non-responders. Immunomonitoring showed a significant increase in WT1-specific CD8+ T cells and signs of general immune stimulation, such as a significant increase of plasma levels of interleukin 2 and of HLA-DR+ CD4+ T-cells. Clinical responses were correlated with elevated levels of activated natural killer cells post-vaccination, but long-term responses were only correlated with an increase in WT1-specific CD8+ T-cell frequencies. There was no significant change post-vaccination in WT1 antibody levels. In conclusion, vaccination with WT1 mRNA-loaded DC elicits immunological and clinical responses in AML patients. DC-based immunotherapy emerges as a feasible and effective strategy to control residual disease and prevent full relapse in AML. Reference: Van Tendeloo VF et al. Proc Natl Acad Sci USA 2010;107:13824-13829.
Active Anti-IFN α Immunotherapy Applied to Chronic Viral and Autoimmune Diseases

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IFN α has been involved in immunosuppression as a master regulatory transcript triggering particularly production of the anti-inflammatory cytokine IL 10. In chronic viral diseases including AIDS and in autoimmune diseases including Systemic Lupus Erythematosus, IFN α is abnormally over-produced.

HIV 1-infected individuals have been subjected to IFN α-Kinoid immunotherapy in a randomized placebo-controlled phase I/II trial (EURIS trial). In patients who developed anti-IFN α antibodies no HIV-related symptoms were observed and their CD4 cell count was stabilized over the 18-month follow-up period. IFN α-Kinoid proved to be both safe and immunogenic and efficient (1).

IFN α-Kinoid was also experimentally assessed in a murine SLE model expressing IFN α conveyed by an IFN α-Adv. Whereas control mice treated with the adjuvant (KLH) or PBS died, IFN α-Kinoid-vaccinated mice in which anti-IFN α Abs were elicited survived over the experimental follow-up period without developing lupus symptoms (2). These clinical and experimental studies stress the safety, immunogenicity and proof of concept of IFN α-Kinoid immunotherapy.

References

Update on Kinoids as an Active Immunotherapy to Combat Pathogenic Cytokines in Viral, Cancer and Autoimmune Diseases: Safety, Immunogenicity

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Kinoids are a derivative of biologically non toxic but immunogenic cytokines prepared either by chemical inactivation of the cytokine (1rst generation) or by coupling the cytokine to a carrier protein such as KLH (2nd generation). Given that abnormal overproduction of cytokines are involved in the pathogenesis of severe chronic diseases, including AIDS (IFN α and IL 10), autoimmune diseases (TNF α and IFN α) and cancer (VEGF and TNF α). Kinoids have been experimentally assayed and are currently under clinical trials. In AIDS, inactivated IFN α has been efficiently used in a phase 2 EURIS trial (1). In Rheumatoid Arthritis TNF α-Kinoid immunotherapy proved to be safe and effective in transgenic hTNF α mice developing arthritis (2) and is currently under phase 1-2 trial in Crohn’s disease and Rheumatoid Arthritis (NCT00808262). VEGF-Kinoid immunotherapy proved to be experimentally safe and effective in preventing the tumor development in a model of SCID or nude mice (3) and the neo vessels sprouting in a murine model of choroidal neovascularization as it occurs in Age-related Macular Degeneration (AMD) and in choroidal melanoma.

New Immune Strategies to Reduce CCR5 Expression and Block HIV Infection

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CCR5 is one of the main co-receptors for HIV and the predominant one for HIV during mucosal infection which represents >95% of new infections worldwide. Antibodies against cell surface receptors can either block ligand binding or cause internalization of the receptor thus decreasing their concentration on the cell surface, either of which would block HIV-1 infection. Thus CCR5 could become an important target for therapeutic and vaccine designs.

The main objective of our vaccine approach is to overcome tolerance to “self” CCR5 epitopes to raise immune responses that will counter the expression of CCR5 on host cells. This has been achieved by the parallel testing of viral vectors, which are able to support antigen presentation of proteins and most importantly support the presentation of conformation dependent epitopes on their surface. As the achievement of strong and long-lasting antibody responses requires a careful timing of administration and a proper adjuvant formulation, especially when conformation-sensitive epitopes are targeted, we focus on specific formulations that promote humoral responses. We used a different combination of immunization routes including intraperitoneal, intranasal, intrarectal and intramuscular. Each immunization with antigens of interest has been performed in the presence of different suitable adjuvant molecules, such as Freund, RIBI, Alum and Montanide.

Preliminary results showed that the use of proper adjuvant formulation and a combination of intraperitoneal plus intranasal immunizations selectively promote CCR5 specific IgG and IgA secretion with expected properties.

Results from these studies provide essential directions for the development of a durable HIV infection prevention measure not restricted by HIV-1 isolate variability. Moreover, this strategy may be combined with other immunization modality to confer more effective protection via multiple targets.

Using Epitopes Recognized by Monoclonal Antibodies as Vaccine Templates

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Bioinformatics analysis of gp120 sequence data demonstrates structural conservation in the 2nd and 3rd sequence-variable loops (V2 and V3) of gp120. Immunochemical data provide evidence of antigenic conservation in V2 and V3. Immunochemical and functional studies of monoclonal antibodies (mAbs) that target quaternary neutralizing epitopes (QNEs) composed of regions of V2 and V3 also provide evidence of cross-reactivity reflective of structural and antigenic conservation in this compound epitope. Since antibodies to these regions can mediate virus neutralization, these regions of the HIV-1 envelope should be included among the targets of an AIDS vaccine intended to induce neutralizing antibodies. In order to design immunogens that target structurally-conserved, sequence-variable regions of a protein, 3D visualization is required; this can be accomplished through physical methods (crystallography and NMR) and through molecular modeling. Indeed, these approaches have revealed generic structures for the V3 loop and suggest related structures recognized by macaque and human QNE mAbs. Crystallography and molecular modeling have recently been applied to the design of V3-scaffold immunogens that contain variants of the generic V3 structure and several such immunogens have been synthesized and tested for antigenicity. Additionally, immunogenicity studies in rabbits show their ability to induce cross-clade neutralizing antibodies. Similar methods are being applied to the design of QNE-scaffold immunogens. The data indicate induction of neutralizing antibodies to conserved epitopes in variable regions, providing a paradigm for further development of rationally-designed HIV vaccines.
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Reconsidering a Live-Attenuated HIV Vaccine?
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Live-attenuated SIVdeltaNef provides solid protection against homologous challenge in the macaque model, although the correlates of protection have not been identified yet. Nevertheless, such an attenuated HIV-1 variant is not considered for further vaccine development because of safety considerations. The attenuated virus causes a chronic infection and the virus may evolve to regain pathogenicity. We made conditionally live HIV-1 and SIV variants that are dependent on doxycycline for replication. These viruses can be turned on and off at will, and could eventually become the next generation of live-attenuated vaccines.

We performed the first tests in macaques with the doxycycline-dependent SIV-rtTA variant. This virus replicates in vivo and demonstrates a partial vaccine effect upon wt SIVmac239 challenge in 8 of 8 animals, with a viral load reduction of 2 to 7 logs. Two of the 8 animals are fully protected and these animals exhibit a typical viral load shoulder of the vaccine strain. Intriguingly, these two animals pick up an identical mutation in the introduced rtTA gene that may provide immune-escape properties to the virus. Similar vaccine protection was observed in animals that continuously received doxycycline versus animals that received doxycycline for 6 months, but not the 8 weeks before challenge. These results demonstrate that persistent replication of the vaccine strain is not necessary for protection, suggesting that induction windows much shorter than 6 months may suffice.

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Fc-Mediated Anti-HIV Effector Function Mediated by New Monoclonal Antibodies That Recognize Epitopes Selectively Exposed During Viral Entry
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Recent SHIV-challenge studies using conformationally constrained immunogens suggest a correlation between protective immunity and the presence of antibodies specific for epitopes that are exposed selectively during viral entry (DeVico, et.al., PNAS, 104:17477-82, 2007). We recently established methods to census memory B cells and isolate monoclonal antibodies (mAbs) from HIV infected people (Guan, et. al. PNAS, 106:3952-7.2009) to directly test this correlation by passive immunization. To this end, we have identified three groups of mAbs specific for gp120 epitopes whose exposures are increased by the binding of CD4 or strictly dependent upon it. Group I mAbs bind approximately 10-fold better to gp120-CD4 complexes than to gp120. Group II mAbs bind 100 to 1000-fold better to gp120-CD4 complexes than to gp120. Group III mAbs only bind to gp120-CD4 complexes with no measurable binding to free gp120. Examples of Group I and Group II mAbs are known (i.e., mAb A32 and mAb 17b, respectively); however, Group III mAbs have not been observed in humans. One Group III mAb, N12-I15, isolated from an HIV-1 controller exhibited unusual properties in functional studies. It is non-neutralizing in the TZM-bl assay but strongly potent in antibody dependent cell mediated cytotoxicity assays (ADCC), which is a measure of Fc-mediated effector function. This mAb potently arms effector cells to kill CD4+ CCR5+ target cells that are sensitized with monomeric gp120, trimeric gp140, or an R5 pseudovirus. The strict dependence of epitope exposure on CD4 binding for this mAb and its ability to strongly facilitate killing of target cells sensitized with trimeric gp140 or an R5 pseudovirus, suggests that it recognizes a novel epitope that is highly exposed during viral entry and that this epitope might be a new target for protective immune responses elicited by vaccination.
Magnitude and Breadth of the Neutralizing Antibody Response in RV144

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RV144 is a recently completed efficacy trial in Thailand of a candidate HIV-1 Env-containing vaccine that provided modest protection against the acquisition of HIV-1 infection in subjects who were mostly at risk of heterosexually acquired infection with CRF01_AE HIV-1 (Rerks-Ngarm S, et al., N. Engl. J. Med. 361:2209-2220, 2009). We assessed the magnitude and breadth of the neutralizing antibody response in a TZM-bl assay against highly sensitive (tier 1) and moderately sensitive (tier 2) strains of virus at two weeks post final immunization in this trial. Moderate neutralizing activity was detected against 3 of 5 tier 1 viruses (MN.3, SF162.LS, MW965.26). No positive neutralization of tier 2 CRF01_AE Env-pseudotyped viruses was detected. The overall neutralizing antibody response as assessed in TZM-bl cells was weaker than the response seen in two earlier clinical trials of protein subunit Env vaccines (Vax003, Vax004) that failed to show a measurable level of protection.

In contrast to the negative results obtained for tier 2 viruses in the TZM-bl assay, plasma samples from RV144 exhibited sporadic positive neutralizing activity against tier 2 CRF01_AE viruses when A3R5 cells (CEM human lymphoblastoid cell origin) were used as targets for infection in the assay. The A3R5 cell line also appears to be about 10-times more sensitive than TZM-bl cells for detecting neutralization by monoclonal antibodies and sera from chronically HIV-1-infected individuals. We are currently using the A3R5 assay to assess the neutralizing antibody response in Vax003 and Vax004 for direct comparison to RV144.

SIV Infection of Natural Hosts

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In recent years there has been a growing appreciation of the importance of “comparative AIDS research” (i.e., studies of HIV and SIV infections in different hosts) as a tool to understand HIV pathogenesis and to improve both prevention and therapy of HIV infection. In this presentation I will provide a state-of-the-art summary of the field of natural, nonprogressive SIV infections of hosts such as sooty mangabeys and African green monkeys.

Natural SIV infections are the result of an evolutionary adaptation that allows a peaceful coexistence of lentiviruses and the primate immune system. This adaptation does not involve control of viremia but, rather, is characterized by phenotypic changes to CD4+ T cells, limited immune activation and preserved mucosal immunity, all of which contribute to the benign phenotype of these infections and, possibly, to the reduced rates of vertical SIV transmission.

In this presentation, I will: (i) summarize the main immunological and virological features of natural, non-pathogenic SIV infections, including the ability to mount robust innate immune responses to the virus; (ii) revisit the main hypotheses that were proposed to explain the apathogenicity of these infections; and (iii) discuss how a different pattern of in vivo infected CD4+ T cells, with central memory cells being relatively spared from direct virus infection, may preserve CD4+ T cell homeostasis and reduce immune activation in SIV-infected natural hosts.
172 Monitoring HIV Drug Resistance as a Guide to Understanding Viral Pathogenesis

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It is relevant that the use of newer more sensitive methods have been key in establishing the role that certain drug-resistance mutations may play in both transmitted and acquired drug resistance. In particular, the use of allele-specific PCR assays (AS-PCR) and pyrosequencing have been very useful in providing new insights. Among the reasons that more ultrasensitive assays may sometimes be needed for more accurate assessments of drug resistance is the differential effect of certain mutations on viral replicative capacity. As an example, the K65R mutation is known to adversely affect HIV replication, and this may be one of the reasons that it is found relatively infrequently among individuals who fail antiretroviral therapy. In contrast, the use of AS-PCR for K65R in subtype C viruses has shown that this method was able to detect the presence of this mutation in an additional 4 of 30 samples who had tested negative by bulk sequencing methods. Now, it also appears as though the transmission of the K65R mutation, while rare, can also be detected in higher numbers by AS-PCR than bulk sequencing, and that this is also more common among subtype C than subtype B viruses. The likely reason is that subtype C viruses are more prone to develop K65R as a consequence of treatment failure and are therefore more likely than subtype B viruses to contain this mutation at the time that transmission takes place. In the case of the M184V mutation, it has also been observed that AS-PCR methods can detect this substitution more efficiently than bulk sequencing among newly-infected individuals. Thus, the reason that some resistance-associated mutations are not commonly observed in newly-infected subjects is not because they impact on the ability of HIV to be transmitted but rather because they may quickly revert to wild-type in the absence of drug pressure and then be rapidly overgrown by wild-type variants.

174 Salvage Therapy in HIV-1 Infected Patients

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Resistance of HIV-1 to antiretrovirals is an issue of clinical relevance even with the availability of five antiretroviral classes. Prevalence of failure to the three original antiretroviral classes is estimated to range from 2.1% to 16% after HAART initiation and it increases over time from treatment baseline.

International guidelines recommend the use of at least two active drugs in constructing a new antiretroviral regimen to obtain virologic success and adding a compound with a different mechanism of action often increases the chances of virologic response. With the introduction of new drug classes and new-generation compounds of older classes in the antiretrovirals armamentarium, the chances of achieving virologic success in patients with resistance to all three original antiretroviral classes are certainly higher than in the past. Patients who experience virologic failure and show resistance to new antiretrovirals are, however, described both in randomized trials and clinical settings. That is why in certain settings patients do not have two fully active drugs, especially in the presence of prior exposure to several suboptimal therapies. In this talk I will review several strategies that might be an effective option to obtain virologic success in patients with triple class resistance as well as treatment strategies for patients who do not have two active drugs to construct a new effective antiretroviral regimen with, after virologic failure.
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HIV-1 Evolution in Primary Infection is Affected by Stochastic Followed by Selective Processes
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Viral evolution in primary HIV-1 subtype B infection was assessed in 11 HIV-1-infected men who have sex with men (MSM): four transmitter:seroconverter transmission pairs and three independent seroconverters. Four hundred and seventy five near full-length HIV-1 genome sequences were generated, including ~10 genomes per specimen at 2-12 visits over the first year of infection in the seroconverters. Single founding variants with nearly homogeneous viral populations were detected in eight of the nine individuals who were enrolled in acute HIV-1 infection. When the transmitter was in chronic infection, the founder variant in the seroconverter was rare in the transmitter’s blood specimen. Although viral diversity underwent a slight contraction over the first 20 to 40 days, mutational patterns indicative of rapid population expansion rather than selection dominated during the first five weeks of infection. Subsequently, selection dominated over the whole proteome. Mutants were detected in the first week and became consensus as early as day 21 post onset of symptoms of primary HIV infection. We found multiple indications of CTL escape mutations while reversions appeared limited. Putative escape mutations were often rapidly replaced with nearby, mutually exclusive mutations, indicating continuing evolution of escape mutants, possibly in adaptation to viral fitness constraints or to evolving variant-specific immune responses.

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Exploring the Potential of CCR5 and CXCR4 Modified CD4 T Cells to Target the HIV-1 Reservoir
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Viruses that use CCR5 (R5 strains) are the major cause of new infections, while viruses that evolve the ability to use CXCR4 can arise later in infection and are associated with accelerated disease progression. Natural resistance to HIV infection can be conferred by the loss of CCR5 due to a Δ32-ccr5 polymorphism that makes individuals homozygous for this allele CCR5-negative. Heterozygosity for Δ32-ccr5 results in slower disease progression in those who become infected. We have conferred genetic resistance to HIV infection through the targeted, permanent disruption of the ccr5 gene in human T cells through the use of high specific zinc-finger nucleases (ZFNs). In vitro, cells with disrupted ccr5 grow normally, are resistant to infection by R5 virus strains, but are sensitive to X4 virus strains. We have recently applied the ZFN gene knockout approach to the HIV coreceptor CXCR4 and find that this also results in a permanent disruption of CXCR4, and resistance to infection with X4-tropic HIV-1. Primary T cells with knockout of both CCR5 and CXCR4 have been created and these T cells exhibit a robust resistance to all tested strains of HIV-1. The current status of the phase I trial testing the safety and feasibility of CCR5 disruption using autologous CD4 T cells treated with CCR5 specific ZFNs will be reviewed.
**Peptide Linkers Between Natural Antibodies and HIV Target**  
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In human serum approximately 1 to 8% of the total IgM and 1 to 2.4% of the total IgG recognize the epitope gal(1,3)gal, which is part of a penta-saccharide present mainly on endothelial cells of all mammals except humans and old world monkeys. These natural antibodies have been shown to participate in the antibody-mediated opsonization of particles and in the induction of the classical cascade of the complement. Moreover, these antibodies can trigger the activation of NK cells via CD16 recognition of the Fc fraction of the antibody and induce an antibody-dependent cellular cytotoxicity (ADCC). We thought it would be useful to temporarily be able to redirect this high level preexisting antibody pool to a new antigen, which rapidly would increase the pool of biologically active antibodies with a pre-determined specificity as an alternative to administering a monoclonal antibody. To explore this possibility we chose to target the receptor-binding region of the envelope protein (gp120) of the human immunodeficiency virus (HIV). The interaction between gp120 and its receptor the CD4 molecule is highly conserved and involves only a limited amount of residues. The envelope glycoprotein binds to 22 residues on the D1 region of the CD4 receptor located between amino acids 25 to 64 and the atomic interactions are well characterized. As previously published, we could show that CD4-derived, gp120-binding, synthetic peptides chemically linked to gal(1,3)gal can redirect these natural antibodies and improve the HIV-1-neutralizing activity of the CD4-derived peptides in vitro and conferred antibody-dependent cellular cytotoxicity after the addition of normal human sera. We have now extended these studies using a sterical mimic of CD4 without sequence homology and peptides derived from CCR5. I will also discuss how we now are employing a random RNA library to select peptides binding to new targets.

**Targeting Cell Cycle to Enhance the Potency of Antiretroviral Therapeutic Agents**  
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HIV exploits cell cycle and key host cell pathways to complete its viral life cycle. As such, both cell cycle and specific host cell pathways could serve as potential therapeutic targets. Two examples of targeting cell cycle and specific host cell pathways will be discussed: targeting CCR5 density via use of agents causing a prolongation of G1- S phase transition; and targeting ribonucleotide reductase in association with induction of prolongation of S phase. CCR5 receptor density is a key factor in terms of its ability to infect target cells. CCR5 receptor density increases with cell activation and is also associated with replication kinetics. We recently postulated and confirmed that cytostatic agents that induced prolongation of G1-S transition were associated with down regulation of CCR5 expression and that this resulted in enhanced potency of both CCR5 antagonist and fusion entry inhibitors. In addition, we demonstrated that this effect also enhanced the potency of antibodies which block viral entry. The potency of all tested entry inhibitors was directly related to CCR5 density. Prototype CCR5 resistant R5 viruses demonstrated wild type susceptibility when tested in cells with reduced but physiologic CCR5 density. Another established target for HIV therapy is HIV reverse transcriptase using nucleoside/nucleotide analogs. These agents compete with natural nucleosides however their efficacy is compromised by development of drug resistance. Resveratrol is an agent which inhibits ribonucleotide reductase and induces a prolongation of S phase. We postulated and confirmed that this reduction of cellular nucleotide pools results in enhanced potency of nucleoside/nucleotide analogs. We further show that this enhanced potency is such that highly resistant HIV isolates to analog alone demonstrate wild type susceptibility when evaluated in combination with Resveratrol. These data suggest that targeting cell cycle and specific host cell pathways could have therapeutic implications and warrants further clinical evaluation especially in the setting of established drug resistance.
Adult T-Cell Leukemia/Lymphoma in Japan: Reminiscences and Perspectives
Kiyoshi Takatsuki and Masao Matsuoka

Adult T-cell leukemia/lymphoma (ATL) was first proposed as a distinct clinical entity based on observations in Japanese patients with T-cell leukemia. Observations typical of ATL include the presence of leukemic cells with multi-lobulated nuclei (flower cells), skin involvement, hypercalcemia, frequent complication with opportunistic infections, and an aggressive clinical course. Cell-mediated immunity is severely impaired in ATL patients, leading to immunodeficiency. Soon after the original report of ATL, Dr. Gallo and his colleagues found human T-cell leukemia virus type 1 (HTLV-1) in a Caribbean patient with T-cell malignancy. Thereafter, researchers have studied HTLV-1, and the mechanism by which this retrovirus causes ATL, extensively.

We found that an antisense transcript of HTLV-1, HTLV-1 bZIP factor (HBZ) was expressed in all ATL cases and promoted their proliferation. Analyses of proviral sequences of ATL cells and HTLV-1-infected cells in carriers showed frequent nonsense mutations in all viral genes except the HBZ gene. Almost all of the nonsense mutations arose from G-to-A mutations, and detailed analysis showed that these mutations were caused by APOBEC3G before retroviral integration. Since HBZ, alone among HTLV-1 genes, was unaffected, these data suggest that HBZ essential for both ATL cells and HTLV-1 infected cells in carriers.

Recent studies reported that more than half of ATL cases expressed FOXP3, indicating that ATL is a neoplastic disease of regulatory T-cells, at least in FOXP3 positive cases. We found that HBZ induced Foxp3 expression in naïve T-cells, and that regulatory T-cells increased in HBZ transgenic mice. T-cell lymphomas developed significantly more frequently in HBZ transgenic mice than in non-transgenic littermates. These data indicate that HBZ is responsible for the regulatory T-cell phenotype of ATL cells. Thus, studies on HBZ shed light on the mechanisms of HTLV-1-associated diseases.

HTLV-1 Research after Three Decades: Insights into Cellular Transformation Mechanisms for ATL (Adult T-Cell Leukemia)
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Human T-cell leukemia virus type 1 (HTLV-1) was first discovered in 1980. This retrovirus is the causative agent of an aggressive T-cell leukemia named Adult T-cell leukemia (ATL). To date the mechanism of how HTLV-1 transforms T-cells is not fully understood; however, it is accepted that the virus encode an oncogenic protein, Tax. I will update our current insights into the transforming routes employed by Tax to cause checkpoint abrogation, DNA damage, and the creation of genomic instability. I will also discuss how the cancer stem cell hypothesis might apply to ATL.
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Risk Indicators for Disease Progression in Asymptomatic HTLV-1 Carriers: A Nationwide Prospective Study in Japan and Expression Profile Analysis Based on the Material Bank
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Definitive risk factors for the development of adult T-cell leukemia (ATL) among asymptomatic human T-cell leukemia virus type I (HTLV-1) carriers remain unclear. Recently, HTLV-1 proviral loads have been evaluated as important predictors of ATL, but a few small prospective studies have conducted. We prospectively evaluated 1218 asymptomatic HTLV-1 carriers (426 males and 792 females) who were enrolled during 2002–2008. The proviral load at enrollment was significantly higher in males than females (median, 2.10 vs 1.39 copies/100 PBMC) (P < .0001), in those aged 40–49 and 50–59 years than that of those aged <40 years (P = .02 and .007, respectively), and in those with a family history of ATL than those without the history (median, 2.32 vs 1.33 copies/100 PBMC) (P=.005). During follow-up, 14 participants progressed to overt ATL. Their baseline proviral load was high (range, 4.17–28.58 copies/100 PBMC). None developed ATL among those with a baseline proviral load lower than approximately 4 copies. Multivariate Cox analyses indicated that a higher proviral load was an independent risk factor for progression of ATL from carrier status. Next we tried to determine risk-indicator genes. In this study, whole genome gene expression profiling was conducted in ATL patients (n=52), HTLV-1 carriers (n=40), and in healthy volunteers (n=21). Five carriers, who later developed ATL were included as “high-risk carriers”. Comparative analyses in terms of diagnosis, proviral load, ATL types, and risk among carriers allowed us to determine potential risk-indicator genes of ATL, including CCR4, CTLA4, MALT1, cMyb, and IL21R, all of which play central roles in regulation of T-cell functions and cell proliferation. Studies are in progress to test if they indeed reflect the transition of cellular phenotypes toward ATL.

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HIV Exposes Major “Trafficking” in HERV- K HML-2 Viruses Which are Critical to the Pathogenesis of HIV Infection and Lymphoma
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We have shown that in HIV Infection the human endogenous retrovirus- K (HML-2) (HERV K) is found in the plasma of patients(Pts) with HIV and HIV associated lymphoma (HIV-L) to concentrations as high as 1010. HERV K virions can be seen in plasma by immunoelectron microscopy, detected by gagRT-PCR and can demonstrate reverse transcriptase activity in the appropriate viral density using iodoxanol gradients. Similar high titer HERV K is found in non-HIVassociated Lymphoma (non-HIV-L). Using primers for RT-PCR which span >1000bp of the HERV K type 1 and type2 env and then cloning and sequencing amplicons, we detect different HERV K species in plasma of HIV, HIVL and non HIVL Pts . We constructed neighbor joining phylogenetic trees (MEGA) (PhyTs) to relate these to the known HERV K species defined in the NCBI and the HERVd data bases. We found 15/171 different Type-1 and 18/115 different Type-2 HERV-K (HML-2) cloned sequence in plasma of HIV, HIV-L and non HIV-L patients. We constructed phylogenetic trees (MEGA) (PhyTs) to relate these to the known HERV K species defined in the NCBI and the HERVd data bases. We found 15/171 different Type-1 and 18/115 different Type-2 HERV-K (HML-2) cloned sequence in plasma of HIV patients. Viruses demonstrated frequent recombination between Type1/Type2 and Type 1/Type2 HERV K. A unique new HERV K type 1 virus called K111 was discovered as a multicopy polyprovirus. PhyTs were similarly constructed from plasma samples taken serially years before and up to the development of HIV L . Pts showed increasing HERV K viral loads prior to HIV L. Multiple HERV Ks appeared with high rates of recombination. Several new viruses with unresolved topologies appear in HIV L. Patients with HIV Diffuse Large B cell (DLBL) lymphoma show a different HERV K pattern than those with HIV Burkitt’s lymphoma (BL). PhyTs are now being constructed from patients with non HIV L . HERV K Rec Antibodies are also elevated in lymphomas. HERV K HML-2 viruses are actively being trafficked in blood in HIV and nonHIV L providing new insights to the pathogenesis of lymphoma.
Beneficial and Detrimental Effects of Human Endogenous Retroviruses (HERVs)

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There has been significant recent progress in the elucidation of structure and function of HERVs, especially of their evolutionarily youngest member, HERV-K. Knowing more about structure and function allows a more solid analysis of the benefits HERVs may possess for humans or the damage they may cause. We will discuss recently recognized properties of HERV-K elements and expressed proteins and describe their established and putative roles in gene regulation, evolution and in cancer.

A number of expressed envelope protein of several HERVs are bone fide fusion machines able to mediate cell entry and pseudotype lentiviruses. Moreover, its profound expression in placental trophoblasts contributes to syncytia formation and the immunosuppressive domains in the transmembrane subunits might subdue maternal immune responses in this organ to protect embryonic tissue.

The accessory Rec protein is highly expressed in several tumors. It not only enhances nucleo-cytoplasmic transport of viral transcripts but also disturbs signaling pathways regulating cellular proliferation. The underlying mechanisms potentially leading to transformation or tumor promotion are already partially revealed.

Moreover, large scale transcriptome analyses disclose more and more cases of significant gene disregulation on the one hand and beneficial domestication of several HERV elements on the other.

Do Modern Human Endogenous Retroviruses Still Replicate?

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The functions of the approximately 98% of the human genome that do not encode human cellular proteins remain to be elucidated. Actively replicating endogenous retroviruses entered the human genome millions of years ago and became a stable part of the inherited genetic material, with retroviral elements presently making up approximately 8% of the modern human genome. These viruses subsequently acquired multiple mutations, leading to the widely-held assumption that they are no longer competent to replicate. However, in studying living patients rather than the standard cell lines, we have recently discovered surprising evidence suggesting that in certain patients with HIV-1 infection or cancer HERV-K (HML-2), an endogenous retrovirus that is a relatively recent entrant into the human genome and has been linked to oncogenesis, might still be capable of replication. Replication and transmission of endogenous retroviruses is difficult to prove using standard techniques, however, as these viruses are already present in the genomes of all human cells. Therefore, we have used a newly devised molecular system in which antibiotic resistance serves as a surrogate marker to assess whether we can passage virus in tissue culture and/or from the blood of patients. Preliminary studies indicate that this may well be the case. Proof that endogenous retroviruses can still replicate in modern humans will lead to a paradigm shift in thinking about these viruses, and will suggest a role for them in reshaping individual genomes. In addition, as increased expression of chromosomal endogenous retroviral sequences has been linked to cancer and autoimmunity, these findings will be relevant to understanding the pathogenesis of significant diseases.
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Awakening of Dormant Tumor Cells by Inflammation
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In two phase carcinogenesis, carcinogen initiated cells may never develop into tumors unless tissue homeostasis is disturbed by tumor promoters, causing inflammation and/or proliferation. Mutatis mutandis, the same applies to viral carcinogenesis, even with highly transforming viruses. Moreover, disseminating cells from frank malignancies may remain dormant over many years, unless “awakened”. This brief survey will discuss mechanisms for awakening. They include:

i). Morphogenetic rearrangements of tissue architecture;
ii). Inflammation, induced by the impact of microorganisms, viruses, or by functional dysregulation;
iii). Changes in the stromal microenvironment.

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KSHV, Angiogenesis and Kaposi Sarcoma
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In the HAART era Kaposi’s sarcoma (KS) remains the second most frequent tumor in HIV-infected patients worldwide, and it is the most common cancer in Sub-Saharan Africa. KS is a multicentric angioproliferative disorder characterized by pathognomic spindle cells of endothelial origin, and is casually associated to human herpesvirus 8, known also as KS associated herpesvirus (KSHV).

KSHV infects endothelial cells, induces the formation of spindle morphology and promotes angiogenesis. We are studying the molecular mechanisms associated to KSHV angiogenesis. We have determined that KSHV induces angiogenesis with two distinct mechanisms. The first is through NF-kappaB activation, via stimulation of the IkappaB kinase (IKK). KSHV selectively triggers the production of high levels of MCP-1, whereas it does not affect the expression of other NF-kappaB-dependent proinflammatory proteins. Interestingly, inhibition of MCP-1 abrogates KSHV angiogenesis. When NFkB is inhibited, infection still results in a residual angiogenic activity, approximately 30-40% of the maximal level. Our experiments have shown that this second mechanism is dependant upon the transcription factor ATF-4. Infact, KSHV infection of endothelial cells results in a significant upregulation of ATF-4. In addition, transfection of ATF-4 in uninfected endothelial cells induces in vitro angiogenic behaviour. Furthermore, ATF-4 has a direct effect on the activation of MCP-1 promoter.

The results show that KSHV promotes angiogenesis by stimulation of two different cellular mechanisms, NFKB and ATF-4, that converge on activating MCP-1. The strict dependence of KSHV angiogenesis on MCP-1 and the elucidation of molecular mechanism involved in this process could result in a better understanding of the angiogenetic process, its involvement in cancer and will help in designing novel therapies to reduce KS growth and vascularization.
VEGF-Notch-EphrinB2 Pathways in AIDS-Related Kaposi’s Sarcoma

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AIDS related Kaposi’s sarcoma display high expression of genes critical for vessel development including VEGF/VEGFRs; arterial-venous specification and maturation (Notch Receptor/ligands and EphrinB2/EphB4) KSHV latency and lytic cyle proteins profoundly regulate VEGF/Notch pathway. In addition KS tumor cells express endothelial precursor cell marker CD133 which is induced by Notch activation. Furthermore, Notch pathway also provides survival signal in KS cells through high expression of Notch ligand Dll4. Similarly EphrinB2 knock down in KS cells inhibits cell proliferation and induces apoptosis. Thus VEGF, Notch, EphrinB2 represent novel targets for KS therapy.

Inhibitors of Notch pathway including decoy soluble Dll4-Fc, and Dll4 neutralizing antibodies have been generated and tested in KS models in vitro and in vivo. Secondly, EphrinB2 inhibitor decoy soluble receptor EphB4 has been generated and tested in KS models in vitro and in vivo.

VEGF positively regulates Notch pathway which negatively regulates VEGF pathway. VEGF and Notch pathways positively regulate EphrinB2 which in turn positively regulates VEGF pathway. Thus targeted combination therapy has great potential to target both KS stem cells as well differentiated cells. Novel inhibitors and surrogate markers of activity should thus be tested in human trials.

HIV Tropism and Decreased Risk of Breast Cancer

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Background: Women with HIV/AIDS had a low risk of breast cancer, but with improving antiretroviral therapies their risk gradually increased to that of the general population. Because HIV binding to the CXCR4 chemokine receptor may induce apoptosis of neoplastic breast cells, and because effective antiretroviral therapy retards emergence of CXCR4-using strains of HIV, we hypothesized that HIV tropism for this receptor would reduce breast cancer risk.

Methods: We conducted a breast cancer nested case-control study among women who participated in HIV/AIDS cohort studies with longitudinally collected risk factor data and plasma. Cases were HIV-infected women (mean age 45.7 years) who had stored plasma, with HIV viral load > 500 copies/mL, and collected within 24 months of breast cancer diagnosis. Three HIV-infected control women, without breast cancer, were matched to each case based on age and plasma collection date. CXCR4-tropism was determined by a phenotypic tropism assay. Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer were estimated by exact conditional logistic regression.

Results: Two (9%) of 22 breast cancer cases had CXCR4-tropic HIV, compared to 19 (29%) of 66 matched controls. Breast cancer risk was significantly and independently reduced with CXCR4 tropism (adjusted odds ratio, 0.10, 95% CI 0.02-0.84) and with menopause (adjusted odds ratio, 0.08, 95% CI 0.001-0.83). Adjustment for CD4+ cell count, HIV viral load, and use of antiretroviral therapy did not attenuate the association between infection with CXCR4-tropic HIV and breast cancer.

Conclusions: Low breast cancer risk with HIV/AIDS is specifically linked to CXCR4-using variants of HIV. These variants are thought to exclusively bind to and signal through a receptor that is commonly expressed on hyperplastic and neoplastic breast duct cells. Additional studies are needed to confirm these observations and to understand how CXCR4 might reduce breast cancer risk.
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Novel Classes of p53 Activators for Cancer Therapy

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The activity and stability of the tumor suppressor protein p53 is negatively regulated in many cancers including virally induced, ADIS-defining Kaposi’s sarcoma and pleural effusion lymphoma. One of the major negative regulators of p53 is an E3 ubiquitin ligase known as MDM2, which binds p53 to block p53-mediated growth inhibitory and apoptotic responses to cellular stress and to target the tumor suppressor protein for proteasomal degradation. Amplification and/or over-expression of MDM2 confer p53 inactivation and tumor development; inhibitors of the p53-MDM2 interaction can activate the p53 pathway and inhibit tumor growth in vitro and in vivo. Thus, antagonizing MDM2 to activate p53 represents a new therapeutic paradigm for cancer treatment. Using a battery of biochemical, biophysical and structural tools in combination with phage-expressed peptide library screening and structure-based rational design, we have recently identified three different classes of potent p53 activators based on L-peptides, miniature proteins, and D-peptides.

In this presentation we describe the discovery of these novel MDM2 antagonists, their structural as well as functional properties, and their application as potential antitumor agents to cancer therapy.

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Sensitizing Hemopoietic Malignant Cells to Glucocorticoid Induced Apoptosis by PK Inhibitors

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Glucocorticoids (GCs) are widely used in the therapy of lymphomas and lymphoblastic leukemias owing to their apoptotic effects on these cancerous cells. A major impediment of GC-based therapy is the gradual acquisition of apoptotic resistance to GC treatment. Also, certain lymphomas and leukemias are a priori resistant to GC. Therefore, a desirable goal is to develop therapeutic strategies that confer GC-sensitivity on otherwise GC-resistant cells. We observed that the broad-acting protein kinase (PK) inhibitor Staurosporine (STS) confers GC-sensitivity on several GC-resistant T and B lymphoma cells. GC-resistant T lymphoma cells express elevated levels of the anti-apoptotic proteins Bcl-2 or Bcl-XL. Transfection with Bcl-2 or Bcl-XL in sensitive cells confers resistance to GC-induced apoptosis. Surprisingly, STS overcomes the anti-apoptotic properties of Bcl-2 but not of Bcl-XL. STS acts at several levels. It induces the expression of the pro-apoptotic Nur77 orphan receptor, which overcomes the anti-apoptotic effects of Bcl-2. STS also leads to phosphorylation of Bim by an ERK-dependent mechanism which results in Bim upregulation. In addition, STS inhibits PI3K/Akt, leading to the activation of GSK3. Inhibition of GSK3 by its specific inhibitor SB216763 or by overexpression of a dominant negative GSK3 attenuated the effect of STS. Our study demonstrates a central role for GSK3α, but not GSK3β, in promoting GC-induced apoptosis. We found that GSK3α is sequestered to the glucocorticoid receptor (GR) in the absence of ligand, but dissociates from the GR complex upon exposure to GC to promote apoptosis. GC-resistance in lymphoma cells can be relieved by inhibiting the PI3K-Akt survival pathway, which inactivates GSK3 by its phosphorylation. Notch1, a transcription factor frequently activated in T acute lymphoblastic leukemia(T-ALL), confers GC resistance through activation of Akt. Indeed, inhibition of Akt is effective in sensitizing T-ALL cells to GC induced apoptosis. Our data demonstrate that lymphoma and leukemia therapy can be significantly improved if GCs are combined with PK inhibitors that shift the cell’s kinome in favor of apoptosis-prone phenotype.
Cross-Talk with the Microenvironment: The Case of Melanoma Brain Metastasis

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Various non-tumor cells in the tumor microenvironment including lymphocytes, macrophages, fibroblasts, endothelial cells and their soluble products shape the malignancy phenotype of the tumor and drive its progression towards metastasis.

This presentation deals with interactions of the brain microenvironment with brain metastasizing melanoma cells.

Brain metastasis confers upon melanoma patients an extremely bad prognosis. The mechanisms underlying homing to and survival of metastatic melanoma cells in the brain are unknown. Our working hypothesis is that interactions of melanoma cells with the brain microenvironment regulate site specific metastasis to this organ.

We generated, from single human melanomas variants that form either local cutaneous tumors or brain metastasis in xenografted nude mice. As these variants have identical genetic backgrounds, any molecular differences between them reflect, most probably, alterations associated with the ability to form brain metastasis. These variants are used to establish a specific molecular signature of melanoma brain metastasis.

Utilizing microarrays, we generated gene expression profiles of the cutaneous and brain-metastasizing melanoma variants. This analysis revealed a set of genes differentially expressed in local and metastatic variants. Surface molecules associated with tumor progression were also found to be differentially expressed on local and metastatic variants.

The two types of variants react differently to signals delivered by the brain microenvironment. This differential reactivity of certain melanoma variants with the brain microenvironment may account for the propensity of such variants to specifically metastasize to this organ site.

This study was supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (Needham, MA, USA)

The Possible Evolutionary Role of Tumors in the Origin of New Cell Types

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Widespread occurrence of tumors and the ability of tumor cells to differentiate in combination with their ability to express genes that are not expressed in normal tissues, may result in the emergence of new cell types in the evolution of multicellular organisms.

We suggest that tumors could be a sort of proving ground (or reservoir) for the expression of newly evolving genes that originate in the course of genome evolution in the DNA of germ cells (i.e., not in tumor cells themselves). The case in which the expression of a newly evolving gene in tumors results in the origin of a new function would be associated with the origin of new feedback and regulatory circuits, as in root nodules in legumes and macromelanophores in Xiphophorus fishes. Tumor cells would differentiate, resulting in a new cell type for the given multicellular species. This cell type would be inherited because of epigenomic mechanisms similar to those in preexisting cell types.

Populations of tumor-bearing organisms with genetically or epigenetically programmed tumors could represent the transition between established species of organisms at different stages of progressive evolution.

Experimental confirmation of the prediction concerning the expression of evolutionarily new and/or silent (neutrally evolving) sequences in tumor cells will be presented.
HPV-Related Cancers in HIV-Infected Subjects
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Aim: The high risk of HPV-related cancers in HIV/AIDS-patients, even under HAART treatment, stimulated our studies on HPV-distribution analyse, as well as on persistence and changes in HPV multiplicity of infections before and during antiretroviral treatment.

Materials and Methods: Prevalence and persistence of mucosal HPV genotypes and HPV16 variants were analysed in a prospective cohort of HIV-positive Italian women along with control population.

Results: HIV-positive women were more likely than HIV-negative women to be infected by HPV at the first examination (P<0.001) and to have a higher period-prevalence of HPV infection over a 3-year follow-up (P<0.001), regardless of CD4+ cell counts and anti-retroviral therapy. ‘High-risk’ and ‘probable high-risk’ HPV’s were predominant in HIV-positive (33.9 %) compared with HIV-negative (13.9 %) women. Among HIV-infected women, with normal cytology as well as with SIL of any grade, the most common genotypes were HPV16 followed by HPV81, -58, -72, -33 and -62. HPV16 isolates from 18 HIV-positive and eight HIV-negative women were classified into variant lineages based on sequencing analysis of the Long Control Region and E6/E7 genes. Whilst the HPV16 G350 European variant was prevalent in both HIV-positive (10.7 %) and -negative women (3.5 %), HPV16 African 2 variants were only detected in HIV-positive women (3.6 %), suggesting different sexual mixing behaviours.

Discussion: The high prevalence of HPV-related lesions in our cohort study of HIV-positive patients under HAART-treatment, is consistent with the reported high standardized incidence rates (SIRs) of HPV-related in situ cervical (SIR 8.9, 95% CI = 8.0-9.9) and anal cancers (SIR 68.6, 95% CI = 59.7-78.4) as well as for invasive oropharyngeal (SIR 1.6, 95% CI = 1.2-2.1). The high prevalence of uncommon viral genotypes and HPV16 variants in HIV-positive women underscores the need for wide-range HPV typing in cervical smears of this population.

Hepatitis C Virus-Specific Immune Response Among Egyptian Healthcare Workers at High Risk of Infection Without Viremia or Seroconversion*
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Hepatitis C Virus (HCV)-specific cell-mediated immunity (CMI) occurs in exposed individuals e.g. IV drug users without detecting viremia or seroconversion. We investigated the HCV-specific CMI response in seronegative, aviremic healthcare workers (HCWs) at the National Liver Institute (NLI), who are at high risk of HCV infection since more than 70% of their patients are HCV-infected. We quantified the CMI responses in 24 Egyptian HCWs with a recent history of a needle stick injury and who remained seronegative and aviremic for at least four months. An enzyme-linked immunospot (ELISPOT) assay was used to quantify interferon gamma (IFN-γ) production in response to 7 HCV genotype 4a overlapping 15mer peptide pools and phenotyped the responding cells by flow cytometry. A positive HCV-specific IFN-γ response (>55 spot forming cells; SFC/million PBMC) was elicited for 2-6 HCV peptide pools in 11 (46%) of the HCW while 13 (54%) subjects responded to one or none of the pools tested with a total mean of 1308 (SEM ±384) and 78 (±21) IFNγ SFC, respectively (p=0.002). CD4 T cells were the main source of IFN-γ as determined by flow cytometry. In summary, the majority of HCW demonstrated HCV-specific T cell responses for multiple HCV peptides without detectable HCV antibodies or RNA suggesting that clearance of low levels of HCV exposures occurs much more frequently than is generally appreciated and supports the concept that an appropriate immunologic stimulus could markedly improve protective immunity. Taken together, these data support the notion that a protective HCV vaccine is feasible.
Newly Identified Circulating Recombinant Form Common in Nigeria

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Background: The patterns of different genetic forms of HIV-1 in Africa are correlated with geography. In Nigeria, two genetic forms have been thought to be responsible for the majority of the infections: subtype G and the circulating recombinant form CRF02_AG. These observations, however, have been derived from small sample sets. For the first time, over 100 patients from Nigeria have been sequenced and classified according to subtype.

Methods: The sera from 199 patients clinically failing antiretroviral drug therapy were used for automated sequencing of the protease gene and part of the reverse transcriptase gene. RNA was reverse transcribed, PCR amplified, sequenced and then phylogenetically analyzed with sequences representing the pandemic.

Results: The most common genetic form identified was CRF02_AG (41.7%), followed by subtype G (25.1%). A newly identified circulating recombinant form, CRF43_G02, a recombinant between CRF02_AG and subtype G, was discovered recently in Saudi Arabia. Surprisingly, CRF43_G02 represented 14.6% of the study population. In the region sequenced, CRF43_G02, is entirely subtype G, but forms a genetic cluster within subtype G that is significant based on bootstrap value. The remaining 18% of the samples were either unique recombinant forms or other subtypes.

Conclusions: Nigeria, the most populous country in Africa, has a diverse HIV-1 population as well, predominantly on subtype G and CRF02_AG. A new recombinant of these two genetic forms, CRF43_G02, represented close to 15% of this population of viruses based on bootstrap value. The remaining 18% of the samples were either unique recombinant forms or other subtypes.

Population Level Drug Resistance Mutations in HIV Type 1 Protease and Reverse Transcriptase in Cameroon: 1995 to 2010 Review

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Objectives: HIV infection in the Central African Region is old and driven by several genetic variants. We analyzed the prevalence of transmitted HIV drug resistance at a population level within fifteen years that can provide information on the effectiveness of first line antiretroviral therapy (ART) in Cameroon.

Methods: HIV-1 protease and reverse transcriptase sequences from Cameroon were obtained from the HIV Sequence Database of the LANL (194) and the CIRCB patient database (126) (from 1995 to 2010) and analyzed for drug resistance mutations and genotype using the Calibrated Population Resistance (CPR) tool of the Stanford University HIV Drug Resistance Database. Of these 320 sequences, 181 were collected from 1995 to 2002 (Group 1) and 139 from 2003 to March 2010 (Group 2). The sequences were classified at that point in time regardless of treatment history of the individual.

Results: Within Group 1, M184V (5.6%), Y181C (6.1%) and M46I (98.3%) were the most frequent mutations, seven (3.9%) showed a 3-class resistance, implying that majority of these sequences would show low-level resistance. Within Group 2, 16.3%, 63.7% and 65.9% had mutations associated to PI, NRTI and NNRTI resistance, respectively, 58.5% for NRTI and NNRTI, and 8.1% for the 3 drug classes. The most frequent DRM identified in Group 2 were M184V (62.2%), K103N (38.5%) and M46I/L (14.3%). Three of the new WHO-approved mutations, I85V in one sequence in Group 1, and L76V (2.9%) and V179F (0.7%) in Group 2 were identified. CRF02_AG was most prevalent within both Groups.

Conclusion: The frequency of mutations at position 46 is fading, while at positions 184 and 103 it is increasing within the population. These results indicate an urgent need to evaluate the Guidelines for first line ART in Cameroon as part of the ART scaling-up process.
Identification of a Human Natural Regulatory T Cell Micro-RNA Signature and Demonstration of the Major Role Played by miR-31, miR-21 and Valproate in Foxp3 Expression

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Regulatory T cells (Treg) are the main mediators of dominant tolerance. Their mechanisms of action and applications are subjects of considerable debate currently. However, a human micro-RNA Treg signature has not been described yet. We investigated human natural Tregs and identified a signature composed of five micro-RNAs (-21, -31, -125a, -181c, and -374) among which miR-31 and miR-125a were under-expressed. We identified a functional target sequence for miR-31 in the 3’ UTR of FOXP3 mRNA and showed using lentiviral transduction of fresh cord blood T cells, that miR-31 and miR-21 had direct negative and indirect positive effects respectively on FOXP3 expression levels. Based on recently published data demonstrating the effect of HDAC inhibitors on FOXP3 expression, we investigated, in the cord blood non-Tregs, the mechanisms by which the aspecific opening of FOXP3 chromatin could lead to an increased expression. We therefore focused on both, potential binding factors to the FOXP3 promoter region rendered accessible via the use of the HDAC inhibitor, valproate, and on possible modifications of expression of the miRs differentially expressed in Tregs. Thus, we uncovered the role of some Ets binding sites located in the FOXP3 promoter and found that Ets-1, and Ets-2 positively regulated FOXP3 expression only when the promoter region was made accessible by valproate treatment. When looking at the miR profile, we found that, following valproate treatment, the non-Tregs exhibited a signature (miR-21, miR-31, and miR-125a) similar to the one of natural Tregs (nTregs). Thus, the epigenetic control of FOXP3 expression is mediated by the accessibility of Ets binding factors, after valproate treatment, to their binding sites in the promoter region allowing thereafter the non-Tregs to acquire a miR expression profile similar to nTregs. This last observation opens up the question of the utility of this compound in the treatment of diseases linked to a deficit or an excess of Tregs, but this needs further investigation.

Role of Heparan Sulfate Proteoglycans in HIV-1 Tat-Induced Transendothelial Migration of Lymphoid Cells

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HIV-1 transactivating factor Tat is actively released by HIV-infected cells and, in its extracellular form, it targets uninfected cells, contributing to tumorigenesis in HIV+ individuals. Heparan-sulfate proteoglycans (HSPGs) act as chemotactic receptors in B-lymphocytes and their expression increases in these cells after neoplastic transformation. Accordingly, Syndecan-1 expression has been proposed as a diagnosis marker for AIDS-related lymphomas. HSPGs are also expressed on endothelial cells (ECs), where they act as receptors for a variety of cytokines and growth factors (including Tat), regulating inflammation and tumor growth.

On these bases, here we decided to evaluate the role of HSPGs/Tat interaction in the processes of chemotaxis and extravasation of B lymphoid cells (LCs).

To this aim, we exploited Burkitt lymphoma cells overexpressing two HSPGs endowed with distinct structural/functional features: Syndecan-1 (a transmembrane receptor) and Glypican (a GPI-anchored receptor).

We thus demonstrated that both Glypican and Syndecan-1 bind Tat and mediate LC adhesion to an EC monolayer. Differently, only the transmembrane HSPG Syndecan-1, but not the GPI-anchored Glypican, mediates Tat-dependent LC chemotaxis and transendothelial migration. Then, we investigated the Syndecan-1-mediated signal transduction pathway involved in mediating these biological activities: only in Syndecan-1, but not Glypican-overexpressing LCs, Tat induces the activation of pp60src, that physically associates with Syndecan-1. In turn, pp60src activation is required for LCs chemotaxis and transmigration, as demonstrated by using specific pp60src inhibitors.

These data provide new insights about the role and mechanisms of action of Tat and HSPGs during HIV infection.
The β Chemokines MDC, TARC and I-309 are an Important Component of the Soluble anti-X4 Activity Secreted by CD4+ and CD8+ T Cells

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Control of persistent viral infection relies particularly on cell-mediated immunity comprised of CD4+ and CD8+ T cells. There is accumulating evidence on the relevance of soluble factor(s) secreted by CD8+ and CD4+ T cells in controlling HIV replication in vivo. While the β chemokines RANTES, MIP1-α and MIP1-β collectively account for the suppression of R5 viruses, yet information is lacking on the identity of the molecules involved in the suppression of X4 viruses. Proteins that inhibit the replication of X4 HIV isolates were purified from the conditioned media (CM) of immortalized CD8+ and CD4+ T cell lines from HIV+ long-term non-progressors subjects (LTNPs) and identified as the β chemokines macrophage-derived chemokine (MDC), thymus and activation-regulated chemokine (TARC) and I-309. These chemokines are secreted primarily by CD4+ T cells but also by CD8+T cells. CD4+ T cells of asymptomatic HIV+ individuals secreted significantly higher levels of MDC and TARC compared with subjects who progressed to AIDS. Recombinant human MDC, TARC and I309 induced a dose dependent inhibition of X4 viruses. A cocktail of neutralizing antibodies against MDC, TARC and I-309 abrogated the inhibition of the replication of X4 viruses mediated by the endogenous chemokines in PBMC and CD8-depleted PBMC cells acutely infected in vitro. While the β chemokines RANTES, MIP1-α and MIP1-β suppress R5 viruses by blocking their entry into host cells the mechanism of inhibition of X4 viruses mediated by MDC, TARC and I-309 is a post entry mechanism of suppression. These molecules represent a major component of the soluble anti-X4 activity of T cells, suggesting that the mechanism whereby CD8+ and CD4+ T cells contribute to the control of HIV-1 replication may relate to the secretion of MDC, TARC and I-309. These results may be relevant to HIV pathogenesis.

Adherence to Highly Active Antiretroviral Therapy Among HIV Infected Children in Kano, Nigeria

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Background: Highly Active Antiretroviral therapy (HAART) in children in Africa has resulted in improved survival and quality of life. However, excellent adherence is one of the most important factors in determining treatment success and preventing resistance. This study determines factors associated with adherence to HAART among HIV-infected children in Kano, Nigeria.

Method: A cross-sectional study using structured questionnaire was conducted at the PEPFAR/HIV clinic in Aminu Kano Teaching Hospital, Nigeria. The study population consisted of children (and their caregivers) who had been taking ART and who access care at the clinic from May to June 2010.

Results: A total of 122 children were included in the study. 64% were males. 80 children (65.6%) were adherent to antiretroviral drugs for the preceding 7 days before the interview. Children whose caregivers reported no missed doses in the previous week (adherent) were more likely to have an HIV viral load <400 copies/ml (75% vs. 45%, P= 0.0001, OR= 0.23, CI: 0.09-0.54). Children whose caregivers reported missing at least one clinic appointment in the last six months are more likely to be non adherent (p= 0.0001, OR= 49.92, CI: 10.60- 235.08). Similarly children whose caregivers are married are more likely to be adherent than those who are not (p=0.001, OR=0.07, CI: 0.02 – 1.33), and children whose caregivers timed their administration of medication to meal times are more likely to be adherent than their counterparts whose administration of medications is not related to meal times (p=0.007, OR= 0.10, CI: 0.02 – 0.53).

Conclusion: Adherence to HAART is positively correlated with no missed clinic appointments, with caregivers being married and administration of medication with mealtimes. Excellent adherence in the previous week is also significantly related to having undetectable viraemia. Adherence intervention by assessing clinic attendance, missed doses and dosing with mealtimes is recommended in this setting.
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The MHC Class II Transactivator CIITA, a Restriction Factor for Human Retroviruses and a Molecule Making the Bridge Between Adaptive and Intrinsic Immunity

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Host defence against pathogens takes advantage of three mechanisms that co-evolved to ensure the best protection for the host: innate immunity, first barrier against pathogens; adaptive immunity, mediated by cells with specific receptor-mediated recognition and memory, and a third type of immunity, “intrinsic immunity” consisting in intracellular mechanisms that restrict pathogen infectivity and, in the case of certain viruses, intracellular pathogen replication. A number of viral restriction factors, are know, such as APOBEC and TRIM family members. These factors are functionally unrelated to molecules of the innate and adaptive immunity. Here I describe a viral restriction factor, the MHC class II transactivator CIITA, exerting its anti-replicative function on human retroviruses, including HIV, HTLV-1 and HTLV-2, and on the same time being one of the major regulator of adaptive immunity via its function on antigen presentation. Interestingly, although CIITA affects human retrovirus replication by inhibiting the function of the viral transactivators Tat, Tax-1 and Tax-2 of HIV-1, HTLV-1 and HTLV-2, respectively, its intimate molecular mechanism is different for the three viral transactivators. These characteristics make CIITA a unique example of viral restriction factor and a unique example of molecule bridging directly adaptive and intrinsic immunity during evolution.

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Donation or Infection: Retrospective Assessment of Transfusion Transmissible HIV Among Sickle Cell Anemia Patients

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Background: Sickle cell anemia is a hereditary disease which manifests itself when present in homozygous state, it predisposes to risk of acquiring transfusion transmissible HIV infection through blood transfusion given to correct the associated victims’ hemolytic anemia crises.
Method: One hundred and twenty sickle cell anemia patients; 41% males and 59% females’ volunteers for this sera study. Volunteers’ blood samples were tested for HIV antibodies using ELISA and biodata were obtained through questionnaire.
Results: 89.2% and 10.8% of volunteers were in age group ≤ 15 years and ≥ 16 years respectively. Prevalence rate 10% was recorded, 66.7% of infection occurred in age group ≤ 15 years while 33.3% 16 years. Prevalence between single and multiple transfused volunteers were 1.67% and 8.33%. At age ≤ 15 years 81.7% had multiple transfusion and 33.7% at age ≥ 16 years. Volunteers’ age, place of residence, parent or guardian, educational status, risk awareness, type of care patronage, and number of transfusion were significantly related to HIV infection rate P < 0.05. Volunteers’ sex was found to be a non-significant correlate of their infection risk P > 0.05. Data were analyzed using SPSS 11.0 by Scheffe’s comparative ANOVA.
Conclusion: HIV transmission risk is significantly related to multiple transfusion in sickle cell patients studied. Government and NGO should prioritize the awareness campaign on importance of premarital screening of the disease and rationale use of safe blood for the management of sickle cell anemia patients.
KEY WORD: HIV, Sickle Cell, Transfusion & Transmission
Adolescents’ Willingness to Participate in HIV Vaccine Clinical Trial Preparedness in Nigeria

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Background: Routine vaccination of recommended vaccines in adolescents/children from 1999 would prevent >14 million disease cases and 33000 deaths over the lifetime of each birth cohort. Data from National sero-prevalence surveys estimate the prevalence of HIV among 15-24 years old to be 5.2%. Therefore including adolescents in HIV vaccine trials makes them an important target for research in primary prevention of HIV infection which they are increasingly at risk of. This study evaluated adolescent perception towards HIV vaccine trial in Nigeria.

Methods: Two hundred and ninety one consenting adolescents were randomly selected for this study. They were recruited from some secondary schools class rooms, university undergraduates' hostels and some traders at the shopping malls within Lagos State Metropolis. Data were collected using semi-structured questionnaire. Information was obtained from knowledge of HIV status, willingness to participate in vaccine trial in future were obtained. Additionally, sexual risk behavior, stigmatization, obtain parental permission (required or not required), and function of efficacy of HIV vaccine and perceived self risk of HIV vaccine were collated and analyzed using EPI INFO 2002 software (CDC, USA).

Result: Of the 291 respondents interviewed, 96% were single. 72.7% who were willing to participate in the HIV vaccine trial (p<0.05), were educated (97.5%) have Knowledge of HIV vaccine (73.5%), and have no perceived risk of HIV vaccine infection (66.2%). Few respondents (31.3%) know their HIV status. Contrarily, those seeking parental permission (66.2%) would significantly reduce willingness to participate (p>0.05).

Conclusion: Efforts should be made on sustained education campaigns on HIV vaccine involving adolescents/parents’ consent, otherwise there would be potential obstacle to hypothetical vaccine acceptance and believe. Sexual high risk behavior is an important factor in the retention of adolescents in future vaccine studies. A number of other ethical and social issues need to be addressed before trials in Nigeria.

Challenges to Pediatric Adherence to ART Treatment and Strategies to Improve Adherence Among a Large Cohort of Uganda Children

Natukunda Marion and Rhonah Nakato

ISSUES: Perfect adherence (>95%) to highly active antiretroviral treatment (HAART) is critical to treatment success, yet little is known about adherence rates to HAART and strategies to improve adherence among children in resource-limited settings.

DESCRIPTION: Mama’s Club Uganda has 2,800 HIV-positive children.1, 200 of these are on HAART, after a rapid scale-up of HIV care and treatment services during the last year. Statistics show that 90% of patients on HAART for 12-15 months have = 95% adherence rates.

LESSONS LEARNED: To support adherence, PIDC a partner organization Mama’s Club works with developed an interdisciplinary strategy and several innovative adherence tools that were rolled out with the scale-up of HAART. All cadres of clinic staff play a critical role in reinforcing and promoting adherence to drugs and refill appointments. An adherence nurse visit, home-based adherence assessment and counseling services. Additional adherence tools developed include pictorial aids and cue cards on the principles of HAART; a patient handout on key guidelines for pediatric HAART administration translated in the local language; a standardized psychosocial pre-HAART assessment form incorporation checklists of “high risk” psychosocial situations, disclosure, and common barriers to adherence; and support groups for adolescents and caretakers. An overview of the above tools will be shared, with an in-depth description of the challenges and successes of adherence in this large, urban pediatric cohort.

RECOMMENDATIONS: Home-based unannounced assessments of adherence are necessary to obtain an accurate picture of true adherence rates among patients on HAART. Adherence support of children and adolescents should involve an interdisciplinary team approach using innovative facility and community-based interventions. Research regarding the effectiveness of these interventions is needed.
Predictors of Adherence for Patients on Highly Active Antiretroviral Therapy in HIV Treatment Program

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**Background:** There is rapid scale-up of treatment program in Nigeria with over 1,000 treatment sites from public and private sector participation. Adherence is needed to ensure optimal treatment outcomes and reduce the chances of drug resistance. Hence, adherence is of great importance towards improving the quality of health of people living with HIV/AIDS. This study assessed determinants of adherence in public sector treatment program.

**Methods:** Questionnaires were administered to 320 HIV patients on treatment for at least 12 months with the questionnaires administered from April-June 2009 in Lagos and Ibadan. Adherence was assessed by comparing their appointment date and date of actual drug refill. A difference of more than 1 week was taken poor adherence.

**Results:** Mean age 29±5.4 years; male 42%; female 58%; 14% had missed their appointment date for at least once in a year during the treatment program; patients treated for other diseases were: 8.2% for TB, 4% for diabetes and 6.4% for hypertension. Predictors of adherence from the multiple logistic regression models include: distance from health facility, with those living ≥3 hours drive from health facility more likely to have poor adherence OR=1.9 95%CI:1.4–2.8; adverse effects OR=1.5 95%CI:1.1–1.9; stigma and discrimination OR=1.4 95% CI:1.1–1.7; unfriendly health facility OR=1.3 95%CI:1.1–1.8 and treatment for other chronic diseases OR=1.2 95%CI:1.1–1.7. However, partner notification and age ≥35 years were protective with OR=0.6 95% CI 0.3–0.9 and OR=0.8 95% CI: 0.5–0.9 respectively. Sex, occupation and type of regimen were not significantly associated with adherence.

**Conclusions:** Adherence education should be scaled-up with increase in treatment sites. Efforts should be made towards ensuring HIV treatment sites are more patient-friendly while increasing the capacity of healthcare workers to support adherence and provide quality healthcare. Partner notification and reduction in stigma and discrimination should be strengthened.

Dysregulated Physiological and Emotional Responses to Stress are Associated with Cytokine and Chemokine HIV Progression Mediators and with CD4+ Count at 36-Month Follow-Up

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The overall goal of our biopsychosocial HIV research program is to identify factors associated with mediators of HIV progression, in order to incorporate these into interventions intended to influence more positive HIV clinical outcomes. In a baseline sample of 200 largely African-American HIV-positive individuals attending an inner-city HIV primary care clinic, we have indeed demonstrated that hypothesized emotional dysregulation (high Type C coping, alexithymia), as well as dysregulated physiological responses to stress (heart rate and blood pressure overreactivity to and slow recovery from experimental emotional stressors) are associated both concurrently and predictively (baseline to 24-month follow-up) with reciprocal immune factors which either amplify the immune activation that is central to HIV progression (IL-6) or which inhibit HIV entry through the CCR5 co-receptor (the beta-chemokines MIP-1α/β) (Temoshok et al., Brain Behavior & Immunity, 2008, 2009).

We now report on cytokine/chemokine, as well as clinical status results at 36-month follow-up of 125 individuals available for analysis. Cytokine/chemokine results are expressed as a stimulation index: HIV p24 (PHA and Candida) antigen-stimulated production of IL-6, and MIP-1α/β, compared to spontaneous production. Using generalized estimating equations to make longitudinal predictions, based on a linear model, and controlling for age, CD4+ count at baseline, medications, and time of measurement, we found the same relationships as previously reported, suggesting a chronic pattern that influences HIV disease progression, as indicated by CD4+ cell count: (1) higher baseline alexithymia scores, and heart rate/blood pressure overreactivity and under-recovery following experimental emotional stress tasks were significantly inversely associated with the MIP-1α stimulation index and with lower CD4+ cell count at follow-up; (2) baseline maladaptive Type C coping was associated with significantly higher IL-6 production at 36 months, as well as lower CD4+ cell count. These findings contribute to the growing evidence we are applying to developing a “natural” biopsychosocial anti-HIV intervention.
Correlation Between Circulating HIV-1 RNA and HIV-1 DNA Copies and the Neutralizing Antibody Response

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Background: Although several studies noted low levels of neutralizing antibodies in Elite Controllers, one recent study ded broadly neutralizing antibodies in 12% of Elite/Viremic Controllers. The objective of this study was to examine the relationship between HIV-1 antigenic load and plasma neutralization.

Methods: Plasma from 111 HIV-infected patients was tested for HIV neutralization against 15 Tier 1 and Tier 2 HIV pseudoviruses. Patients belonged to one these 4 cohorts: HIV-1 Natural Viral Suppressors (NVS, HIV viral loads <400 copies/ml without HAART), Low Viral Load (LVL, HIV-1 viral loads of 500-20,000 copies/ml without HAART), Medium/High Viral (MHVL, HIV-1 viral loads >20,000 copies/ml without HAART), HAART (patients on HAART with suppressed viral loads for > 1 year). Broadly neutralizing was defined as neutralization of >75% of Tier 2 viruses.

Results: For Tier 2 viruses, the LVL demonstrated greater ID80 titers compared to the NVS (p=.0007), MHVL (p=.0004), and HAART (p=.0019) groups. There was a quadratic correlation between HIV-1 DNA copies and HIV-1 neutralization titers (at the lowest proviral copy numbers, HIV neutralization was diminished, increasing steadily around a proviral copy number of 100, and again diminishing at higher values). There was also a correlation between plasma viral load and the presence of broadly neutralizing antibodies in those with HIV-1 RNA between 1000 and 5000 copies/ml to those <1,000 or >5,000 copies/ml (p=.021 and .04, respectively).

Conclusions: There was a quadratic correlation between HIV-1 DNA copies and HIV-1 neutralization, and a similar correlation between HIV-1 RNA copies and broad neutralization. These results indicate that low but persistent HIV antigen (approximately 103 copies of HIV-1 RNA/ml plasma) correlates with high levels of HIV neutralizing/broadly neutralizing activity. These findings may have important implications in understanding the HIV humoral response.

Emergence of Exhausted B Cells in Asymptomatic HIV-1-Infected Patients Naïve for HAART is Related to Reduced Immune Surveillance

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HIV-1 infection induces a general status of perturbation on immune system cells leading patients to be susceptible to opportunistic infections and tumours. Immune cells of HIV-1-infected patients show several functional defects associated to the chronic cell-hyperactivation and cell-exhaustion. Most of these impairments characterize advanced stage of HIV-1 disease (CD4+ T cell lymphopenia and ongoing HIV-1 replication).

To investigate if signs of B cells exhaustion and impaired viral immune-surveillance were present in asymptomatic HIV-1-infected patients with preserved CD4+ T cell counts and Highly Active Antiretroviral Therapy (HAART)-untreated.

Forty-three asymptomatic HAART-untreated HIV-1-infected patients were evaluated for immunological parameters defining immune exhaustion of B cells, and for Torque Teno Virus (TTV) viral load. Twenty aviremic HAART-treated patients and 34 healthy individuals were used as control groups. Peripheral blood samples were analyzed by Flow cytometry for the identification of B cell subpopulations. IL-7 serum levels were quantified by ELISA. TTV viral load was determined by real-time PCR. Mann-Whitney U-test and Spearman rank correlation coefficient test were used for statistical analysis.

Asymptomatic HIV-1-infected patients showed dramatic expansion of exhausted tissue-like memory B cells, which correlated with HIV-1 and TTV viral loads. They also displayed increased IL-7 levels. Normal B cell subsets, IL-7 levels and TTV viral load were observed in aviremic HAART-treated patients.

Taken together these results show that asymptomatic HIV-1-infected patients showed the emergence of exhausted B cell elements associated with an impaired control of TTV replication. Successfully HAART-treated patients showed normal B cell subpopulations frequency and TTV viral load. Early application of HAART may prevent the loss of immune functions.
Low Post-Seroconversion CD4 Count and Rapid Decrease of CD4 Density Identify HIV+ Fast Progressors

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Background: CD4 expression in HIV infection is paradoxical: HIV entry requires high cell-surface CD4 densities, but replication requires CD4 down-modulation. However, is CD4 density in HIV+ patients affected over time? Do changes in CD4 density correlate with disease progression? Here, we examined the role of CD4 density for HIV disease progression by longitudinally quantifying CD4 densities on CD4+ T-cells and monocytes of ART-naive HIV+ patients with different disease progression rates.

Methods: We defined three groups of HIV+ patients by their rate of CD4+ T-cell loss, calculated by the time between infection and reaching a CD4 level of 200 cells/µl: fast (<7.5 years), intermediate (7.5–12 years) and slow progressors (>12 years). Mathematical modeling permitted us to determine the maximum CD4+ T-cell count after HIV seroconversion (defined as “post-seroconversion CD4 count”) and longitudinal profiles of CD4 count and density. CD4 densities were quantified on CD4+ T-cells and monocytes from these patients and from healthy individuals by flow cytometry.

Results: Fast progressors had significantly lower post-seroconversion CD4 counts than other progressors. CD4 density on T-cells was lower in HIV+ patients than in healthy individuals and decreased more rapidly in fast than slow progressors. ART did not normalize CD4 density.

Conclusions: Thus, post-seroconversion CD4 counts define individual HIV disease progression rates that may help to identify patients who might benefit most from early antiretroviral therapy (ART). Early discrimination of slow and fast progressors suggests that critical events during primary infection define long-term outcome. A more rapid CD4 density decrease in fast progressors might contribute to progressive functional impairments of the immune response in advanced HIV infection. The lack of an effect of ART on CD4 density implies a persistent dysfunctional immune response by uncontrolled HIV infection.

Virological Characterization of HIV-1 Acute Infection by Ultra-Sensitive Next Generation Sequencing

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Aim: To establish HIV-1 RNA and proviral DNA dynamics at early times after seroconversion, to evaluate V3 quasispecies (viral diversity and proportion of X4 variants) and to assess correlations between early quasispecies composition and clinical evolution.

Methods: Nineteen HIV-acutely infected patients were enrolled within 4 wks from seroconversion (T0). Nine patients remained free of therapy, 10 started HAART according to standard guidelines. HIV RNA, proviral DNA and CD4 were monitored along a 6 month follow-up. Viral quasispecies was assessed at T0 by V3 ultra-deep pyrosequencing (UDPS); prediction of co-receptor usage was performed by PSSM.

Results: At T0, HIV-1 RNA and proviral DNA were positively correlated (r=0.494, p=0.032). No correlation between HIV-1 RNA and CD4 was observed. Diversity and proportion of X4 variants were positively correlated (r=0.677, p=0.001). The median proportion of X4 variants in the circulating HIV quasispecies was 0.3% (range: 0.0–56.25%), with only 9 out of 19 patients displaying X4 variants below 0.3%. At T0, CD4 cell counts were lower in patients who started treatment (p=0.028). In addition, a significant higher diversity was observed, at this time, in patients who underwent early treatment as compared to those who remained free of therapy (p=0.028). The decline of both HIV-1 RNA and HIV-1 DNA was significantly higher in HAART treated patients as compared to the untreated group (p<0.001 and p=0.040).

Discussion: UDPS may represent a breakthrough in the study of HIV pathogenesis, providing quantitative evaluation of viral diversity and X4 frequency in viral quasispecies. Filling of proviral DNA circulating reservoirs is an early event during acute infection and HAART is able to significantly counteract this phenomenon. The observed strong correlation between viral diversity and X4 frequency is consistent with the enrichment of X4 variants along virus evolution within each infected individual.
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Profiling Responses to a Lassa Vaccine Delivered to SIV-Infected Rhesus Macaques
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Lassa hemorrhagic fever infects more than 300,000 West Africans a year resulting in approximately 4000 fatalities. An attenuated Lassa vaccine candidate, ML29, protects against Lassa challenge in guinea pigs, marmosets, and rhesus macaques. However W. Africa has a high HIV prevalence and the impact of vaccination with ML29 in the context of AIDS is a concern. Here we determined whether ML29 could be safe and immunogenic in SIV-infected monkeys.

Eleven rhesus macaques, infected a year earlier with 10 TCID50 SIVmac251 and vaccinated with SIVgag-p27, were used in this study. Four SIV-negative macaques were used as controls. Six monkeys were vaccinated sc with ML29, 3 were ig vaccinated and 3 were not given the vaccine. None of the vaccinated animals showed signs of virulent arenavirus infection: high viral loads, high AST/ALT, dehydration, petechial rash, or respiratory distress occurring within 25 days after inoculation. Animals that succumbed later had classical symptoms of AIDS (swollen ileocaecal lymph nodes and SIV loads) and died at the same rate as SIV animals not given the vaccine.

8 of the 11 monkeys showed excellent virus-specific cell-mediated immune responses by intracellular staining for IFN-γ flow cytometry. All vaccinees eventually showed cell-mediated immune responses comparable to controls. All monkeys also developed antibody responses, though none were neutralizing. Bioinformatic profiling confirmed that responses to vaccination were similar in SIV and uninfected monkeys and were more similar to benign than to virulent arenavirus infection.

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A CD4-Independent gp41 Variant of HIV-1(BaL)
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HIV-1 infection normally requires interactions of the viral surface (gp120) glycoprotein with both cell surface CD4 and a chemokine coreceptor (usually CCR5 or CXCR4). Genetic variability, generally in gp120 or the gp41 ectodomain, can result in altered coreceptor use, fusion kinetics, and neutralization sensitivity.

Here we describe an R5 HIV variant that can infect T cell lines with low levels of cell surface CCR5. This is correlated with an ability to infect cells in the absence of any CD4, an increased sensitivity to a neutralizing antibody recognizing the coreceptor binding site of gp120, and an increased resistance to the fusion inhibitor T20. Surprisingly, these properties were determined in part by the cytoplasmic tail of gp41, a region not previously shown to influence coreceptor use. These data suggest that HIV infection of cells negative for CD4 can be facilitated by gp41 sequences that, although not exposed on the cell surface, induce allosteric changes in gp120 that facilitate exposure of the CCR5 binding site. We have constructed a SHIV using the Env of this variant that can infect rhesus macaques mucosally and will present data on infection, pathogenesis, and the host immune response to infection.
Derivation and Characterization of Novel Anti-HIV Envelope Monoclonal Antibodies from Macaques Vaccinated With a Constrained HIV Gp120 Immunogen and Challenged with SHIV162p3
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An approach to the development of an effective vaccine to HIV will be to generate antibodies that recognize conserved HIV envelope domains and thereby afford protection against heterologous infection. We have been exploring the approach of targeting epitopes located in the HIV-1 envelope glycoprotein (gp120) that are exposed on transition state structures formed after attachment to the CD4 receptor. These so-called CD4-induced (CD4i) epitopes comprise some of the most conserved and functionally important domains on the viral envelope including the co-receptor binding site; further, cognate antibodies appear in most HIV infected individuals and are cross-reactive for neutralizing and other antiviral activities against heterologous HIV strains in vitro. Towards this goal, rhesus macaques were vaccinated with a gp120 immunogen that is constrained into a transition state structure by covalent linkage to macaque CD4 (rhesus full-length sig-nal-chain; rhFLSC) in order to favor the immunogenicity of CD4i epitopes. These animals developed conventional cross-reactive neutralizing activities and exhibited non-sterilizing control of heterologous mucosal challenge with SHIV162P3 that correlated with stronger responses to CD4i epitopes in the rhFLSC-vaccinated animals. Further, viremia associated with infection significantly boosted conventional neutralizing and CD4i responses. To census and characterize the antibody specificities in these animals, we employed a new algorithm to isolate monoclonal antibodies (Mabs) from rhesus macaques. So far, we have isolated several anti-CD4i epitope Mabs from vaccinated/challenged animals that showed the most broadly neutralizing responses following vaccination and/or challenge. Antibodies with other specificities for the HIV envelope have also been identified. The variable (V)-gene usage, CDR-H3 length, potency and breadth of neutralization exhibited by various Mabs will be presented.

In Vivo Binding and Retention of CD4-Specific DARPin 57.2 in Macaques
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The recently described Designed Ankyrin Repeat Protein (DARPin) technology can produce highly selective ligands to a variety of biological targets at a low production cost. To investigate the in vivo use of DARPins for future application to novel anti-HIV strategies, we identified potent CD4-specific DARPins that recognize rhesus CD4 and followed the fate of intravenously injected CD4-specific DARPin 57.2 in rhesus macaques. The human CD4-specific DARPin 57.2 bound macaque CD4+ cells and exhibited potent inhibitory activity against SIV infection in vitro. DARPin 57.2 or the control E3_5 DARPin was injected into healthy SHIV-RT-infected rhesus macaques and the fate of cell-free and cell-bound CD4-specific DARPin was evaluated. DARPin-bound CD4+ cells were detected in the peripheral blood as early as 30 minutes after the injection, decreasing within 6 hours and being almost undetectable within 24 hours. The amount of DARPin bound was dependent on the amount of DARPin injected. CD4-specific DARPin was also detected on CD4+ cells in the lymph nodes within 24 hours. More extensive analysis using blood revealed that DARPin 57.2 bound to all CD4+ cell types (T cells, monocytes, dendritic cells) in vivo and in vitro with the amount of binding directly proportional to the amount of CD4 on the cell surface. Cell-free DARPins were also detected in the plasma, decreasing rapidly after 30 minutes. We demonstrate that the CD4-specific DARPin can rapidly and selectively bind its target cells in vivo, warranting further studies on possible clinical use of the DARPin technology.
Self-Renewing Monocytoid Cells (SRMC) Derived from Cultured Blood Monocytes as Targets and Hosts for Viruses

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Self-renewing monocytoid cells (SRMC) are small, recently identified cells that develop de novo within cultured human blood monocyte-derived macrophages termed “nurse macrophages” (NM). SRMC can differentiate into NM that produce another generation of SRMC; the NM/SRMC cycle can continue in vitro for several generations. NM can also produce, within themselves, cells of other lineages, including CD4+ T-lymphocytes, and cells expressing hematopoietic stem cell markers. (See abstracts by S. Gartner et al and Y. Liu et al.) Moreover, SRMC are highly susceptible to HIV entry leading to productive infection, and the virus-infected SRMC develop into classic HIV-expressing multinucleated giant cells. Because NM can produce lymphoid cells, we assessed infection of this cell system with HTLV-I and EBV. We hypothesized that HTLV-I infection of NM, either directly, or via initial SRMC infection, might lead to generation of virus-transformed T-cells with a more immature phenotype. For the HTLV-I studies, both purified SRMC and NM-containing primary macrophage cultures were used. Our preliminary results show that SRMC are highly susceptible to HTLV-I. Cell-free infection of SRMC was readily achieved, and the infected cells were able to differentiate into macrophages that maintained virus expression. Cocultivation of NM-containing macrophage cultures with irradiated HTLV-I-producing cells yielded populations with surface markers characteristic of immature T-cells and unlike typical transformants, could be expanded in the absence of IL-2. Exposure of purified SRMC to cell-free EBV led to enhanced development of NM from SRMC and the appearance of cells expressing EBV antigens. EBV+ cells also expressed CD19 or CD11b, markers of B-cell or macrophage lineage, respectively. Cells expressing both CD19 and CD11b were also seen, suggesting a possible NM origin for the CD19+ cells. The pluripotency of this SRMC/NM cell system provides for generation of a broad spectrum of highly susceptible and permissive hosts for infectious agents.

HIV Infection Risk Association with FCG Receptor Polymorphism

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Antibody-dependent cellular cytotoxicity (ADCC) is a mechanism for HIV-specific antibodies to target infected cells for destruction by cytotoxic effectors. The Fc portion of IgG antibodies bind cytotoxic effector cells through a family of Fc receptors that include FCGR3A (CD16). Alternately, antibody binding to Fc receptor delivers an activating signal to the effector cells. Thus there is a balance between effector cell stimulation and target cell killing during ADCC.

Single nucleotide polymorphisms (SNP) in the Fc receptor gene FCGR3A alter the amino acid sequence and encode high or low functioning proteins. We predicted that elite controller groups (HIV+ individuals with durable virus suppression without taking antiretroviral drugs) would have increased frequencies of the high functioning alleles, compared to HIV progressors or uninfected controls.

Genomic DNA was analyzed from individuals with no HIV infection, Elite controllers and HIV progressors on HAART for a known polymorphism in the FCGR3A gene that has functional relevance to ADCC function. The rs396991 polymorphism results in a T (F amino acid) or a G (V amino acid) allele at the SNP position. PCR was used to amplify a region of FCGR3A and an analysis of rs396691 was done using the ABISnapShot multiplex kit. An allele frequency of T (0.82) and G (0.18) for normal donors, T (0.75) and G (0.25) for elite controllers and T (0.58) and G (0.42) for HIV progressors. These results of higher frequencies of high functioning alleles in HIV progressors suggest that this allele may be responsible for chronic lymphocyte activation that is a feature of progressing HIV disease.
Characterization of Binding Affinity and Epitope Dynamics of Anti-HIV-1 Antibodies

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A preventive vaccine is potentially the most effective way to control the HIV pandemic. Such a vaccine needs to successfully harness humoral immunity and produce cross-reactive anti-envelope antibodies that mediate direct virus neutralization and/or Fc receptor-dependent killing. For these antibodies to carry out their functions in clearing HIV infection, they must bind the virus and prevent it from infecting target CD4+ cells. The capacity of an antibody to do this is dependent on the timing, duration and extent of cognate epitope exposure before and during the attachment and entry processes. The goal of this study was (i) to quantify antibody binding to HIV, and (ii) to characterize when and for how long antibody epitopes are exposed before and during virus-cell fusion. We studied the binding properties and epitope dynamics of previously characterized antibodies b12, 2G12, A32, C11, and 17b, as well as 5 novel antibodies isolated from an HIV-1 controller cohort (N5-O1.1, N12-O3.1, N12-I2, N12-I15, and L9-B1). To directly quantify antibody binding to virus in solution, we developed a fluorescence correlation spectroscopy (FCS) methodology that uses fluctuations in fluorescent signals to measure diffusion and reaction kinetics of fluorescently-labeled anti-envelope Mabs as they attach to HIV-1JRFL, HIV-1Bal, and HIV-1NL4-3 pseudoviruses and infectious molecular clones. We have also developed methods to visualize the temporal appearance and disappearance of cognate epitopes during virus-cell fusion using live-cell confocal microscopy. In this case, viral particles are labeled with a novel SNAP-tag technology that permits tracking of particles during different stages of fusion with CD4+ target cells, and concurrent imaging of epitopes that become exposed on the HIV envelope. Findings regarding the binding of new human anti-envelope Mabs to free and bound particles will be presented.

Characterization of Nurse Macrophage-Derived Cells That Express Stem Cell Markers

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We have observed that long-term cultures of human blood monocyte-derived macrophages contain populations of adherent cells we refer to as “nurse macrophages” (NM) that are capable of generating, within themselves, other kinds of cells. One type of cell produced is a previously unknown small cell (~6 µm) that exhibits a monocyte/macrophage immunophenotype and other features characteristic of macrophages such as adherence. These small cells lack the hematopoietic stem cell markers CD34, CD117 and CD133, and ~50% express CD135, a marker thought to distinguish multipotent progenitors. We named them self-renewing monocytooid cells (SRMC) because they could differentiate into NM that produce another generation of SRMC. The development of SRMC within NM, and their exit via budding, was captured by confocal microscopy. We used flow cytometry and confocal microscopy to identify and quantitate the cell populations produced in MDM cultures during 6 weeks or more of culture. No exogenous growth factors were used. Cells bearing CD34, CD117 or CD133 were detected at frequencies of <1% of the total nonadherent population, persisted for 2 months or more and their numbers often increased with time. Importantly, confocal microscopy showed cells with these markers developing within NM and budding from them. Because the majority of nonadherent cells in these cultures are maturing macrophages, we used 3.0 µm transwell filters to select out the small cells for further characterization. Filtered cells expressing the macrophage marker CD11b along with CD90, CD117, CD133 or CD135 were observed, suggesting their recent exit from NM. Surprisingly, we also observed nuclear expression of the embryonic stem cell markers NANOG and Oct-4 in some filtered cells. Molecular characterization and differentiation studies of NM-derived populations are underway. Our findings suggest that NM can be a source of multipotent stem and progenitor cells.
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Novel Functional mAbs Against HIV-1 Gp120 from HIV Controller
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Despite over 20 years efforts, there is still no AIDS vaccine. Identification of new functional mAbs against conserved epitopes of HIV-1 Env should help guide the development of a vaccine. We previously reported strong memory B cell responses against HIV-1 Env in HIV-1 natural viral suppressor (NVS) patients. To test whether these B memory cells are protective, it is necessary to clone and characterize their Abs.

Using a novel practical method for rapid cloning human mAbs from memory B cells, novel antibodies were cloned and mapped by a series of assays including binding to different Env antigens in ELISA, competition of Env proteins binding to cellular CD4 or CCR5 receptors by flow cytometer, competition ELISA. Neutralization assay and ADCC assay were used to test their function.

Fifty new mAbs were isolated by our method. 46 are against the conserved epitopes of CD4-induced (CD4i) CCR5 binding site or CD4 binding site (CD4bs) and 4 are V3 specific. Most of these CD4bs and CD4i mAbs neutralize clade B, C and/or A viruses together with mediating ADCC activity. 3 of the 4 V3 mAbs contain strong ADCC function and potent neutralizing activity against tier 1 clade B viruses. Interestingly, 6 novel non-neutralizing CD4i mAbs showed very potent ADCC function in vitro.

In conclusion, a novel rapid method for direct expression clone of human mAb from memory B cell was successful in practice and a large panel of novel functional mAbs against conserved epitopes of HIV-1 Env were identified. Unique non-neutralizing mAb capable of potent ADCC has been identified for the first time. Passive immunization of these novel functional mAbs will help guide the development of AIDS vaccine.

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HIV Persistence in Dendritic Cells - Contribution of NK Cells and Pivotal Role of HMGB1
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Persistent viruses are able to subvert host antiviral immunity, thereby facilitating viral dissemination. In particular, HIV-1 has evolved ways to exploit dendritic cells (DCs) by blocking the “editing process” that physiologically allows NK cells to keep in check the quality of DCs through their elimination when they are altered. In the context of viral infections, NK-mediated killing of infected DCs is an essential step for early control of viral replication and avoidance of dissemination. In this study, we addressed the question of the impact of HIV-1 infection on DCs’ susceptibility to NK killing.

We report that uninfected iDCs are susceptible to NK-mediated killing, which involves the TRAIL/DR4 pathway. In contrast, HIV-1-infected DCs become resistant to NK-mediated killing. This is due to the dramatic upregulation of two anti-apoptotic molecules, c-FLIP and c-IAP2, in infected DCs, inducing their resistance to NK (TRAIL)-mediated apoptosis. Interestingly, the alarmin HMGB1, expressed at the synapse between NK cells and DCs, was found to play a pivotal role in this process, and inhibition of its activity by glycyrrhizin or specific antibodies restored NK-dependent killing of infected DCs through the inhibition of c-FLIP and c-IAP2 upregulation. Moreover, the crosstalk between NK cells and infected DCs resulted in a dramatic increase in viral replication in DCs, that was associated with a strong impairment of their ability to induce Th1 polarization.

Overall, these observations provide new insights into how HIV hijacks DCs to promote viral persistence and dissemination, and uses NK-DC interaction to promote the survival of infected DCs, thus establishing long-term reservoirs. In addition, they challenge the question of the in vivo involvement of HMGB1 in the triggering of viral replication, the in vivo expression of HMGB1 being correlated with viral load and disease progression.
222 HIV-1 p17 Activates PTEN and Inhibits Akt Signaling Pathway in B Cells: Evidence for a Variant with Different Effects on Signaling and Cell Growth

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The HIV-1 matrix protein p17 is a structural protein that can act in the extracellular environment as a virokine to deregulate several immune functions, through a specific interaction with a cellular surface receptor (p17R). At the moment signal transduction pathways triggered by p17/p17R interaction are not completely characterized yet. In this study we map the structure-function relationship of p17, analyzing the specific signal transduction pathways induced by different functional epitopes involved in p17/p17R interaction.

We demonstrate that p17 displays two distinct functional epitopes involved in p17R binding capable of selectively activating or deactivating a variety of signalling pathways. In particular, we show that p17 induces a transient activation of the transcriptional factor AP-1 in Raji cells and it upregulates MAPK/ERK and downregulates PI3K/AKT signal cascades, which are the major intracellular signalling pathways involved in AP-1 activation and in cellular functions such as cell growth, survival and tumourigenesis. Our data also demonstrate that the effect of p17 on AKT signaling is due to its capability to keep PTEN, a phosphatase that regulates the PI3-K/AKT pathway, in an active state through the Ser/Thr kinase ROCK, recently reported to control PTEN activity. Moreover, we show that among the different p17 variants one named S75X triggers ,on contrary to p17, an activation of PI3K/Akt signalling pathway and a consequent increase of cell proliferation which could be correlate with the development of a large fraction of human cancers in HIV+ patients.

In summary, this study demonstrates the complexity of p17 binding to and signalling through its receptor, and suggests that a conformational change of the protein due to specific escape mutations may play an important role in receptor binding, signaling and cell growth.

223 The Newly-Discovered Human Endogenous Retrovirus HERV-K 111 is Transcriptionally-Active Only Upon HIV Infection

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We previously reported finding the RNA of a particle-coding human endogenous retrovirus type K, HERV-K (HML-2), in the plasma of HIV-1 patients. We found that the HERV-K (HML-2) RNA was contained in HERV-K viral particles as observed by immuno-electron microscopy. Surprisingly, a novel HERV-K (HML-2) provirus was discovered, termed K111, which seems to be transcriptionally active exclusively during HIV infection. Using real time RT-PCR specific for the K111 env sequence, we corroborate the detection of K111 RNA in plasma of HIV patients and not in breast cancer or lymphoma patients. K111 RNA was also found in the supernatants of cultured cell lines and PBMCs infected with R5 and X4 HIV strains. A K111 counterpart was found to be present in Chimpanzees. The human K111 provirus was fully amplified and sequenced. K111 proviruses were detected in all human DNA samples tested (140 individuals and 10 cell lines) and may produce only the Np9 oncoprotein. K111 is polymorphic in humans, represented by a minimum of at least 6 copies in each individual. K111 soloLTRs can also be found in the centromeric region. All these variants are integrated into tandemly repetitive D22Z3 sequences, which have been found uniquely in the centromere of chromosome 22. The degree of variability in the different K111 forms is only appreciated in HIV infected patients, where these proviruses are transcriptionally active. Furthermore, the discovery of the first centromeric HERV-K (HML-2) described so far might have only been possible due to a unique effect of HIV on chromatin remodeling of the centromere of chromosome 22, exposing K111 to active transcription.
The MHC-II Transactivator, CIITA, Inhibits Tat-Mediated HIV-1 LTR Transactivation and Virus Replication in Human U937 Monocytic Cells

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We have previously shown that the MHC-II transactivator, CIITA, inhibits the replication of HIV-1 in human T cells by targeting the viral transactivator Tat.

The aim of this study was to analyze the anti-viral function of CIITA in a monocyte-macrophage model of HIV-1 infection. To this purpose U937 cell clones, named plus and minus sustain efficiently or poorly HIV-1 replication, respectively (Franzoso et al. 94, J.Exp. Med. 180:1445). Noteworthy, unstimulated minus clones express MHC-II molecules on their cell surface whereas plus clones do not. We here demonstrate that this phenotype correlates with the expression, or the absence, of CIITA protein in minus and in plus clones, respectively. Interestingly, plus cells allowed Tat-dependent LTR transactivation, whereas the minus cells did not. In order to better understand the role played by CIITA, we stably transfected plus cells with a CIITA expression vector and selected several clones. Quite strikingly, the transcriptional activity of Tat on the HIV-1 LTR was inhibited in all plus-CIITA clones. Furthermore, preliminary experiments of HIV-1 infection of plus-CIITA clones suggest that CIITA-mediated functional block of Tat correlates with the inhibition of viral replication. Therefore, CIITA suppresses HIV-1 LTR transactivation by Tat also in a model of monocytic cell infection. These findings demonstrate that CIITA has a dual function against HIV-1: on the one hand, it positively modulates the adaptive immune response to the infection by promoting antigen presentation and, on the other hand, it acts as an endogenous restriction factor against viral transcription. Thus, modulation of CIITA expression in HIV-1-infected individuals should be investigated as a strategy to boost the immune response and, simultaneously, to inhibit HIV-1 replication and spreading.

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Engineered Lactobacilli as a System to Screen Potent RANTES Mutants Acting as Live CCR5 Antagonist HIV-1 Blockers
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As AIDS preventatives, topical microbicides represent an alternative and complementary option to a vaccine, and CCR5 (the main HIV-1 coreceptor) is a target of major interest. The live microbicide concept, based on the engineering of commensal bacteria to deliver anti-HIV-1 agents in vivo and in situ, is the landscape in which this work has been envisaged and devised. The successful production of the wild type chemokine RANTES (a natural ligand of CCR5) and the C1C5 RANTES mutant (a CCR5 antagonist) in lactobacilli and the evidence for a superior expression/folding by lactobacilli, as compared to mammalian cells, set the standards for lactobacilli as a platform to integrate design and selection of conceptually novel full-length RANTES derivatives with potent anti-HIV-1 activity and CCR5 antagonism (crucial to avoid pro-inflammatory activity). Two lactobacilli strains have been implemented, providing vaginal and intestinal applicability, respectively. Hot spot mutations spanning through RANTES aa sequence were retrieved or designed ex novo to achieve CCR5 antagonism and potent anti-HIV-1 activity. The lactobacilli-induced partial proteolytic cleavage occurring between RANTES aa positions 12 and 13 is also being resolved by mutagenesis. Lactobacilli codon-optimized cDNAs encoding for RANTES mutants were inserted into vectors suitable for lactobacilli protein secretion. Protein quantity and forms secreted by engineered lactobacilli were analyzed by ELISA and Western blot, respectively. Qualitative anti-HIV-1 activity was tested by acute infection assays on partially purified proteins. These procedures flowed in iterative cycles, with a stepwise integration of successful mutations in single CCR5 antagonist RANTES derivatives. The system is designed to collectively yield a small number of lead compounds presenting ideal features as live microbicides. The most effective leads will be purified and accurately tested for: i) CCR5 antagonism, ii) anti-HIV-1 activity, iii) protein full-length, and iv) correct disulphide bonding.

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Kinome Profiling of Bovine Leukemia Virus-induced Ovine Leukemia: An Approach for Identifying Altered Signaling Pathways and Drugable Targets in Cancer
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Screening new therapeutic agents which specifically inhibit phosphorylation of key cell signaling molecules has become a major focus in cancer research. It is critical, however, that the animal model or cell system used to screen therapeutic agents accurately reflect the biology of the target cancer cells. With the development of species-specific kinome arrays and their application in the Bovine Leukemia Virus (BLV) ovine leukemia model, it was possible to analyze changes in kinase activities associated with B cell transformation. Removal of cancer cells from the host and passage in tissue culture significantly altered phosphorylation patterns that define transformation of this specific cell lineage, suggesting that cell signaling closely reflects responses to the external environment. Thus, high-throughput kinome analysis in this unique large animal model of leukemogenesis provides an opportunity to identify critical phosphorylation events governing onset and progression of malignancy and define whether key cell signaling events that characterize primary cancer cells are accurately reflected in in vivo or in vitro screening models.
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Glycosylation Profiling of the HIV-1 gp120-CD4 Full Length Single Chain Complex
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The HIV-1 envelope glycoprotein gp120 is a major target for HIV-1 vaccine. A typical gp120 carries more than 20 N-glycans that account for about half of the molecular mass of the envelope glycoprotein. Compelling experimental data have shown that glycosylation of gp120 can have a profound effect on the processing, maturation, and immunogenicity of gp120. Moreover, the dense carbohydrate shield forms a strong barrier that helps protect HIV from immune recognition and limits effectiveness of antibody neutralization. The glycosylation patterns on several HIV-1 gp120, including strains of JR-FL, IIIB, and SF2, have been analyzed. These studies revealed a significant difference in glycosylation patterns among different HIV-1 strains. Therefore, characterizing the glycosylation profiles and correlating the glycosylation patterns with the immunogenicity are essential first steps towards HIV-1 vaccine design. We have initiated a project on characterizing the glycosylation profile of the full-length HIV-1Bal gp120/CD4 single chain complex, a candidate vaccine. Our data indicated that the nature of glycans from the CHO-expressed and HEK293 cell line expressed HIV-1Bal gp120 was significantly difficult. In addition, we were able to achieve selective de-glycosylation, which led to novel glycoforms that demonstrated enhanced binding to antibody 2G12. The results suggest that neighboring complex type N-glycans partially occlude the epitope (a high-mannose N-glycan cluster) of 2G12. A mass spectrometry-based (LC/MS/MS), glycoproteomic approach is being used to characterize the glycosylation sites and the nature of N-glycans on each site, which will define how individual N-glycans modulate the immunogenicity of the complex.

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HIV-1 Replication through hHR23A-Mediated Interaction of Vpr with 26S Proteasome
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HIV-1 Vpr is a virion-associated protein. Its activities link to viral pathogenesis and disease progression of HIV-infected patients. In vitro, Vpr moderately activates HIV-1 replication in proliferating T cells, but it is required for efficient viral infection and replication in vivo in non-dividing cells such as macrophages. How exactly Vpr contributes to viral replication remains elusive. We show here that Vpr stimulates HIV-1 replication at least in part through its interaction with hHR23A, a protein that binds to 19S subunit of the 26S proteasome and shuttles ubiquitinated proteins to the proteasome for degradation. The Vpr-proteasome interaction was initially discovered in fission yeast, where Vpr was shown to associate with Mts4 and Mts2, two 19S-associated proteins. The interaction of Vpr with the 19S subunit of the proteasome was further confirmed in mammalian cells where Vpr associates with the mammalian orthologues of fission yeast Mts4 and S5a. Consistently, depletion of hHR23A interrupts interaction of Vpr with proteasome in mammalian cells. Furthermore, Vpr promotes hHR23A-mediated protein-ubiquitination, and down-regulation of hHR23A using RNAi significantly reduced viral replication in non-proliferating MAGI-CCR5 cells and primary macrophages. These findings suggest that Vpr-proteasome interaction might counteract certain host restriction factor(s) to stimulate viral replication in non-dividing cells.
**VEGFR2 and αvβ3 Recruitment and Cross-Talk at the Basal Aspect of HIV-1 Tat-Adherent Endothelial Cells Drives pp60src/ERK1/2 Activation, Cytoskeleton Organization and Pro-angiogenic Activation**

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HIV-1 Tat protein released by infected cells accumulates in the extracellular matrix. In this substrate-immobilized form it promotes endothelial cell (EC) adhesion and consequent FAK/RhoA/pp60src/NF-kB activation and pro-angiogenic activation. All these effects, except pp60src activation, are dependent on αvβ3 engagement and activation by Tat.

Here we demonstrate that EC adhesion to substrate-immobilized Tat induces the recruitment in the ventral plasma membrane (VPM) of αvβ3, paxillin, focal adhesion kinase (FAK), pp60src and the vascular endothelial growth factor receptor-2 (VEGFR2). As expected, paxillin and αvβ3 co-localize in the focal adhesion plaques of ECs adherent to the αvβ3-ligands Tat, vitronectin (VN) and fibrinogen (FG), but not to the αvβ1-ligand collagen. At variance, VEGFR2 recruited at the VPM of Tat- (but not FG-) adherent ECs localizes in lipid rafts where it undergoes phosphorylation. In turn, VEGFR2 activation triggers pp60src and ERK1/2 phosphorylation. The VEGFR2/pp60src/ERK1/2 signaling pathway eventually leads to a proper cytoskeleton organization and pro-angiogenic activation of Tat-adherent ECs.

In conclusion, VEGFR2 and αvβ3 are recruited in VPM of Tat-adherent ECs, in lipid rafts and focal adhesion plaques, respectively. VEGFR2 undergoes phosphorylation and triggers a signal transduction pathway (including pp60src and ERK1/2) that is distinct from the pathway activated by αvβ3. And is required for cytoskeleton re-organization and pro-angiogenic activation of ECs. These results provide new biochemical and biological insights in the cross-talk between integrin, angiogenic growth factors and tyrosine kinase receptors during angiogenesis.

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**The MHC Class II Transactivator, CIITA, is a Viral Restriction Factor for Human Oncogenic Retroviruses**

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We show that CIITA, the master regulator of MHC-II gene expression, inhibits the replication of HTLV-1 by targeting the viral transactivator Tax-1. CIITA and Tax-1 physically interact in vivo and the first 108 amino acids of Tax-1 are necessary for this binding. Two adjacent regions (1-252 and 253-410) of CIITA bind independently to Tax-1, but only region 1-252 mediates Tax-1 inhibition, in agreement with the fact that CIITA residues from positions 64-124 are required to block Tax-1 transactivation. The CIITA inhibitory action on Tax-1 function correlates with the nuclear localization of CIITA and is independent of the transcription factor NF-YB, previously involved in CIITA-mediated inhibition of HTLV-2 Tax-2. CIITA severely impairs the physical and functional interaction of Tax-1 with the cellular co-activator PCAF, which is required for the optimal activation of HTLV-1 promoter. Accordingly, CIITA-inhibited transactivation of the viral LTR promoter by Tax-1, is rescued by the over-expression of PCAF.

Together with the previously reported inhibition of other human retroviruses, HIV-1 and HTLV-2, these findings support the idea that CIITA might have evolved as a general defense mechanism of the host against retroviruses, not only because it activates the immune response against the infectious agents, but also because of its intrinsic capacity to act as an endogenous viral restriction factor.

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Plasmacytoid Dendritic Cells Accumulate and Secrete Interferon Alpha in Lymph Nodes of Chronically Infected, Treatment-Naïve HIV-1 Patients

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During the chronic phase of HIV-1 infection, circulating plasmacytoid dendritic cells (pDC) progressively decline, and express higher interferon alpha (IFNα) levels, which is associated with disease progression. To elucidate the fate and function of pDC during chronic, untreated HIV-1 infection, we analyzed the expression levels and function of several tissue-homing markers on circulating pDC from HIV-1 patients (n = 17) and uninfected controls (n = 11). We also determined pDC frequency, expression levels of activation/maturation markers and IFNα, and the rate of cell death of pDC in lymph nodes untreated HIV-1 patients (n = 18) and healthy controls (n = 11). We found significantly higher CCR7 and CD62L (p<0.05) and CD103 (p<0.01) levels on circulating pDC from HIV-1 patients. All other markers were unchanged. Higher CCR7 levels led to increased migration in response to CCR7 ligands (p<0.01). In addition, higher CCR7 levels and migratory potential correlated directly with HIV-1 viremia (p<0.001, r = 0.79; and p = 0.01, r = 0.7), and inversely with pDC frequency in blood (p = 0.008, r = -0.6; and p = 0.04, r = -0.69). We also found and accumulation of pDC in LN of HIV-1 patients (p = 0.002). LN-homed pDC of HIV-1 patients expressed higher CD40 (p<0.0001), lower BDCA2 (p<0.03), but stable 83 and CD86 levels (p>0.05). Moreover, these cells secreted markedly higher IFNα levels compared to controls (p<0.01), which correlated with higher rates of cell death (p = 0.04, r = 0.48). These results demonstrate that untreated HIV-1 infection is characterized by elevated CCR7 and CD62L expression on circulating pDC, which relocate to lymph nodes, acquire an activated but immature phenotype, and express elevated amounts of IFNα before undergoing cell death. Chronic IFNα expression in lymph nodes of HIV-1 patients may impair differentiation and immune function of bystander CD4+ T cells, contributing to the mechanisms of pathogenesis.

Interleukin-21 (IL-21) is a Determinant of H1N1 Vaccine Induced Antibody Response in HIV-Infected Persons

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Background: The cytokine IL-21 is secreted mainly by CD4+ T cells including CXCR5+T follicular helper cell subset (Tfh). IL-21 has been shown to augment cytotoxic granule accumulation in CD8 T cells and to facilitate B cell differentiation and immunoglobulin secretion. Recently, HIV infected patients were reported to have dysregulated IL-21 production. This study investigated the role of IL-21 in H1N1 vaccine induced antibody responses in HIV infected persons.

Methods: 17 HIV infected aviremic persons were evaluated pre- and 28 days post- a single intramuscular 15μg dose of inactivated, monovalent A/California/07/2009 H1N1 vaccine (Sanofi Pasteur). Nine of 17 patients (53%) developed a protective antibody titer (>40 Hemagglutinin inhibition units) and were designated as vaccine responders.

Results: Compared to vaccine non-responders, at 28 days post vaccination, responders manifested increases a), in serum IL-21 levels by ELISA (pg/ml) over baseline (116 ± 3.9 vs. 128.9 ± 10.8; P =0.0018), b), in IL-21R expression on CD20+ total B cells (15.1 ±6.3 vs. 26.7±7.8; p = 0.003), and c), in the frequencies of B cell activation factor (BAFF) binding receptors (BCMA and TACI) on total B cells. Serum H1N1 antibody titers correlated with IL-21 levels (r= 0.83; p= <0.0001). At baseline, serum IL-21 levels correlated with frequency of CD4+CXCR5+ cells (r = 0.64; p=0.004) and with frequencies of CD4+ T cells and of CD4+CXCR5+ Tfh cells induced to express intracellular IL-21 following PMA+ionomycin stimulation (r = 0.67; p =0.003 and r = 0.49; p =0.046 respectively).

Conclusion: IL-21 producing CD4+ T cells could deliver important B cell help for differentiation and production of protective antibodies after H1N1 vaccination. Pre-immunization frequency of CD4+CXCR5+ Tfh cells may be important determinants of vaccine induced stimulation of IL-21 production and subsequent H1N1 antibody response.
CME Information

Overview

In the 25 years since the first reports of the disease, AIDS has become a global epidemic. Worldwide, an estimated 38.6 million people are living with HIV, nearly half of them women and girls between the ages of 15 and 24. And though the spread of the virus has slowed in some countries, it has escalated or remained steady in others. In 2005, more than 4 million people were newly infected with HIV; 25 million have died of AIDS since the epidemic began.

Despite improved treatments and better access to care for people in the hardest-hit parts of the world, most experts agree that the pandemic is still in the early stages. With a vaccine probably decades away, the best hope for stemming the spread of HIV now lies in prevention, treatment, and education.

The 11th Annual International Meeting of the Institute of Human Virology will target physicians, scientists and other health care professionals involved in research, patient care, public health, and disease prevention. This meeting provides three full days of continuing educational courses and comprehensive scientific developments in infectious diseases: advances in HIV therapeutics, HIV and lymphoma genesis, controlling replication of human retroviruses, latency updates, Hepatitis C, HIV entry, development of HIV vaccines, pathophysiology, diagnosis, treatment, and prevention.

Target Audience

Clinicians and researchers in infectious disease, internal medicine, microbiology, and retro-virology, as well as health professionals in disease prevention and public health, from a variety of practice settings and work situations worldwide.

Learning Objectives

At the conclusion of this course, participants should be able to:

• Summarize and evaluate progression and anti progression factors in patients with HIV documented infections
• Outline new clinical developments in HIV treatment as they relate to available therapeutics in all medication classes
• Differentiate lymphoma genesis in the context of HIV infection including B-cell activation
• Formulate a hypotheses discussing replication control of the human retroviruses
• Discuss new clinical evidence on HIV viral latency including progression and manipulation
• Identify clinical advances in the treatment of the Hepatitis C virus
• Recall and interpret early events associated with HIV Infection
• Review the HIV therapeutic class of entry inhibitors
• Discuss advances in proteomics in HIV infection and therapeutics
• CME Sponsorship

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Presentations in this continuing medical education activity may contain references to unlabeled or unapproved uses of drugs or devices. The audience is advised to consult the full prescribing information of all drugs or devices prior to use.