

7th International
Discussion Meeting on
HIV Dynamics and Evolution



April 28-30, 2000
University of Washington and
Fred Hutchinson Cancer Research Center
Seattle, Washington, USA
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Schedule Overview

Morning, Friday, April 28, 2000

- 7:00 Registration
- 8:15 Opening Remarks by Lee Hartwell and Gerald Learn
- 8:30 Session 1: Evolution and Global Variation of HIV & Related Viruses
- 9:45 Break
- 11:45 Lunch

Afternoon, Friday, April 28, 2000

- 1:00 Session 2: HIV Evolution and Transmission
- 3:05 Break
- 4:15 Session 3: Evolution and Drug Resistance

Evening, Friday, April 28, 2000

- 6:45 Banquet Skyline Level of the Space Needle - - Speaker: Steven Austad,

Busses leave for Space Needle at 6:45

Morning, Saturday, April 29, 2000

- 8:45 Session 4: Lessons from Other Viruses
- 9:45 Break
- 10:50 Session 3: Evolution and Drug Resistance Continued
- 11:40 Lunch

Afternoon, Saturday, April 29, 2000

- 12:40 Session 5: Viral Variability and the Immune Response
- 3:10 Break
- 3:25 Session 6: Mathematical Modeling of HIV Dynamics & Evolution
- 7:00 Cocktail Hour & Poster Session

Morning Sunday, April 30, 2000

- 8:30 Session 7: Evolutionary Genetics of HIV
- 9:45 Break
- 11:50 End of talks

Schedule

Morning, Friday, April 28, 2000

7:00-8:15

Registration

8:15-8:30

Lee Hartwell, *Fred Hutchinson Cancer Research Ctr., Seattle, USA*: Opening Remarks

Gerald Learn, *University of Washington, Seattle, USA*: Opening Remarks

Session 1: Evolution and Global Variation of HIV & Related Viruses

Chairs: David Robertson & Frederick Bibollet-Ruche

8:30-8:55

Paul Sharp, *University of Nottingham, UK*: Complex Origins of SIV From Red-Capped Mangabeys

8:55-9:20

Frederick Bibollet-Ruche, *University of Alabama, Birmingham, USA*: Molecular Characterization of Primate Lentiviruses from DeBrazza, Mona and Blue Monkeys

9:20-9:45

Vanessa Hirsch, *National Institutes of Health, Rockville, USA*: Characterization of SIV from the L'Hoest Superspecies

9:45-10:05 Break

10:05-10:30

Anne-Mieke Vandamme, *University of Leuven, Belgium*: Dating the Origin of HIV-1 Group M and HIV-1 Group M/SIV_{cpz} Separation

10:30-10:55

Eric Delaporte, *IRD, Montpellier, France*: Identification and Molecular Characterization of a New Primate Lentivirus (SIV_{col}) from Colobus Monkeys in Cameroon

10:55-11:20

Pierre Roques, *CRSSA, Fontenay-aux-Roses, France*: Molecular Relationships Between HIV-1 Group N and SIV_{cpz} (*Pan troglodytes troglodytes*) Strains

11:20-11:45

David Robertson, *Oxford University, UK*: The HIV Nomenclature

11:45-1:00 Lunch

Afternoon, Friday, April 28, 2000

Session 2: HIV Evolution and Transmission

Chairs: Marcia Kalish & Tom Folks

1:00-1:25

Michaela Muller-Trutwin, *Institut Pasteur, Paris, France*: Viral Dynamics During Primary SIV Infection in its Natural Host

1:25-1:50

Gabriella Scarlatti, *Ospedale San Raffaele, Milan, Italy*: Early Detection Of Molecular And Biological Heterogeneity In HIV-1 Isolates From Children With Slow And Fast Disease Progression

1:50-2:15

Thomas Leitner, *Karolinska Institute, Solna, Sweden*: Competing Subpopulations after Multiple HIV-1 Mother-to-Child Transmission

2:15-2:40

E. Michelle Long, *Fred Hutchinson Cancer Research Center, Seattle, USA*: Gender Differences In HIV-1 Diversity At Time Of Infection

2:40-3:05

Linqi Zhang, *Aaron Diamond AIDS Research Center, New York, USA*: Genetic Characterization of the Rebounding HIV-1 after Cessation of HAART

3:05-3:25 Break

3:25-3:50

Tae-Wook Chun, *National Institutes of Health, Bethesda, USA*: Relationship Between Pre-Existing Viral Reservoirs and the Re-Emergence of Plasma Viremia Following Discontinuation of Highly Active Antiretroviral Therapy

3:50-4:15

Zvi Grossman, *National Cancer Institute, Bethesda, USA*: Proximal Immune Activation and HIV Transmission: Implications for HIV Transmission and Evolution

Session 3: Evolution and Drug Resistance

Chair: Andrew Leigh Brown

4:15-4:40

François Clavel, *INSERM, Paris, France*: HIV Drug Resistance and Fitness

4:40-5:05

Ronald Swanstrom, *University of North Carolina, Chapel Hill, USA*: Dynamic Behavior of HIV-1 Populations While Under Strong Selective Pressure

5:05-5:30

Jaap Goudsmit, *University of Amsterdam, The Netherlands*: Bottleneck Transmission of Nucleoside Analogue-Resistant HIV-1 and the Establishment of New HIV-1 RT Wild Types

5:30-5:55

Charles Boucher, *University Hospital, Utrecht, The Netherlands*: Difference In Resistance Contribute More Strongly To The Evolution Of Zidovudine Resistance In HIV-1 Infected Patients Than Difference In Replication Capacity

Evening, Friday, April 28, 2000

6:45-10:00 Banquet

Skyline Level, Space Needle

Busses leave for Space Needle at 6:45

Speaker: Steven Austad, *University of Idaho, Moscow, USA*: The Curse of Tithonus: Why Evolution Makes Us Age and What Molecular Biology Can do About It

Morning, Saturday, April 29, 2000

Session 4: Lessons from Other Viruses

Chair: Avidan U. Neumann

8:45-8:55

Avidan U. Neumann, *Bar-Ilan University, Ramat-Gan, Israel*: Introduction to Hepatitis C Virus

8:55-9:20

Derek Smith, *University of New Mexico, Albuquerque, USA*: Variable Efficacy Of Repeated Annual Influenza Vaccination

9:20-9:45

Margaret Koziel, *Harvard University, Cambridge, USA*: Immunopathogenesis of Hepatitis C Viruses

9:45-10:00 Break

10:00-10:25

Jean-Michel Pawlotsky, *Hopital Henri Mondor, Creteil, France*: Hepatitis C Virus Quasi-Species Intra-host Evolution

10:25-10:50

Avidan U. Neumann, *Bar-Ilan University, Ramat-Gan, Israel*: Clinical Implications of Hepatitis C Virus Dynamics

Session 3: Evolution and Drug Resistance (Continued)

Chair: Andrew Leigh-Brown

10:50-11:15

Jeanette Whitcomb, *ViroLogic, South San Francisco, USA*: HIV-1 Hypersensitivity to NNRTI is Associated with Reduced Susceptibility to NRTI

11:15-11:40

Andrew Leigh Brown, *University of Edinburgh, Scotland*: Identifying Mutations with Small Effects on Drug Resistance

11:40-12:40 Lunch

Afternoon, Saturday, April 29, 2000

Session 5: Viral Variability and the Immune Response

Chair: Bette Korber

12:40-1:05

Doug Nixon, *Aaron Diamond AIDS Research Center, New York, USA*: A Novel Single Cell Flow Based Killing Assay To Measure CTL Responses To Viral Variants

1:05-1:30

David Watkins, *University of Wisconsin-Madison, USA*: Tat-Specific CTL Responses Select for Escape Variants During Acute SIV Infection

1:30-1:55

Frances Gotch, *Chelsea and Westminster Hospital, London, UK*: Immune Responses to HIV in Uganda

1:55-2:20

Philip Goulder, *Massachusetts General Hospital and Harvard Medical School, Charlestown, USA*: Qualitative Differences Between HIV-Specific CTL Responses as Reflected by the Presence or Absence of Epitope-Specific Escape Mutation

2:20-2:45

Gerald Learn, *University of Washington, Seattle, USA*: The Use of an Inferred Epidemic Ancestral Sequence as a Vaccine Immunogen

2:45-3:10

Bette Korber and Brian Gaschen, *Los Alamos National Laboratory, USA*: Global Variability of Defined CTL Epitopes

3:10-3:25 Break

Session 6: Mathematical Modeling of HIV Dynamics & Evolution

Chairs: John Mittler & Alan Perelson

3:25-3:50

Dimitar Dimitrov, *FCRDC, Frederick, USA*: Comparative Analysis of HIV/SHIV Dynamics in Cultured Cells, Primary Infections of Monkeys and Humans Off Therapy

3:50-4:15

Bob Grant, *University of California San Francisco, USA*: Viremia Increases During Treatment Interruption After Long Term Virologic Failure

4:15-4:40

William Hlavacek, *Los Alamos National Laboratory, USA*: Follicular Dendritic Cells and HIV Dynamics

4:40-5:05

Duncan Callaway, *Cornell University, Ithaca, USA*: Intermittent Viremia in HIV-1 Infected Patients Receiving Antiretroviral Therapy: A Dynamic Point of View

5:05-5:30

Ruy Ribeiro, *Los Alamos National Laboratory, USA*: Measuring T-Cell Turnover

7:00-9:00 Cocktail Hour & Poster Session

Helix Café, FHCRC

Session 7: Evolutionary Genetics of HIV
Chair: Allen Rodrigo

8:30-8:55

Igor Rouzine, *Tufts University, Boston, USA*: Stochastic Evolution of a Single Locus in a Virus Population: An Analytic Review

8:55-9:20

Simon Frost, *University of Edinburgh, Scotland*: Genetic Drift and Within Host Metapopulation Structure in the Evolution of HIV

9:20-9:45

Allen Rodrigo, *University of Auckland, New Zealand*: Evolutionary Analyses of Serially Sampled Populations

9:45-10:10 Break

10:10-10:35

Daniel A. Vasco and Keith A. Crandall, *Brigham Young University, Provo, USA*: Applications of Coalescent Statistical Methods to HIV Data Analysis

10:35-10:00

Rasmus Nielsen, *Harvard University, Cambridge, USA*: Detecting selection in the HIV-1 genome using the dn/ds ratio.

11:00-11:25

Yun-Xin Fu, *University of Texas at Houston, USA*: Estimating the Generation Time of Within-Host HIV Populations

11:25-11:50

Jim Mullins, *University of Washington, Seattle, USA*: Measuring In-vivo migration and Compartmentalization of HIV

Abstracts for Oral Presentations

Oral Presentation Abstracts

Morning, Friday, April 28, 2000

Session 1: Evolution and Global Variation of HIV & Related Viruses

Chairs: David Robertson & Frederick Bibollet-Ruche

Complex Origins of SIV from Red-Capped Mangabeys

Paul Sharp

Institute of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, U.K.

Knowledge of the evolutionary history of the simian immunodeficiency viruses is far from complete. From phylogenetic analyses, the known SIVs fall into five, approximately equidistant, major lineages: SIV_{cpz} (including HIV-1), SIV_{sm} (including SIV_{mac} and HIV-2), SIV_{agm}, SIV_{hoest}/SIV_{mnd}, and SIV_{syk}. Within this phylogeny there are several clades within which there appears to have been host-dependent evolution of SIVs (i.e., 'co-speciation' of host and viral lineages), but also instances where cross-species transmissions have occurred. Unanswered questions include the extent of cross-species transmission, the time scale of this evolutionary tree, and the origins of SIV_{cpz}.

SIV has now been isolated from several red-capped mangabeys (*Cercocebus torquatus*) from across their natural range (Nigeria, Cameroon, and Gabon). Full length sequences for two SIV_{rcm} isolates, and partial sequences for others, indicate diversity similar to that seen among SIV naturally infecting other species, such as vervets, or l'Hoest's monkeys.

It might be expected that SIV_{rcm} would be most closely related to SIV_{sm} from the closely related species *Cercocebus atys*. However, preliminary phylogenetic analyses based on fragments of the *gag* and *pol* genes gave surprising results (Georges-Courbot et al., 1998, J.Virol. 72:600-608). In neither region was SIV_{rcm} closely related to SIV_{sm}: in *gag* SIV_{rcm} was equidistant from SIV_{sm} and SIV_{agm}; while in *pol* it was most closely related to SIV_{cpz}. However, the full-length sequences reveal that SIV_{rcm} contains a *vpx* gene, a feature unique to the SIV_{sm} lineage. Using the full-length sequences, we have performed maximum likelihood analyses of multiple overlapping windows of a concatenated

proteome. The results of these analyses suggest that SIV_{rcm} was generated by recombination between an SIV_{sm} -like virus and an ancestor of the SIV_{cpz} lineage.

Molecular Characterization of Primate Lentiviruses from DeBrazza, Mona, and Blue Monkeys.

F. Bibollet-Ruche*¹, V. Cournaud², X. Pourrut², E. Mpoudi³, J. Mwenda⁴, V. Hirsch⁵, E. Delaporte², M. Peeters², F. Gao⁶, G. M. Shaw¹, And B. H. Hahn⁶.

1: Howard Hughes Med. Inst.; 2: IRD, Montpellier, France; 3: PRESICA Project, Yaounde, Cameroon; 4: IRP, Nairobi, Kenya; 5: NIAID/NIH, Rockville, MD; and 6: Univ. of Alabama at Birmingham.

In a recent sero-survey of wild born primates in Cameroon, we identified three DeBrazza monkeys (*Cercopithecus neglectus*) to harbor HIV/SIV cross-reactive antibodies. To characterize the infecting viruses, we used regular and long PCR methods to amplify viral sequences from uncultured PBMC DNA. This approach yielded subgenomic *pol* and *nef* fragments for two viruses ($SIV_{deb}CM1$, and $SIV_{deb}CM40$) and a full length genome ($SIV_{deb}CM5$) for the other. Sequence analysis revealed that CM1, CM40 and CM5 were closely related to each other, indicating that these were genuine De Brazza monkey viruses. Moreover, phylogenetic analyses of the various SIV_{deb} protein sequences showed that this virus clusters with SIV_{syk} with significant bootstrap values, albeit rather distantly. Since DeBrazza and Sykes' monkeys are both members of the *Cercopithecus* genus, these data, along with some specific characteristics in the TAR secondary structure, suggested that their viruses had evolved in a host-dependent fashion. However, two other previously reported viral strains, i.e., SIV_{lhoest} from L'Hoest monkeys and SIV_{sun} from Sun-tailed monkeys (both also classified within the *Cercopithecus* genus), did not group with SIV_{syk}/SIV_{deb} . To investigate these relationships, we amplified and phylogenetically analyzed nuclear gene sequences (ERV LTR and G6PD sequences) from a variety of different Old World monkey species. These studies revealed that sequences from members of lhoesti group of monkeys (L'hoest Preuss' and Sun-tailed monkeys) formed a tight-knit cluster, only distantly related to the other *Cercopithecus* species. Taken together, these data suggest that SIV_{syk} and SIV_{deb} might represent the true *SIVcercopithecus* lineage. We have recently characterized SIVs from Blue (*Cercopithecus mitis*) and mona (*Cercopithecus mona*) monkeys, and partial sequence and phylogenetic analyses are in full agreement with this hypothesis.

Characterization of SIV from the L' Hoest Superspecies

Vanessa Hirsch

Dating the origin of HIV-1 group M and HIV-1 group M/SIV_{cpz} separation

Anne-Mieke Vandamme¹, Korbinian Strimmer², William W. Hall³, Eric Delaporte⁴, Souleymane Mboup⁵, Martine Peeters⁴, and Marco Salemi¹.

1: Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium; 2: MIPS/GSF, Max-Planck-Institut für Biochemie, D-82152 Martinsried, Germany; 3: Department of Medical Microbiology, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4, Ireland; 4: Laboratoire Retrovirus, IRD, Montpellier, France; 5: African Network of HIV variability, Senegal.

Dating events in the history of SIV/HIV are not straightforward since different HIV-1 lineages and their currently closest simian counterpart, SIV_{cpz} from *Pan troglodytes troglodytes*, have different evolutionary rates. That is, they do not follow a molecular clock. Using a newly developed method, called site stripping for clock detection (SSCD), which allows selection of nucleotide sites in a set of aligned sequences evolving at an equal rate in different lineages, we investigated particular dates in the history of HIV-1. Two separate gene regions were used to estimate dates on the nodes of the HIV-1 tree, a set of *pol* sequences isolated in 1998, and a set of *env* sequences, isolated in 1992. The molecular clock was calibrated using all available *pol* and *env* strains of known isolation year. The results from both datasets were very concordant. Subtype B originated in the early 1970s, and the separation between the B and D subtype dates from the early 1950s. The most recent common ancestor of group M has to be placed in the 1930s. Interestingly, our analysis uncovered sites in the *pol* and *env* region for which HIV-1 group M and SIV_{cpz} strains accumulate mutations following the same molecular clock. As a consequence, it was possible to estimate that the most recent common ancestor of HIV-1 group M and SIV_{cpz} dates from around 1800. These results shed new light on the origin of AIDS pandemic and seem to indicate a long time interval between HIV-1 origin and the first reported waves of the AIDS epidemic.

Identification and Molecular Characterization of a New Primate Lentivirus (SIV_{col}) from Colobus Monkeys in Cameroon.

V. Courgnaud¹, F. Liegeois¹, X. Pourrut¹, F. Bibollet-Ruche², E. Mpoudi³, **E. Delaporte¹**
And M. Peeters¹.

1: Laboratoire Retrovirus, IRD, Montpellier, France. 2: Howard Huges Med. Inst. Univ. of Alabama at Birmingham, Birmingham, AL. 3: Projet PRESICA, Yaounde, Cameroon

To date, based on sequence relatedness, primate lentiviruses can be classified into five major lineages approximately equidistant. In order to study primate lentivirus diversity, a sero-survey of wild born primates in Cameroon was conducted. This screening identified two Colobus monkeys (*Colobus guereza*) with HIV/SIV cross-reactive antibodies. To characterize these viruses, we PCR amplified viral sequences from PBMC DNA. Using primers highly conserved among all known primate lentiviruses, we obtained a small fragment in *pol* gene for the 2 viruses (SIV_{col}CGU1 and SIV_{col}43). Sequence analysis confirmed that the 2 viruses were closely related to each other. Then, using strain specific primers, we amplified the complete genome of SIV_{col}CGU1 by targeting unintegrated circular DNA. SIV_{col}CGU1 has been fully characterized and had a genomic organization characteristic for most primate lentiviruses (lacking *vpu* and *vpx*). Comparisons of the predicted protein sequences encoded by the eight common genes revealed that SIV_{col} was very distinct from all other known SIVs/HIVs isolates: on average we observed 50% amino acid sequence difference for conserved protein like Pol to 75% for Env or Nef /representatives of each of the five lineages of primate lentiviruses. Phylogenetic analyses confirmed that SIV_{col} is genetically distinct from others SIVs and clusters independently, forming thus a novel (sixth) lineage. Among Cercopithecidae monkeys (Old world monkeys), two groups are recognized, the Colobinae and the Cercopithecinae and, so far, all Cercopithecidae monkeys from which lentiviruses have been isolated belong to the Cercopithecinae subfamily. Therefore, SIV_{col} from Colobus (*Colobus guereza*) monkey is the first primate lentivirus identified in the Colobinae subfamily and represents the most divergent primate lentivirus reported to date.

Molecular relationship between HIV-1 group N and SIV_{cpz} (*Pan troglodytes troglodytes*) strains.

P. Roques*¹, S. Souquiere², M. Müller Trutwin³, A. Ayouba⁴, E. Nerrienet⁴, D. L. Robertson⁵, D. Dormont¹, P. Mauclore⁴, F. Barré-Sinoussi³ And F. Simon².

1: Service de neurovirologie, CEA, France; 2: CIRMF, Gabon; 3: Institut Pasteur, France; 4: Centre Pasteur du Cameroun; 5: Zoology Dept., Oxford University, UK.

Introduction: Up until now, three HIV-1 lineages (groups M, N and O) are known. Studies have shown a close relationship between SIV_{cpz} from common chimpanzees (subspecies *Pan troglodytes troglodytes*) and HIV-1 group N. However, only three SIV_{cpz} (P.t.t.) strains (GAB, US and Cam3) have been fully characterized. These sequences, in association with the full-length sequence of HIV-1 group N YBF30, support the hypothesis of transmission of SIV_{cpz} to humans. **Objective:** To study the group N epidemiology, and to better define the HIV-1/SIV_{cpz} relationship. New group N and SIV_{cpz} strains were derived and phylogenetic analysis performed. **Methods:** 5464 Human HIV-1 positive samples collected in Cameroon (1997-99) were tested using a group M, O, and N-specific V3 peptides EIA. Using the same peptide assay, more than 120 wild caught young chimpanzees captured in Cameroon, Gabon and Congo were tested. **Results:** 97% of the human samples reacted with the M group peptide, 2% with the O group peptide and three samples (0.1%) with group N peptide. Fragments of coding sequence for integrase, gp41, V3 *env* regions and *vif* were amplified for these three new group N strains (designed YBF106, YBF115 and YBF116). In addition, the full-length sequence of YBF106 was obtained. Phylogenetic analysis showed a monophyletic clustering of all five group N strains in all regions analyzed. Among the chimpanzees studied, a high rate of positive sera was found in regard to the age of tested animals. Three *P. t. troglodytes*, from Cameroon and Gabon were positive, reacting specifically with the N peptide. Two of these SIV_{cpz} strains, Cam3 and Cam5, were isolated and fully sequenced. The HIV-1 group N and SIV_{cpz} strains form a monophyletic cluster in *env* region while in *vif* and the *gag* region the SIV_{cpz} and human strains are more divergent. **Conclusions:** These new sequences confirm a primate-human cross-species transmission and the circulation of related SIV/HIV group N strains in humans and chimpanzees. Lack of infection of juvenile chimps points to the need for epidemiological studies of adult primate populations which may allow a better understanding of the missing links in the origins of HIV groups M and O.

The HIV Nomenclature

D.L. Robertson

The HIV nomenclature has been important to our understanding of the AIDS pandemic. In particular it has implications for research including epidemiological tracking, vaccine design, and as a foundation for understanding whether there is a relationship between biology and genetic diversity. Due to the continued discovery of divergent viral strains plus the high prevalence of recombination, which obscures our ability to understand the “true” evolutionary relationships between lineages, the HIV nomenclature has the potential of becoming increasingly confusing. Thus, a meeting took place at the Santa Fe Institute, New Mexico (September 1999) to discuss HIV nomenclature. Questions that arose included: What criteria should be used to define a novel subtype? How should recombinants with the same form be named? Should subtypes “E” and “I” that appear to exist as complex genotypes with no full parental strains be called subtypes or recombinant subtypes? As a result of the discussion at the meeting, and subsequent communications, a unified proposal was created (HIV-1 Nomenclature Proposal: a Reference Guide to HIV-1 Classification by Robertson DL, Anderson J, Bradac JA, Carr JK, Foley B, Funkhouser RK, Gao F, Hahn BH, Kuiken C, Learn GH, Leitner T, McCutchan F, Osmanov S, Peeters M, Pieniazek D, M.L. Kalish, Salminen M, Sharp PM, Wolinsky S, and Korber B, available at <http://hiv-web.lanl.gov>). While it was agreed there are shortcomings in the current nomenclature system it was felt that remaining consistent with the present system was of vital importance. In particular, four categories should be used to describe the major HIV-1 lineages: groups, subtypes, sub-subtypes and Circulating Recombinant Forms (CRFs). The HIV-1 group O lineage also exhibits comparable diversity to group M, and an important question is whether group M-like subtypes can be identified within it. New strains of this lineage have furthered our understanding of its evolution (Characterization and Phylogenetic Analysis of 48 Newly Derived HIV-1 Group O Strains from Patients Living in France and Cameroon by Roques P, Robertson DL, Souquiere S, Damond F, Ayouba A, Farfara I, Depienne C, Nerrienet E, Dormont D, Brun-Vézinet F, Simon F, Maucière P [submitted]). While group O can be sub-divided into major “subtype”-like clades to provide reference points, the group O branching pattern is clearly different to that of group M. This difference between the group O and M tree structures appears to be epidemiological in origin, i.e., the result of sampling from a Cameroonian-centered group O endemic as opposed to sampling from the pan-African/global group M epidemic.

Afternoon, Friday, April 28, 2000**Session 2: HIV Evolution and Transmission****Chairs: Marcia Kalish & Tom Folks****Viral dynamics during primary SIV infection in its natural host****O. M. Diop¹ A. Gueye² M. Dias-Tavares² C. Kornfeld² A. Faye¹ P. Ave³ M. Huerre³ S. Corbet² F. Barre-Sinoussi² & M. C. Müller-Trutwin²**

1: Laboratoire de Rétrovirologie, Institut Pasteur, Dakar, Sénégal; 2: Unité de Biologie des Rétrovirus and 3: Unité d'Histopathologie, Institut Pasteur, Paris, France.

In contrast to pathogenic HIV/SIV infections, chronic SIV_{agm} infection in African Green Monkeys (AGM) is characterized by persistently low peripheral and tissue viral loads that correlate with the lack of disease observed in these animals. Here we report data on the dynamics of acute SIV_{agm} infection in AGMs (*sabaeus*) that exhibit remarkable similarities with viral replication patterns observed in peripheral blood during the first two weeks of pathogenic SIV_{mac} infections. Plasma viremia was evident at day 3 p.i. in AGMs, and rapid viral replication led subsequently to peak viremias by days 7 to 10 characterized by high levels of antigenemia (1.2 - 5ng p27 / ml of plasma), peripheral DNA viral load (10^4 - 10^5 DNA copies / 10^6 PBMC) and plasma RNA viral load (2×10^6 - 2×10^8 RNA copies/ml). The lymph node (LN) RNA and DNA viral load patterns were similar to those in blood with peaks observed between day 7 and day 14. These values in LNs (ranging from 3×10^5 - 3×10^6 RNA copies / 10^6 LNC and 10^3 - 10^4 DNA copies / 10^6 LNC) were at no time point higher than those observed in the blood. Both in LN and blood, rapid and significant decreases were observed in all infected animals after this peak of viral replication. Within three to four weeks post-infection, antigenemia was no longer detectable and peripheral viral loads decreased to values similar to those characteristic of the chronic phase of infection (10^2 - 10^3 DNA copies / 10^6 PBMC and 2×10^3 - 8×10^5 RNA copies / ml of plasma). In LNs, viral loads declined to 5×10^1 - 10^3 DNA copies and 10^4 - 3×10^5 RNA copies per 10^6 LNC at day 28 p.i. and continued to decrease until day 84 p.i. (<10 - 3×10^4 RNA copies/ 10^6 LNC). Despite extensive viremia during primary infection, neither follicular hyperplasia nor CD8+ cell infiltration into LN germinal centers was detected. Altogether, these results indicate that the non-pathogenic outcome of SIV_{agm} infection in its natural host is rather associated with a rapidly induced control of viral replication in response to SIV_{agm} infection, than with a poorly replicating virus or a constitutive host genetic resistance to virus replication.

Early detection of molecular and biological heterogeneity in HIV-1 isolates from children with slow and fast disease progression.

Francesca Salvatori*, Åsa Björndal°, Fabrizio Mammano, Robert Fredriksson°, Eleonora Tresoldi*, François Clavel, Eva Maria Fenyö°, and **Gabriella Scarlatti***.

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The aim of our study was to identify differences in genetic and phenotype variability according to slow or fast disease progression in children infected from their seropositive mother. Between 2 and 8 sequential primary isolates derived from 9 children with slow or fast HIV-1 disease progression were studied. The biological phenotype of PBMC-derived isolates was determined in U87.CD4 cell lines expressing the chemokine receptors CXCR4, CCR5, CCR3, CCR2, and CCR1. Plasma derived viruses were tested with Tropism Recombination Test (TRT) which relies on homologous recombination of RT-PCR amplified *env* sequences. HIV-1 V1-C2 *env* fragments amplified from isolates were screened for diversity by heteroduplex mobility assay (HMA). Sequences were obtained of the *env* regions V2-C2 and V3. We detected high intra-sample genetic heterogeneity in the V1/V2 and C2 domains of the gp120, as traced by HMA. Two of 4 fast progressors had a heterogeneous viral population within 4 months from birth, suggesting transmission of multiple viral variants from mother to child. Viral heterogeneity increased after 6 months of age in slow progressors. Likewise, we detected phenotype variation over time. With the exception of one child, the first isolate from all children had a R5 phenotype. Later on and independently from the rate of disease progression, X4 viruses emerged in 7 out of 9 cases. Passage of dualtropic isolates in the U87.CD4 cell lines revealed that these were composed of mixtures of monotropic and dualtropic viral variants. Analogously, virus amplified from plasma had similar characteristics. The combined net charge of the amino acid sequences of V2-C2/V3 domains was lower in R5 than X4 monotropic isolates. Interestingly, dualtropic R5X4 viruses showed a R5-like genotype in V2-C2 and a X4 genotype in V3. Early viral heterogeneity may be more frequent than described, and largely dependent on which region of the virus is analyzed. Viral phenotypic variation increased over time, with frequent emergence of X4 monotropic or dualtropic viruses. Interactions between different parts of the envelope molecule are likely to occur, and may influence coreceptor utilization, perhaps in a charge-dependent manner.

Competing subpopulations after multiple HIV-1 mother-to-child transmission

Thomas Leitner, Francesca Salvatori and Gabriella Scarlatti

Mother-to-child transmission displays a special case of transfer of an infectious agent. The passage of the agent can take several routes into its new host, and it may do so during a long period of time. Here, we report on a case where at least three different HIV-1 variants were transmitted from mother to child. We have characterized the natural history of the child and the evolution of the virus from prepartum to death using phylogenetic and biological analyses. The transmission occurred on at least two different time points, one during early pregnancy of X4 virus and another close to or at delivery of R5 virus. During the course of the infection three subpopulations, established by the individually transmitted maternal variants, competed with each other in the blood compartment. Immediately after birth only a monophyletic R5 population could be detected, but already after one month, the X4 virus emerges and establishes a second subpopulation. Towards the end of the disease progression, at 33 months, subpopulation two completely takes over and becomes rather homogeneous. At 38 months the child dies. Due to the competition between the subpopulations apparent biological characters of the virus shift in the child. It is interesting to speculate whether the competition between distinct subpopulations expanded the genetic possibilities of the virus, rapidly adapting it to become more pathogenic, and apparently aggravated the disease progression.

Gender Differences In HIV-1 Diversity At Time Of Infection

E. Michelle Long^{1,2}, Harold Martin³, Joan Kreiss³, Stephanie M.J. Rainwater¹, Ludo Laureys³, Denis J. Jackson^{3,4}, Joel Rakwar⁴, Kishorchandra Mandaliya⁵, and Julie Overbaugh¹.

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For an HIV-1 vaccine to be most effective, it may be important to identify and characterize the viruses that are transmitted, particularly to individuals living in endemic areas, such as sub-Saharan Africa. Several studies have shown that the virus population in the blood from recently infected adults was homogeneous, even when the virus population in the index case was genetically diverse. In contrast to those results with mainly male cohorts in America and Europe, in several cases a heterogeneous virus population has been found early in infection in African women. Thus, we more closely compared the diversity of transmitted HIV-1 in additional Kenyan men and women who became infected through heterosexual contact. We found that 20 out of 32 women were infected by multiple virus variants (63%). In contrast, all of the ten men studied were

infected with a single viral species. Moreover, a heterogeneous virus was present in the women before their seroconversion and in most instances, viral variants highly related to those observed at seroconversion were detected in the earlier pre-seroconversion sample. The genetic relatedness of these variants strongly suggests that the complex population was derived from a single index case, indicating that diversity was most likely to be the result of transmission of multiple variants. Our data indicate that there are important differences in the transmitted virus populations in women and men even when cohorts from the same geographic region who are infected with the same subtypes of HIV-1 are compared. Current studies are focused on determining the biological phenotype and coreceptor specificity of viral variants found in these Kenyan women at time of infection.

Genetic Characterization of the Rebounding HIV-1 after Cessation of HAART

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Aaron Diamond AIDS Research Center, The Rockefeller University, New York.

After discontinuation of HAART, almost all patients exhibit prompt viral rebound with doubling times of 1.5-2.5 days. To determine the source of the rebound virus, we conducted an extensive study to understand its genetic relationship to viruses found at primary infection, as well as to those in lymphoid tissues and the latent reservoir during treatment. We developed a new, easy technique to determine the genetic relatedness of viruses based on the known extensive length polymorphism in V1, V2, V4 and V5 regions of *env*. A total of 65 sequential plasma samples, along with viral sequences from tissue biopsies and latent reservoirs were studied from 4 acutely infected patients before, during, and after HAART. Despite complete suppression of plasma viremia for 3 years, replication-competent HIV-1 was successfully isolated from resting memory CD4 cells in each case. After cessation of therapy, all 4 patients had viral rebound. In 2 patients who did not have genetic evidence of residual HIV-1 replication during treatment, the rebound virus was identical in length to those within the latent reservoir and at primary infection. In two patients, however, the rebound virus was dramatically different from the latent reservoir virus, and further studies showed evidence of persistent, low-level viral replication despite sustained suppression of plasma viremia below the detection limit. The rebound virus was in fact identical to minor quasispecies detected in biopsies of a lymph node and tonsil taken during the treatment course, suggesting a possible source. Interestingly, as these two patients remained off therapy, the initial rebounding virus was gradually overtaken by a strain identical to that found in the latent reservoir and at primary infection, demonstrating its replicative advantage. Similar studies are now being conducted on additional cases to confirm our initial conclusions that (1) in the setting of complete HIV-1 suppression by HAART, the viral rebound is likely due to the activation of the virus from the

latent reservoir and (2) in patients with incomplete suppression by chemotherapy, the viral spread upon cessation of treatment is probably triggered by ongoing, low-level replication of HIV-1.

Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia following discontinuation of highly active antiretroviral therapy

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The persistence of a pool of latently infected, resting CD4+ T cells carrying replication-competent HIV has been well documented in human immunodeficiency virus (HIV)-infected individuals who are receiving highly active antiretroviral therapy (HAART) and in whom successful suppression of plasma viremia has been achieved. This latent viral reservoir is considered to be a major impediment to complete eradication of HIV in infected individuals receiving HAART due to its long half-life and the ineffectiveness of currently available drugs in eliminating these infected cells; however, the pathogenic significance of this viral reservoir as well as its role in the rebound of plasma viremia following discontinuation of HAART is largely unknown. We have previously demonstrated that there was no correlation between the kinetics of viral rebound in plasma upon cessation of HAART and the size of the latent HIV reservoir prior to discontinuation of therapy in infected individuals in whom prolonged periods of successful suppression of plasma viremia had been achieved. Using heteroduplex mobility and tracking assays, we demonstrate in this study that the detectable pool of latently infected, resting CD4+ T cells does not account entirely for the early rebounding plasma HIV in infected individuals in whom HAART has been discontinued. The rebounding plasma virus was genetically distinct from both the cell-associated HIV RNA and replication-competent virus within the detectable pool of latently infected, resting CD4+ T cells in the majority of patients examined. These results suggest the existence of other persistent HIV reservoirs that could prompt rapid emergence of plasma viremia following cessation of HAART and underscore the necessity to develop therapies directed toward such populations of infected cells.

Proximal Immune Activation and HIV Transmission: Implications for HIV Pathogenesis and Evolution

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We have proposed that HIV replication occurs in multiple local bursts, associated with chronic immune activation in response to antigens and inflammatory signals, and that antiretroviral treatment substantially reduces the size and frequency of such bursts but does not generally prevent the steady-state regeneration of infected memory cells. In a system in which HIV transmission is predominantly local, there are opportunities for HIV to move among T cell clones of different specificity due to patterns of cross-reactivity among co-stimulated T cells. We envision disease progression during the asymptomatic phase as a progressive penetration of HIV into the memory component of an increasing number of clones. This is further enhanced by the secondary activation of infected, HIV-specific memory cells in any response in which HIV is produced. Under treatment, in particular, such HIV-specific cells may facilitate the expansion of drug resistant variants with relative rapidity. The local nature and clonal segregation of virus transmission limit competition for predominance among viral mutants and facilitate diversity. The more restricted diversity during the acute phase and late in progression can be correlated to different predominant modes of virus transmission. The possibility that HIV-specific CD4 and CD8 T cells may exert opposite effects on HIV variant selection should be considered, as CD4 cells play a dual role in HIV pathogenesis.

Session 3: Evolution and Drug Resistance**Chair: Andrew Leigh Brown****HIV drug resistance and fitness**

François Clavel

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Resistance mutations that are selected in the course of virologic failure of antiretroviral therapy in HIV disease confer a high selective growth advantage to the virus in the presence of antiretroviral drugs. However, some mutations or combinations of mutations are deleterious for the overall replicative capacity of the virus, which is sometimes referred to as HIV fitness. These effects are particularly perceptible for mutations in the HIV protease that mediate resistance to protease inhibitors, leading to abnormal patterns of cleavage of the precursors of HIV structural and enzymatic proteins. As a consequence, mutant viral particles display a significantly reduced infectivity. Interestingly, these defects can be partially or, in some instances completely, compensated by secondary mutations emerging either in PR or in some of its natural Gag substrates. Some resistance mutations in reverse transcriptase can also impair HIV drug-free fitness, but generally to a lesser extent. The impact of the reduction of HIV replicative potential on the clinical and immunological profile of the disease is not fully understood. Several lines of evidence suggest that, indeed, loss of viral fitness is translated into a parallel loss in HIV virulence. In patients failing antiretroviral therapy, a disconnection between the high levels of resistant virus replication and the persistence of high CD4 counts has been described. This disconnection appears to fade with time, but can persist for about 3 years after the start of treatment failure. In patients with established HIV drug resistance in which treatment is interrupted, the resistant virus is most often replaced by a wild-type, drug sensitive counterpart, a sign that its replicative fitness is reduced. In parallel, a strong rebound in viremia, associated with a marked decrease in CD4 counts is observed, a sign that wild-type virus has also recovered higher virulence characteristics. The mechanisms through which impaired HIV fitness can lead to higher CD4 counts in spite of high virus replication levels is still debated. In the SCID-hu mouse model, protease inhibitor-resistant viruses fail to replicate in thymic implants while they replicate almost normally in cultures of stimulated PBLs, suggesting that resistance-associated replication impairment can strikingly differ according to the cell or tissue compartment that harbors the virus. In treated patients displaying a disconnected profile, high CD4 counts appear to be indeed associated with a higher thymic output, as measured by quantitative analysis of the amounts of TRECs (T cell receptor excision circles) a marker of recent thymic emigration of peripheral T cells. These results suggest that although combined with multiple other parameters of immune reconstitution, the loss of viral

fitness that can result from some patterns of resistance mutations may reduce HIV pathogenicity and disease progression.

Dynamic Behavior of HIV-1 Populations While Under Strong Selective Pressure

Ronald Swanstrom

Bottleneck Transmission of Nucleoside Analogue-resistant HIV-1 and the Establishment of new HIV-1 RT Wild Types

Jaap Goudsmit

Substantial reductions in mortality and morbidity among HIV-1 infected individuals are the result of widespread use of combination antiretroviral therapies. Among participants of the prospective Amsterdam Cohort Studies in homosexual men, the mortality rate dropped from 15% in 1994 to 1% in 1998, while coverage by therapy combining reverse transcriptase (RT) and protease inhibitors rose from 4% to 64%. Maintenance of this success requires that viruses in the drug-naïve seropositive population remain therapy sensitive. We therefore analyzed the RT and protease genes of viruses present at the point of seroconversion of people newly infected in the period 1990-1998. Three of 43 individuals tested (7%) harbored viruses with AZT-resistant conferring mutations, all of which were replaced by AZT-sensitive virus within a year after seroconversion. The build-up of AZT-resistance is described by a mathematical model that takes into account the coverage of drug regimes selecting AZT-resistance, the lag time in which resistance is gained and lost, the death rate of people infected with resistant virus, and the replacement of resistance-selecting regimes by more potent treatments that substantially reduce viral load. Our model indicates that the frequency of resistance in a population is determined largely by the number of individuals on insufficient or failing therapy and only modestly influenced by secondary transmission of AZT-resistant strains. However, viruses with a variant RT arise rapidly in most, if not all individuals infected with a 215Y AZT-resistant virus. These RT variants with improved fitness contain a D, S or N at position 215 and are shown to be equally fit in vitro to 215T mutants. Such AZT-sensitive mutants are only one mutational step removed from the 215Y mutation and evidence is obtained in the Amsterdam Cohort Studies for the first transmission of a 215D mutant that was shown to be stable during follow-up. These data point to the establishment of new transmissible and drug-sensitive HIV-1 wild types due to transmission of nucleoside analogue-resistant virus.

Difference in resistance contributes more strongly to the evolution of zidovudine resistance in HIV-1 infected patients than difference in replication capacity.

W. Keulen, R. de Boer, L. de Graaf, B. Berkhout, R. Jeeninga, J. Whitcomb, R. Schuurman and **C. Boucher.**

The evolution of drug resistance in HIV-1 infected patients is directed by several factors such as mutational frequency of the viral RT enzyme, replication capacity and drug susceptibility of the selected virus variants. In this study, we determined these factors for nine different AZT resistant variants containing either the individual mutations (M41L, K70R, T215F/Y) or several combinations of mutations. Pairwise competition experiments in primary cells demonstrated a strong reduction of the replication capacity of the M41L and the M41L+K70R variant. Interestingly, only minor differences were observed between the replication capacity of the other variants. Analysis of the drug susceptibility showed increasing AZT resistance that coincided with the accumulation of the resistance conferring mutations. In addition, cross- resistance to ddC and ddI was determined for the complete set of variants and demonstrated low level of cross-resistance for the T215Y variant. Based on the data, we propose that due to the minor differences in replication capacity, differences in resistance contribute more strongly to the evolution of AZT resistant variants during AZT mono-and combination therapy. In combination with the nucleotide substitution bias of the RT enzyme, differences in resistance can explain the observed evolution of AZT resistant variants in vivo.

Morning, Saturday, April 29, 2000

Session 4: Lessons from Other Viruses

Chair: Avidan U. Neumann

Introduction to Hepatitis C Virus

Avidan U. Neumann,

Bar-Ilan University, Ramat-Gan, Israel

Variable efficacy of repeated annual influenza vaccination

Derek J. Smith^{1,2,3}, Stephanie Forrest^{2,4}, David H. Ackley⁴, and Alan S. Perelson^{2,5}.

1: Erasmus University, 2: Santa Fe Institute, 3: Popular Power, 4: University of New Mexico, and 5: Los Alamos National Laboratory.

As the influenza virus evolves, the vaccine is updated to track the evolution and public health recommendations are that at-risk individuals are revaccinated annually. Vaccine efficacy in repeat vaccinees has been difficult to determine definitively. A meta-analysis of 19 repeat vaccination studies showed that on average repeat vaccinees were protected at least as well as first-time vaccinees; however, in a subset of 12 of the studies, there was statistically significant unexplained heterogeneity: In some years repeat vaccinees were better protected than first-time vaccinees, in other years they were not as well protected [Beyer, W.E.P. et al (1999) Arch Intern Med 159, 182-188]. We have proposed the 'antigenic distance hypothesis' as an explanation of this heterogeneity [Smith, D.J. et al. (1999) PNAS 96, 14001-14006]. The hypothesis is that variation in repeat vaccine efficacy is due to differences in antigenic distances among vaccine strains and between the vaccine strains and the epidemic strain in each outbreak. The hypothesis was tested by analyzing influenza outbreaks that occurred during the Hoskins [Hoskins, T.W. et al., (1979) Lancet i, 33-35] and Keitel [Keitel, W.A. et al. (1997) Vaccine 15, 1114-1122] repeated vaccination studies; antigenic distances were calculated from historical hemagglutination inhibition assay tables, and a computer model of the immune response was used to predict the vaccine efficacy in individuals given different vaccinations. The model accurately predicted the observed vaccine efficacies in repeat vaccinees relative to the efficacy in first-time vaccinees (correlation 0.87). These results have implications for the selection of influenza vaccine strains, and also for vaccination strategies for other antigenically variable pathogens that might require repeated vaccination.

The Immunopathogenesis Of Hepatitis C Virus

Margaret James Koziel, MD.

Beth Israel Deaconess Med. Ctr. And Harvard Medical Sch., Boston MA, USA

Hepatitis C virus (HCV) is notable for the high rate of chronic infection, which occurs in nearly all individuals who become infected. The immune response to HCV is polyclonal and multispecific, both in terms of antibody and cellular immune responses. In hepatitis C, numerous antibodies develop during the course of chronic infection, but it has been difficult to determine which antibody response correlates best with recovery from acute viral infection. Emerging evidence indicates that cellular immunity mediated by both CD4+ and CD8+ T cells is more important in recovery from acute HCV. Individuals who recover from acute HCV infection appear to have quantitatively more vigorous CD4+ proliferative responses against one or more HCV proteins compared to those individuals who develop chronic disease. Recent data also suggests that HCV-specific cytotoxic T lymphocyte (CTL) responses may play a critical role in recovery, and may persist for years after acute infection. However, HCV-specific CTL can be readily isolated from the liver and PBMC of chronically infected individuals, and recognize multiple epitopes. These CD8+ CTL may also cause tissue damage once chronic infection is established. Cytokines produced by these and other cells likely play a role in liver fibrosis. Mechanisms of viral escape include interference with host cell proteins and mutations in B- and T cell epitopes

Hepatitis C Virus Quasi-species Intra-host Evolution

Jean-Michel Pawlotsky.

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Hepatitis C virus (HCV) genome is a 10-kb long single-strand linear RNA molecule. Like for many RNA viruses, the HCV genome is highly variable, due to uncorrected errors made by the RNA-dependent RNA polymerase during replication. HCV replication in human hosts triggers numerous non specific and specific antiviral responses, including immune responses, which exert strong pressures towards viral evolution. The treatment of chronic hepatitis C is based on the administration of interferon (IFN) α , a molecule with both non specific antiviral effects and immunomodulatory effects. The administration of IFN provides a model of exacerbation of host-virus interactions, because IFN acts by increasing the antiviral pressure on the virus. Nevertheless, HCV evolution appears to be constrained by various structural and functional features. The genetic evolutions of genomic regions representative of the whole genome upon

various conditions and their structural and functional consequences will be described. These regions include : (i) the 5' non coding region, a highly structured and well-conserved region containing a stable stem-loop structure acting as an internal ribosome entry site ; (ii) the hypervariable region 1 (HVR1), a variable region located at the 5' end of the E2 envelope glycoprotein gene that encodes a major neutralizing HCV epitope, a cytotoxic epitope and, possibly, conserved structures involved in the virus life-cycle ; (iii) the non structural 5A (NS5A) protein, that plays a role in the regulation of HCV replication and acts as a transcriptional activator in vitro.

Clinical implications of HCV dynamics

Avidan U Neumann

Bar-Ilan University, Ramat-Gan, Israel

The viral kinetics of Hepatitis C virus (HCV) during treatment with IFN is very different of that of HIV during current anti-retroviral treatment. HIV shows a viral decline with a half-life of about 1-2 days after a delay of 1-2 days. In contrast, for HCV a more rapid, dose-dependent, decline occurs already at 6-10 hours after the first injections, followed by a slower 2nd phase decline comparable in some patients to that of HIV. Furthermore, in HIV the decline slope between days 1-7 slows during the first month of treatment, while for HCV this further slowing down is not observed. Modeling of the HCV kinetics shows that the difference is due to different mechanisms of anti-viral effect, blocking infection for HIV while blocking production for HCV. The HCV models allow to directly estimate parameters that can not be estimated from HIV kinetics, such as the absolute effectiveness of the drug and the clearance rate of free virus. In addition, the early kinetics of HCV decline has a better predictive value for the success of treatment than HIV and can be used to make clinical decisions. Thus, allowing us to introduce individualized treatment approaches based on viral dynamics.

Session 3: Evolution and Drug Resistance (Continued)**Chair: Andrew Leigh Brown****HIV-1 Hypersensitivity to NNRTI is Associated with Reduced Susceptibility to NRTI**J. Whitcomb¹, W. Huang¹, S. Deeks², T. Wrin¹, E. Paxinos¹, K. Limoli¹, R. Hoh², N. Hellmann¹, C. Petropoulos¹

1: ViroLogic, Inc, S.S.F., CA, 2: UCSF Gen Hosp, S.F., CA

Background: We have observed increased NNRTI susceptibility (hypersensitivity) among viruses containing NRTI resistance mutations. This study investigates the relationship between NRTI and NNRTI susceptibilities in virus from HIV-1 infected patients and genetically engineered HIV-1 viruses. **Methods:** Quantitative measurements of drug susceptibility were obtained for 331 NRTI/NNRTI naïve (naïve) and 447 NRTI experienced/NNRTI naïve (NRTI exp) patient viruses using PhenoSense™HIV. Regression analyses and non-parametric statistics were used to evaluate correlations between NRTI and NNRTI susceptibility. Hypersensitivity and reduced susceptibility were defined as fold change, ≤ 0.4 and ≥ 2.5 , respectively, compared to a drug sensitive reference virus. In addition, the genotypes of viruses with either *i*) wild-type, *ii*) NNRTI hypersensitivity, *iii*) low level reduced NNRTI susceptibility (2.5 – 10 fold) or *iv*) high level reduced NNRTI susceptibility (>10 fold) were determined. **Results:** Hypersensitivity to NNRTIs (DLV, EFV, NVP) was observed in a significantly greater percentage of viruses from NRTI-exp patients (29, 26, 21%) compared to naïve patients (5, 9, 11%). In NRTI-exp patients, regression analyses demonstrated significant inverse correlations ($p < 0.0001$) between reduced NRTI susceptibility (ZDV, 3TC) and increased NNRTI susceptibility. Viruses with NNRTI hypersensitivity and viruses with high level reduced NNRTI susceptibility had significantly more NRTI resistance mutations than viruses with wild-type susceptibility or low level reductions in NNRTI-susceptibility. The inverse correlation between NRTI- and NNRTI-susceptibility in patient virus was further demonstrated by phenotypic testing of serial plasma samples from an NNRTI naïve patient following interruption of a failing NRTI-containing treatment regimen: fading NRTI resistance over time was associated with loss of NNRTI hypersensitivity. **Conclusions:** Reduced susceptibility to NRTIs was significantly correlated with NNRTI hypersensitivity. This observation may provide an explanation for the superior virologic response to NNRTI containing salvage regimens in NRTI experienced patients. The clinical implications of NNRTI hypersensitivity require additional study.

Identifying Mutations with Small Effects on Drug Resistance

A.J. Leigh Brown*, H.M. Precious, J. Whitcomb, J.K. Wong, M. Quigg, W. Huang, E. Daar, R.T. D'Aquila, P.Keiser, E.Connick, N. Hellmann, C. Petropoulos, D.D. Richman and S.J. Little

Recently, significant numbers of individuals with primary HIV infection have been found to be harboring viral strains with reduced susceptibility to antiretroviral drugs. In one study, HIV from 16% of such antiretroviral-naïve individuals was shown to have a susceptibility to non-nucleoside reverse transcriptase inhibitors (NNRTIs) of between 2.5 and 10-fold lower than a wild-type control. No mutations that had previously been associated with antiretroviral resistance were observed in the reverse transcriptase (RT) domain of these strains. We have analyzed all variable amino acid sites in this dataset and have identified 2 novel sites influencing susceptibility to NNRTIs: amino acid 135 and amino acid 283 in RT. The 8 different combinations of amino acids at these sites were found to have a 14-fold range in mean susceptibility to both nevirapine and delavirdine. In vitro mutagenesis of the control strain combined with an HIV drug resistance phenotypic assay confirmed the significance of amino acid variation at these sites for susceptibility to these drugs.

Afternoon, Saturday, April 29, 2000

Session 5: Viral Variability and the Immune Response

Chair: Bette Korber

A Novel Single Cell Flow Based Killing Assay to Measure CTL Responses to Viral Variants

Doug Nixon

Tat-Specific CTL Responses Select for Escape Variants During Acute SIV Infection

Todd M. Allen[‡], David H. O'Connor[‡], Peicheng Jing^{*}, John L. Dzuris[§], Bianca R. Mothé^{*}, Ed Dunphy^{*}, Max E. Liebl[†], Thorsten U. Vogel^{*}, Carol Emerson^{*}, Nancy Wilson^{*}, Kevin J. Kunstman[#], Xiaochi Wang^{||}, Austin L. Hughes^{*}, Ronald C. Desrosiers⁺, John D. Altman^{||}, Steven M. Wolinsky[#], Alessandro Sette[§], David I. Watkins^{*||**}

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Strong cytotoxic T lymphocyte (CTL) responses emerge early in HIV and SIV infections coincident with declining plasma viremia. Here we show that this initial CTL response selects for viral escape variants within an immunodominant Tat epitope. By eight weeks post-infection, the majority of the replicating virus escapes recognition by these CTL. In contrast, no variation occurs in three other previously identified CTL epitopes in Gag, Env, and Vif. These findings imply that replication of wild-type virus is initially controlled by Tat-specific CTL and suggests that induction of CTL active during acute viremia might be an important component of an effective HIV vaccine.

[‡]TMA and DHO contributed equally to this work.

Immune Responses to HIV in Uganda.

Frances Gotch, Pontiano Kaleebu, Alleluiah Rutemberwa, Jill Gilmour, Jimmy Whitworth and David Yirrell.

We are investigating the prevalence of inter-genic and intra-genic inter-subtype recombination events in HIV.1 in Ugandan populations of patients. We are evaluating the implications such data may have in our analyses of cross-clade cytotoxic T lymphocyte (CTL) activity in the same group of patients. We have shown that the majority of HIV.1 infected patients show cross-reactivity in their CTL responses as assessed using chromium release assays, and using ELISPOT assays employing both specific peptides and recombinant vaccinia viruses. All patients have been designated as being infected with a specific clade of HIV.1 based on envelope heteroduplex mobility analyses. We are however aware that in areas such as Uganda where HIV prevalence is high and where different subtypes of HIV.1 co-circulate, inter-subtype recombination is likely to take place. Such recombination events may have implications for our evaluation of the putative efficacy of cell mediated immune responses. We have examined sequence from the V3/V4 region of envelope and from a 700bp fragment of the *gag* gene encompassing the majority of p17 and p24 in 87 patients from two cohorts of HIV infected persons in Uganda. Our data shows 29.8% of viruses to be *env/gag* (inter-genic) recombinants, and 9.2% to be *gag/gag* (intra-genic) recombinants - the majority with breakpoints close to the p17/p24 junction. Our data is in concordance with previously published data from Rwanda. We have identified 15 different subtype assortments. The implications of the data will be discussed.

Qualitative Differences Between HIV-Specific CTL Responses as Reflected by the Presence or Absence of Epitope-Specific Escape Mutation

Philip Goulder

The Use of an Inferred Epidemic Ancestral Sequence as a Vaccine Immunogen

Gerald Learn¹, Allen Rodrigo², Fusheng Li¹, Matthew Rain¹ and James I. Mullins¹

1: Department of Microbiology, University of Washington, Seattle, WA 98195-7740 and 2: University of Auckland, School of Biological Sciences, Auckland, New Zealand

A primary concern in designing an AIDS vaccine to protect against infection by HIV-1 is which strains should be chosen to best provide protection against the myriad of evolving variants. Evolutionary trees of HIV-1 sequences sampled from infected individuals typically form a star-burst

pattern with most of the variants roughly equidistant from the center of the tree and also approximately equidistant from any other circulating strain. To designate single variants that would be more closely related to any of the diverse circulating viruses we propose to choose ancestral viruses representative of the center of the evolutionary radiation; these would appear to have given rise to the vast range of viral diversity within an infected population. It is very unlikely that we could ever identify this ancestral virus even from stored specimens near the start regional AIDS epidemics. However, computational methods can be used to infer an ancestral viral sequence. -- We have recently constructed an ancestral sequence for the envelope gene (*env*) of HIV-1 subtype B which is on average more closely related to any given circulating virus than any other variant; nearly all known targets of the cellular immune response (99.2%) are represented within this ancestral sequence. Most recently, we have extended this procedure to include the Subtype E *env* gene sequence common in HIV-1 from Thailand and other countries in the epidemic in Southeast Asia.

Global Variability of Defined CTL Epitopes

Bette Korber and Brian Gaschen

Session 6: Mathematical Modeling of HIV Dynamics & Evolution**Chairs: John Mittler & Alan Perelson****Comparative Analysis Of HIV/SHIV Dynamics In Cultured Cells, Primary Infections In Monkeys And Humans Off Therapy**

D.S. Dimitrov, I. Sidorov, R.A. Lempicki, J. Kovacs, R.T. Davey, H.C. Lane, J. Lifson and M.A. Martin

Analysis of HIV dynamics in acute infections is important for understanding mechanisms of pathogenesis and evaluation of vaccines. By titering HIV-1 in cultured cells we previously experimentally quantitated and mathematically described the dynamics of HIV-1 infection using a simple model based on virus dissemination by subsequent cycles of infection (1) and a more sophisticated model based on a system of differential equations (2,3). We found that an effective infection rate constant, k , ranging from 0.2 to 1.8 day⁻¹ reproducibly and consistently characterized the dynamics of the initial stage of virus dissemination for a number of virus/cell systems (1). We and others (M. Feinberg and his collaborators) estimated values of k in the same range or even higher for primary SIV or SHIV infections in monkeys and found that they may or may not be correlated to clinical outcome (4 and citations therein). Recently, similar but somewhat lower values of k ranging from 0.12 to 0.91 day⁻¹ were measured in humans with HIV-1 infections after interruption of HAART (5 and citations therein). The lower values of k could be due to immune responses, lower number of susceptible cells, lower susceptibility to infection or/and other reasons. These similarities and differences between the values of k (and several other parameters characterizing the dynamics of acute infections) for cultured cells and in vivo infections, and correlations with other virological and immunological parameters may help in the elucidation of the mechanisms of virus pathogenesis and evaluation of vaccines.

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Viremia Increases During Treatment Interruption After Long Term Virologic Failure.

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BACKGROUND: Immunological and clinical failure of protease inhibitor containing regimens is still rare despite high rates of virological failure in clinical practice. Persistent partial suppression of viremia after the appearance of drug resistant HIV-1 correlates with the amount of CD4+ preservation. **METHODS:** To study the virological basis of persistent partial virological responses, we prospectively followed subjects in long term virological failure who either discontinued (N=18) or continued antiretroviral therapy (N=6). **RESULTS:** Subjects had received PI containing therapy for a median 35 months and had been in virologic failure for a median 28 months. Prior to stopping antiretroviral therapy, these subjects had substantial levels of viremia (median 4.55 log₁₀ cpm) that remained lower than their pre-protease inhibitor baseline (median -0.31 log₁₀ cpm; range -0.61 to +0.02). Subjects typically had multi-drug resistant HIV-1 prior to stopping therapy, which was overgrown by drug sensitive variants after median 5.5 weeks (IRQ 4-7 weeks) in 17/18 subjects, demonstrating decreased fitness of the drug resistant HIV-1 in vivo. Greater degree of viral suppression at baseline and lower PI resistance were independently associated with more rapid overgrowth of wild-type HIV-1. Viremia increased after stopping therapy (median change +0.48 log₁₀ cpm) and prior to loss of drug resistance suggesting that antiretroviral therapy had continued partial activity against the resistant HIV-1 population. After overgrowth of drug resistant HIV-1, viremia increased an additional median +0.48 log₁₀ cpm, despite marked decreases in circulating CD4+ target cells, demonstrating the increased capacity of drug sensitive HIV-1 to achieve high level viremia. **MODELING:** A simple mathematical model in which drug resistant and sensitive HIV-1 populations, that differ with respect to infectivity, compete for target CD4+ cells was developed. Preliminary analysis of this model indicates that resistant virus infectivity that is substantially less than wild-type could account for the data, including 0.4 log₁₀ persistent suppression of resistant viremia during therapy and a 5 week delay to wild-type rebound after treatment interruption. **CONCLUSION:** These data indicate that partial viremia suppression and persistent CD4+ gains after virological failure of PI containing regimens is dependent on continued exposure to antiviral therapy that are partially active against drug resistant HIV-1 and maintain selection for less fit viral populations.

Follicular Dendritic Cells and HIV Dynamics

William S. Hlavacek, Carla Wofsy, Nikolaos I. Stilianakis, Daan W. Notermans, Sven A. Danner, and Alan S. Perelson

The follicular dendritic cell (FDC) network is a major site of HIV-1 accumulation in infected patients. We have used mathematical models for HIV-1 dynamics, which combine earlier models for viral and cellular dynamics with a physicochemical model for the reversible multivalent binding of virions to receptors on FDC, to analyze blood and lymphoid tissue data. We consider two questions. How long does virus persist on FDC? And to what extent does FDC-associated virus influence the dynamics of viral decay during treatment? We find that (1) a small fraction of virus may remain on FDC indefinitely, (2) potentially infectious virus is released from FDC continuously during treatment, (3) release of virus from FDC influences the entire time course of viral decay and requires us to revise upward previous model-based estimates of the rate constants for death of productively infected cells and clearance of virus, and (4) drug regimens that contain only protease inhibitors may be more effective at reducing the load of infectious virus than combinations of protease and reverse transcriptase inhibitors. These results suggest that the interaction of HIV-1 with FDC may be an important target for therapeutic intervention.

Intermittent viremia in HIV-1 infected patients receiving antiretroviral therapy: a dynamical point of view.

Duncan Callaway and Alan S. Perelson

Despite the demonstrated potency of antiretroviral regimens, HIV-1 infected patients, in whom therapy reduces viral load to below 50 HIV RNA copies/mL, exhibit periods of intermittent viremia. This phenomenon could be accounted for by a patient's poor adherence to the drug protocol, or occasional expansion of the target cell pool due to secondary infection. In this study, we offer an alternative explanation based upon the consequences of in vivo viral population dynamics. We develop a mathematical model that mimics the effect of drug therapy in HIV infected patients and exhibits a robust, low steady state viral load (5 orders of magnitude lower than viral load in the absence of drug therapy). This steady state is achieved by including a drug resistant target cell compartment, in addition to the standard primary compartment used in earlier models. In this model, simulation of therapy results in damped oscillatory viral dynamics with a period of approximately 3-6 months. Though for the majority of the period of oscillation the viral load is below 50 copies/mL, the peaks of the oscillations can be as large as 200 copies/mL, which is similar in value to viral loads observed during intermittent episodes of plasma viremia in clinical

studies. Based upon numerical studies of the model, we predict that as the time since the initiation of therapy increases, the likelihood of observing “blips” in viral load decreases. Furthermore, the model suggests that patients in whom the initial decay in viral load is slow should be less likely to exhibit blipping behavior. The results of this model stress the importance of even a small population of drug resistant target cells, and the role they play in preventing the eradication of HIV-1 infection from individual patients.

Measuring T-cell turnover

Ruy M. Ribeiro and Alan Perelson

Recently, a new technique was developed, involving deuterium enriched glucose, that allows the *in vivo* measurement of the turnover of cells. We will present data from a study which measured the CD4⁺ and CD8⁺ T cell turnover in three different groups: healthy individuals, HIV infected patients and subjects undergoing highly active antiretroviral therapy. We have used simple dynamical models to analyse these data. These models for the labeling and unlabeleding of DNA strands during replication of the cell give estimates for the proliferation and death rates of T cells. This presentation will focus on work in progress and the latest results will be discussed.

Morning, Sunday, April 30, 2000**Session 7: Evolutionary Genetics of HIV****Chair: Allen Rodrigo****Stochastic Evolution of a Single Locus in a Virus Population: An Analytic Review**

I.M. Rouzine and J.M. Coffin.

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A broad range of problems of population genetics is analyzed with a view to virological applications, such as growth competition assay and fixation of advantageous alleles in vitro, and evolution of HIV within and between infected patients. We start from a one-locus, two-allele model of haploid populations including the following factors: random drift (as represented by a finite population size, N), purifying selection (selection coefficient, s) and mutations (mutation rate, μ). Evolutionary behavior, at any population size, is analyzed by a unified method using a stochastic equation of diffusion type, and by the Monte-Carlo simulation. We calculate quantitative parameters suitable for comparison with experiment such as the probability density of the mutant frequency, as well as the expectation values and variances of the mutant frequency and the intra-population genetic distance. The analysis shows that that evolution becomes almost deterministic at population sizes much larger than the inverse mutation rate, $N \gg 1/\mu$. The smallest populations, $N \ll 1/s$, exhibit behavior described by "neutral model". The two limits are joined by a broad interval of population sizes, $1/s \ll N \ll 1/\mu$, termed the "selection-drift" regime. In this regime, weakly polymorphous populations behave essentially randomly, while the dynamics of highly polymorphous populations is almost deterministic and controlled by selection pressure. We discuss the biological impact of these findings. For general evolution, we suggest that most biological species may evolve while in the selection-drift regime, in which case both selection and random drift are equally important for the fixation speed of new advantageous mutations. For HIV in vivo, we applied a specially designed (and almost model-independent) linkage disequilibrium test to show that an effective HIV population, in a typical untreated patient, is larger than $1/\mu \sim 10^5$ infected cells, i.e., either at the border or within the deterministic regime (1). We also studied different models of random sampling during transmission between patients to show that the observed high level of genetic diversity in the pro gene is, most likely, due to strong differences in the best-fit sequence between individuals, 16%. Such a strong difference is consistent with cytotoxic immune response combined with co-selection (2).

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Genetic Drift and Within Host Metapopulation Structure in the Evolution of HIV

Simon D. W. Frost and Andrew J. Leigh Brown

The highly variable way in which drug resistance evolves in different HIV-infected individuals has led to the suggestion that HIV evolution may have a strong stochastic component. Genetic drift in the frequency of drug resistant virus prior to therapy can lead to variability between individuals in the rate of evolution of resistance during early therapy. Genetic drift may also generate variability in the rate of evolution of highly fit resistant virus during ongoing therapy, as these mutants may disappear by chance before they reach a frequency where fixation is effectively certain. The importance of genetic drift can be quantified by determining the variance effective population size, which indicates the amount of noise in gene frequencies over a generation. To indirectly estimate the variance effective population size of HIV within the infected host, we have analysed data on the outgrowth of lamivudine resistant mutants, M184I and M184V in reverse transcriptase, during lamivudine monotherapy. By extrapolating back to the initiation of therapy, we show that there is considerable variation in the relative frequencies of M184I and M184V prior to therapy. Using a simple but robust stochastic model, we show that this variation is consistent with a variance effective population size of a million or less i.e. at least 10-100 times lower than the actual population size. We hypothesised that this difference between the actual and the effective population size may have arisen as a consequence of a metapopulation structure of infected cells within the host, where small subpopulations of infected cells, which are formed by one or a few founders, have a high rate of turnover, due to a limited supply of target cells within subpopulations and the short lifetime of individual infected cells. A low variance effective population size arises as founding infected cells produce more daughter infected cells than those in subpopulations where infection has already been established. To test whether such a metapopulation structure exists within solid tissue, where the majority of viral replication occurs, we have analysed sequence data from the V1/V2 region of the envelope gene obtained from provirus isolated from multiple splenic white pulps taken from six HIV infected individuals and one SIV-infected macaque. We show that the pattern of genetic variation within and between virus isolated from splenic white pulps is consistent with a metapopulation model of colonisation and extinction. Our results show that genetic drift is sufficient to generate significant differences in the evolution of single resistance mutations and that this drift may arise as a consequence of a high turnover of small subpopulations of infected cells.

Evolutionary Analyses of Serially Sampled Populations

Allen G. Rodrigo¹, Alexei Drummond¹, Roald Forsberg², and David Nickle³

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The analysis of serially sampled populations presents a relatively new challenge for evolutionary geneticists. In this presentation, we introduce the concept of the “measurably evolving population”, and describe a likelihood ratio test for the accumulation of substitutions from one sampling time to the next. We also described a new method – serial sample UPGMA (sUPGMA) — that allows the phylogeny of serially sampled sequences to be reconstructed under the assumption of a molecular clock. Finally, we discuss the estimation of population and mutation parameters under different models appropriate for measurably evolving populations.

Applications of of Coalescent Statistical Methods to HIV Data Analysis

Daniel A. Vasco and Keith A. Crandall

Intra-host HIV evolution within patients is a dynamic process dependent upon the interaction of the virus and the host. Viral population parameters such as generation time, growth rate and genetic diversity are determined by this interaction. This interaction can cause the effective population size and genetic diversity of the viral population to change rapidly over time. Therefore, it is important to have efficient and fast estimators of these parameters which can take into account more realistic features of the varying host environment of the virus. In my talk I discuss several coalescent statistical methods based upon summary statistics estimated from HIV genealogies in varying host environments. Such summary statistics include the number of segregating sites in the sample, the pairwise differences between sequences, the estimated number of segregating sites along a branch of a genealogy and some other more recently developed measures. By combining phylogeny reconstruction and coalescent theory powerful methods of analysis can be developed which include: parameter estimation, statistical tests of neutrality and hypothesis testing. However, coalescent and phylogenetic theory that includes varying host environments is essentially a nonlinear estimation problem which makes computation of statistical inferences challenging. Some problems include: inferring estimation bias, determining consistency of the estimators, Monte Carlo estimation of the sampling variance of estimated parameters determined from a single genealogical history, as well as demonstrating existence of minimum variances for such histories. A case study illustrating these problems is presented using set of 1200 HIV sequences serially sampled from three patients over a three week time interval.

Detecting selection in the HIV-1 genome using the dn/ds ratio.

Rasmus Nielsen, Ziheng Yang, Nick Goldman and Anne-Mette Krabbe Pedersen.

Methods based on estimating the nonsynonymous/synonymous rate ratio provide a powerful and direct method for testing hypotheses regarding selection at the molecular level. Newly developed codon based likelihood methods can be applied to test hypotheses regarding molecular evolution. By allowing the rate of nonsynonymous substitution to vary along a sequence, it is possible to detect positive selection, even when the ratio of nonsynonymous to synonymous substitutions on average is much less than one. The new likelihood methods are applied to the HIV-1 *env*, *vif* and *pol* genes. Previous analyses of the *vif* and the *pol* gene using heuristic methods did not reveal evidence for positive selection. However, the new likelihood ratio test reveal strongly significant evidence for positive diversifying selection in the *vif* and *pol* genes in addition to the *env* gene. Subsequent to the detection of positive selection, an empirical Bayes method is used to identify which nucleotide sites are being targeted by selection.

Estimating the generation time of within-host HIV population

Yunxin Fu

I will present a simple method for estimating the mutation rate and generation time of fast evolving populations, such as HIV, taking advantage of DNA polymorphisms in longitudinal samples. The estimator is unbiased under a number of population models, including population structure and variable population size over time. From a longitudinal sample of DNA sequences of the *env* gene of human immunodeficiency virus type 1 (HIV-1) from a single patient, we estimated using this method that mutation rate per site per year is 9.89×10^{-3} and the length of one generation is 0.9 to 1 day.

Measuring In-Vivo Migration and Compartmentalization of HIV

Jim Mullins

Abstracts for Poster Presentations

Reanalysis of full-length HIV-1 group M subtype K and sub-subtype F2 with an MS-DOS based bootscanning program

Gert Van der Auwera, Wouter Janssens, Leo Heyndrickx, and Guido van der Groen

Recently, five new complete HIV-1 group M genome sequences were published (Triques et al., *Aids Res. Hum. Retroviruses* 2000;16:139-151). One of these clustered consistently with subtype F sequences, while two others were identified as representatives of a subcluster within the subtype F clade called F2, and the two remaining sequences were described as a new subtype K. We reanalyzed these sequences by means of bootscanning and phylogeny using a newly developed MS-DOS based bootscanning program. Although our analysis confirms the existence of the new subtype K, it does not support the F2 cluster in that it indicates that the F2 sequences are in fact recombinants between subtype F and a still unidentified subtype. If the F2 sequences continue to be treated as a subcluster of subtype F, future analysis of other subtype F related isolates may be seriously hampered, as subtype F would not be monophyletic.

Viral Fitness in Different HIV-1 Infected Cell Compartments

Georg A. Funk, Marek Fischer, Beda Joos, Richard W. Cone, Milos Opravil, Bruno Ledergerber and Sebastian Bonhoeffer

Mathematical models have lead to fundamental new insights into the replication dynamics of HIV-1 in vivo and its pathogenesis. Continuing this approach, we present a population dynamical model describing CD4+ cells, actively, latently, persistently and defectively HIV-1 infected cells, respectively, and free virus in 21 patients under anti-retroviral treatment. The model was numerically solved and simultaneously fitted to clinical data of HIV-1 infected cell compartments. We found that <1% of the activated CD4+ cells in the blood were HIV-1 infected before treatment. Our estimated half-lives of free virus and productively infected cells further support previously published data. Further, we estimated the basic reproductive ratio of the virus in different HIV-1 infected cell compartments. Although the burst size was higher in persistently infected cells than in actively virus producing cells, our model indicated considerably higher basic reproductive ratios of the virus in actively virus producing cells compared to the other HIV-1 infected cell compartments together, because most of the newly infected cells become actively virus producing cells.

Molecular Clock In The Recent Evolutionary History Of HIV-1

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The molecular clock hypothesis is widely used to estimate dates of species divergence and to reconstruct phylogenetic relationships among organisms. In the present study, we attempted to test whether the molecular clock is operational in the recent history of HIV-1 subtype B and, if so, to reconstruct the evolutionary history of the global HIV-1 subtype B epidemic. We analyzed evolutionary distances to the common ancestor of HIV-1 *env* (V3) and *pol* (prot and partial RT) sequences obtained from several outbreaks of the global HIV-1 epidemic – those in the US as well as among homosexual men and injecting drug users (IDUs) in The Netherlands. The onset of all of these epidemics is known based on intensive retrospective epidemiological data, so we had an opportunity to study which phylogenetic model and which genomic region of HIV would provide the most reliable estimate for the time of recent HIV-1 introduction into a human population. Our analysis of evolutionary distances of the US V3 sequences to their reconstructed common ancestor revealed that, according to both the ML and NJ methods, there is a significant positive correlation between the sampling years of sequences and their nucleotide distances to the common ancestor. There was a strong correlation between evolutionary distances of individual sequences to the common root, estimated by both methods (correlation – 0.81, $p < 0.001$). The years of HIV-1 introduction into the US, calculated by both methods based on nucleotide distances, were 1953 and 1967 (for the NJ and ML methods, respectively), which clearly has to be considered an overestimation of the age of the US epidemic (known to start in the mid-70's). Subsequent separate analysis of synonymous and nonsynonymous distances revealed the reason for this discrepancy. There was a highly significant positive correlation between the sampling years of the US V3 sequences and their synonymous distances to the common ancestor ($p < 0.00001$), while nonsynonymous distances of the sequences to the common ancestor were not significantly increasing ($p > 0.1$) during the observation period (1983-1994). The extrapolation of the regression line of synonymous distances back to the date when no synonymous heterogeneity was present in the US HIV-1 population allowed to estimate 1976 (95% CI 1974-1977) as a year of HIV-1 introduction into the USA, which is in agreement with epidemiological data on the history of HIV-1 epidemic in the US. Similar to our findings for the US epidemic, both the ML and NJ methods, when based on nucleotide distances, robustly underestimated the date of virus introduction into Dutch homosexual men and IDUs. Subsequent separate analysis of nonsynonymous and synonymous distances revealed that this underestimation is resulting from nonsynonymous distances, which were not increasing significantly in both virus populations over the observation period. At the same time, analysis of synonymous distances provided accurate estimates for the years of virus introductions into both risk groups, that were in complete

agreement with epidemiological data (1977, 95% CI 1976-1979, and 1980, 95% CI 1979-1981, for homosexual men and IDUs, respectively). In contrast to the V3 region, analysis by either the ML or NJ method of nucleotide distances of *pol* sequences from Dutch homosexual men and IDUs provided accurate estimates for the years of virus introductions in these risk groups.

Determinants of HIV-1C Regulatory Evolution

Monty A. Montano

The past ten years of HIV-1 genome research has seen extraordinary advances in our understanding of the extent of HIV-1 genetic diversity within individuals and populations and has identified the global presence of at least 5 major subtypes (A-E). While it is well recognized that the global epidemic is quickly becoming dominated by HIV-1C, the potential link between epidemic expansion, genetic-subtype variation, and an improved biological fitness has only recently begun to be fully recognized and should now be considered a research priority. Many studies now suggest a capacity for HIV-1C to be a comparatively more aggressive virus than other subtypes. These features include a preferential use of the HIV-1 co-receptor CCR5, an increased propensity for activation in response to elevated levels of cytokines (e.g., TNF- α), an increased viral load compared to other co-circulating subtypes, and an apparent increase in perinatal transmission in utero. We present evidence, based on studies in our laboratory and others, that may collectively support a role for regulatory evolution of HIV-1C that may provide a viral genetic component to the apparent differential expansion of HIV-1C globally.

Absence of genetic diversity reduction in the HIV-1 integrated proviral LTR sequence population during successful combination therapy.

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By measuring over time levels of unintegrated and integrated cell-associated viral DNA we have previously investigated the reservoir of HIV-1 present in PBMCs of 10 infected patients on highly active anti-retroviral therapy (HAART) (Ibañez et al., AIDS 13: 1045-1049, 1999). Concordant with the decline of virus in plasma, a significant decrease in total HIV-1 DNA was observed after 48 weeks of therapy. However, no statistically significant reduction was detected when copy numbers of integrated HIV-1 DNA were compared. We present here the HIV-1 integrated provirus sequence variation in the PBMCs from four of these patients (B, C, D and H). Integrated proviral fragments

of LTR taken from four time points were PCR amplified from PBMCs. The study period included a naïve phase and 1-2 years of therapy. Endpoint dilution of PBMC DNA was performed before nested PCR to make products derived from a single provirus, which were then directly sequenced. Ten individual clones were sequenced for each time point sample. The mean intrasample genetic distances at the beginning of treatment was 1.7%, 2.0%, 1.1% and 2.6% for patients B, C, D and H, respectively. After 48 weeks of therapy no significant change in the intrasample genetic distances was noted (1.6%, 2.3%, 0.4% and 2.3%, respectively). Moreover, samples collected between 48 and 108 weeks of therapy had very similar genetic diversity (1.4%, 1.9%, 0.4% and 2.0%, respectively) compared with the corresponding baseline or 48 weeks samples. Phylogenetic reconstruction of all LTR sequences showed that clones clustered in a patient-specific manner. Phylograms for each patient revealed an intermingling of sequences from the four time points, suggesting an absence of genetic change in this locus after the introduction of treatment. In conclusion, the data presented here imply that 1-2 years of successfully HAART does not significantly reduce the genetic repertoire of the integrated reservoir of HIV-1 present in latently infected CD4 T cells.

Linking *env* proteins from the HIV1 virus to co-receptor usage

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We have carried out an analysis of the *env* protein sequences available in the Los Alamos HIV Database for which we know the cell co-receptor usage (Dorms et al., 1998). We performed a multiple sequence alignment of these 47 proteins (typically over 800 residues long). The alignment is based on a model described by Lamers et al. (1996). We utilized eight different methods to predict the secondary structure of these proteins and considered the consensus predictions. Next we applied a classification method to correlate the amino acids to the information regarding the co-receptor usage. We also correlate the secondary structures to the latter.

CTL Responses and Viral Evolution During the Acute Phase of SIV Infection

David H. O'Connor*, Todd M. Allen*, Edward J. Dunphy*, Peicheng Jing*, Bianca R. Mothe*, Kevin J. Kunstman[#], Xiaochi Wang[¶], John L. Dzuris[§], David T. Evans[%], Ronald C. Desrosiers[%], John D. Altman[¶], Alessandro Sette[§], Steven M. Wolinsky[#], and David I. Watkins[^]

Massive viral replication and the subsequent generation of robust cytotoxic T-lymphocyte (CTL) responses characterize the acute phases of simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) infections. These early events are difficult to study in HIV-infected patients since the exact time of infection and the composition of the infecting virus is often unknown. The SIV-infected rhesus macaque model facilitates analysis of the evolution of the virus in the face of this strong immune response during primary infection. In a previous study, we showed that SIV-specific CTL select for amino acid variation during chronic infection in ten of ten CTL epitopes. Given the magnitude of CTL responses and the extent of viral replication during acute viremia, we reasoned that similar selection for CTL epitope variants should occur during the first month of infection. We infected 10 Mamu-A*01 positive rhesus macaques with pathogenic, molecularly cloned SIVmac239 and monitored CD8 responses using seven MHC-Class I tetramers folded with newly identified Mamu-A*01-restricted epitopes. Antigen-specific CD8 positive lymphocyte responses peaked between three and four weeks post-infection coincident with a decline in plasma viremia. We are currently investigating whether the robust Mamu-A*01-restricted responses selected for CTL epitope variants during the acute phase.

A Rapid Competition Assay Shows A Direct Correlation Between HIV-1 Fitness And Disease Progression

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Rapid evolution of human immunodeficiency virus type 1 (HIV-1) gives rise to drug resistance, escape from immune responses and failure of most single-strain vaccines. Previous studies have suggested that isolates of different clades or even isolates of the same subtype may show variations in replication efficiency/fitness when infecting primary cell isolates. However, few studies have examined the impact of HIV fitness on disease progression. In this study we have developed a competition assay in PBMC to measure the ex vivo fitness of any primary HIV-1 isolates. For viral fitness studies, we established conditions for dual HIV-1 infections of PBMC and a sensitive heteroduplex tracking assay (HTA) to measure relative virus production. Briefly, HIV-1 *env* DNA (a ~1.6 Kb fragment corresponding to gp120-coding region) was PCR-amplified from infected cells using a pair of universal HIV-1 primers. *Env* DNA products from the co-infections were then annealed to reference probes for both clades. Production of both HIV-1 isolates in each

competition was analyzed by HTA and compared with initial inoculums to calculate a relative fitness value for each isolate. Using a pair wise fitness comparison of various nonsyncytium-inducing (NSI)/CCR5-tropic (R5) and syncytium-inducing (SI)/CXCR4-tropic (X4) HIV-1 isolates, four control strains with moderate ex vivo fitness were selected and competed against primary HIV-1 isolates from an HIV-infected Belgian cohort. HIV-1 isolates from long-term survivors (LTS) were out-competed by control strains and were significantly less fit than HIV-1 isolates from patients with accelerated progression to AIDS (PRO). In addition, ex vivo HIV-1 fitness in both PRO and LTS showed direct and independent correlations with viral load, suggesting that HIV-1 fitness together with viral load may be a strong predictor for the rate of disease progression. Based on these preliminary findings, HIV-1 fitness appears to influence and predict HIV-1 disease progression.

HIV tropism and drug treatment

Roland R Regoes and S. Bonhoeffer

The human immunodeficiency virus (HIV) targets macrophages in the beginning of the infection, and in the further course gradually shifts towards a preference for T cells. The preference of HIV variants for certain target cell types is referred to as tropism. Some anti-retroviral agents selectively inhibit HIV phenotypes depending on their tropism. We analyze mathematical models, which describe the *in vivo* interaction of HIV phenotypes, differing in their tropism, with two target cell types (macrophages and T cells). We determine the circumstances, under which the virus population specializes for a certain target cell type, and compare the dynamics of treated with untreated infections. In particular, we investigate the conditions for a treatment-induced phenotype switch.

Uncovering the origin of viral epidemics with a new method to calibrate clock-like phylogenetic trees.

Marco Salemi, Korbinian Strimmer, William W. Hall, and Anne-Mieke Vandamme

When all the viral strains in a phylogenetic tree exhibit the same evolutionary rate, i.e. they evolve according to the molecular clock hypothesis, the estimation of the time of origin of the most recent common ancestor for each clade of the tree can be easily computed. In fact, in such a tree, the degree of sequence divergence, as measured by the number of substitutions along a branch, is linearly proportional to the time of divergence. However, several observations suggest that viruses such as HIV or HCV do not follow a molecular clock. To overcome these limitations, we developed

a new method, called site stripping for clock detection (SSCD), which allows selection of nucleotide sites in a set of aligned sequences evolving at an equal rate in different lineages. The method was validated employing a dataset of HCV patients all infected by the same donor in 1977 and it was able to exactly date the “known” origin of the HCV infection in that cohort. On the other hand, molecular clock dating on the dataset without using the SSCD procedure gave wrong estimates. Our results show that the new method can be reliably used to construct clock-like phylogenetic trees and to calculate divergence times at ancestral nodes. Identifying the sites that distort the molecular clock, the SSCD procedure also allows us to potentially investigate the selective forces leading to differential evolutionary rates.

Inferring Phylogenies for Large, Serially Sampled Data Sets

Daniel Shriner, Allen Rodrigo and James I. Mullins

Although computational power is increasing in terms of speed and memory, the size and complexity of phylogenetic analyses are also increasing. This problem is especially evident in the case of serial sampling, e. g., when following HIV-1 infected patients longitudinally. Whereas there are numerous simulation studies that have examined the behavior of various algorithms for inferring phylogenies, few have examined phylogenetic inference for large data sets (where large is greater than 20). Hence, we investigated computational procedures for inferring phylogenies of 50 and 100 sequences. We addressed three issues: 1. the utility of backbone constraints to account for the time structure present in serially sampled data sets; 2. trees with long diameters and short branches, which are common with serially sampled data sets; and 3. large data sets. We evaluated 10 combinations of optimality criteria and branch swapping algorithms. The results suggest that building a neighbor-joining tree using maximum likelihood distances and then swapping using the subtree-pruning and regrafting algorithm under maximum likelihood is the best compromise between accuracy and speed.

Phylogenetic Evidence for Recombination within a HIV-1 Singly Infected Individual

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Whereas the *env* gene has been extensively studied in terms of its molecular evolutionary dynamics, much less is known about how other regions of the HIV-1 genome evolve. These studies were initiated with the aim of examining how the six auxiliary genes contemporaneously evolve. Initial phylogenetic analyses revealed topological incongruence among the auxiliary genes. Subsequent analyses using parametric bootstrapping support the notion of frequent

recombination, leading to essentially independent assortment of auxiliary genes, within the quasispecies of a singly infected individual.

Evidence for transmission networks based on phylogenetic analysis of demographic and temporal clustering among incident HIV-1 infections, within a prospective injecting drug user cohort in Bangkok, Thailand.

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Background and Objective: During 1995-96, 1,209 HIV-1-negative IDUs attending 15 methadone treatment clinics in Bangkok were enrolled into a prospective cohort study. The overall HIV-1 incidence rate through 1998 was 5.8 per 100 person-years (PY) of follow up. During August-December 1996, the incidence rate doubled to about 12 per 100 PY. Of 130 seroconvertors identified, sequence analysis of the C2-V4 region of gp120 showed that 103 (79%) were infected with HIV-1 subtype E and 27 (21%) with HIV-1 subtype B. The objective of the study was to explore potential HIV-1 transmission networks in relation to temporal and demographic factors.

Methods: All samples used for this study were collected at approximately 3-4 months and again at 12 months following the estimated date of seroconversion (EDS). The C2-V4 region was amplified by nested PCR. Multiple alignments were performed using GCG (Wisconsin package). Phylogenetic analyses were performed with the neighbor-joining distance method using CLUSTAL, PHYLIP, and MEGA programs. Tree topologies were confirmed with maximum-likelihood trees using fastDNAmI and with maximum- parsimony trees using PHYLIP. Amino-acid alignments were generated using DNASIS. **Results:** 1) 6 phylogenetically significant clusters (bootstrap values >80%) were noted for 20/103 subtype E strains which were demographically (same clinic) and/or temporally (similar EDS) associated. Seroconvertors from clinic 9 (n=5), clinic 6 (n=3), and clinic 7 (n=2) were part of 3 strong clinic-based clusters. Seroconvertors from clinics 9 (n=3), 14 (n=2), and 6(n=1) comprised 3 mixed-clinic clusters. 4/6 significant clusters involved temporally associated HIV-1 strains from IDUs who seroconverted during the incidence spike of late 1996.2) 2 phylogenetically significant clusters (bootstrap values >80%) were noted among subtype B strains. One cluster of 2 strains appears to be both demographically (clinic 14) and temporally (EDS late 1996) linked. 3) The deduced amino acid sequences from each of the major phylogenetic clusters showed distinct amino acid patterns that were unique identifiers for each group. **Conclusions:** Phylogenetic analysis using several inference methodologies are suggestive of distinct HIV-1 transmission networks both among IDUs attending the same clinic, or between different clinics in Bangkok. We also note that the clusters of transmission mainly involve subtype E strains from clinics 9, 6, and 14 and are estimated to have occurred during late 1996. A better

understanding of such networks and associated behavioral factors can help improve and focus intervention strategies to reduce HIV-1 transmission.

Adjusting for Assay Detection Limit in Model-Based Estimation of HIV RNA Decay During HAART

Florin Vaida and Tony Fitzgerald

TBA

HIV-1 Populations in Blood and Semen

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Different representations of groups of viral sequences between blood and semen has been found in some patients (Delwart et al.,1997), including drug resistant vs. sensitive populations (Kroodsma et al.,1994). In order to understand the mechanisms underlying this nonuniform distribution, particularly to determine if virus in the two compartments evolve separately, viral populations from blood and semen were compared using longitudinal samples from five patients.

Four out of five patients showed consistently lower nucleotide diversity in semen, whereas one patient showed similar levels of diversity within the two compartments. Using the Sladkin-Maddison method for assessing compartmentalized structure, (Poss et al.,1998), four patients showed evidence of compartmentalization at one or more time points. Three out of four patients showed very similar rates of viral evolution in the two compartments, with the other having a lower evolutionary rate in semen. No signature amino acid residues were found in semen vs. blood viral populations. X4 viruses were only found in one patient, and only in the blood plasma.

Our results suggest that there maybe limited transfer between the two compartments during infection. This results in somewhat parallel evolutionary tracks, however, the biological significance of these two tracks remain obscure. We found no evidence that specific virus populations targeted the blood versus the semen, however, this conclusion is tempered by the fact that we examined only about 7% of the viral genome.

Molecular Epidemiology and Phylogenetic Analysis of HIV-1 *pol* Genotypes from STD Clinics in Mumbai

Womack, C.A., Joshi, S., Deshpande, A., Sahni, S., Maniar, J.K., Saple, D.G., Bond, V.C., and Essex, M.

Reevaluation Of Amino Acid Variability Of The HIV-1 Gp120 And Prediction Of New Discontinuous Epitopes

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To elucidate the evolutionary mechanisms of the HIV-1 gp120 envelope glycoprotein at the single site level, the degree of amino acid variations and the numbers of synonymous and nonsynonymous substitutions were examined from 186 nucleotide sequences for the gp120 (subtype B). Analyses of amino acid variabilities showed that the level of variability was very different from site to site in both conserved (C1-C5) and variable (V1-V5) regions previously assigned. To examine the relative importance of positive and negative selection for each amino acid position, the numbers of synonymous and nonsynonymous substitutions that occurred at each codon position were estimated by taking phylogenetic relationships into account. Among the 414 codon positions examined, we identified 33 positions where nonsynonymous substitutions were significantly predominant. These positions where positive selection may be operating, which we call 'putative Positive Selection sites (PS sites)', were found not only in the variable loops but also in the conserved regions (C1-C4). In particular, we found seven PS sites at the surface positions of the α -helix (positions 335-347 in the C3 region) in the opposite face for the CD4 binding. Furthermore, two PS sites in the C2 region and four PS sites in the C4 region were detected in the same face of the protein. These PS sites found in the C2, C3 and C4 regions were apart in the amino acid sequence but close together in the three-dimensional structure. This observation suggests the existence of discontinuous epitopes in the protein surface including this α -helix, although the antigenicity of this area has not been reported yet.

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