Meeting Report of Colloquium on Systems Biology and HIV Vaccine Development

February 8-10, 2010, Dolce Atlanta Peachtree Conference Centre, Atlanta, GA, USA

Background
The idea for this meeting arose from initial discussions between Alan Bernstein of the Global HIV Vaccine Enterprise and Juan Carlos Lopez, Chief Editor, *Nature Medicine*. Development and planning of the meeting was undertaken by a scientific organising committee (see below). Logistical and financial management of the meeting was undertaken by Nature Publishing Group’s (NPG’s) Nature Conferences team.

Scientific Organising Committee
The Scientific Organising Committee (SC) for the meeting was:

- **Alan Bernstein** (Global HIV Vaccine Enterprise (USA))
- **Bali Pulendran** (Emory University, USA)
- **Rafick Sekaly** (Vaccine and Gene Therapy Institute –Florida, USA)
- **Clare Thomas** (*Nature*, UK)

(Note that, until one month before the meeting, Clare Thomas was an Editor for *Nature Medicine*.)

The Scientific Organising Group developed the aims and scope of the meeting program, discussing the topics to be covered, the program sessions, and identifying the speakers and keynotes to be invited. The final program is attached below.

Speakers
The final program included 27 speakers, 24 from the US, one from Canada, one from France and one from the UK.

Selected attendees and stakeholders
In addition to the presenters, the meeting was designed to include around 10 further attendees selected from their applications to attend the meeting.

Promotion of the availability of these places via the meeting website attracted 35 applicants. From these, the SC selected 12 attendees. Of these 12 attendees, 9 were from the US, two from France and one from Australia. The attendees were invited to present a poster of their work, an invitation eight of them took up. The posters were presented during the after-dinner reception on the first evening. (Poster abstracts attached below – Appendix A.)

The SC also invited a number of ‘stakeholders’ - representatives of companies and organisations involved in HIV vaccine development to attend. Six stakeholders accepted the invitation: two from NIH, and one each from ANRS, France; Global HIV Vaccine Enterprise; Gates Foundation; and Sanofi.

A full list of the delegates is attached below (Appendix B).
Roundtable sessions
In addition to the time allowed in the program for presentations and discussions, the SC scheduled two round table discussions. As part of the preparation for the meeting, speakers were asked to submit questions on what they felt were the major issues around the systems biology approach to HIV vaccine development. These questions were collated (see below) to from the basis of the roundtable discussions.

On-site logistics
The weekend before the meeting coincided with the heaviest snowfall in DC in over 50 years, and eight expected attendees (including five speakers) had to cancel as they were unable to travel. The final day of the meeting coincided with a second storm on the East Coast, and eight attendees stayed in Atlanta an additional night until they were able to travel home.

Scientific summary
This meeting was designed to discuss whether systems biology approaches might aid HIV vaccine design.

Overall, the discussions were interesting, but there was not a consensus whether systems biology approaches could help to *design* vaccines. What was clear is that systems approaches will be very useful to provide insights into pathogenesis and mechanisms of disease, and it will also be useful to use these approaches to evaluate vaccine trials and identify potential mechanisms of protection. It is unclear whether ‘signatures’ of protection obtained from pre-clinical testing or signatures of virus control in elite controllers or other human cohorts or even signatures of protection obtained from other vaccines would be the same signatures that would indicate a protective HIV vaccine. There was some debate about this, but people seemed split.

As this was a discussion meeting it wasn’t intended to disseminate new data and much of what was presented was already published. There were a few exceptions: Rafick Sekaly showed that elite controllers express high levels of heat shock proteins compared with non-controllers; Virginia Pascual showed that gene expression profiling on fibromyalgia patients can offer clues about the pathogenesis of this disease (their gene expression signatures are more reminiscent of what happens after viral infection compared with other rheumatological diseases), Julie McElrath explained how collaborations are being set up with a number of different investigators to use systems biology approaches to analyze samples from the recent modestly successful phase III Thai trial, Anne O’Garra presented some new transcriptional profiling data on TB patients, Elias Haddad presented a paper on a new molecule involved in T cell exhaustion.
Program

Monday, February 8

5:45 p.m  **Welcome and Introductions/Opening Remarks**  
Clare Thomas, *Nature*, UK  
Alan Bernstein, Global HIV Vaccine Enterprise, USA  
Bonnie Mathieson, Office of AIDS Research, National Institutes of Health, USA

6:00 p.m.  **Keynote lecture**  
*Vaccines and immune memory*  
Rafi Ahmed, Emory University, USA

7:00 p.m.  **Dinner**

8.00 p.m.  **Welcome Reception and Poster Session**

Tuesday, February 9

**Session 1 - The power of systems biology in immunology**  
Moderator: Ron Germain, Rockefeller University, USA

8:45 a.m.  **Computing the immune system: Technologies and research organizations needed to generate new understanding and gain predictive capacity**  
Ron Germain, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA

9:10 a.m.  **Using the tools of systems biology to enable rational vaccine design**  
Alan Aderem, Institute for Systems Biology, USA

9:35 a.m.  **Systems approaches to macrophage biology: Mechanisms, networks, models and phenotypes**  
Shankar Subramaniam, University of California, San Diego, USA

10:00 a.m.  **Coffee break**

**Session 2 - Response to infection**  
Moderator: Bali Pulendran

10:20 a.m.  **Functional genomics of natural SIV infections**  
Guido Silvestri, University of Pennsylvania, USA

10:45 a.m.  **Integrative genomic analysis of T cell immunity in humans**  
Nicholas Haining, Harvard Medical School, USA

11:10 a.m.  **Can systems, iterative, and computational biology shed much needed light on our understanding of AIDS pathogenesis and vaccines?**  
Michael Katze, University of Washington, USA

11:35 a.m.  **Systems biology approaches characterize the host response in human tuberculosis**  
Anne O’Garra, MRC National Institute for Medical Research, UK

12:00 a.m.  **Transcriptional profiles of the host response to systemic infection**  
Stephen Popper, Stanford University, USA

12:25 p.m.  **Break and Lunch**

**Session 3 - What can we learn from the genome?**  
Moderator: Jacques Banchereau, Baylor Institute for Immunology Research, USA

2:00 p.m.  **Rare genetic variants and the control of HIV-1**  
David Goldstein, Duke University, USA

2:25 p.m.  **Connecting genomics to pathogenesis**  
Dana Gabazuda, Dana-Farber Cancer Institute, USA

2:50 p.m.  **Coffee break**
Session 4 - Signatures of protection and prognosis I (HIV)
Moderator: Jacques Banchereau

3:20 p.m. Systems biology approaches in HIV pathogenesis and vaccine research
Richard Koup, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA

3:45 p.m. T Cell receptor clonotypic correlates of outcome
Danny Douek, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA

4:10 p.m. Correlates of immunity in HIV infection: Systems analysis of HIV-specific responses
Elias Haddad, McGill University, Canada

4:35 p.m. Coffee break

5:00 p.m. Roundtable discussion: Identification of important questions for the field
Moderators: Rafick Sekaly and Jacques Banchereau

7:00 p.m. Conference Dinner

Wednesday, February 10

Session 5 - Signatures of protection and prognosis II (cancer and autoimmunity)
Moderator: Jacques Banchereau

8:00 a.m. Signatures of the immune response to illuminate lymphoma pathogenesis and predict survival
Louis Staudt, National Cancer Institute, National Institutes of Health, USA

8:25 a.m. Prognostic signature development in lung cancer
David Beer, University of Michigan, USA

8:50 a.m. A systems biology approach linking anatomic pathology, immune gene array profiles, and prognosis: Potential for patient selection for vaccines and other immunotherapies
James Mulé, H. Lee Moffitt Cancer Center and Research Institute, USA

9:15 a.m. Harnessing genomics for diagnosis and treatment of autoimmune diseases
Jacques Banchereau, Baylor Institute for Immunology Research, USA

9:40 a.m. Coffee break

Session 6 - Systems vaccinology
Moderator: Clare Thomas

10:00 Systems vaccinology
Bali Pulendran, Emory University, USA

10:25 Systems biology of T cell memory
Rafick Sekaly, Vaccine & Gene Therapy Institute - Florida, USA

Session 7 - Systems biology in clinical trials
Moderator: Rafick Sekaly

10:50 a.m. Immune signature of a sustained virus control
Brigitte Autran, Université Pierre et Marie Curie, France

11:15 a.m. Who does what, and to whom?
Michael Lederman, Case Western Reserve University, USA

11:40 a.m. Defining vaccine-induced responses in clinical trials using systems biology approaches
Julie McElrath, Fred Hutchinson Cancer Research Center, USA

12:05 p.m. High throughput blood profiling approaches for measuring the immune status of HIV patients
Damian Chaussabel, Baylor Institute for Immunology Research, USA

12:30 p.m. Lessons learned by monitoring cancer vaccines
List of Potential Discussion Questions

- What are the best ways to organize dedicated systems research groups to make the most progress in the fastest manner, and to do so in a cost-effective manner?
- How can the entire community of biomedical investigators be empowered (and convinced) to employ quantitative systems approaches in their research and to co-operate to make the contributions of whole greater than the sum of its parts?
- How can diverse, large-scale datasets be made to meet higher standards and be made available to the larger community in close to a real-time manner?
- How do we deal with the transition from "conventional" microarray to full transcriptome sequencing?
- Where do we draw the line between things that we do in our labs and things that are done in core facility/centers?
- How can we improve the "visualization" of the systems biology results?
- How low can we really go-- i.e., what is the lowest number of cells that can be analyzed and still obtain reliable results?
- What are the key questions in HIV/AIDS research that would mostly benefit from a systems biology approach? Which of these questions would benefit from a collaborative approach?
- What genetic/genomic resources need to be generated to move the field forward-- I am thinking about transcriptome controls for different cell subsets and/or tissues in various NHP species, for instance?
- Given the limited success of translating gene expression-based predictors of cancer outcome into clinical tests in oncology, what is the best way to develop signature-based predictors as correlates of immunity?
- What is the best way for immunologists to capitalize on developments in chemical genomics to develop targeted drugs that augment T cell function in chronic viral infection?
- Why have traditional approaches to understanding the virology and immunology of AIDS been such dismal failures?
- How can we mount a much needed interdisciplinary approach to solve the AIDS vaccine problems?
- What are the most important biological questions that need to be answered for HIV vaccine design and development? Which -omics approaches are most likely to result in actionable answers? What are the consequent critical issues for study design?
- Is natural resistance to HIV related to adaptive or innate immune mechanisms, or intrinsic defense mechanisms.
- What resources are required to allow integration of different 'omic' levels (inherited genome, transcriptome, proteome, etc) in the search for biological correlates of viral control?
- Why has it been so difficult to develop a gene expression-based signature that is robustly prognostic for lung cancer?
Appendix A - Poster Abstracts (in alphabetical order)

COMPUTATIONAL MODELING OF THE IMMUNE RESPONSES INDUCED BY HPV VACCINATION: LESSONS LEARNED AND PERSPECTIVES TOWARDS AN HIV IN-SILICO PROJECT

G.-2, F. Gueyffier.1,2, J-P Boissel 1,2
1IMTh, Institut de Médecine Théorique, Lyon, France; 2CNRS, UMR5558, Laboratoire de Biométrie et Biologie Évolutive, Villeurbanne, France

In spite of the fact that the induction of humoral immune responses against HIV (through prophylactic vaccination) has not, so far, led to any on-the-field satisfactory reduction of transmission (or transition-to-disease) rates, it would seem to remain, when compared to other experimental strategies (such as those targeting cell-mediated immunity), the most promising approach towards an HIV vaccine. The rapid establishment of latent viral reservoirs, HIV’s capacity to escape from the host’s CTL-oriented responses, and the central role of the rapidly depleted CD4 T cells in the establishment of cell-mediated responses, lead us to think that the battle against HIV should be preferentially fought away from the chronic infection field, and, ideally, through the prevention of the earlier acute infection phase.

HPV infections can nowadays be successfully prevented through the induction of a strong systemic humoral response. It is thought that the production of highly specific IgG neutralizing antibodies, which are capable of transducing the mucosal epithelium, impede HPV to complete its viral life cycle. In this project, we devised a simulation platform for quantitatively studying: (i) the immune reactions following the intramuscular administration of the HPV vaccine known as Gardasil® (first sub-model); and (ii) the fate of a ‘virtual’ epithelial tissue that can be confronted to different scenarios of viral challenge both in the presence and in the absence of protective antibodies (second sub-model).

The first sub-model consists of a system of 14 delay differential equations, which describe the behavior (over time) of 14 variables representing the fate of three cellular (APCs, helper T and B cells) and two molecular (HPV-VLPs, anti-HPV antibodies) entities. The second sub-model is based on a multi-agent system in which computational objects (representing biological entities) freely interact according to both a set of rules and a set of parameters (all of which have a biological sense).

This project has permitted us to come up with original solutions for coping with the two major pitfalls of this kind of modeling: (i) the possible lack of relationship between simulations and reality; and (ii) the possible gaps in the biological knowledge (or in the parameters) used as raw material. The formulation of an HIV in-silico project, in which similar computational modeling techniques would be employed, might represent a great opportunity for providing alternative insights to the field. However, for doing so, it would be necessary to implement a series of complementary modeling tools for taking into account other key aspects in the search of an HIV vaccine, such as the need for inducing the production of broadly reactive neutralizing antibodies.

SINGLE-CELL MICROTOOLS FOR SYSTEMS-LEVEL PROFILING OF IMMUNE RESPONSES TO HIV

J. Christopher1,2, Navin Varadarajan1, Adebola O. Ogunniiyi1, Qing Han1, Boris Julg2, Douglas Kwon2, Bruce D. Walker2
1Department of Chemical Engineering, MIT, Cambridge, MA, USA; 2Ragon Institute of MGH, MIT, and Harvard, Boston, MA, USA

Existing technologies for assessing the functional responses of HIV-specific cells have been insufficient for establishing correlates of protection. This poster will describe the development of a collection of new analytical techniques that use microfabricated arrays of subnanoliter containers (10^5-10^6) to determine multiple attributes concurrently from many single live cells in parallel. These arrays make it possible to assess both functional and immunophenotypic characteristics, including antibody specificity, cytokine profiles, cytotoxicity, and lineage-specific, surface-expressed markers. The resulting measures are comparable to a combination of flow cytometry, ELISpot, and functional assays for proliferation and cytotoxicity, but each measure is connected to the same individual cell. Demonstrations of the utility of these single-cell methods for studying cytotoxic T cells and antibody-secreting B cells from peripheral blood and mucosal biopsies from elite controllers will be presented. Together, the integrated single-cell analyses enabled by these microtools should enable new systems-based approaches to clinical monitoring of cellular responses to HIV or candidate vaccines.

GLOBAL GENOMIC ANALYSIS REVEALS RAPID CONTROL OF A ROBUST INNATE RESPONSE IN SIV-
INFECTED SOOTY MANGABEYS

Steven E., Qingsheng Li, Shari N. Gordon, Nichole R. Klatt, Lijie Duan, Luoling Xu, Nicholas Francella, Abubaker Sidahmed, Anthony J. Smith, Elizabeth M. Cramer, Ming Zeng, David Masopust, John V. Carls, Longsi Ran, Thomas H. Vanderford, R. Benjamin Isett, Don A. Baldwin, James G. Else, Silvia I. Staprans, Guido Silvestri, Ashley T. Haase, David J. Kelvin

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Functional genomics were used to characterize host-gene expression during acute and chronic SIV infection of sooty mangabeys (SMs) and rhesus macaques (RMs). SIV infection of SMs was consistently associated with a robust innate immune response during the acute phase, including widespread up-regulation of 21 interferon-stimulated genes (ISGs). While SMs exhibited rapid resolution of ISG expression and immune activation, this response was maintained during chronic infection (> 6 mos) in SIVmac239-infected RMs. Real-time PCR validation of 969 array data points demonstrated remarkable concordance (r=0.6614, p<0.0001). Upregulation of mRNA encoding alpha, beta, and theta defensins were detected in the lymph nodes of SMs but not RMs, and mRNA expression was validated by immunohistochemistry. A systems biology approach identified the lymphocyte exhaustion markers LAG3 and TIM3/HAVCR2 as significantly correlated with immune activation in SIVmac239-infected RMs. These results suggest that the absence immune activation observed in chronically infected SMs is due to immunomodulation of a robust acute response, and not, as has been previously suggested, by hyporesponsiveness to SIV. SMs may control infection of immunological niches by selective expression of defensins and restriction factors. The induction of lymphocyte exhaustion markers TIM3 and LAG3 during acute infection of RMs but not SMs suggests that dysfunctional lymphocyte maturation may occur as early as 10 days post-infection in susceptible hosts and provide targets for elucidating the mechanism of pathogenic lymphocyte immune activation in SIV/HIV infection.

EFFECT OF HLA-C EXPRESSION LEVELS ON HIV PATHOGENESIS

Rasmi, Richard Apps, Ying Qi, Xiaojiang Gao, Victoria Male, Colm O'hUigin, Geraldine O'Connor, Dongliang Ge, Jacques Fellay, Jeffrey N Martin, Joseph Margolick, James J Goedert, Susan Buchbinder, Gregory D Kirk, Maureen P. Martin, Amalio Tenti, Steven D Deeks, Bruce D Walker, David Goldstein, Daniel W. McVicar, Ashley Moffett, Mary Carrington

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A variant located 35kb upstream of the HLA-C gene (-35C/T) was previously shown to associate with HLA-C mRNA expression and the steady-state level of plasma HIV RNA levels. We show that the -35C allele is a proxy for high HLA-C cell surface expression using a HLA-C specific antibody, and further show that HLA-C has dual temporal effects against HIV disease: (1) exceptional early control of HIV-1 replication, independent of the natural killer cell activating KIR2DS receptors, and (2) slow progression to AIDS outcomes that may be dependent on presence/absence of activating KIR2DS. We propose a model in which HLA-C expression levels affect both early and late immune responses through partially distinct mechanisms, illustrating the complex primary role of variation in these genes as major host determinants of HIV disease outcome.
SLOW INFECTION KINETICS: A FUNDAMENTAL BARRIER TO T-CELL BASED HIV VACCINES?

Miles P. Davenport
Complex Systems in Biology Group, Centre for Vascular Research, University of New South Wales, Sydney, NSW, Australia

The metaphor of acute infection as a ‘race between infection and immunity’ is often used to understand the outcome of infection and the benefits of vaccination: Pathogens can grow with a doubling time of as little as one hour, whereas mammalian lymphocytes require 4-5 hours for division, giving pathogens an early growth advantage. In this scenario, vaccination benefits the host by increasing the number of specific lymphocytes and giving a ‘head start’ in the race. One implication of this paradigm is that slowly growing pathogens should be more easily overcome, as they can be rapidly ‘outpaced’ by the immune response. Kinetic analysis of viral-immune dynamics in SIV/SHIV infection shows that the virus is slow growing (with a doubling time of ~12 hours), but that this slow viral growth is accompanied by a 10-day delay in the initiation of the CD8+ T cell response, and slow subsequent growth in T cell numbers. The link between slow pathogen growth and a delayed and slow immune response is not limited to SIV/SHIV infection, and has been observed in a variety of other models of chronic infection. Thus, rather than facilitating immune control, slow pathogen growth appears a mechanism for allowing the establishment of chronic infection.

We have studied the dynamics of antigen presentation in murine models of acute infection in order to understand the dynamics of antigen presentation in vivo. Comparison of the timing of antigen presentation in acute HSV versus influenza challenge of mice suggests that antigen presentation is delayed until a sufficient level of infection/inflammation is achieved. Thus, the delay in CD8+ T cell activation in SIV/SHIV may be due to a delay in achieving a ‘critical mass’ of infection required to stimulate dendritic cell maturation and migration.

There are many vaccination efforts aimed at developing T cell-based vaccines for a variety of chronic infections, which also happen to involve slow growing pathogens. Further modeling and kinetic analysis of the link between pathogen growth rate, immune kinetics, and the implications of this for vaccination are urgently required.

THE STRONG HIV SUPPRESSIVE CAPACITY OF CD8+ T CELLS FROM HIV CONTROLLERS IS ASSOCIATED WITH GAG-SPECIFIC CD8+ T CELL RESPONSES

Asier Sáez-ón, So Youn Shin1, Martine Sinet2, Alejandra Urrutia2, Pierre Versmisse1, Faroudy Boufassa3, Françoise Barré-Sinoussi1, Christine Rouzioux4, Olivier Lambotte5, Alain Venet5, Gianfranco Pancino6; for the ANRS EP36

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The development of anti-HIV T cell-based vaccines is a current major objective in the strategy to halt the AIDS pandemic. For this purpose, the understanding of the mechanisms underlying effective HIV-specific CD8+ T cell responses is of great importance. One of the most appealing models for such efficient responses is found today in HIV controllers (HICs), rare individuals able to control HIV infection to undetectable levels for more than ten years in the absence of therapy. Spontaneous viral control in HICs is usually associated to strong functional HIV-specific CD8+ T cell responses. Accordingly, we have shown that CD8+ T cell suppression in HICs strongly suppress ex vivo HIV-1 infection of autologous CD4+ T cells, suggesting a crucial role of this response in vivo. We have further characterized the HIV-suppressive capacity of CD8+ T cells in 19 HICs.

HIV suppressive capacity in 14/19 HICs was strong, stable and broad, being partially effective even on other primate lentiviruses. HIV suppressive capacity of CD8+ T cells from HICs correlated strongly with the frequency of the HIV-specific CD8+ T cell response and, in particular, with the frequency of Gag-specific CD8+ T cells. Actually, depletion of Gag-specific CD8+ T-cells abrogates HIV suppression. This is especially relevant when it is considered that no other differences were observed between CD8+ T-cells of different specificities in these experiments (phenotype, capacity to secrete cytokines, avidity or potential to proliferate).

Our results suggest the importance of Gag responses in the antiviral potency of CD8+ T cells from HICs. Thus, the anti-HIV potency of CD8+ T cells probably depends on the concurrence of magnitude, quality and, possibly, specificity. Our results underline the convenience of assessing CD8+ T-cell function in a context closer to what the cells find in vivo, i.e. infected cells, rather than in response to peptide stimulation.
MUCOSAL IMMUNOBIOLOGY OF HIV-1 INFECTION

Ruizhong, Phillip D. Smith

1Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA and 2VA Medical Center, Birmingham, AL, USA

The mucosal surfaces of the gastrointestinal, genitourinary and pulmonary tract exclude harmful elements from the body and provide first-line defense against microorganisms. However, mucosal tissues also are common sites of infection by gastrointestinal, genital and pulmonary pathogens, and many microorganisms enter the host through these tissues. Virtually all HIV-1 infections, except those acquired intravenously, are transmitted via the gastrointestinal and genital mucosae, and these mucosal tissues are important sites of HIV-1 replication, yet the early events in HIV-1 mucosal infection have not been fully elucidated.

To address the paucity of information pertaining to the immunobiology of mucosal HIV-1 transmission, we have established explant models using primary human vaginal, rectal and intestinal tissues to recapitulate heterosexual, homosexual, and vertical (mother-to-child) HIV-1 transmission in vitro. These models retain the in situ spatial relationship of the epithelium and lamina propria and, thus, mimic natural HIV-1 infection at mucosal sites. We also have established novel protocols for the routine isolation, purification, and culture of human vaginal, rectal and intestinal cells. Studying HIV-1 infection in primary mucosal cells (macrophages, lymphocytes, dendritic cells, and epithelial cells) isolated from these tissues will provide important information regarding HIV-1 replication in the relevant mucosal cells.

Using human explant model systems and primary mucosal cells, we have shown that (1) human intestinal dendritic cells participate in the translocation of HIV-1 from the apical surface into the intestinal mucosa and transmit HIV-1 to lamina propria and peripheral blood lymphocytes; (2) HIV-1-specific antibodies can block cell-free HIV transcytosis through human rectal mucosa and model colonic epithelium; (3) macrophages in human vaginal mucosa, unlike their counterparts in intestinal mucosa, are permissive to HIV-1 infection; (4) intestinal macrophages are non-permissive to HIV-1 due to NF-κB inactivation; and (5) glycan composition of the HIV-1 envelope potently influences HIV-1 entry into target cells. Understanding the early events of HIV-1 mucosal infection, especially at the molecular level, using explant models and primary cells in a systems biology approach should provide critical information for the design and development of novel vaccine and preventive strategies.

APPLICATION OF AITCHISON SPACE GEOMETRY TOWARD SYSTEMS LEVEL DISSECTION OF HIV IMMUNOPATHOGENESIS AND VACCINE DEVELOPMENT

Ashwin Tumne

Pittsburgh Retrovirus Laboratory, Department of Infectious Diseases & Microbiology, University of Pittsburgh, Pittsburgh, PA, USA

A large amount of biomedical research and development requires the analysis of proportional or frequency data. Such data sets are replete in HIV research, from simple experiments expressed as percentages of a control, to systems level analysis involving experimental approaches such as quantitative protein mass spectrometry, multicolor flow cytometry, time-dependent phosphorylation cascades and global gene expression profiling. Of particular interest here is the generalization of an analysis space for such datasets and their subsequent integration in a systems level molecular analysis of biological phenomenon. Aitchison first described a geometric approach for analyzing multi-dimensional datasets that are inherently proportional within a discrete finite vector space. These approaches are generalized here for a variety of systems level experimental platforms important to HIV vaccine research and development. Current research in the field typically encompasses the intersection of proteomics, transcriptomics and interactomics as they relate to immune biomarker identification, cytokine profiling, inflammatory signaling pathways, and gene regulation. Such datasets and their accompanying analyses can be modeled in the framework of Aitchison geometry, allowing for the quantification of multifaceted intracellular events by simple metrics within the analysis space. A generalization of Aitchison geometry to infinite dimensional Hilbert space facilitates a richer dissection of the molecular biology defining two distinguishable phenotypes, particularly in quantifying at a population level selection pressures regulating gene expression during transitions from one disease phenotype to another. Several examples of experimental datasets from HIV and non-HIV fields are given here to demonstrate the application of Aitchison space geometry in different experimental settings. The results of such analyses is the quantitative framing of transitions between phenotypes as changes in Euclidean distances and angles within geometric objects representing the total system rather than individual numerical calculations which may not satisfy symmetry and triangle equality requirements for proper closure of the dataset as a true system. The application of Aitchison space geometry in both experimental design and data analysis may open the door for novel dissections of biological phenomenon and less ambiguous approaches to HIV vaccine development.
### Appendix B - Delegates List

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