Reflecting on a quarter century of HIV research

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The AIDS pandemic is caused by human immunodeficiency virus, which was discovered at the Institut Pasteur in 1983. In May 2008, scientists met in Paris to discuss the progress and setbacks of 25 years of research in this field and to debate future directions.

For the 'one-off' meeting "25 years of HIV," held 19–21 May, 2008, at the Institut Pasteur, Paris, France, Simon Wain-Hobson (Paris) brought together scientists specializing in various aspects of human immunodeficiency virus (HIV). The topics under discussion ranged from structural studies of viral proteins to the changing nature of the AIDS pandemic. A gradual 'zooming out' from microscopic to global was achieved through sequential sessions describing virus–host cell interactions, immunopathogenesis, correlates of viral control and the evolution of vaccine design.

However, the conference started at the beginning, as Luc Montagnier (Paris) and Robert Gallo (Baltimore, USA) revisited the pioneering work of the 1980s[1–3]. Montagnier described the discovery of HIV-1 by his group[1] and the initial interactions with Gallo's laboratory[3]. HIV-1's inherent genetic variability, generated through recombination and error-prone reverse transcription, facilitates evasion of immunity and drug control; on those grounds, he proposed the development of therapeutic vaccines aimed at functionally eradicating infection by targeting conserved epitopes. Such a vaccine might be effective when used alongside measures to suppress other correlates of disease progression, including oxidative stress and chronic immune activation. Gallo echoed the need to

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understand and counter the overactivation of immunity in HIV-1 infection, which is the best predictor of disease course. He noted heterologous challenge as a vital component of vaccine trials with monkeys and cast doubt on the potential efficacy of vaccines based solely on cell-mediated immunity. Speaking more generally, he emphasized the requirement for transparency, openness and collaboration among researchers, and the rapprochement between the two opening protagonists was exemplary in this respect.

Virus–host cell interactions

A common theme echoed throughout the meeting was the need for more understanding of basic HIV-1 biology. Aspects of HIV-1 envelope–host cell interactions, viral budding and host restriction mechanisms were discussed, which provided a 'taste' of ongoing research in this area. Anthony Fauci (Bethesda, Maryland, USA) presented experiments identifying the activated form of integrin $\alpha_4\beta_7$, a gut-homing marker for lymphocytes, as a previously unknown ligand for the HIV-1 envelope glycoprotein gp120 (ref. 4). Binding to $\alpha_4\beta_7$ by a conserved tripeptide in gp120's V2 region activates LFA-1, a second integrin that aids the cell-to-cell spread of HIV-1 by stabilizing virological synapses. These synapses are supramolecular assemblies reminiscent of immunological synapses, characterized by cytoskeletal rearrangements and polarization of specific cellular and viral proteins such as LFA-1, CD4, gp120 and Gag[5]. Activated $\alpha_4\beta_7$ localizes together with such proteins at adhesive junctions between HIV-1-infected and uninfected cells. The link among $\alpha_4\beta_7$, the

virus and the gut is intriguing because the gut mucosa represents a key site of HIV-1 replication and seems to be central to immunopathogenesis.

Olivier Schwartz (Paris) also considered cell-to-cell transfer of HIV-1. Beyond conventional virological synapses, he reported transfer by means of filopodia- or pseudopodia-like membrane protrusions and from one cell to multiple targets simultaneously, with several sites of Gag polarization in single cells. Although cell-to-cell spread of HIV-1 remains to be demonstrated *in vivo*, HIV-1 target cells are in frequent contact in lymph nodes and mucosae. Viral budding from sites of Gag polarization was also discussed (Wesley Sundquist, Salt Lake City, Utah, USA). To facilitate viral exit from cells, HIV-1 and other enveloped viruses appropriate the cellular 'ESCRT' (endosomal sorting complex required for transport) pathway, which normally directs membrane fission events such as abscission, the final step of cytokinesis. The HIV-1 Gag p6 protein recruits two ESCRT pathway proteins to sites of viral budding: Tsg101, part of the ESCRT-I complex, and ALIX, which interacts with both ESCRT-I and the CHMP4 proteins of the ESCRT-III complex. He presented detailed structural and biochemical characterization of ESCRT pathway components and their interactions, from which models of ESCRT-mediated membrane fission can be proposed[6,7].

In cells, retroviral replication is restricted by intrinsic host factors, including certain APOBEC3 and TRIM proteins. Evolutionary genomics has shown how positive selection has shaped these proteins throughout primate

evolution, reflecting their probable long-term involvement in defense against pathogens (as discussed by Amalio Telenti, Lausanne, Switzerland)[8]. Tom Hope (Chicago, USA) used live imaging techniques to investigate restriction by TRIM5α, which mediates a post-entry block to HIV-1 replication in Old World monkey cells[9], whereas Michael Malim (London) discussed viral inhibition by the host cytidine deaminases APOBEC3G and APOBEC3F. When HIV-1 Vif activity is bypassed, these host proteins induce lethal cytidine-to-uridine editing ('hypermutation') of nascent viral cDNA. However, the observation of editing-independent antiviral effects raises questions of whether restriction relates mainly to deaminase activity, and Malim presented evidence that inhibition of viral cDNA accumulation correlates better with antiviral function[10]. Nevertheless, APOBEC3-induced mutation might have a substantial effect on HIV-1 pathogenesis if induced in 'nonlethal' amounts (such as in circumstances in which the opposing APOBEC3-Vif functions were appropriately balanced) potentially contributing to viral genetic diversification and drug or immune escape.

Considerable involvement of RNA in virus-host interactions is becoming apparent. Monsef Benkirane (Montpellier, France) specifically considered the interaction of microRNA and RNA silencing with viral replication. Primary microRNA transcripts are processed by the type III RNases Drosha and Dicer and the RNA-induced silencing complex. The resulting microRNA silences target mRNA, often in association with P-bodies, which are sites of RNA storage and processing. Knockdown of Drosha and Dicer enhances HIV-1 replication and derepresses latent viruses, which indicates the innate restriction capacity of this pathway. Conversely, HIV-1 infection leads to downregulation of specific microRNAs that translationally repress PCAF (a cofactor of the viral transactivator Tat), which demonstrates viral manipulation of cellular microRNA[11]. Notably, both HIV-1 transcripts and APOBEC3G or APOBEC3F (which associate with various proteins of RNA metabolism and specific RNAs; Michael Malim[12]) also localize to P-bodies.

As the work presented above reflects, progress in understanding basic virus–host cell interactions continues to provide insight into basic cellular processes and may inform future therapeutic strategies.

Insights into HIV-1 immunopathogenesis

Several key advances in understanding HIV-1 immunopathogenesis concern the destruction of gut-associated lymphoid tis-

Participants of the meeting: front row (left to right), Daniel Douek, Andrew McMichael, Gary Nabel, Brigitte Autran, Ashley Haase, Simon Wain-Hobson, Anthony Fauci, Luc Montagnier and Robert Gallo. Photo: Institut Pasteur.

sue during acute HIV-1 infection. Although HIV-associated enteropathy was initially described in 1984 (ref. 13), the rapidity and scale of the mucosal pathology was not appreciated until later[14]. Considerable loss of most $CD4^+CCR5^+$ memory T cells from the gastrointestinal lamina propria, their main anatomical reservoir, occurs within weeks of infection. Thus, understanding the early events in infection is crucial.

Transmission through genital mucosa is the most common route of infection globally. Ashley Haase (Minneapolis, Minnesota, USA) investigated vaginal transmission in the macaque simian immunodeficiency virus (SIV) model. Even with large viral inocula, foci of infected cells in the vaginal mucosa are rare, which demonstrates the structural and immunological defense this mucosa provides. However, a mucosal 'outside-in' signaling mechanism triggered by the virus specifically recruits viral target cells, inadvertently fueling expansion and dissemination of these small founder populations. Andrew McMichael (Oxford) presented studies of acute HIV-1 infection. Consistent with the observations noted above obtained with macaques, phylogenetic analysis of single HIV-1 genomes from early infection indicates that most systemic infections are established by single founder viruses[15]. Furthermore, by combining large-scale sequencing and detailed immunological analyses, the team from the Center for HIV-AIDS Vaccine Immunology has been able to identify early cytotoxic T lymphocyte responses around the time of peak vire-

mia in acute infection; however, immune escape mutations rapidly follow many such responses, which probably renders them useless for viral control.

Does the destruction of $CD4^+$ T cells in gut-associated lymphoid tissue during acute infection contribute to the progression of HIV-1 disease, given that the gut is also depleted of $CD4^+$ T cells in nonpathogenic SIV infection of nonhuman primates? The pandemic HIV-1 strains are closely related to SIVs from chimpanzees and gorillas, which shows the zoonotic origin of the pandemic. Over 30 African primate species carry SIV infections (Martine Peeters, Montpellier, France); however, unlike infection with HIV-1 or macaque SIV, these infections are typically nonpathogenic in their natural hosts. Françoise Barré-Sinoussi (Paris) and Daniel Douek (Bethesda, Maryland, USA) compared progressive infection (such as with HIV-1 and macaque SIV) with nonpathogenic infection (such as with African green monkey SIV), reiterating chronic immune activation status as the best correlate of disease progression. Differences in the functional profiles of plasmacytoid dendritic cells during early infection of African green monkeys and macaques correlates with the resolution or persistence of systemic immune activation (Barré-Sinoussi). Furthermore, critical events of early infection in the gut may dictate immune activation status at later time points (Douek). 'Preferential' depletion of the interleukin 17–producing T helper cell subset of $CD4^+$ T cells from gut-associated lymphoid tissue, which protect

against fungal and bacterial infection, has been noted in HIV-1-infected humans but not in SIV-infected African green monkeys or sooty mangabeys. This immune deficit correlates with greater gut permeability and translocation of microbial products, such as lipopolysaccharide and peptidoglycan, which also correlates with systemic immune activation[16]. Furthermore, Petronela Ancuta (Montreal) and Richard Koup (Bethesda, Maryland, USA) described 'preferential' infection of specific T cell subsets by HIV-1, which possibly contributes to pathogenesis. Thus, the nature of structural and immunological damage to gut-associated lymphoid tissue during acute infection influences persistent immune activation in the chronic phase, which in turn correlates with disease progression.

Correlates of viral control

Variability in the extent of viral control and in disease progression among infected individuals is well described, yet correlates of control remain to be fully characterized. Several speakers addressed this issue from various angles. Richard Koup assessed the contribution of T cells with various phenotypic profiles to viral control. Despite the lack of correlation between the overall magnitude of the CD8^{+} T cell response and plasma viral load, HIV-1 nonprogressors maintain higher proportions and frequencies of CD8^{+} T cells expressing many functional markers (such as cytokines (interferon-γ, tumor necrosis factor and interleukin 2), chemokines (CCL4 (MIP-1β)) and markers of degranulation (CD107a mobilization)) than do progressors[17]. Thus, as with other persistent viral infections (including cytomegalovirus and Epstein-Barr virus), effective CD8^{+} T cell responses to HIV-1 infection tend to be characterized by an abundance of polyfunctional cells. Bruce Walker (Boston, USA) introduced data from the International HIV Controller Study, which recruits 'elite controllers' (HIV-1-infected people who consistently maintain viral loads below 50 RNA copies per ml in plasma without therapy) and aims to identify correlates of viral control by studying sufficient numbers of such people. Preliminary results suggest associations between viral replicative capacity *in vitro* and viral control *in vivo* and that T cells restricted by known protective HLA alleles (such as HLA-B57) constrain replicative capacity more effectively than do those restricted by other alleles, which therefore suggests a link among virology, host genetics and immune function.

How host genetic variation influences the disease course of HIV-1 infection was explored further by Mary Carrington

The Eiffel tower at night. Photo: Emma Jo Bowles

(Frederick, Maryland, USA) and Sunil Ahuja (San Antonio, Texas, USA). They emphasized synergistic interactions between the HLA class I and killer immunoglobulin-like receptor (KIR) loci and between the CCR5 and CCL3L1 genotypes, respectively. HLA class I molecules (HLA-A, HLA-B and HLA-C) bind both T cell receptors and KIRs. Notably, alleles encoding HLA-Bw4-80I in combination with both the activating KIR3DS1 receptor and inhibitory KIR3DL1*h receptor (where '*h' indicates 'highly expressed allotype') protect against high viral loads and rapid progression[18]. Similarly, a particular single-nucleotide polymorphism in the promoter of the gene encoding HLA-Cw correlates with higher surface expression of HLA-Cw and lower viral load[19]. However, later in infection, the protective effects of this single-nucleotide polymorphism are lost in people who express KIR2DS. Thus, various HLA-KIR haplotypes have complex influences on innate and adaptive responses to HIV-1, conferring distinct temporal protection during HIV-1 infection. Susceptibility to HIV-AIDS is also influenced by polymorphisms in *CCR5*, which encodes

an HIV-1 coreceptor, and variation in copy number of *CCL3L1*, which encodes an HIV-suppressive chemokine ligand for CCR5. People with fewer than the population average copy number of *CCL3L1* have enhanced susceptibility, with the effect greatest in those also carrying 'risk' *CCR5* genotypes. CCR5 and CCL3L1 are direct and indirect participants, respectively, in HIV-1 entry, yet entry-independent effects are also apparent, as cell-mediated immune function and CD4^{+} T cell recovery in people with viral suppression induced by highly active antiretroviral therapy also correlate with particular *CCL3L1-CCR5* combinations[20,21]. In general, determination of host genetic susceptibility traits might provide useful prognostic information and may have value in the evaluation of vaccine trials.

AIDS vaccines

The withdrawal, mid-trial, of the Merck 'STEP' vaccine aimed at stimulating HIV-1-specific T cells loomed large over the conference[22]. Delegates debated several issues surrounding vaccine development: the pros and cons of therapeutic versus prophylactic vaccines; whether neutralizing antibodies or T cells (or both) should be stimulated; and what sort of vectors should be used to deliver the vaccine. Gary Nabel (Bethesda, Maryland, USA) described this situation as being at a 'crossroads'. He emphasized that in the absence of natural protective immunity to HIV-1, rational design of a successful vaccine will require deep understanding of the virus. Three approaches to vaccines were described.

What constitutes an effective T cell response to HIV-1 remains incompletely understood, although accumulating evidence suggests that a multifunctional response to several epitopes is desirable[17,23]. The global diversity of HIV-1 and its ability to escape immunological pressures presents a further challenge for vaccine design. Targeting T cells against conserved epitopes, whose mutation probably incurs a 'fitness cost' to the virus, could circumvent this obstacle. Vaccines aimed at priming broad T cell responses against conserved regions are being developed by Gary Nabel and Tomas Hanke (Oxford). Designing vectors that induce strong immunity is another important issue, particularly given the problems surrounding the adenovirus vector used in the Merck vaccine. Brigitte Autran (Paris) presented data from a trial of a therapeutic T cell vaccine that uses an engineered canary-poxvirus vector. Viral loads were higher in vaccinees than controls; this vaccine boosted HIV-specific CD4^{+} T cells, potentially creat-

ing targets for viral replication, whereas HIV-specific CD8^{+} T cells were not induced. Pierre Charneau (Paris) discussed lentivirus-based vaccine vectors incorporating the HIV-1 DNA 'flap' sequence[24], which maximizes gene-transfer efficiency. Vaccines using such vectors can transduce nondividing cells, such as immunogenic dendritic cells. Herpesvirus vectors, which are being deployed by Ronald Desrosiers (Boston, USA), provide sustained infection and antigen presentation. A herpesvirus of rhesus monkeys has been identified and has been engineered into recombinant forms to coexpress SIV genes. Vaccines using the vectors described above are in various stages of development, can induce T cell and in some cases humoral immune responses, and generate protection against viral challenge in macaques to various extents.

Eliciting broadly reactive neutralizing humoral immunity is considered a goal for an effective vaccine. However, HIV-1 is a problematic target for antibodies because of its highly variable gp120 envelope, its masking glycan shield and its capacity for rapid escape. Structural studies of one broadly neutralizing antibody (b12) bound to HIV-1 gp120 have benefited vaccine design. Peter Kwong (Bethesda, Maryland, USA), in collaboration with Gary Nabel, showed that b12 contacts a region of gp120 that is functionally constrained because of the nature of gp120-CD4 binding[25]. Creating stable conformational mimics of this site and using them as immunogens might generate effective neutralizing antibodies. Immunologically 'cloaking' regions surrounding the site with various SIV gp120 sequences in heterologous prime-boost regimens may further focus the antibody response on the vulnerable area. Dennis Burton (La Jolla, California, USA) and Alexandra Trkola (Zurich) discussed what constitutes an effective neutralizing antibody response to HIV-1 *in vivo*. Although this issue (like that of HIV-specific T cells) is incompletely understood, two points made were that in vaccine trials, the efficacy of antibodies is more apparent with low-dose viral challenge, and that much less neutralizing antibody is needed for protective effects in the acute phase of infection than in the chronic phase[26].

Given the importance of mucosae for viral transmission, providing protection against infection in these anatomical sites is desirable. Intramuscular administration of SIV genes with a DNA–modified vaccinia virus Ankara 'prime-boost' procedure could induce potent SIV-specific T cell responses at mucosal sites and considerably diminish disease progression after vaginal challenge (Marie-Claire Gauduin, Boston, USA). Ashley Haase discussed using anti-inflammatory microbicides to prevent vaginal transmission. Such microbicidal agents could diminish the virus-induced signaling cascades that recruit HIV-1-susceptible T cells into the mucosa.

Overall, there was still optimism that a successful vaccine could be developed, perhaps by incorporating components of each of the approaches described above. Cell-mediated immunity alone may struggle to contain a rapidly mutating virus, and although neutralizing antibodies specific for conformationally invariant epitopes may be induced, sustaining effective humoral responses probably requires T cell help. Additionally, slowing the spread of the virus through mucosal barriers may assist in the generation of immune protection. As several speakers mentioned, effective cooperation among the various arms of immunity may follow from closer collaboration among vaccine researchers.

Perspectives and concluding remarks

The conference ended with sobering talks by Jean-Paul Moatti (Geneva), Malegapuru Makgoba (Durban, South Africa) and Peter Hale (Eschborn, Germany) on the history, present state and future of the global pandemic. Emphasis was placed on better basic science (and more funding for it), transparency among researchers and the continued development of vaccines to effectively combat AIDS. Harking back to the pathogenesis of this prototypic lentiviral infection, Simon Wain-Hobson described HIV-1 as 'remorseless'; to succeed in controlling the pandemic, the research community will need to be implacable.

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