Human Immunodeficiency Virus Type 1 Infection, Mucosal Immunity, and Pathogenesis: Comments and Conference Summary

Sharon M. Wahl, Phillip D. Smith, and Edward N. Janoff

Infection with human immunodeficiency virus type 1 (HIV-1) has now been reported in virtually every country of the world. Responding to the challenge of this devastating pandemic, basic and clinical investigators in many regions of the world have rapidly advanced our understanding of the biology, immunology, and virology of systemic HIV-1 infection. Although the vast majority of HIV-1 infections are acquired through mucosal transmission, information regarding the immunobiology of mucosal HIV-1 infection has been more difficult to obtain due to the difficulties of studying mucosal tissues in vivo and primary mucosal cells in vitro. Many of these difficulties have now been overcome, leading to new insights into the pathogenesis of mucosal HIV-1 infection. To address this emerging and important area of study, this symposium brought together basic and clinical investigators whose work focuses directly or indirectly on mucosal HIV-1 disease.

The work discussed by these investigators covered the following seven areas: mucosal transmission, primate models of transmission and disease, mucosal cells and tropism, viral tropism, mucosal defense and HIV-1, pathogenesis of opportunistic mucosal pathogens, and mucosal immunity and vaccination. The salient points of many of these observations are presented individually in the following articles, and highlights of presentations by Sten Vermund, Deborah Anderson, Norman Letvin, Andrew Lackner, Mario Stevenson, Eric Hunter, Jan Orenstein, Rosemary Soave, Kent Sepkowitz, Barney Graham, and Jay Berzofsky are summarized below.

Sten Vermund (University of Alabama at Birmingham) emphasized the magnitude of the AIDS epidemic and summarized recent epidemiologic trends. In 1989, the cumulative number of AIDS cases in the United States was 100,000, but by 1997, the number had increased dramatically to 600,000. By 1993, AIDS had become the leading cause of death among persons between 25 and 44 years of age living in the United States. The rate of increase reached a plateau during 1996–1997, likely due to the effective use of newer antiviral therapies. In particular, a dramatic 50% reduction (from 60,000 to 30,000) in yearly deaths due to AIDS in the United States has been attributed to highly active anti-retroviral therapy (HAART).

Deborah Anderson (Harvard Medical School, Boston) reviewed how disease stage, the presence of sexually transmitted diseases and genital tract inflammation, and anti-retroviral therapy affect the level of HIV-1 in genital secretions. HIV-1 is present at high levels in genital tract secretions during the acute primary infection stage and is followed by reduced levels during the subsequent period of clinical latency and by increased levels during late-stage disease. In addition, the presence of certain genital tract infections is associated with substantial increases in the level of HIV-1 in genital secretions. The virus in these secretions appears to be carried by both T cells and macrophages. The ability of T cells to adhere to genital tract epithelium offers a potential mechanism by which virus is transferred to the recipient partner. Of importance, accumulating evidence suggests that therapy with antiretroviral agents, such as zidovudine and the new protease inhibitors, markedly diminishes HIV-1 levels in genital secretions, although residual HIV-1 DNA and RNA may occasionally be detected.

Other factors that may be related to increases in the levels of HIV-1 in genital secretions in women include hormonal contraception, cervical ectopy, and pregnancy. In general, the level of HIV-1 in genital tract secretions mirrors that of the blood, although the absolute level is lower, possibly due to the presence of inhibitors of replication or neutralizing antibodies. The roles of epithelial cytokines, chemokines, chemokine receptors, and intraepithelial lymphocytes are important topics that warrant critical investigation.

Norman Letvin (Harvard Medical School) underscored the importance of cytotoxic T lymphocyte (CTL) activity in eliminating viral spread through the lysis of virus-infected target cells and the release of cytokines (and possibly chemokines) with antiviral activity. The emergence of CD8 \(^{+}\) lymphocytes in primary infection correlates with the clearance of virus during the initial viremia that characterizes acute infection. These cells have been identified in virtually all body compartments, including the mucosa. In addition, the long-term nonprogression
of HIV-1 infection that occurs in some persons correlates with the high frequency of HIV-1-specific CTLs.

Dr. Letvin’s group has characterized the time course, anatomic compartmentalization, clonality (focused vs. diverse epitope recognition), and magnitude of the CTL response in acute infection in the simian immunodeficiency virus (SIV) model. In pilot studies, CTL activity for cells expressing SIVmac Gagp11C, a peptide fragment that is the dominant epitope in the CTL recognition site, was generated rapidly (72 h) in mucosa of vaginally inoculated animals. Moreover, these CD8+ CTLs appeared to traffic from mucosal inductive sites to regional and then peripheral lymph nodes. Analyses of the T cell receptor V\(\beta\) repertoire during primary SIV infection have revealed expansion of selected V\(\beta\) (V\(\beta\) 14, 7, and 23)-expressing CD8+ T cells and that this expansion is oligoclonal. These CTLs exhibited specificity and lysis in novel experiments in which CD4+ cells were labeled with peptide/major histocompatibility complex class I/\(\beta\) 2 complex to which tetrameric Mamu-A*/01/p11C avidin complex was bound. These studies reinforce the need to induce CTL activity in the mucosa, particularly through mucosal vaccination.

Andrew Lackner (Harvard Medical School) emphasized that tissues, in addition to blood, are important sites of HIV replication. Despite the remarkable size of the intestinal mucosa as a lymphoid organ, little is known about viral replication in this unique immunologic environment. To address this issue, Dr. Lackner and colleagues inoculated groups of rhesus macaques with molecular clones of SIV, including SIVmac239, SIVmac239/316, SIVmac239Y\(\Delta\)nef, and SIVmac239\(\Delta\)nef, and then evaluated harvested intestinal lymphocytes to characterize CD4 and CD8 expression and tissue sections to determine the number and phenotype of SIV-infected cells. Infection with each strain, except SIVmac239\(\Delta\)nef, caused a marked decrease in the number of CD4+ lymphocytes in the jejunum, ileum, and colon as early as 7 days after inoculation despite minimal reductions in the number of these cells in the blood, spleen, and lymph nodes at the same early time points (days 7, 14, and 21). Coinciding with the reduction in tissue CD4+ T cells, more SIV-infected cells were present in the intestinal mucosa than in peripheral lymph nodes. Moreover, the number of infected cells declined over time in parallel with the progressive decline in the number of mucosal CD4+ lymphocytes. In addition, using Ussing chambers to analyze short-circuit current changes in the duodenum, jejunum, and ileum of SIV-infected animals, the investigators showed that alterations in chloride resistance coincided with the CD4+ T cell depletion, implicating SIV-induced CD4+ T cell depletion in the intestinal dysfunction associated with SIV disease. Thus, the intestinal mucosa is a major site of SIV replication and CD4+ depletion in early infection, underscoring the fundamental role of the mucosa in SIV disease pathogenesis.

Mario Stevenson (University of Massachusetts, Worcester, MA) proposed that the ability of HIV-1 to infect terminally differentiated macrophages required functional Vpr protein. His studies previously demonstrated that Vpr facilitates nuclear targeting of the viral reverse transcription complex. Additional properties of HIV-1 Vpr, which are unrelated to its role in macrophages, include the induction of cell cycle arrest and an association with the DNA repair enzyme, uracil DNA glycosylase (UDG). Members of the HIV-2/SIV\(\text{sm}\) lineage contain an additional gene, termed vpx, which shares considerable sequence homology with vpr.

Dr. Stevenson reported that Vpx is necessary for macrophage infection, whereas Vpr governs cell cycle arrest and UDG association. Thus, mutations in vpx prevent infection of macrophages by SIV\(\text{sm}\) but do not influence the ability of the virus to induce cell cycle arrest or to associate with UDG. Using SIV\(\text{sm}\) PBj variants containing mutations in vpr and vpx, his laboratory examined mucosal transmissibility and in vivo replication properties of these mutants relevant to macrophages. Mucosal coinfection of macaques with wild-type and vpx mutant viruses resulted in selective transmission of wild-type viruses, whereas intravenous transmission was comparable between the 2 isolates. In addition, replication and pathogenicity of vpx mutant viruses following either intravenous or intrarectal infection were markedly impaired relative to wild-type or vpr mutant viruses. These studies demonstrate that both efficient mucosal transmission and replication of SIV\(\text{sm}\) PBj require an intact vpx gene and suggest an important role for macrophages in these processes.

Eric Hunter (University of Alabama at Birmingham) emphasized the importance of structural motifs in the HIV-1 gp41 fusion peptide, the hepad repeat region (leucine zipper region), which is the highly conserved membrane-spanning domain that plays a key role in envelope-mediated virus entry and cell-cell fusion. The HIV-1 gp41 hepad repeat domain is not involved in assembly oligomerization of the HIV-1 glycoprotein complex in the rough endoplasmic reticulum, but it can induce oligomerization when fused to a monomeric protein. The hepad repeat domain is critical for biologic activity, as reflected in the ability of peptides corresponding to this region to block fusion and entry. Mutations that block oligomerization also block both the biologic activity of envelope and the inhibitory activity of peptides. Point mutations in the hepad repeat domain or leucine zipper region cause destabilization of the helix, leading to the inability of peptides to inhibit fusion and the inability of mutated glycoproteins to mediate fusion or replications. One peptide, DP178/T20, is a potent inhibitor of HIV-1 in vitro and in animal models and is currently in phase 1 clinical trial.

A highly conserved tryptophan-rich region of gp41 is also crucial for envelope biologic activity, specifically virus entry. Mutations of individual tryptophans have no significant effect on cell-cell fusion, but each dramatically affects virus entry. In contrast, deletion of this region or substitution of multiple tryptophans with alanine residues blocks both cell fusion and infectivity. Virus incorporation studies indicate that even single
amino acid changes in this region severely inhibit packaging of glycoprotein into virions, resulting in defects in infectivity. Thus, major conformational changes are involved in virus entry and are potential targets for the inhibition of the initial entry of HIV-1 into the mucosa.

Jan M. Orenstein (George Washington University, Washington, DC) summarized recent information on Microsporidium species, which is one of the most promiscuous opportunistic pathogens affecting patients with advanced HIV-1 infection. These protozoan parasites cause a remarkable array of symptoms and pathology. Enterocytozoon bieneusi resides exclusively in the gastrointestinal tract. The organism infects epithelial cells but does not infect macrophages or disseminate. Diarrhea and biliary tract involvement define its spectrum of disease. In contrast, Encephalitozoon intestinalis, which also infects intestinal epithelial cells, may disseminate to kidney and brain, particularly microglial cells, the preferred targets. Consequently, examination of urine and stool for ova and parasites is a sensitive diagnostic test for E. intestinalis. Microsporidian infections of the brain may mimic the ring-enhancing brain lesions of toxoplasmosis by computed tomography, but tissues of virtually all organs, including those of the respiratory, musculoskeletal, cardiac, and endocrine systems may be involved in disseminated microsporidiosis. Virtually no cell is inhospitable to the organism, which can be identified in septated vacuoles by Gram’s stain, Brown and Brenn stain, fluorochrome dye, or electron microscopy. The disseminated Encephalitozoon species are susceptible to treatment with albendazole (400 mg orally twice a day).

Rosemary Soave (Cornell Medical School, New York City) discussed Cryptosporidium infection in association with HIV-1 infection. In contrast to Microsporidium species, Cryptosporidium species has been recognized since 1907. However, this environmentally hardy, waterborne, epidemic-associated protozoan came to medical attention with the HIV-1/AIDS epidemic. Whereas immunocompetent persons, particularly young children and adult travelers, may experience significant but self-limited enteric illness with Cryptosporidium infection, symptoms may be more severe and persistent with concomitant HIV-1 infection. The magnitude and duration of intestinal symptoms is related to the extent of immune dysfunction of the host. In this regard, use of effective antiviral therapy appears to have decreased the incidence and prevalence of symptomatic Cryptosporidium infections in HIV-1–infected persons.

Infection with Cryptosporidium species, like E. bieneusi, is typically limited to the intestine. The parasite occupies a unique intracellular, but extracytoplasmic, niche in epithelial cells, a location that may limit immune recognition and clearance of the organism. The development of antibody responses to Cryptosporidium antigens may be related to host immune status. Preliminary data suggest that ELISA IgG and IgA antibody responses to the parasite may not correlate with specific antigen reactivity by Western blot. The presence of parasite-specific antibodies detected by immunoblot is associated with a favorable clinical response, whereas the absence of such antibodies correlates with a poorer outcome. Current investigations seek to characterize the mechanisms of epithelial cell–Cryptosporidium interaction and the factors that promote persistent parasitosis.

Kent Sepkowitz (Sloan-Kettering Memorial Cancer Center, New York City) reviewed the impact of HAART on the epidemiology of HIV-1 and AIDS. Nationwide, as in New York City, the number of AIDS-related deaths has declined, and the death rate has fallen below the incident rate. HIV-1/AIDS is no longer the leading cause of death among 25- to 44-year-old men. Although the spectrum of opportunistic infections and processes among HIV-1–infected patients admitted to the hospital is similar to the spectrum prior to the HAART era (1995–1997; e.g., about one-third of patients with Pneumocystis carinii and 5%–12% each with cytomegalovirus, esophagitis, wasting, lymphoma, and Mycobacterium avium complex), the incidence of these infections appears to be appreciably decreased even though case-fatality rates are similar. Most of these infections now occur in persons who have not received prophylactic therapy. HAART can affect the natural history as well as the incidence of opportunistic infections. Although progressive multifocal leukoencephalopathy may progress during therapy, molluscum contagiosum, Kaposi’s sarcoma, parvovirus B19 infection, wasting syndrome, cryptosporidiosis, and microsporidiosis either regress or resolve. Seroreversion is noted with hepatitis B infection, and cytomegalovirus retinitis may stabilize. However, selected patients, including those with cytomegalovirus retinitis, hepatitis B infection, and M. avium complex infection, show transient exacerbation of pathogen-related symptoms. Thus, HAART exerts both quantitative and qualitative effects on the incidence and natural history of several opportunistic infections, including important mucosal infections.

Barney Graham (Vanderbilt University, Nashville, TN) summarized efforts toward production of an effective vaccination against HIV-1. In addition to avoiding contact with HIV-1–containing blood and body fluids, vaccination holds the greatest promise for controlling the HIV-1 pandemic. The goals of immunization include preventing HIV-1 infection, preventing HIV-1–associated disease, eliminating persistent infection, and reducing or eliminating transmission. Whether, in some instances, immunization may promote viral replication and disease progression should be resolved. SIV appears to reach regional lymph nodes within 18 h of primary infection, and both SIV and HIV-1 show systemic dissemination within 4–5 days. Consequently, the goal of immunization is to prevent the early dissemination of virus to multiple nodes, from which immune clearance of the virus by either antibody or CTLs could be problematic.

With these issues in mind, the goals of the AIDS Vaccine Evaluation Group (AVEG) have been to evaluate the safety
and immunogenicity of candidate HIV-1 vaccines. To date, these efforts have included primarily Phase I and some Phase II but no Phase III efficacy trials. Of the vaccine approaches evaluated by AVEG (subunit and chemical adjuvants, poxvirus vectors, mucosal immunization, nucleic acid vaccines, and cytokine adjuvants), mucosal vaccines have the advantage of acting at the earliest stage and site of HIV-1 entry. One trial followed patients who were administered oral followed by parenteral antigen (trial 018 with monovalent HIV-1 MN V3 peptides on heptalyysyl encapsulated in microparticles); another two trials used a parenteral followed by oral or rectal schedule with the same antigen noted above (trial 023) or with TY GagVLP (trial 019). None of the three scenarios showed immunogenicity or specific antibody in secretions. Current trials include multiple combinations of routes and sites with recombinant canarypox expressing HIV-1 MN/LAI gag/env (trial 027) or recombinant Salmonella typhi expressing HIV-1 Lai gp120 given orally (trial 028). Ultimately, live vectors, which utilize their own mechanisms of mucosal entry, may show the most promise for effective mucosal protection.

Jay A. Berzofsky (National Cancer Institute, Bethesda, MD) discussed research into determining if HIV-1 vaccines might induce CTL activity. Protective immunity against HIV-1 will require HIV-1–specific responses at mucosal sites, including CTL activity. Mucosal CTL responses have received little investigative attention, and whether vaccines might induce such responses is not known. To address this issue, Dr. Berzofsky and colleagues have constructed “cluster peptides” composed of multideterminant T helper peptides from HIV-1 envelope glycoprotein and peptide 18 of the V3 loop of HIV-1 gp160, the principal neutralizing determinant of HIV-1RIIB. Intrarectal immunization of BALB/c mice with cluster peptide plus the mucosal adjuvant cholera toxin resulted in prolonged (6 months) HIV-1 peptide–specific CTL activity in Peyer’s patches, lamina propria, and spleen. Subcutaneous administration of the multideterminant vaccine resulted in HIV-1–specific CTL only in the spleen. The CTL targets (P815 cells) were pulsed with either the HIV-1 peptide or expressed endogenous whole envelope glycoprotein. Generation of the HIV-1 peptide–specific CTLs was dependent on interleukin-12 and interferon-γ. Mice vaccinated intrarectally were protected from challenge with a recombinant vaccine virus expressing HIV-1RIIB gp160. Thus, a synthetic multideterminant HIV-1 peptide vaccine appears capable of inducing an HIV-1–specific, protective, mucosal CTL response in this animal model.

Summary

As the primary site of HIV-1 infection, the mucosa remains an understudied tissue system. This symposium sought to address this issue, highlighting the mucosa, its role in and susceptibility to transmission, and particularly, its importance in prevention and as a target for therapy. Bringing together basic and clinical investigators from immunology, virology, molecular biology, mucosal immunology, and vaccinology laid the foundation for new approaches and collaborations. These endeavors will set the stage for controlling the HIV-1 pandemic by identifying and implementing new advances in prevention and treatment of this devastating infection into the twenty-first century.