AIDS Rev 2000; 2: 144-154

Sexual HIV-1 Transmission and Mucosal Defense Mechanisms

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Abstract

Mucosal exposure to HIV-1 constitutes its primary mode of transmission. Greater than 75% of HIV-1 infections worldwide occur through heterosexual intercourse. Through the use of nonhuman primate models, the early events of mucosal HIV-1 transmission are beginning to be elucidated. Further, only recently have acquired and innate mucosal defense systems been studied in terms of HIV-1 transmission. This review will discuss the early events leading to HIV-1 acquisition, those factors that may affect HIV-1 transmission, and the role the mucosal immune system plays in modulating HIV-1 transmission.

Key words

HIV-1. Transmission. Sexually transmitted diseases. Mucosal immune system. Genital tract

Introduction

Heterosexual contact, the predominant mode of transmission of HIV-1, has caused 75% of the infections worldwide[1]. Mucosal surfaces of both the gastrointestinal and urogenital tracts provide portals of entry for pathogens such as HIV-1. Immunity generated at these mucosal surfaces provides a first line of defense against HIV-1 infection. Factors which compromise the integrity of these tissues, such as physical trauma or damage from a vigorous host immune response, can potentially enhance susceptibility to infection as well as increase the likelihood of transmission. The host's defense at mucosal surfaces is mediated by 1) acquired immunity (antibody production and cytotoxic and helper T-cell responses) and 2) innate immunity (epithelial cells and innate defense factors). Only recently have acquired and innate mucosal defense mechanisms been studied in terms of HIV-1 transmission. This review will focus on the early events after HIV-1 transmission, the factors influencing HIV-1 transmission through mucosal exposure, and the mucosal immune response.

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Mucosal HIV-1 transmission

Male-to-female heterosexual transmission of HIV-1 is two to eight times more efficient compared to female-to-male, with a male-to-female *per* contact infectivity estimated to be 0.0009 (95% CI 0.0005-0.001)[2,3]. Receptive anal intercourse results in an estimated *per* contact infectivity of 0.0082 (95% CI 0.0024-0.0276)[4]. The reason for the increased rate of infection from penile-anal sex compared to penile-vaginal sex may be due to the differences in the architecture of the rectum/colon and vagina/cervix. The rectum/colon are lined with simple columnar epithelial cells that are involved in transportation and adsorption of molecules, secretion, and protection. These cells form tight junctions that prevent the free flow of molecules from the lumen into the substrata. Transmission of HIV-1 is thought to occur through micro-tears of the epithelial lining, allowing access of the virus to the underlying immune cells in the intraepithelial spaces and the *lamina propria,* the area directly beneath the epithelial layer (Fig. 1).

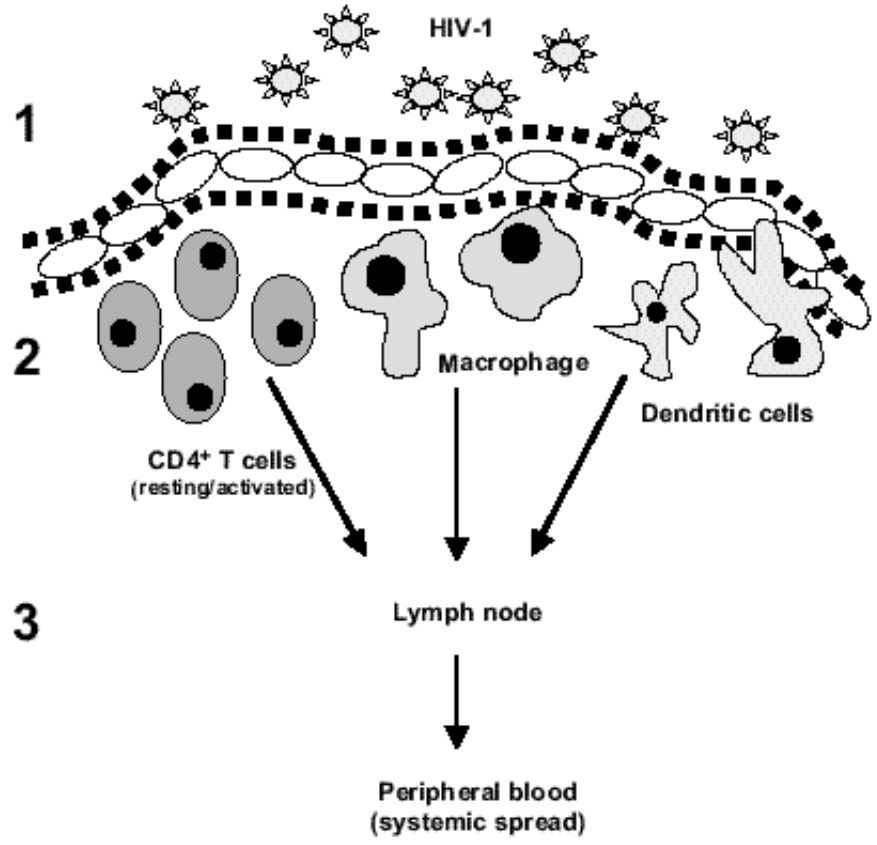

Fig. 1. *HIV-1 mucosal transmission: 1) Cell-free HIV-1 is deposited on the luminal side of the mucosal epithelium where virions are transported into the lamina propria by transcytosis, through microtears in the epithelium, or by local immune cells. 2) Various HIV-1 target cells are located within the intraepithelial spaces or the lamina propria, and include CD4+ T cells (resting and activated), macrophages, and/or dendritic cells. 3) After local infection, these immune cells travel via the draining lymphatics into the local lymph nodes where subsequent systemic spread of HIV-1 occurs.*

However, it has recently been shown *in vitro* that in the absence of trauma, infectious HIV-1 can transcytose through intestinal epithelial cell lines in micro-vesicles[5]. Moreover, anti-HIV-1 antibody can neutralize the virus and render it non-infectious as it passes through these cells[6]. HIV-1 enters into these cells, not by using the traditional CD4 and coreceptor, but via the interaction between the HIV-1 gp120 and galactosylceramide[7,8]. However, the apical side of intestinal epithelial cells is shrouded with a 500 nm-thick layer of integral membrane glycoproteins called the glycocalyx that serves as a protective diffusion barrier, inhibiting the interaction between gp120 and galactosylceramide[9]. An alternative conduit for HIV-1 transmission by penile-anal sex may be the M cell. M cells lack the glycocalyx, are specialized for endocytosis, and indicate areas rich in immune cells (see below). Using explanted rabbit and mouse mucosa, HIV-1 was shown to adhere to and be transcytosed through the M cells[10]; however, this has yet to be shown with human mucosa. Other studies have shown that intestinal epithelial cell lines can be productively infected *in vitro*[7,11]; however, there has been no conclusive evidence of HIV-1 infection of intestinal epithelial cells *in vivo*.

Male-to-female HIV-1 transmission occurs more efficiently relative to female-to-male, possibly because of extended exposure to seminal fluid or greater availability of HIV-1 targets. The vagina and ectocervix are covered by stratified, squamous, non-keratinized epithelial cells, 150 - 200 nm in thickness. These epithelial cells do not form tight junctions like the simple columnar epithelial cells, but rather consist of several layers, thus forming a protective barrier. The superficial cells are continually desquamated into the vaginal lumen providing an additional protective mechanism. The endocervical epithelium is composed of mucus-secreting, simple columnar epithelial cells. Polarized ectocervical epithelial sheets obtained from tissue explants have been shown to be refractory to HIV-1 infection and unable to transcytose HIV-1 after addition of the virus to the apical side of the tissue[12]. Further, simian immunodeficiency virus (SIV) inoculations of Rhesus macaques showed that 10,000 times more SIV was needed to establish infection through non-traumatic vaginal challenge compared to intravenous challenge, suggesting that the vaginal epithelial layer is an effective, but not absolute, barrier for HIV-1/SIV infection[13,14]. In contrast, cervical epithelial cell lines have been shown to be infected *in*

Table 1. *HIV-1 transmission models*

Investigator	Model	Initial Target Cell
Collins[18]	HIV-1/Ectocervical explant	$CD4^+$ T cells/Macrophage
Greenhead[12]	HIV-1/Ectocervical explant	Macrophage/Dendritic cells
Howell[17]	HIV-1/Uterine/cervical explant	Macrophage/Dendritic cells
Miller[14]/Hu[22]	SIV/Rhesus macaque	Dendritic cell
Spira[19]	SIV/Rhesus macaque	Dendritic cell
Zhang[20]	SIV/Rhesus macaque	Resting and activated $CD4^+$ T cells

vitro[15], but recent work indicates that primary cervical epithelial cells and cell lines are refractory to cell-free and cell-associated HIV-1 infection[16]. While female-to-male HIV-1 transmission does readily occur, little is known about this type of transmission. The urethra is lined with pseudostratified columnar epithelium, while the tip of the penis is lined with stratified squamous epithelium. Studies in primates suggest that SIV can be transmitted across the intact mucosa of the foreskin and glans of the penis to infect the local immune cells[14].

The *lamina propria* is typically rich in immune cells and provides a second defensive barrier against patho-gens (as described below); moreover, it also serves as an area rich in target cells for HIV-1. After virus has crossed the epithelium, these underlying immune cells become infected or carry the virus to the local lymph nodes (Fig. 1). The first infected cells have been shown to be T cells, macrophages, and/or dendritic cells depending on the system studied (Table 1). Studies done using explanted tissues from the female genital tract demonstrated that the macrophage and dendritic cells were infected within the first week[12,17]. Other studies using explanted female genital tract tissues found that $CD4^+$ T cells and, to a lesser extent, macrophages were infected with HIV-1[18]. Using primate models rather than tissue explants, investigators have found that after non-traumatic vaginal inoculation, either resting and activated intraepithelial and *lamina propria* T cells or dendritic cells within the vaginal epithelium were infected with SIV[19-21]. These studies have also reported rapid dissemination to local lymph nodes within 2 days post-inoculation. More recently, Hu *et al.*[22] have shown that a 60-minute non-traumatic intravaginal exposure to SIV resulted in the infection of dendritic cells within the vaginal epithelium and dissemination to the draining lymph nodes within 18 hours. Collectively, these data suggest that dendritic cells, $CD4^+$ T cells, and macrophages in the local epithelium are important liaisons between virus entry and dissemination throughout the body.

Recent work has shown that immune cells from the human genital tract have the capacity to be infected with either non-syncytium-inducing (NSI) or syncytium-inducing (SI) variants[12,23]. The selection for the NSI isolate to preferentially replicate may lie in the presence of a greater percentage of $CCR5^+$ mucosal T cells that produce IL-2, the cytokine representative of an activated, memory T helper (Th)1 phenotype[23,24]. Mucosal T cells with the Th1 phenotype appear to be more permissive to HIV-1 infection compared to those with the Th2 phenotype[25]. Moreover, mucosal epithelial cells constitutively express stromal derived factor-1 (SDF-1), the ligand for CXCR4 (SI co-receptor)[26]. This constitutive expression of SDF-1 down-modulates the CXCR4 co-receptor on $CD4^+$ intraepithelial and *lamina propria* lymphocytes, without affecting CCR5 expression. The down-modulation of CXCR4 may further explain the higher percentage of $CCR5^+$ mucosal T cells. Because HIV-1 targets activated, memory $CCR5^+/CD4^+$ T cells, the gastrointestinal tract, which is rich in this cell type, is a major site of virus replication and T-cell depletion[27-29].

It has been noted that different HIV-1 genotypes are isolated from the genital tract and peripheral blood. Comparisons of HIV-1 sequences from both seminal fluid and cervical/vaginal lavage to peripheral blood indicate that there are distinct differences between the HIV-1 genotypes in plasma and genital tract from men and women[30-33]. This compartmentalization indicates that HIV-1 replicates locally in the genital tract and is not of peripheral origin. Further, the sexually transmitted virus is from the genital tract reservoir. These data suggest that factors present in the genital tract, while not affecting virus in the periphery, may influence sexual HIV-1 transmission.

Factors that influence HIV-1 transmission

The precise mechanisms influencing HIV-1 transmission or susceptibility are not known, but several factors are associated with an increased risk in transmission. The prominent factor identified to date is viral load. There is a strong correlation between HIV-1 plasma RNA levels and the ability to detect HIV-1 RNA in cervical/vaginal lavage, semen, and anal-rectal swabs[30,34-39]. Both HIV-1 cell-associated DNA and cell-free RNA have been quantified from these secretions. Even though HIV-1 genotypes found in the peripheral blood differ from those found in the urogenital secretions, higher viral load in the periphery is associated with higher viral load in the cervical/vaginal lavage, seminal plasma, and elutions from anal-rectal swabs. Two recent studies show that peripheral blood viral load was the primary predictor of the risk for heterosexual transmission[40,41]. Collectively, these data suggest that when the viral load is high, it is generally high in both the blood and mucosal secretions. Indeed, highly active antiretroviral therapy (HAART) has been shown to decrease HIV-1 RNA levels in plasma as well as in these mucosal compartments. After one month of therapy, signifi-

cant drops in HIV-1 RNA levels were detected in both plasma and mucosal secretions[42-44]. While these results are promising, additional studies have found that, even though HIV-1 RNA titers drop, cell-associated HIV-1 appears to be unaffected by HAART[45-47]. These data suggest that HAART is effective in reducing the viral RNA titers, but cells harboring HIV-1 provirus still remain and may continue to produce infectious HIV-1.

Immune activation has long been associated with inducing HIV-1 replication[48]. A local inflammatory response appears to influence HIV-1 replication locally, but not systemically. For example, anal-rectal samples taken from men showed higher HIV-1 RNA levels associated with anal-rectal inflamation compared to controls[38]. The increase in anal-rectal HIV-1 RNA levels were not duplicated in the plasma, indicating local HIV-1 production and further suggesting the compartmentalization between the mucosa and the peripheral blood. Further, Lawn *et al.*[49] showed that women who had undergone surgery for cervical dysplasia had a 2-log increase in cervical/vaginal HIV-1 RNA titers, but not in plasma, 2 weeks post surgery. This increase was associated with local immune activation due to parallel increases in inflammatory cytokines such as tumor necrosis factor-α and IL-6 in cervical secretions, but not plasma. Sexually transmitted diseases (STDs) are known to induce a vigorous immune response and have been implicated in the transmission of HIV-1[50]. A population-based survey among persons in four African cities has found that ulcerative pathogens, such as syphilis and/or herpes simplex virus-2 (HSV-2), were more predominant in cities that had a high prevalence of HIV-1[51]. Moreover, several studies measuring HIV-1 shedding have reported that both ulcerative and non-ulcerative STDs can increase the HIV-1 RNA titers or the ability to detect HIV-1 RNA in these mucosal secretions[52-55]. In most of these studies in which successful STD treatment was given, viral load concomitantly decreased. These data indirectly suggest that therapy for STDs may decrease the transmission of HIV-1. Indeed, this was seen in a STD treatment study in Tanzania, showing that aggressive STD therapy reduces the transmission of HIV-1[56]. A second study in Uganda appeared to have a contradictory conclusion[57]. Several differences between these studies could explain the disparate outcomes. These variables include the stages of the HIV-1 epidemic, the nature of the STD intervention, and the STD profile[58]. Collectively, these data indicate that STDs act as immune stimulators and drive HIV-1 shedding as well as acquisition. When STDs are successfully treated, viral shedding may be reduced, decreasing the risk of HIV-1 transmission.

Another possible factor influencing HIV-1 transmission is use of hormonal contraceptives. Estrogen and progesterone regulate humoral and cellular mucosal immunity in the female reproductive tract[59]. There is little evidence that hormonal changes during the menstrual cycle affect HIV-1 RNA shedding[60]. However, when given as a contraceptive, progesterone influences the physiology of the vagina, cervix, and endometrium by causing thinning of the epithelium and thickening of the cervical mucus. Two macaque studies provide evidence that within 30 days after treatment with progesterone, the vaginal squamous epithelial layer becomes thinner[61,62]. Moreover, Marx *et al.*[61] showed an enhanced susceptibility to SIV challenge and elevated plasma RNA levels as compared to the control macaques. Recently, estrogen has been shown to protect macaques against a vaginal SIV challenge primarily due to thickening of the vaginal epithelium[63]. When hormonal contraceptive use has been studied in human populations, two of three reports suggest that HIV-1-infected women have increased shedding of infected cells[64-66]. Several studies examined the use of hormonal contraceptives and the incidence of HIV-1 infection. Three studies did not find a significant association between HIV-1 infection and oral contraceptive use[67-69]. Another study showed lifetime duration of oral contraceptive use was significantly greater in HIV-1-infected women; however, this association was found in women with concurrent genital ulcers[70]. Martin *et al.*[71] show injectable, but not oral, contraceptive use was significantly associated with increased incidence of HIV-1 infection, while Plummer *et al.*[72] found oral contraceptive use was associated with increased risk of infection. Collectively, these findings imply that hormonal contraception affects HIV-1 infection; however discrepancies remain. Clearly, further research is needed to elucidate the impact that hormonal contraceptive use has on HIV-1 transmission.

Acquired and innate mucosal defense mechanisms against HIV-1 transmission

The current lack of a preventative vaccine or effective microbicide is reflective of the limited understanding of mucosal immunity which, until recently, had been overlooked in terms of both acquisition/transmission and pathogenesis of HIV-1. Recent data suggest that HIV-1 disease is more a disease of the mucosa than peripheral lymphoid tissues. Given that HIV-1 is predominantly transmitted via the genital mucosa, a better understanding of the basic mechanisms of mucosal immunity is intrinsic to the development of improved antiviral and preventative therapies.

Mucosa-associated lymphoid tissues. The mucosa-associated lymphoid tissues (MALTs) consist of tissues at various mucosal surfaces that have organizational and functional similarities in their lymphoid elements[73]. MALTs serve two arms of mucosal immunity: 1) the induction and amplification of mucosal immune responses and 2) the effector mechanisms of local immunity[74]. Mucosal immune responses are initiated primarily in mucosal inductive sites, such as the Peyer's patch (PP) in the intestine and the nasal-associated lymphoid tissues in the oropharyngeal cavity[75]. Activated lymphocytes derived from these inductive mucosal surfaces can recirculate and specifically localize to mucosal effector sites such as the salivary gland and the urogenital tract, where immune protection can be mediated[75]. With this functional linking of the various mu-

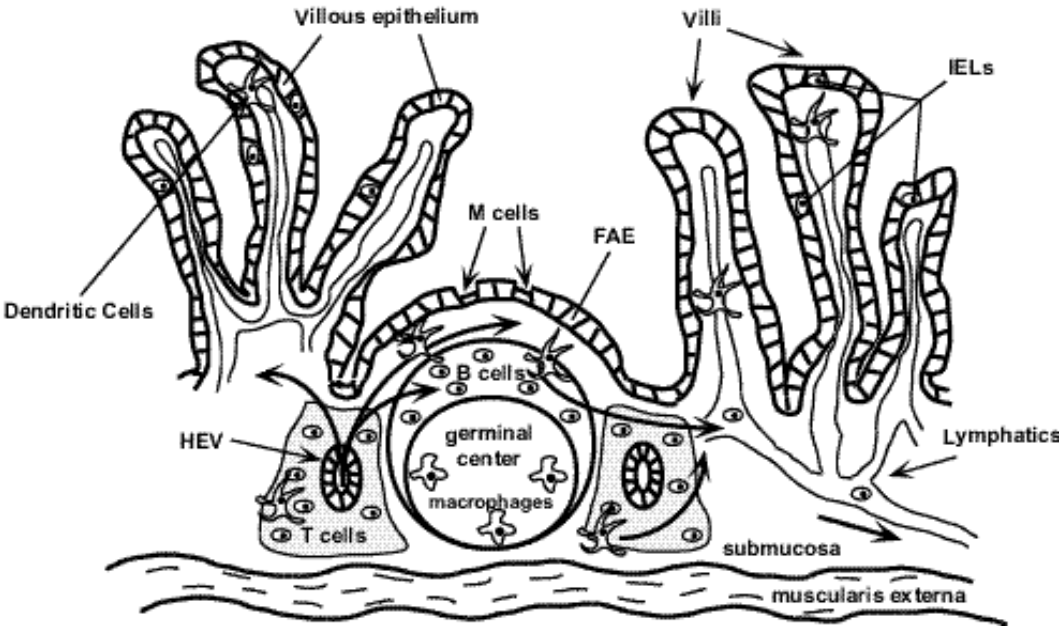

Fig. 2. *Structural and functional features of a Peyer's patch. The lymphoid follicle is composed of three parts: follicular area (B-cell zone and germinal center), interfollicular area beneath villi (T-cell zone), and follicle-associated epithelium. The curved arrows on the left indicate the influx of lymphocytes across the high endothelial venules which mediate selective immigration of lymphocytes. The arrows on the right indicate the exit pathway of activated lymphocytes or primed dendritic cells into the submucosal lymphatics. (Abbreviations: HEV, high endothelial venule; FAE, follicle-associated epithelium; and IEL, intraepithelial lymphocyte).*

cosal tissues, immunity initiated at one mucosal surface can potentially protect other mucosal sites[74]. Defense against mucosal pathogens through the induction and amplification of a localized specific immune response includes both humoral and cell-mediated immunity.

The gut-associated lymphoid tissue (GALT) has been the most extensively studied of all the MALTs. It consists of both organized lymphoid aggregates characterized by the PP as well as non-organized lymphoid elements in the epithelium and the *lamina propria*[74]. Most basic features of GALT are also found in other mucosal tissues, including the genital tract[76]. With the exception of the tonsils[77] and the transformation zone of the cervix[78], there is generally a lack of lymphoid aggregates in the oral cavity and urogenital tract. In humans, the best described mucosal inductive site is the PP (Fig. 2). These lymphoid aggregates are found in the distal small intestine (ileum) and colon and extend through the *lamina propria* and submucosa. Unlike typical lymph nodes, PPs lack afferent lymphatics and thus sample antigen from the intestinal lumen *via* the overlying epithelium; however, PPs do have efferent lymphatics which drain to the mesenteric lymph nodes (MLNs)[74]. Within the PP, a specialized epithelium overlying the lymphoid follicles efficiently samples and transports antigen from the intestinal lumen to the underlying macrophages and lymphocytes[79]. This follicle-associated epithelium (FAE) contains specialized cells (M cells) which have unique microfolds and increased numbers of cytoplasmic vesicles that allow efficient transport of particulate antigen from the apical (lumen) to the basolateral (follicle) surface[80-82]. Once antigen is transported into the lymphoid follicle, macrophages process and present antigen peptides to the local T cells[83] which in turn activate the precursors of IgA-secreting plasma cells[84,85]. B and T cells activated in the PP migrate via the efferent lymphatics into the MLNs and, subsequently, into the thoracic duct and blood[86-89]. After recirculation, these activated lymphocytes eventually extravasate into mucosal tissues[73,84,90] through specialized postcapillary venules found in the *lamina propria* of mucosal tissues such as the intestine[91-93]. Having entered the effector sites, primed B and T cells are equipped to mount a local immune response which includes the production of antigen-specific IgA.

Humoral Immunity. In terms of humoral immunity, much more is known about antibody production in the female genital tract than in the male genital tract[94]. Recent studies indicate that both systemic (serum-derived IgG) and secretory (secretory IgA or S-IgA) immunity are present in cervicovaginal secretions[94-96]. Interestingly, the major isotype in vaginal secretions is IgG, although both serum-derived and secretory IgA are also present[95]. S-IgA, a unique molecular form of the IgA isotype, is distinct from serum IgA and present in high concentrations in mucosal secretions[97]. Unlike the monomeric IgA predominating in serum, the S-IgA found in mucosal secretions such as saliva is mainly polymeric and contains an additional polypeptide which is termed the secretory component (SC)[97]. In the uterus and endocervix, the presence of SC on the luminal and glandular epithelium and IgA plasma cells in the submucosa suggests that IgA is actively transported

by SC through the epithelium[94]. In the endometrium and cervicovaginal mucosa, immunoglobulin-containing cells are rare but primarily of the IgG isotype; however, IgA-containing cells are present and S-IgA transport can occur[98]. Studies examining the presence of albumin in vaginal secretions indicate that a significant proportion of IgG is derived from serum transudation through the vaginal epithelium[95]. However, specificity patterns to proteins from mucosal pathogens indicate that mucosal IgG displays antigen specificity which differ from that of serum IgG, suggesting local IgG production[96]. In some instances, greater numbers of IgG plasma cells have been observed in the vaginal submucosa[98,99]. These results suggest a significant role for systemic and local IgG-associated immunity and imply that IgG may complement S-IgA as an immune barrier to mucosal pathogens[96].

In fact, recent data indicate that total IgG levels in mucosal fluids (saliva, rectal wash, and cervicovaginal secretions) were elevated in HIV-1-infected individuals[100]. Local IgG and IgA production was detected in cervicovaginal secretions[100]. In all mucosal specimens, the response to HIV-1 was predominantly IgG, with highest titers detected in the cervicovaginal secretions[100]. The specific IgA response appeared weaker in the mucosa than serum[100]. In another study, IgG and IgA specific to HIV-1 gp120 were detected in both sera and cervicovaginal secretions from HIV-1-infected women[101]. A high specific activity to gp160 was detected in IgG and IgA from cervicovaginal secretions as compared to serum, suggesting local synthesis[101]. Although data suggest that serum IgA from HIV-1-infected individuals is capable of neutralizing HIV-1[102,103], or generating antibody-dependent cellular cytotoxicity (ADCC) against HIV-1-infected target cells[104], data demonstrating that S-IgA can neutralize HIV-1[105,106] or mediate ADCC activity are limited[104]. Recently, it was demonstrated that HIV-1-specific IgA in the absence of IgG could be detected in the serum of exposed seronegative partners of HIV-seropositive persons and that sera from these individuals was capable of generating HIV-1 neutralizing activity[107]. Another study demonstrated that neutralizing IgG directed against HIV-1 envelope glycoprotein could completely block HIV-1/SIV chimeric virus infection in pigtailed macaques[108]. In contrast, studies in women who are highly exposed but persistently seronegative (HEPS) suggest that IgA in genital secretions may play a role in preventing transmission[107,109-111]. These women were more likely to have HIV-1-specific IgA in vaginal secretions than were infected women; furthermore, HIV-1-specific IgG appeared to be lacking in serum and vaginal secretions from HEPS women, but was present in serum and secretions from infected women[107,109,111]. Neutralizing activity demonstrated in serum and purified serum IgA from HEPS women, may also reflect the ability of mucosal IgA to neutralize HIV-1[107]. More recently, protection in HEPS women in the absence of HIV-1-specific vaginal IgA or IgG with no HIV-1 neutralizing activity in vaginal secretions has been observed in another cohort of HEPS women, thus suggesting that resistance is not solely based on HIV-1-specific humoral immunity[112]. Though the results from these studies are promising in terms of the development of a vaccine capable of generating neutralizing IgG and IgA antibodies, it remains unclear if the generation of HIV-1-specific mucosal antibodies in humans can protect against transmission of virus.

Cellular Immunity. In addition to IgA plasma cells, the effector limb of the GALT also includes numerous T cells which are distributed in both the epithelium and the *lamina propria*. Although the majority of $CD4^+$ T cells resides within the *lamina propria* (lamina propria lymphocytes or LPLs), the majority of $CD8^+$ and a small number of $CD4^+$ T cells cross the basement membrane and distribute between epithelial cells (hence "intraepithelial" lymphocytes or IELs). Memory T cells from inductive sites such as PPs can be activated by new contact with relevant antigen by *lamina propria* dendritic cells and presumably by enterocytes within the intestinal epithelium. $CD4^+$ *lamina propria* T cells control local inflammatory responses and contribute to the final differentiation of IgA plasma cells. Intraepithelial T cells secrete cytokines and are strongly cytotoxic. Although the majority of these cells are $CD8^+$ T cells, the IEL compartment can contain mast cells, natural killer (NK) cells,[113,114] and dendritic cells[115,116]. Thus, this population of cells within the epithelium has the innate cytolytic activity of NK cells, the classical MHC-restricted cytotoxicity of T cells, and the antigen-presenting abilities of dendritic cells. Although the functions of intraepithelial T cells are not completely understood, they appear to play a role in regulating the absorptive and secretory function of the epithelium[74], as well as in the surveillance and repair of damaged epithelial tissue[117].

Since no organized inductive sites such as PPs have been identified in the female genital tract, the induction of localized immune responses has not been extensively studied and is thus not well characterized[94,118]. Although macrophages can be found in the endometrium, cervix, and vagina, an additional class of antigen-presenting cells - Langerhans' cells - are also found in the ectocervix and vagina[78,119]. Unlike the M cells in the PP of the intestine, uptake of antigens across the vaginal epithelium is thought to be mediated by Langerhans' cells[94]. Once activated, these cells migrate from the genital epithelium to the draining lymph nodes where antigens can be presented to T lymphocytes, thus initiating an immune response[118]. In addition, $CD8^+$ T lymphocytes are numerous within the epithelium of the ectocervix, vagina, and transformation zone[78,120]. Substantial numbers of $CD4^+$ and $CD8^+$ cells are also observed in the stroma of the transformation zone, often in lymphoid aggregates; however, relatively few T cells are found in the stroma of the ectocervix and vagina[78]. Despite the presence of T cells in the genital mucosa, their function in STDs such as HIV-1 is poorly understood[121].

Recent studies indicate recovery of HIV-1-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) from the vaginal epithelium of SIV-infected macaques[122] as well

as recovery of $CD4^+$ and $CD8^+$ CTLs from the cervix of HIV-1-infected women[121]; however, it remains to be determined whether induction of CTL activity within the genital epithelium is protective against sexual transmission. Earlier studies indicated that peripheral blood mononuclear cells (PBMCs) from seronegative heterosexual partners of HIV-1-infected individuals proliferated in response to HIV-1 antigens[109,123]. More recent studies in HEPS women who lack the CCR5 HIV-1 coreceptor resistance phenotype (Δ32 mutant allele)[124,125] suggest that T cell immunity may play a role in protection against mucosal exposure to HIV-1[111,126-130]. In some HEPS individuals, systemic Th cell responses specific for the HIV-1 envelope were demonstrated[111,126]. Although the frequency of virus-specific CTL is thought to play a role in delaying disease progression, it is unclear whether the presence of HIV-1-specific CTL protects against sexual transmission[131]. Interestingly, HIV-1-specific CTL activity has been detected in blood from HEPS individuals[127-129]. More recently, HIV-1-specific CTL responses were detected in both blood and $CD8^+$ T cells from isolated cervical mononuclear cells from HEPS women[130]. When present, the frequency of CTL activity in HIV-1-infected women was greater in blood than the cervix; conversely, in HEPS women, HIV-1-specific CTL frequency was greater in the cervix than blood[130]. Although less is known about cellular immunity in the male genital tract, recent studies indicate the presence of SIV-specific CTL in the semen of chronically-infected macaques[132] as well as HIV-1-specific CTL in the semen of HIV-1-infected men[133]. Whether the presence of lentivirus-specific CTL in the male genital tract is protective is currently unknown. However, these data suggest that both Th and CTL cellular immunity may play a role in protecting the genital tract from mucosal HIV-1 infection.

Innate defenses. Innate defense mechanisms of the mucosa include those derived from the host as well as the local environment, with which epithelial cells intimately interact[134]. Upon injury or infection, epithelial cells respond by communicating with underlying immune cells through the production of cytokines, including chemokines such as IL-8[134], and antimicrobial products which include humoral factors (lactoferrin, lysozyme, and peroxidase) and peptides[135,136]. Major antimicrobial products produced by epithelial cells include the human β-defensins (hBD) and secretory leukocyte protease inhibitor (SLPI)[136]. These products are secreted by epithelial cells in the gastrointestinal tract, genitourinary tract, and the oral cavity, mucosal sites where exposure to HIV-1 occurs. In addition to the antimicrobial activity of humoral factors such as lysozyme and peroxidase, defensins (β-sheet, 30-40 amino acid peptides) mediate their activity by permeabilizing microbial cell membranes[136]. Consequently, defensins are capable of neutralizing a variety of bacteria and enveloped viruses[136]. Alpha defensins, produced by neutrophils, demonstrate inhibitory activity against HIV-1-induced cytopathogenicity in the $CD4^+$ human T-cell line MT-4[137]. Since defensins are structurally and functionally similar to peptides involved in cell-membrane fusion, they may block viral and cell membrane fusion during virus entry[137,138]. However, the role that hBD from mucosal epithelia may play in HIV-1 infection/transmission is currently unknown.

On the other hand, studies have also demonstrated that native and recombinant SLPI, a 12 kDa neutrophil elastase inhibitor having antibacterial and anti-inflammatory properties, is capable of inhibiting HIV-1 infection of adherent monocytes or PBMCs with laboratory-adapted strains (BaL and IIIB)[139, 140]. Evidence suggests that SLPI targets a host cell molecule rather than the virus itself and that inhibition occurs prior to reverse transcription[141,142]. Interestingly, it was recognized relatively early in the AIDS pandemic that saliva was capable of inhibiting HIV-1 (LAV) infection of activated PBMCs[143]. The presence of SLPI in saliva, along with other anti-HIV-1 factors such as neutralizing antibody, may explain the general lack of oral transmission of HIV-1 as well as the ability of saliva to neutralize HIV-1 infectivity *in vitro*[141]. In contrast to saliva, breast milk contains levels of SLPI below the in vitro HIV-1 inhibitory concentration and appears unable to inhibit HIV-1 infection when tested *in vitro*[139,144]. Therefore, it has been proposed that the difference in SLPI levels between saliva and breast milk may help explain the apparent lack of HIV-1 transmission by saliva as compared to the high incidence of postnatal HIV-1 transmission through breast milk[145-147]. On the other hand, SLPI levels in colostrum, the milk produced during the first few days after parturition, are equivalent to those in saliva and able to inhibit *in vitro* HIV-1 infection, thus suggesting protection against transmission during early breastfeeding[144]. However, a recent study examining HIV-1-infected, breastfeeding mothers indicated no difference in SLPI levels in colostrum and milk between transmitting women and those who did not transmit HIV-1 to their infants[148]. These results suggest that the higher levels of SLPI in colostrum may not necessarily provide protective antiviral activity and that other factors in breast milk (amount of free virus, number of HIV-1-infected cells, and HIV-1-specific humoral and cellular immunity) may ultimately determine the risk of HIV-1 transmission via breastfeeding[148]. Although significant levels of SLPI occur in semen and cervical secretions, it is presently unknown what role, if any, this protease inhibitor may play in sexual HIV-1 transmission[149,150].

In addition to innate defenses provided by the epithelium, the bacterial flora and pH of the local mucosal microenviroment can provide an additional protective barrier in sites such as the vagina. Lactobacilli, gram-positive bacteria found in the healthy vagina, can mediate protection by competing with pathogens for adherence to common bacterial receptors on the vaginal epithelium as well as releasing antimicrobial compounds such as lactic acid and hydrogen peroxide[151]. By maintaining an acidic environment (pH 2.8-4.2) as well as production of hydrogen peroxide, lactobacilli appear to control the microbial flora of the healthy vagina[152]. Interestingly, in cases of bacterial vaginosis where the bac-

terial flora is abnormal, the levels of hydrogen peroxide-producing lactobacilli are lower[151,152]. In fact, studies indicate that lactobacilli can inhibit bacteria associated with bacterial vaginosis[152]. Even further, the presence of lactobacilli at high concentrations is viricidal to HIV-1[153]. At lower lactobacilli levels, the addition of peroxidase and a halide restores anti-HIV-1 activity, suggesting that the survival of HIV-1 in the vagina may be influenced by the lactobacilli - peroxidase - halide system[153]. In addition, HIV-1 infection is associated with abnormal flora and bacterial vaginosis, suggesting that loss of lactobacilli and/or the presence of bacterial vaginosis may increase susceptibility to HIV-1 infection[154]. In fact, a more recent study in a population of HIV-1-seronegative sex workers indicated that the absence of vaginal lactobacilli was associated with an increased risk of acquiring HIV-1 infection[155]. Altogether these data suggest that treatment of bacterial vaginosis and promotion of vaginal lactobacilli colonization could potentially reduce the risk of sexual HIV-1 acquisition[155].

Summary

The current lack of a preventative vaccine for HIV-1 not only points to the complex nature of this virus, given its ability to mutate in response to pressure from the immune system and antiretroviral therapy, but also to a general lack of knowledge of a most basic process - the interaction of the virion with the genital mucosa and the subsequent events of infection. In fact, until recently, much effort was focused upon the peripheral immune response against HIV-1 and the ensuing disease course in HIV-1-infected individuals. However, studies in humans and nonhuman primates now suggest that mucosal sites, the intestine in particular, may play a formative role in pathogenesis. Further, studies in HEPS women suggest a protective role for both antibodies and CTLs in the genital tract; however, it is not clear whether systemic and/or mucosal immunization is capable of inducing protective immunity in individuals who are not “highly exposed”. Consequently, this fact should be considered during vaccine development and interpretation of results in studies with high risk individuals. On the other hand, the presence of STD pathogens and innate mucosal factors such as SLPI may well alter susceptibility to HIV-1 infection. Only recently have scientists begun to examine these in terms of sexual HIV-1 transmission. Thus, a further understanding of the mechanisms underlying mucosal HIV-1 transmission is important for discovering new methods for prevention. Even with the advent of HAART, infected persons still shed HIV-1 into their mucosal secretions. Preliminary studies show that successful treatment of STDs is clearly effective in reducing HIV-1 transmission in areas that have a low HIV-1 prevalence rate; however, in regions with non-treatable STDs and high prevalence rates, STD intervention appears to be ineffective at reducing transmission. In terms of new therapeutic strategies, the development of a microbicide with anti-HIV-1 properties may be a more feasible and cost-efficient vaccine alternative. A product which effectively neutralizes HIV-1 before it crosses the genital epithelium and is disseminated should, theoretically, protect against a variety of HIV-1 strains. Consequently, a thorough knowledge of mucosal transmission and how the host’s immune system responds to infection is critical for development of effective vaccines and microbicides capable of halting the spread of HIV-1.

References

1. Joint United Nations Program on HIV/AIDS. AIDS epidemiology update. Geneva: World Health Organization, December 1999.
2. Nicolosi A, Correa Leite M, Musicco M, *et al*. The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. Epidemiology 1994; 5: 570-5.
3. Padian N, Shiboski S, Glass S, *et al*. Heterosexual transmission of human immunodeficiency virus (HIV) in northern California: results from a ten-year study. Am J Epidemiol 1997; 146: 350-7.
4. Vittinghoff E, Douglas J, Judson F, *et al*. Per-contact risk of human immunodeficiency virus transmission between male sexual partners. Am J Epidemiol 1999; 150: 306-11.
5. Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nat Med 1997; 3: 42-7.
6. Bomsel M, Heyman M, Hocini H, *et al.* Intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. Immunity 1998; 9: 277-87.
7. Yahi N, Baghdiguian S, Moreau H, *et al.* Galactosyl ceramide (or a closely related molecule) is the receptor for human immunodeficiency virus type 1 on human colon epithelial HT29 cells. J Virol 1992; 66: 4848-54.
8. Bhat S, Mettus R, Reddy E, *et al.* The galactosyl ceramide/sulfatide receptor binding region of HIV-1 gp120 maps to amino acids 206-275. AIDS Res Hum Retroviruses 1993; 9: 175-81.
9. Neutra M.HIV transmission and immune protection at mucosal surfaces. Adv Exp Med Biol 1998; 452: 169-75.
10. Amerongen H, Weltzin R, Farnet C, *et al*. Transepithelial transport of HIV-1 by intestinal M cells:a mechanism for transmission of AIDS. J Acquir Immune Defic Syndr 1991; 4: 760-5.
11. Fantini J, Yahi N, Chermann J. Human immunodeficiency virus can infect the apical and basolateral surfaces of human colonic epithelial cells. Proc Natl Acad Sci USA 1991; 88: 9297-301.
12. Greenhead P, Hayes P, Watts P, *et al*. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. J Virol 2000; 74: 5577-86.
13. Sodora D, Gettie A, Miller C, *et al*. Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. AIDS Res Hum Retroviruses 1998; 14: S119-23.
14. Miller C. Localization of simian immunodeficiency virus-infected cells in the genital tract of male and female rhesus macaques. J Reprod Immunol 1998; 41: 331-9.
15. Tan X, Pearce-Pratt R, Phillips D. Productive infection of a cervical epithelial cell line with human immunodeficiency virus:implications for sexual transmission. J Virol 1993; 67: 6447-52.
16. Dezzutti C, Guenthner P, Cummins Jr. J., *et al*. Lack of evidence for productive HIV-1 infection of primary cervical and prostate epithelial cells. Keystone Symposia: Innate and Acquired Immunity at Mucosal Surfaces, Taos, NM, 2000 Abstract 208.
17. Howell A, Edkins R, Rier S, *et al.* Human immunodeficiency virus type 1 infection of cells and tissues from the upper and lower human female reproductive tract. J Virol 1997; 71: 3498-506.
18. Collins K, Patterson B, Naus G, Landers D, Gupta P. Development of an *in vitro* organ culture model to study transmission of HIV-1 in the female genital tract. Nat Med 2000; 6: 475-9.
19. Spira A, Marx P, Patterson B, *et al.* Cellular targets of infection and route of viral dissemination after an intravaginal inoculation

of simian immunodeficiency virus into rhesus macaques. J Exp Med 1996; 183: 215-25.
20. Zhang Z, Schuler T, Zupancic M, *et al.* Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. Science 1999; 286: 1353-7.
21. Hu J, Pope M, Brown C, O'Doherty U, Miller C. Immunophenotypic characterization of simian immunodeficiency virus-infected dendritic cells in cervix, vagina, and draining lymph nodes of rhesus monkeys. Lab Invest 1998; 78: 435-51.
22. Hu J, Gardner M, Miller C. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. J Virol 2000; 74:6087-95.
23. Hladik F, Lentz G, Delpit E, McElroy A, McElrath M.Coexpression of CCR5 and IL-2 in human genital but not blood T cells: implications for the ontogeny of the CCR5+ Th1 phenotype. J Immunol 1999; 163: 2306-13.
24. Patterson B, Landay A, Andersson J, *et al.* Repertoire of chemokine receptor expression in the female genital tract:implications for human immunodeficiency virus transmission. Am J Pathol 1998; 153: 481-90.
25. Tanaka Y, Koyanagi Y, Tanaka R, *et al.* Productive and lytic infection of human CD4+ type 1 helper T cells with macrophage-tropic human immunodeficiency virus type 1. J Virol 1997; 71:465-70.
26. Agace W, Amara A, Roberts A. Constitutive expression of stromal derived factor-1 by mucosa epithelia and its role in HIV transmission and propagation. Curr Biol 2000; 10: 325-8.
27. Lapenta C, Boirivant M, Marini M, *et al.* Human intestinal *lamina propria* lymphocytes are naturally permissive to HIV-1 infection. Eur J Immunol 1999; 29: 1202-8.
28. Veazey R, DeMaria M, Chalifoux L, *et al.* Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science 1998; 280: 427-31.
29. Veazey R, Tham IC, Mansfield K, *et al.* Identifying the target cell in primary simian immunodeficiency virus (SIV) infection:highly activated memory CD4(+) T cells are rapidly eliminated in early SIV infection *in vivo*. J Virol 2000; 74: 57-64.
30. Coombs RW, Speck CE, Hughes JP, *et al.* Association between culturable human immunodeficiency virus type 1 (HIV-1) in semen and HIV-1 RNA levels in semen and blood: evidence for compartmentalization of HIV-1 between semen and blood.J Infect Dis 1998; 177: 320-30.
31. Kiessling AA, Fitzgerald LM, Zhang D, *et al.* Human immunodeficiency virus in semen arises from a genetically distinct virus reservoir. AIDS Res Hum Retroviruses 1998; 14: S33-41.
32. Overbaugh J, Anderson R, Ndinya-Achola J, Kreiss J. Distinct but related human immunodeficiency virus type 1 variant populations in genital secretions and blood. AIDS Res Hum Retroviruses 1996; 12: 107-15.
33. Poss M, Rodrigo A, Gosink J, *et al.* Evolution of envelope sequences from the genital tract and peripheral blood of women infected with clade A human immunodeficiency virus type 1. J Virol 1998; 72: 8240-51.
34. Hart C, Lennox J, Pratt-Palmore M, *et al.* Correlation of human immunodeficiency virus type 1 RNA levels in blood and the female genital tract. J Infect Dis 1999; 179: 871-82.
35. Goulston C, McFarland W, Katzenstein D. Human immunodeficiency virus type 1 RNA shedding in the female genital tract. J Infect Dis 1998; 177: 1100-3.
36. Iversen A, Larsen A, Jensen T, *et al.* Distinct determinants of human immunodeficiency virus type 1 RNA and DNA loads in vaginal and cervical secretions. J Infect Dis 1998; 177: 1214-20.
37. Vernazza P, Gilliam B, Dyer J, *et al.* Quantification of HIV in semen: correlation with antiviral treatment and immune status. AIDS 1997; 11: 987-93.
38. Kiviat N, Critchlow C, Hawes S, *et al.* Determinants of human immunodeficiency virus DNA and RNA shedding in the anal-rectal canal of homosexual men. J Infect Dis 1998; 177: 571-8.
39. Anderson D, Politch J, Tucker L, *et al.* Quantitation of mediators of inflammation and immunity in genital tract secretions and their relevance to HIV type 1 transmission. AIDS Res Hum Retroviruses 1998; 14: S43-9.
40. Pedraza M, del Romero J, Roldan F, *et al.* Heterosexual transmission of HIV-1 is associated with high plasma viral load levels and a positive viral isolation in the infected partner. J Acquir Immun Defic Syndr 1999; 21: 120-5.
41. Quinn T, Wawer M, Sewankambo N, *et al.* Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med 2000; 342: 921-9.
42. Gupta P, Mellors J, Kingsley L, *et al.* High viral load in semen of human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. J Virol 1997; 71:6271-5.
43. Uvin S, Caliendo A, Reinert S. Effect of highly active antiretroviral therapy on cervicovaginal HIV-1 RNA. AIDS 2000; 14:415-21.
44. Kotler D, Shimada T, Snow G, *et al.* Effect of combination antiretroviral therapy upon rectal mucosal HIV RNA burden and mononuclear cell apoptosis. AIDS 1998; 12: 597-604.
45. Zhang H, Dornadula G, Beumont M, *et al.* Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. N Engl J Med 1998; 339: 1803-9.
46. Lampinen T, Critchlow C, Kuypers J. Association of antiretroviral therapy with detection of HIV-1 RNA and DNA in the anorectal mucosa of homosexual men. AIDS 2000; 14: F69-75.
47. Vernazza P, Troiani L, Flepp M, *et al.* Potent antiretroviral treatment of HIV-infection results in suppression of the seminal shedding of HIV. The Swiss HIV Cohort Study. AIDS 2000; 14:117-21.
48. Wahl S, Orenstein J. Immune stimulation and HIV-1 viral replication. J Leukoc Biol 1997; 62: 67-71.
49. Lawn S, Subbarao S, Wright Jr. T, *et al.* Correlation between human immunodeficiency virus type 1 RNA levels in the female genital tract and immune activation associated with ulceration of the cervix. J Infect Dis 2000; 181: 1950-6.
50. Fleming D, Wasserheit J. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 1999; 75: 3-17.
51. Buve A. Factors determining differences in rate of spread of HIV in sub-Saharan Africa:results from a population based survey in four African cities. 7th Conference on Retroviruses and Opportunisitic Infections, San Francisco, CA, 2000 [Abstract S28].
52. Fiscus S, Vernazza P, Gilliam B, *et al*. Factors associated with changes in HIV shedding in semen.AIDS Res Hum Retroviruses 1998; 14: S27-31.
53. Cohen M, Hoffman I, Royce R, *et al.* Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet 1997; 349: 1868-73.
54. Ghys P, Fransen K, Diallo M, *et al.* The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. AIDS 1997; 11: F85-93.
55. Uvin S, Hogan J, Caliendo A, *et al.* Bacterial vaginosis decreases suppression of female genital tract HIV-1 RNA levels. 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 2000 [Abstract 678].
56. Grosskurth H, Mosha F, Todd J, *et al.* Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. Lancet 1995; 346: 530-6.
57. Wawer M, Sewankambo N, Serwadda D, *et al.* Control of sexually transmitted diseases for AIDS prevention in Uganda:a randomised community trial. Rakai Project Study Group. Lancet 1999; 353: 525-35.
58. Grosskurth H, Gray R, Hayes R, Mabey D, Wawer M.Control of sexually transmitted diseases for HIV-1 prevention:understanding the implications of the Mwanza and Rakai trials. Lancet 2000; 355: 1981-7.
59. Wira C, Kaushic C, Richardson J. Role of sex hormones and cytokines in regulating the mucosal immune system in the female reproductive tract.In:Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J, eds. Mucosal Immunology. San Diego: Academic Press, 1999: 1449-61.
60. Mostad S, Jackson S, Overbaugh J, *et al.* Cervical and vaginal shedding of human immunodeficiency virus type 1-infected cells throughout the menstrual cycle. J Infect Dis 1998; 178: 983-91.
61. Marx P, Spira A, Gettie A, *et al.* Progesterone implants enhance SIV vaginal transmission and early virus load.Nat Med 1996;2: 1084-9.
62. Hild-Petito S, Veazey R, Larner J, Reel J, Blye R.Effects of two progestin-only contraceptives, Depo-Provera and Norplant-II, on

the vaginal epithelium of rhesus monkeys. AIDS Res Hum Retroviruses 1998; 14: S125-30.
63. Smith S, Charkraborty P, Baskin G, Marx P. Estrogen protects against vaginal transmission of SIV. 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 2000 Abstract 119.
64. Clemetson D, Moss G, Willerford D, *et al.* Detection of HIV DNA in cervical and vaginal secretions. Prevalence and correlates among women in Nairobi, Kenya. JAMA 1993; 269: 2860-4.
65. Mostad S, Overbaugh J, DeVange D, *et al.* Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. Lancet 1997; 350: 922-7.
66. Kreiss J, Willerford D, Hensel M, *et al.* Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA. J Infect Dis 1994; 170: 1597-601.
67. Saracco A, Musicco M, Nicolosi A, *et al.* Man-to-woman sexual transmission of HIV: longitudinal study of 343 steady partners of infected men. J Acquir Immun Defic Syndr 1993; 6: 497-502.
68. Sinei S, Fortney J, Kigondu C, *et al.* Contraceptive use and HIV infection in Kenyan family planning clinic attenders. Int J STD AIDS 1996; 7: 65-70.
69. Kapiga S, Lyamuya E, Lwihula G, *et al.* The incidence of HIV infection among women using family planning methods in Dar es Salaam, Tanzania. AIDS 1998; 12: 75-84.
70. Plourde P, Plummer F, Pepin J, *et al.* Human immunodeficiency virus type 1 infection in women attending a sexually transmitted diseases clinic in Kenya. J Infect Dis 1992; 166: 86-92.
71. Martin Jr. H, Nyange P, Richardson B, *et al.* Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1.J Infect Dis 1998; 178: 1053-9.
72. Plummer F, Simonsen J, Cameron D, *et al.* Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. J Infect Dis 1991; 163: 233-9.
73. Bienenstock J, McDermott M, Befus D, *et al*. A common mucosal immunologic system involving the bronchus, breast and bowel. Adv Exp Med Biol 1978; 107: 53-9.
74. Croitoru K, Bienenstock J. Characterics and functions of mucosa-associated lymphoid tissue. In: Ogra P, Mestecky J, Lamm M, Strober W, McGhee J, Bienenstock J, eds. Handbook of Mucosal Immunology. San Diego: Academic Press, Inc., 1994: 141.
75. McGhee J, Lamm M, Strober W. Mucosal immune responses:an overview. In: Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J, eds. Mucosal Immunology. San Diego:Academic Press, 1999: 485-506.
76. Kelsall B, Strober W. Gut-associated lymphoid tissue: antigen handling and T-lymphocyte responses. In: Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J, eds. Mucosal Immunology. San Diego: Academic Press, 1999: 293-317.
77. Bernstein J, Gorfien J, Brandtzaeg P. The immunobiology of the tonsils and adenoids. In:Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J, eds. Mucosal Immunology. San Diego: Academic Press, 1999: 1339-1362.
78. Edwards J, Morris H. Langerhans' cells and lymphocyte subsets in the female genital tract. Br J Obstet Gynaecol 1985; 92:974-82.
79. Owen R, Nemanic P. Antigen processing structures of the mammalian intestinal tract:an SEM study of lymphoepithelial organs. SEM Symp 1978; II: 367.
80. Owen R, Jones A. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology 1974; 66: 189-203.
81. Owen R.Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine:an ultrastructural study. Gastroenterology 1977; 72: 440-51.
82. Smith M, Peacock M.Microvillus growth and M-cell formation in mouse Peyer's patch follicle-associated epithelial tissue. Exp Physiol 1992; 77: 389-92.
83. MacDonald T, Carter P. Isolation and functional characteristics of adherent phagocytic cells from mouse Peyer's patches. Immunology 1982; 45: 769-74.
84. Craig S, Cebra J. Rabbit peyer's patches, appendix, and popliteal lymph node B lymphocytes:a comparative analysis of their membrane immunoglobulin components and plasma cell precursor potential. J Immunol 1975; 114: 492-502.
85. Befus A, O'Neill M, Bienenstock J. Immediate IgA precursor cells in rabbit intestinal *lamina propria*. Immunology 1978; 35:901-6.
86. Bienenstock J. Review and discussion of homing of lymphoid cells to mucosal membranes: the selective localization of cells in mucosal tissues. In: Strober W, Hanson L, Sell K, eds. Recent Advances in Mucosal Immunity. New York:Raven Press, 1982: 35.
87. Bienenstock J. Gut and bronchus associated lymphoid tissue:an overview. Adv Exp Med Biol 1982; 149: 471-7.
88. Reynolds J, Pabst R.The emigration of lymphocytes from Peyer's patches in sheep. Eur J Immunol 1984; 14: 7-13.
89. McDermott M, Bienenstock J. Evidence for a common mucosal immunologic system. I.Migration of B immunoblasts into intestinal, respiratory, and genital tissues. J Immunol 1979; 122:1892-8.
90. Gowans J, Knight E.The route of recirculation of lymphocytes in the rat. Proc R Soc London (Biol) 1964; 159: 257.
91. Gallatin W, Weissman I, Butcher E. A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature 1983; 304:30-4.
92. Navarro R, Jalkanen S, Hsu M, *et al.* Human T cell clones express functional homing receptors required for normal lymphocyte trafficking. J Exp Med 1985; 162: 1075-80.
93. Jalkanen S, Jalkanen M, Bargatze R, Tammi M, Butcher E.Biochemical properties of glycoproteins involved in lymphocyte recognition of high endothelial venules in man.J Immunol 1988; 141: 1615-23.
94. Bernstein D, Milligan G.Mucosal immunity of the genital tract.In: Stanberry L, Bernstein D, eds. Sexually Transmitted Diseases: Vaccines, Prevention, and Control.San Diego:Academic Press, 2000: 97-122.
95. Hocini H, Barra A, Belec L, *et al.* Systemic and secretory humoral immunity in the normal human vaginal tract.Scand J Immunol 1995; 42: 269-74.
96. Berneman A, Belec L, Fischetti VA, Bouvet JP. The specificity patterns of human immunoglobulin G antibodies in serum differ from those in autologous secretions. Infect Immun 1998; 66:4163-8.
97. Mestecky J, Moro I, Underdown B. Mucosal immunoglobulins. In:Ogra P, Mestecky J, Lamm M, *et al.* eds. Mucosal immunology. San Diego: Academic Press, 1999: 133-152.
98. Bjercke S, Brandtzaeg P. Glandular distribution of immunoglobulins, J chain, secretory component, and HLA-DR in the human endometrium throughout the menstrual cycle. Hum Reprod 1993; 8: 1420-5.
99. Crowley-Nowick P, Bell M, Edwards R, *et al.* Normal uterine cervix:characterization of isolated lymphocyte phenotypes and immunoglobulin secretion. Am J Reprod Immunol (Copenhagen) 1995; 34: 241-7.
100. Raux M, Finkielsztejn L, Salmon-Ceron D, *et al.* Comparison of the distribution of IgG and IgA antibodies in serum and various mucosal fluids of HIV type 1-infected subjects. AIDS Res Hum Retroviruses 1999; 15: 1365-76.
101. Belec L, Tevi-Benissan C, Dupre T, *et al.* Comparison of cervicovaginal humoral immunity in clinically asymptomatic (CDC A1 and A2 category) patients with HIV-1 and HIV-2 infection.J Clin Immunol 1996; 16: 12-20.
102. Kozlowski P, Chen D, Eldridge J, *et al*. Contrasting IgA and IgG neutralization capacities and responses to HIV type 1 gp120 V3 loop in HIV-infected individuals. AIDS Res Hum Retroviruses 1994; 10: 813-22.
103. Burnett P, VanCott T, Polonis V, Redfield R, Birx D. Serum IgA-mediated neutralization of HIV type 1. J Immunol 1994; 152:4642-8.
104. Black K, Cummins Jr. J, Jackson S. Serum and secretory IgA from HIV-infected individuals mediate antibody-dependent cellular cytotoxicity. Clin Immunol Immunopathol 1996; 81: 182-90.
105. Bukawa H, Sekigawa K, Hamajima K, *et al.* Neutralization of HIV-1 by secretory IgA induced by oral immunization with a new macromolecular multicomponent peptide vaccine candidate. Nat Med 1995; 1: 681-5.
106. Moja P, Tranchat C, Tchou I, *et al.* Neutralization of human immunodeficiency virus type I (HIV-1) mediated by parotid IgA of HIV-1-infected patients. J Infect Dis 2000; 181: 1607-13.
107. Mazzoli S, Lopalco L, Salvi A, *et al.* Human immunodeficiency virus (HIV)-specific IgA and HIV neutralizing activity in the serum of exposed seronegative partners of HIV-seropositive persons. J Infect Dis 1999; 180: 871-5.

108. Shibata R, Igarashi T, Haigwood N, *et al.* Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med 1999; 5: 204-10.
109. Mazzoli S, Trabattoni D, Lo Caputo S, *et al.* HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. Nat Med 1997; 3: 1250-7.
110. Beyrer C, Artenstein A, Rugpao S, *et al.* Epidemiologic and biologic characterization of a cohort of human immunodeficiency virus type 1 highly exposed, persistently seronegative female sex workers in northern Thailand. Chiang Mai HEPS Working Group. J Infect Dis 1999; 179: 59-67.
111. Kaul R, Trabattoni D, Bwayo J, *et al.* HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. AIDS 1999; 13:23-9.
112. Dorrell L, Hessell A, Wang M, *et al.* Absence of specific mucosal antibody responses in HIV-exposed uninfected sex workers from the Gambia. AIDS 2000; 14: 1117-22.
113. Schrader J, Scollay R, Battye F. Intramucosal lymphocytes of the gut: Lyt-2 and thy-1 phenotype of the granulated cells and evidence for the presence of both T cells and mast cell precursors. J Immunol 1983; 130: 558-64.
114. Tagliabue A, Befus A, Clark D, Bienenstock J. Characteristics of natural killer cells in the murine intestinal epithelium and *lamina propria*. J Exp Med 1982; 155: 1785-96.
115. Maric I, Holt P, Perdue M, Bienenstock J. Class II major histocompatibility complex antigen (Ia)-bearing dendritic cells (DC) in the epithelium of the rat intestine. J Immunol 1996; 156:1408-1414.
116. Kelsall B, Strober W. Dendritic cells of the gastrointestinal tract. Springer Semin Immunopathol 1997; 18: 409-20.
117. Boismenu R, Havran W. Modulation of epithelial cell growth by intraepithelial gamma delta T cells. Science 1994; 266: 1253-5.
118. Anderson D. The importance of mucosal immunology to problems in human reproduction. J Reprod Immunol 1996; 31: 3-19.
119. Bjercke S, Scott H, Braathen L, Thorsby E.HLA-DR-expressing Langerhans'-like cells in vaginal and cervical epithelium. Acta Obstet Gynecol Scand 1983; 62: 585-9.
120. Miller C, Vogel P, Alexander N, *et al*. Localization of SIV in the genital tract of chronically infected female rhesus macaques. Am J Pathol 1992; 141: 655-60.
121. Musey L, Hu Y, Eckert L, *et al*. HIV-1 induces cytotoxic T lymphocytes in the cervix of infected women.J Exp Med 1997;185: 293-303.
122. Lohman B, Miller C, McChesney M.Antiviral cytotoxic T lymphocytes in vaginal mucosa of simian immunodeficiency virus-infected rhesus macaques. J Immunol 1995; 155: 5855-60.
123. Kelker H, Seidlin M, Vogler M, *et al.* Lymphocytes from some long-term seronegative heterosexual partners of HIV-infected individuals proliferate in response to HIV antigens. AIDS Res Hum Retroviruses 1992; 8: 1355-9.
124. Liu R, Paxton W, Choe S, *et al.* Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996; 86: 367-77.
125. Samson M, Libert F, Doranz B, *et al.* Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996; 382: 722-5.
126. Furci L, Scarlatti G, Burastero S, *et al.* Antigen-driven C-C chemokine-mediated HIV-1 suppression by CD4+ T cells from exposed uninfected individuals expressing the wild-type CCR-5 allele. J Exp Med 1997; 186: 455-60.
127. Rowland Jones S, Dong T, Fowke K, *et al.* Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. J Clin Invest 1998; 102: 1758-65.
128. Bernard N, Yannakis C, Lee J, *et al.* Human immunodeficiency virus (HIV)-specific cytotoxic T lymphocyte activity in HIV-exposed seronegative persons. J Infect Dis 1999; 179: 538-47.
129. Goh W, Markee J, Akridge R, *et al.* Protection against human immunodeficiency virus type 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects. J Infect Dis 1999; 179: 548-57.
130. Kaul R, Plummer F, Kimani J, *et al.* HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J Immunol 2000; 164: 1602-11.
131. Haas G, Hosmalin A, Hadida F, *et al*. Dynamics of HIV variants and specific cytotoxic T-cell recognition in nonprogressors and progressors. Immunol Lett 1997; 57: 63-8.
132. Letvin N, Schmitz J, Jordan H, *et al.* Cytotoxic T lymphocytes specific for the simian immunodeficiency virus. Immunol Rev 1999; 170: 127-34.
133. Quayle A, Coston W, Trocha A, *et al*. Detection of HIV-1-specific CTLs in the semen of HIV-infected individuals. J Immunol 1998; 161: 4406-10.
134. Fujihashi K, Ernst P. A mucosal internet: epithelial cell-immune cell interactions. In: Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J, eds. Mucosal Immunology. San Diego: Academic Press, 1999: 619-30.
135. Pruitt K, Rahemtulla B, Rahemtulla F, *et al.* Innate humoral factors. In: Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J, eds. Mucosal Immunology. San Diego: Academic Press, 1999: 65-88.
136. Lehrer R, Bevins C, Ganz T. Defensins and other antimicrobial peptides. In: Ogra P, Mestecky J, Lamm M, *et al.* eds. Mucosal Immunology. San Diego: Academic Press, 1999: 89-99.
137. Nakashima H, Yamamoto N, Masuda M, *et al*. Defensins inhibit HIV replication in vitro. AIDS 1993; 7: 1129.
138. Monell C, Strand M. Structural and functional similarities between synthetic HIV gp41 peptides and defensins. Clin Immunol Immunopathol 1994; 71: 315-24.
139. McNeely T, Dealy M, Dripps D, *et al.* Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity *in vitro*. J Clin Invest 1995; 96:456-64.
140. Shugars D, Sauls D, Weinberg J. Secretory leukocyte protease inhibitor blocks infectivity of primary monocytes and mononuclear cells with both monocytotropic and lymphocytotropic strains of human immunodeficiency virus type I. Oral Dis 1997; 3:S70-2.
141. Shugars D. Endogenous mucosal antiviral factors of the oral cavity. J Infect Dis 1999; 179: S431-5.
142. McNeely T, Shugars D, Rosendahl M, *et al*. Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. Blood 1997; 90: 1141-9.
143. Fultz P. Components of saliva inactivate human immunodeficiency virus. Lancet 1986; 2: 1215.
144. Wahl S, McNeely T, Janoff E, *et al.* Secretory leukocyte protease inhibitor (SLPI) in mucosal fluids inhibits HIV-I.Oral Dis 1997;3: S64-9.
145. Shugars D, Wahl S. The role of the oral environment in HIV-1 transmission. J Am Dent Assoc 1998; 129: 851-8.
146. Becquart P, Garin B, Sepou A, *et al.* High incidence of early postnatal transmission of human immunodeficiency virus type 1 in Bangui, Central African Republic. J Infect Dis 1998; 177:1770-1.
147. Semba R, Kumwenda N, Hoover D, *et al.* Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis 1999; 180: 93-8.
148. Becquart P, Gresenguet G, Hocini H, Kazatchkine M, Belec L. Secretory leukocyte protease inhibitor in colostrum and breast milk is not a major determinant of the protection of early postnatal transmission of HIV. AIDS 1999; 13: 2599-602.
149. Moriyama A, Shimoya K, Kawamoto A, *et al.* Secretory leukocyte protease inhibitor (SLPI) concentrations in seminal plasma: SLPI restores sperm motility reduced by elastase. Mol Hum Reprod 1998; 4: 946-50.
150. King A, Critchley H, Kelly R. Presence of secretory leukocyte protease inhibitor in human endometrium and first trimester decidua suggests an antibacterial protective role. Mol Hum Reprod 2000; 6: 191-6.
151. Hitchcock P. Topical microbicides. In: Stanberry L, Bernstein D, eds. Sexually Transmitted Diseases. San Diego: Academic Press, 2000: 149-66.
152. Klebanoff S, Hillier S, Eschenbach D, Waltersdorph A.Control of the microbial flora of the vagina by H_2O_2-generating lactobacilli. J Infect Dis 1991; 164: 94-100.
153. Klebanoff S, Coombs R. Viricidal effect of Lactobacillus acidophilus on human immunodeficiency virus type 1:possible role in heterosexual transmission. J Exp Med 1991; 174: 289-92.
154. Sewankambo N, Gray R, Wawer M, *et al.* HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet 1997; 350: 546-50.
155. Martin H, Richardson B, Nyange P, *et al.* Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition.J Infect Dis 1999; 180: 1863-8.