Innate Immunity Including Epithelial and Nonspecific Host Factors: Workshop 1B

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Adv Dent Res 23(1):122-129, 2011

ABSTRACT

The majority of HIV infections are initiated at mucosal sites. The oral mucosal tissue has been shown to be a potential route of entry in humans and primates. Whereas HIV RNA, proviral DNA, and infected cells are detected in the oral mucosa and saliva of infected individuals, it appears that the oral mucosa is not permissive for efficient HIV replication and therefore may differ in susceptibility to infection when compared to other mucosal sites. Since there is no definitive information regarding the fate of the HIV virion in mucosal epithelium, there is a pressing need to understand what occurs when the virus is in contact with this tissue, what mechanisms are in play to determine the outcome, and to what degree the mechanisms and outcomes differ between mucosal sites. Workshop 1B tackled 5 important questions to define current knowledge about epithelial cell-derived innate immune agents, commensal and endogenous pathogens, and epithelial cells and cells of the adaptive immune system and how they contribute to dissemination or resistance to HIV infection. Discovering factors that explain the differential susceptibility and resistance to HIV infection in mucosal sites will allow for the identification and development of novel protective strategies.

The oral epithelium is a complex structure serving as an interface between the external environment and the diffuse lymphoid tissue and immune cells in the underlying connective tissues. Workshop 1B attempted to address the state of knowledge about the innate immune and related protective properties of oral mucosal epithelium and how they might control HIV-1 infection during clinical exposures to infectious virus. What we know about this topic pales in comparison to what we need to learn. This article reports on a workshop wherein 5 major underinvestigated research areas were presented, each of which must be addressed for better understanding of the potential of oral mucosa to serve as a reservoir for infectious virus and a cryptic site of latent HIV-1 infection.

DOI: 10.1177/0022034511399917

WORKSHOP QUESTIONS

Question 1: What is the role of epithelial cells in HIV-1 infection?

Question 2: How is the mucosal epithelial response to HIV-1 modified or regulated by the presence of normal commensals and endogenous pathogens?

Question 3: What are the roles of salivary and epithelial innate immune effector molecules such as secretory leukocyte protease inhibitor (SLPI), defensins, and cytokines and chemokines during infection with HIV and copathogens?

Question 4: How do interactions among mucosal epithelial cells, dendritic cells, T cells, and natural killer (NK) cells contribute to systemic dissemination or resistance to HIV-1 infection?

Question 5: What is the role of the fetal/infant oral epithelium in HIV mother-to-child transmission?

QUESTION 1

What is the role of epithelial cells in HIV-1 infection?

A Janus-Faced Barrier to HIV-1 Entry to the Submucosa

Cells forming the squamous mucosal epithelium that lines the oral cavity and oropharynx, including the tonsils, are connected to one another by tight, adherens, desmosomal, and gap junctions. Depending on the tissue, the thickness of this type of epithelium can range from 1 to 20 or more cell layers. For particles the size of viruses, the intercellular spaces in healthy, uninflamed squamous epithelia would appear to restrict passage from the outer mucosal surface, across the basement membrane, into the connective tissues. Formed from multiple overlapping layers of cells, the squamous epithelium is occasionally interspersed with resident immune cells, including Langerhans cells (LCs), which

Key Words

HIV/AIDS, infectious disease, SLPI, oral epithelium, HIV replication, HIV transmission.

are dendritic cells expressing the cell surface receptor langerin (CD207), intraepithelial lymphocytes, and tissue macrophages. Epithelial cells also form a barrier against HIV-1 infection by producing antiviral substances, including SLPI and human β-defensins (hBDs). How these function to restrict HIV-1 infection of the epithelium *in vivo* remains to be studied.

Several lines of evidence suggest that squamous epithelial cells can capture and harbor infectious HIV-1. Indeed, oral exposure to HIV-1 can be frequent in nursing infants and in men who have sex with men. Consequent to oral exposures, however, the efficiency of systemic infection is low. Given that the oral route infrequently results in primary clinical infections of uninfected individuals, it is unlikely that oral epithelial cells become productively infected and pass virus promiscuously through or across the epithelium to LCs or permissive cells in the lamina propria. When *trans*-infection of permissive cells occurs, albeit at a low level, the pathway is likely to mirror mechanisms suggested for the vaginal epithelium, which is anatomically similar except for the superficial presence of antigen-sampling dendritic cells. Infection of the genitourinary tract is a common route of acquisition of primary infection. Since both epithelia produce SLPI and hBDs constitutively, it is unclear whether the high rate of vaginal infection can be best explained because of more efficient *trans*-infection than that in the oral cavity or more frequent clinical exposures. Nonetheless, for virus to systemically disseminate from an oral focus, a low level of *trans*-infection of HIV-1 by the squamous keratinocytes that line the oral cavity and oropharynx would appear to contribute.

Epithelial Cells Harbor HIV-1 and *Trans*-Infect Permissive Cells

Like squamous keratinocytes from other tissues, primary and immortalized oral keratinocytes can harbor infectious HIV-1 (Vacharaksa *et al*., 2008). The virus remains infectious since harbored virus can *trans*-infect permissive reporter cells (TZM-bl) and peripheral blood mononuclear cells for up to 24 to 48 hours and there is no preference for X4- or R5-tropic HIV-1. Levels of HIV-1 in cell culture supernatants are below the limits of detection, and HIV-1 RNA and infectious virus can be recovered from experimentally lysed cells. Furthermore, transfer of harbored HIV-1 to permissive cells appears to require intercellular contact (Giacaman *et al*., 2008), suggesting that keratinocytes and leukocytes form a virologic synapse.

To understand HIV-1 infection in the context of the oral and oropharyngeal environment, we modeled the effects of key local factors on the fate of the virus. By studying the kinetics of salivary inactivation of HIV-1, we identified a window of opportunity for escape into oral keratinocytes. Indeed, when HIV-1 was incubated in the presence of saliva for 5 or 15 minutes with oral keratinocytes, virus was rapidly harbored, and it resisted trypsin and was able to *trans*-infect TZM-bl reporter cells. Hence, the antiviral activity of saliva appears insufficient to prevent keratinocytes from disseminating HIV-1 to permissive cells.

Harboring and *trans*-infection of HIV-1 might occur *in vivo*. Simian immunodeficiency virus 1 (SIV-1) disseminates from an oral focus 24 hours after atraumatic exposure to the oral mucosa (Milush *et al*., 2004), although it is difficult to compare the size and infectivity of the inocula to human exposures. Evidence of systemic dissemination begins with the appearance of gastrointestinal infection 4 days postinoculation. As signs of infection disappear from the oral epithelium, they appear and increase in systemic sites (Kosub *et al*., 2008). Proviral DNA can be also detected in oral keratinocytes (Liuzzi *et al*., 1996), and HIV-1 gag RNA has been demonstrated in buccal cells and oral biopsies (Qureshi *et al*., 1997; Rodriguez-Iñigo *et al*., 2005). Given that human experiments cannot be performed, animal models such as humanized mice must be fully exploited to understand the complex interactions between HIV-1 and the oral tissues.

Evidence for Productive Infection of Oral Epithelial Cells

Productive HIV-1 infection in permissive cells requires entry through CD4 and a fusion coreceptor, usually CCR5 or CXCR4, but oral epithelial cells do not express these canonical entry receptors. However, the viral envelope protein gp120 binds to noncanonical cell surface molecules, including the C-type lectin DC-SIGN (dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin), the glycosphingolipid galactosylceramide, and proteoglycans such as syndecan-1. Since oral epithelial cells express galactosylceramide and proteoglycans, HIV-1 could enter cells through these alternative mechanisms. Yet, the literature remains ambiguous; some studies report no evidence of productive HIV-1 infection in oral epithelial cells (Quinones-Mateu *et al*., 2003; Vacharaksa *et al*., 2008) while others suggest otherwise (Han *et al*., 2000; Moore *et al*., 2003; Moutsopoulos *et al*., 2007). These contradictory findings may resolve in consideration of the oral epithelial cell lineage and experimental conditions used. Internalization of X4- or R5-tropic HIV-1 appears to occur without preference into oral epithelial cells, and the purported roles of these cognate noncanonical receptors remain unclear. Therefore, the fundamental issue of whether epithelial cells can be productively infected with HIV-1 remains controversial. The consensus of evidence suggests that adult keratinocytes internalize and integrate HIV-1 in the absence of CD4 without becoming productively infected (Vacharaksa *et al*., 2008).

QUESTION 2

How is the mucosal epithelial response to HIV-1 modified or regulated by the presence of normal commensals and endogenous pathogens?

HIV and Coinfecting Secondary Pathogens

Coinfection with non-HIV pathogens is thought to influence the severity and rate of disease progression in HIV-positive individuals, reducing survival and increasing the risk of HIV-1 transmission. Likewise, the frequency of secondary coinfections in HIV-positive individuals implies that HIV-1 induces a profound perturbation of the mucosae, particularly in the oral cavity (Challacombe and Naglik, 2006). Therefore, HIV-1 infection can be hypothesized to directly affect the oral epithelium, resulting in alterations that promote colonization and infection by commensal and pathogenic organisms. Examples of copathogens

include human herpesvirus 6 and 8, herpes simplex virus 1/2 (HSV-1/2), Epstein-Barr virus, human papilloma virus, several protozoan parasites, bacteria, and the fungus *Candida albicans*. Although the composition of the oral flora is relatively unaffected by HIV infection, HIV-positive individuals endure increased colonization and tissue invasion of oral epithelium by coinfecting pathogens (Myint *et al*., 2002). Coinfection might be attributable to reduced saliva flow rates, which are often observed in early HIV-1 disease (Lin *et al*., 2003). Rhesus macaque infection with SIV and humans with HIV-1 produce similar disease progression, with similar coinfections (George *et al*., 2008). The oropharynx of SIV-infected animals shows loss of CD4+ cells in the oral mucosa, repression of genes regulating biogenesis as a result of viral invasion, and increased apoptosis (George *et al*., 2008, 2009). SIV infection may thus inhibit the ability of the oral mucosa to repair damage, resulting in impaired host responses and increased invasion by secondary pathogens.

Effects of Secondary Pathogens on Oral Epithelium in the Context of HIV-1 Disease

Infection and/or inflammation of the mucosa caused by secondary pathogens may promote HIV-1 susceptibility. Secondary pathogens may increase expression of HIV entry receptors CD4, CCR5, and DC-SIGN through increased production of proinflammatory cytokines such as TNF-α and IL-1β (Jotwani *et al*., 2004). Infection with *Porphyromonas gingivalis*, a common gram-negative bacterium associated with periodontal disease, was recently shown to upregulate CCR5 expression in oral keratinocytes through pathways common to activation of protease-activated receptors and Toll-like receptors (TLRs) (Giacaman *et al*., 2007). By coinfecting with *P. gingivalis*, oral keratinocytes selectively increased the harboring and *trans*-infection of R5-tropic virus to permissive cells without affecting the infectivity of HIV-1 (Giacaman *et al*., 2008). Hence, HIV-1 infection in the presence of coinfecting pathogens could create an R5-specific mucosal gatekeeper, selectively opening the entry of R5-tropic virus into keratinocytes and simulating the viral tropism of most primary clinical infections. A CCR5 mucosal gatekeeper could explain the predominance of R5-tropic primary infections since more than 80% of infections occur by a mucosal route. *P. gingivalis* may also induce HIV-1 reactivation via chromatin modification owing to the secretion of the bacterial metabolite butyric acid (Imai *et al*., 2009). Butyric acid inhibits histone deacetylases, causing histone acetylation and dissociation of the histone deacetylase 1 and AP-4 (transcription factor) corepresser complex from the HIV-1 long terminal repeat promoter. Although such regulatory and pathogen activation mechanisms have been observed in ACH-2 and U1 cells (derived from human CD4+ T-cell and macrophage cell lines, respectively), whether similar mechanisms occur in oral epithelial cells remains to be determined. If so, periodontal and other oral diseases could increase the risk of reactivation of HIV-1 in infected individuals, assuming that the oral epithelium is a site of latent infection.

HSV-2 is a common secondary pathogen coinfecting HIV-positive individuals, and HIV-1 and HSV-2 coinfection in patients leads to enhanced sexual transmission and increased replication of HIV-1 (Freeman *et al*., 2006). The genital mucosa of coinfected female sex workers show increased HIV-1 and HSV-2 viral shedding and increased circulating cells expressing CCR5 and DC-SIGN (Rebbapragada *et al*., 2007). HIV-1 infection also causes depletion of immature dendritic cells (iDCs), which may be responsible for reactivation of HSV production. Synergy between HIV-1 and HSV-2 was also suggested since anti-HSV compounds promote concomitant reduction in HIV-1 RNA viral load (Schacker *et al*., 2002). Given that the oral mucosa is frequently infected with HSV, coinfection with HIV-1 may affect acquisition and transmission from oral foci.

HIV-1 may affect both the host and the fungus *C. albicans*. *C. albicans*, as isolated from HIV-positive and AIDS patients, showed greater adherence to buccal epithelial cells from healthy patients, whereas buccal epithelial cells from HIV-positive and AIDS patients permitted increased adherence of *C. albicans* from healthy patients (Sweet *et al*., 1995). This dual effect suggests that complex interrelationships occur *in vivo* among HIV-1, epithelial cells, and secondary copathogens.

The responses of oral keratinocytes to HIV-1 in the presence and absence of coinfecting microorganisms can also affect signaling to proximal cells. For example, HIV-1 appears to downregulate interferon-related genes in oral keratinocytes, but this may be inhibited by prior infection with *P. gingivalis* (Asrani *et al*., 2009, unpublished data). Hence, we must learn whether coinfection of mucosal epithelia with HIV-1 and other pathogens results in production of cytokines that are proinflammatory or that signal for mucosal anergy. In primate models, SIV elicits mucosal epithelial immune responses (Li *et al*., 2009). In human infection, HIV-1-induced mucosal anergy appears to enable primary viral infections to replicate before an effective T-cell response can be launched (Bren *et al*., 2009). Keratinocyte-initiated T-cell responses must be elucidated.

Pathogen Recognition and HIV-1 Infection

Innate immune recognition of mucosal pathogens in humans involves a family of 10 TLRs. Ligation of pathogens to TLRs triggers a cascade of events involving the activation of NF-κB and MAPK signaling pathways and the production of proinflammatory cytokines involved in immune activation (Takeda and Akira, 2005). TLR-pathogen interactions could play an indirect role in regulating HIV through the activation of HIV long terminal repeat sequences by host transcription factors (Bentwich *et al*., 1995). Cytokine-mediated induction of the HIV long terminal repeat through NF-κB activation may elevate viral expression as observed in coinfected individuals (Báfica *et al*., 2004). Chronic HIV-1 infection (CD4+ < 200 cells/mL) in the absence of HAART was associated with significantly increased expression of TLR2, TLR3, TLR4, TLR6, TLR7, and TLR8 mRNA, and TLR responsiveness was increased (Lester *et al*., 2008). After TLR2 stimulation, TLR2 surface expression and viral production increased in HIV-positive patients (Heggelund *et al*., 2004). TLR2 and TLR4 signal recognition of most bacterial and fungal mucosal pathogens, which leads to production of proinflammatory cytokines. Activation of TLR-mediated pathways also appear to regulate or promote HIV-1 replication, potentially

promoting HIV-1 disease (Lane *et al*., 1999). Since HIV-1 infection of the oral mucosal epithelium occurs in the presence of the existing microbiota, we must determine the conditions that cause proinflammatory or anergic responses in the clinical setting.

QUESTION 3

What are the roles of salivary and epithelial innate immune effector molecules such as SLPI, defensins, and cytokines and chemokines during infection with HIV and copathogens?

The relative infrequency of oral HIV-1 infection can be posited to involve (1) a thick multilayered mucosal surface (first line of defense against microbial invasion), (2) low salivary HIV-1 titers, and (3) endogenous antiviral factors present in oral secretions. Many endogenous inhibitors of HIV-1 in saliva have been proposed (*e.g.*, amylase, lactoferrin, proline-rich peptides, salivary mucins, thrombospondin and SLPI; reviewed in Shugars and Wahl, 1998). Remarkably, these agents are also found in seminal fluid and vaginal secretions, routinely harvested from sites more susceptible to HIV-1 infection (Shugars, 1999; Kazmi *et al*., 2006). Furthermore, fresh human saliva cannot inactivate HIV-1 rapidly enough to prevent entry of infectious virus into oral keratinocytes (Herzberg presentation at Sixth World Workshop). More information is clearly needed to understand whether salivary-innate immune factors efficiently contribute to the resistance against acquisition of HIV-1.

Mucosal epithelial cells also express antimicrobial peptides with antiretroviral activity. In addition to SLPI, oral epithelial cell–derived hBDs (reviewed in Diamond *et al*., 2009) inhibit HIV-1 infection of immunocompetent cells *in vitro* (Quinones-Mateu *et al*., 2003; Sun *et al*., 2005). The lack of hBD and SLPI expression in fetal oral mucosae, when compared with adult oral mucosae, appears to render underlying immunocompetent cells more susceptible to HIV-1 infection (Tugizov presentation at Sixth World Workshop). Although transcytosis of the virions was not appreciably different in adult and fetal mucosal cells, the ease with which HIV-1 infects the underlying lymphocytes in the fetal mucosal model, along with the loss of protection by use of specific antibodies to hBDs and SLPI in the adult oral mucosae, strongly suggests that these peptides protect against HIV-1 infection. Interestingly, a notable difference between oral epithelia and most other epithelia is the constitutive expression of hBDs. These defensins are expressed only in the presence of infection or inflammation in most tissues, including skin, trachea, and gut epithelium (Liu *et al*., 1998; Ong *et al*., 2002). However, both hBD-2 and hBD-3 are expressed in normal uninflamed gingival tissue (Dale *et al*., 2001), perhaps due to chronic exposure to specific oral commensal bacteria that promote hBD expression (Weinberg, unpublished data). What is less clear is whether the target cells for antiviral activity are the hBD-producing oral epithelial cells or the proximal immunocompetent cells.

The hBDs may also thwart other viral infections. Since 2000, epidemiologic reports have suggested that oral human papilloma virus replication is increased in chronic HIV-1 infection in those taking HAART for long periods. Interestingly, these individuals have been shown to express less hBDs in their oral mucosae when compared with age-matched healthy controls (Sun *et al*., 2005; confirmed by Weinberg *et al*.).

During HIV-1 disease, levels of histatin-5, a potent antimycotic agent, decrease in saliva of HIV-positive persons, with subsequent overgrowth of *C. albicans* (Torres *et al*., 2009). *C. albicans* also efficiently and rapidly degrades histatin-5, resulting in loss of its anticandidal potency (Meiller *et al*., 2009), suggesting synergistic loss of antifungal activity in HIV-positive patients. In HIV-infected patients with oropharyngeal candidiasis, Fidel *et al*. identified reduced nondefensin- and non-SLPI-associated oral epithelial cell antimycotic activity (Steele *et al*., 2000), which could contribute to susceptibility to oropharyngeal candidiasis. A strong candidate for the effector moiety has been identified as annexin-A1, which affects growth-associated signaling cascades within cells (Lilly *et al*., 2010). Finally, *C. albicans* has been shown to promote a predominantly proinflammatory cytokine response in oral epithelial cells (Steele and Fidel, 2002; Weindl *et al*., 2007; Moyes *et al*., 2010), which is attenuated in oral epithelial cells from HIV-positive individuals (Lilly *et al*., 2006).

QUESTION 4

How do interactions among mucosal epithelial cells, dendritic cells, T cells, and NK cells contribute to systemic dissemination or resistance to HIV-1 infection?

Although most studies have investigated HIV-1 transmission via genital and gastrointestinal epithelium, HIV-1 transmission across the oral mucosa may show similarities. Transmission of HIV-1 across the mucosal tissues can occur in a variety of ways (Hladik and Hope, 2009). First, cell-free and cell-associated HIV-1 is likely to become trapped in mucus overlaying the epithelial surface. Here, cell-associated HIV-1 may attach to host epithelial cells and release free virus. Cell-free virus may then bind directly to epithelial cells (via galactosylceramide, lactosylceramide, syndecans, or gp340) and be transcytosed to basal layers, or it may penetrate between gaps in the outer epithelium, where they might be captured by resident LCs and/or CD4+ intraepithelial T cells (Hladik and Hope, 2009) (via C-type lectins and canonical HIV receptors). LCs may directly sample virus on the surface in the vaginal mucosa (Neutra *et al*., 2001; Hladik *et al*., 2007), where the mean number of LCs in the upper third of the epithelium is significantly greater than that in the oral epithelium (Hussain and Lehner, 1995). The paucity of LCs in the outer third of the oral epithelium may contribute to the relative protection from direct HIV-1 infection. In LCs, the virus is ushered into endocytic compartments or Birbeck granules (depending on the mucosal site), but productive infection is difficult to detect (Hladik and Hope, 2009), in contrast to CD4+ intraepithelial T cells and stromal DCs. CD4+ intraepithelial T cells can directly transfer HIV-1 to DCs or tissue macrophages; these intercellular interactions can also stimulate viral production within the T cell. Indeed, the transfer of HIV-1 from cell to cell may be bidirectional, depending on the cell types involved. Bidirectional transfer may explain the presence of integrated HIV-1 DNA in buccal keratinocytes in chronically infected

individuals (Rodriguez-Inigo *et al*., 2005). HIV-infected LCs, DCs, and T cells eventually migrate into draining lymph nodes to augment cell activation and virus production and establish acute infection.

Acute infection with HIV-1 results in a rapid depletion of memory CD4+ T cells in the lamina propria, particularly in the gastrointestinal tract (Veazey *et al*., 1998), as well as a consequent expansion/influx of CD8+ T cells (Veazey *et al*., 2001), which can provide robust responses despite chronic HIV-1 infection (Shacklett *et al*., 2009). However, the CD8+ T cells fail to clear the infection, indicating that other factors are required for HIV-1 containment or control of infection. HIV-1 infection of NK cells has been reported (Harada *et al*., 2007), and NK cell responses to HIV-1 peptides were recently shown to correlate with protection against maternal-infant transmission (Tiemessen *et al*., 2009). Although NK cells may be selectively depleted during the early stages of infection (Li and Xu, 2008), the role of NK cells in the HIV-1 infection process is still unclear. The role of T-regulatory and T-helper 17 cells in HIV-1 pathogenesis remains controversial. Both cell types are present in mucosal tissues of HIV-infected individuals, but studies in monkeys indicate that the loss of the T-regulatory–T-helper 17 balance is related to SIV progression (Broliden *et al*., 2009). Mucosal γδ T cells have also been implicated in HIV-1 infection (Lehner *et al*., 2000), and further studies are needed to precisely define the mechanisms of participation.

QUESTION 5

What is the role of the fetal/infant oral epithelium in HIV mother-to-child transmission?

Oral transmission is a potentially important but poorly understood route of HIV infection. The oropharyngeal epithelium of the fetus/neonate may serve as a portal of entry for HIV mother-to-child transmission, whereas oral transmission of HIV in adults rarely occurs (Page-Shafer *et al*., 2006). Hence, HIV transmission differs between adults and fetuses/neonates, but the molecular mechanisms underlying differences are not well understood.

Using *ex vivo* tissue explants, Tugizov *et al*. (presentation at Sixth World Workshop) demonstrated that HIV-1 transmigrates across fetal oropharyngeal and intestinal epithelia. HIV-1 also efficiently transcytosed through adult and fetal polarized oral epithelial cells, yet virions emerging after transcytosis from adult cells were not infectious, whereas highly infectious virions were recovered from fetal epithelial cells. Innate immune molecules may contribute to antiviral resistance in adult epithelial cells since expression of hBD-2 and hBD-3 and SLPI was substantially higher in adult epithelial cells than in fetal epithelial cells. Neutralization of these proteins in adult oral epithelial cells restored infectivity, suggesting that these proteins protect against viral transmission by the adult oral epithelium. During mother-to-child transmission through fetal oral epithelium, the lack of expression of these proteins may therefore contribute to preservation of infectious virus.

Mother-to-child transmission of HIV may occur *in utero* before birth (prenatal), during labor and delivery (perinatal), or after birth (postnatal) (Volmink and Mahlati, 2005). In prenatal mother-to-child transmission, HIV-1 RNA/DNA and proteins were detected in placental trophoblasts, endothelial cells, and villous Hofbauer cells by 8 weeks of gestation, suggesting transmission by the transplacental route (Lewis *et al*., 1990; Soeiro *et al*., 1992; Sheikh *et al*., 2000). An alternative prenatal route could be transamniotic, where cell-free HIV-1 virus and/or HIV-infected cells may penetrate into the amniotic sac and infect the fetus from the oropharyngeal cavity or gastrointestinal tract. For example, HIV-1 p24 antigen was detectable in amniotic fluid from 8 of 10 HIV-positive women (Jaspan *et al*., 2004) and, in an earlier study, from 5 of 13 women (38%) and in serum from 3 of 13 fetuses (23%) (Viscarello *et al*., 1992). Furthermore, HIV-1 was detected in the amniotic fluid of HIV-positive women at 32 weeks of gestation (Mundy *et al*., 1987) and in gastric aspirates of a 15-week-old fetus (Sprecher *et al*., 1986). These data are consistent with mother-to-child transmission of HIV *in utero* from transmucosal sites.

Factors that promote intrauterine infection and inflammation may contribute to the dissemination of HIV-1. The prevalence of HIV-1 infection and mother-to-child transmission is directly related to the rate of upper and lower genital tract infections, such as bacterial vaginosis, candidiasis, gonorrhea, chlamydia, trichomoniasis, and HSV-1 (Taha and Gray, 2000; Chi *et al*., 2006). Intrauterine infection may induce acute or subclinical chorioamnionitis and an intra-amniotic inflammatory response involving the activation of a number of cytokines and chemokines in the amniotic fluid, including TNF-α, IFN-γ, MCP-1, IL-6, IL-8, IL-1, and IL-2 (Stringer and Goldenberg, 2000; Chi *et al*., 2006). These changes may trigger cervical ripening, rupture of the fetal membranes, and preterm labor, which may facilitate mixing of cell-free HIV-1 and HIV-infected immune cells from cervicovaginal secretions with amniotic fluid. These HIV-infected immune cells may circulate and interact with the mucosal surfaces of the oropharynx and gastrointestinal tract of the fetus. During birth, the neonate is exposed to HIV-1 containing cervicovaginal fluids, which can interact with the neonatal oropharyngeal and gastrointestinal mucosal surfaces. In the newborn, breast-feeding from an HIV-infected mother can increase risk of mucosal acquisition in the presence of infection, allergy, and inflammation of the breast. Conditions such as acute and subclinical mastitis and mammary abscesses may lead to an increase in proinflammatory cytokines and facilitate release of cell-free and cell-associated HIV-1 into breast milk (Semba, 2000; Willumsen *et al*., 2000).

FUTURE DIRECTIONS

Definitive information about the fate of the HIV virion in mucosal epithelium is absent. We must learn what occurs after the virus contacts this tissue, what mechanisms determine the outcome, and to what degree the mechanisms and outcomes differ between mucosal sites. Greater understanding of vulnerable versus resistant mucosal sites may help identify strategies to bolster vulnerable sites. Potential approaches to address these issues include:

- comparative analysis of HIV-1 in fetal and adult tissues to uncover factors contributing to susceptibility and resistance to mucosal infection by HIV-1, defining mechanisms such as

innate keratinocyte antiretroviral activities, the effector cells for resistance, and cell-to-cell routes of virus transfer and dissemination;
- elucidation of ontogenic factors in HIV-1 susceptibility, including maturity and efficacy of antiretroviral mechanisms during gestation, birth, early life, midlife, and late life;
- comparison of the anatomic structure of mucosal epithelia with function during HIV-1 exposure, rest, and active HIV infection, with mucosa of interest including oral, intestinal, colorectal, vaginal, and transitional cervical; and
- conduct genomics and proteomics discovery-based studies on the respective epithelium from non-HIV/naïve and HIV-positive/HAART individuals.

To understand the mechanisms of HIV-1 infection, more robust models are needed. Humanized mice, selective knockout mice, and other *ex vivo* and *in vitro* relevant models (*e.g.*, organotypic epithelial cultures) should be used to test hypotheses. Models should be designed to be readily translated into tests of therapeutic outcomes.

We need to further investigate how coinfecting mucosal microbes (*i.e.*, the endogenous microbiome, including oral biofilms) modify the fate of HIV-1. Resident commensal and pathogenic bacteria, fungi, and viruses must be considered as copathogens. More precisely, signaling pathways in epithelial cells that are modulated by interactions with oral biofilms and specific commensal and pathogenic microbes are required. For example, the NF-κB and MAPK pathways appear activated by *Candida*, and the MAPK pathways are required to discriminate between the yeast and the hyphal form (Moyes *et al*., 2010). Attention to this issue would address the following questions:

How does the epithelial response to HIV-1 in the context of copathogens affect interactions with and transfer of infectious virions to proximal immune cells?

How do the mechanisms of fate of HIV-1 in oral tissues in the presence of the microbiome compare with other tissues with their respective resident flora?

How does the resident microbiome contribute to resistance/vulnerability to HIV-1 infection, and how does this manifest in different mucosal sites?

We must learn how inflammatory mediators from epithelial cells contribute to acute and chronic responses to HIV alone or in conjunction with the resident microflora. In this polymicrobial context, how do epithelial cells modulate the function of immune cells during HIV-1 infection, and, conversely, how do immune cells alter the function of epithelial cells and resistance during chronic exposures to HIV-1? Further scientific scrutiny must focus on the roles of salivary and epithelial innate immune effector molecules such as SLPI, defensins, and cytokines and chemokines during infection with HIV-1 and copathogens. We must learn how genetics contributes to the expression of innate host-derived antiretroviral agents that foster resistance to mucosal HIV-1 infection. Attention to this issue would address the following questions:

Can single-nucleotide polymorphisms and/or copy number polymorphisms in genes encoding salivary and epithelial innate immune effectors contribute to our understanding of interpersonal variability toward mucosal infections?

How do innate antiretroviral effector molecules produced by keratinocytes affect the ability of local immune cells to control or be directed to inhibit HIV-1 infectivity and secondary coinfecting pathogens (*e.g.*, human papilloma virus)?

How does chronic HIV-1 and/or HAART alter expression of innate responses—that is, cytokines, chemokines, and innate antiretroviral molecules—and dictate mucosal immune outcomes?

What are the immunoregulatory properties of keratinocyte effector molecules, and how do they contribute to mucosal protection?

These major areas must be addressed to better understand the role of the oral mucosae in preventing/promoting HIV infection. Through a better understanding of the mechanisms underlying HIV infection of human mucosal sites, we may one day use the knowledge to generate novel prophylactic strategies against HIV infection.

REFERENCES

Báfica A, Scanga CA, Schito M, Chaussabel D, Sher A (2004). Influence of coinfecting pathogens on HIV expression: evidence for a role of Toll-like receptors. *J Immunol* 172:7229-7234.

Bentwich Z, Kalinkovich A, Weisman Z (1995). Immune activation is a dominant factor in the pathogenesis of African AIDS. *Immunol Today* 16:187-191.

Bren GD, Trushin SA, Whitman J, Shepard B, Badley AD (2009). HIV gp120 Induces, NF-κB dependent, HIV replication that requires procaspase 8. *PLoS One* 4:e4875.

Broliden K, Haase AT, Ahuja SK, Shearer GM, Andersson J (2009). Introduction: back to basics. Mucosal immunity and novel HIV vaccine concepts. *J Intern Med* 265:5-17.

Challacombe SJ, Naglik JR (2006). The effects of HIV infection on oral mucosal immunity. *Adv Dent Res* 19:29-35.

Chi BH, Mudenda V, Levy J, Sinkala M, Goldenberg RL, Stringer JS (2006). Acute and chronic chorioamnionitis and the risk of perinatal human immunodeficiency virus-1 transmission. *Am J Obstet Gynecol* 194:174-181.

Dale BA, Kimball JR, Krisanaprakornkit S, Roberts F, Robinovitch M, O'Neal R, *et al*. (2001). Localized antimicrobial peptide expression in human gingiva. *J Periodontal Res* 36:285-294.

Diamond G, Beckloff N, Weinberg A, Kisich KO (2009). The roles of antimicrobial peptides in innate host defense. *Curr Pharm Des* 15: 2377-2392.

Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ (2006). Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* 20:73-83.

George MD, Verhoeven D, Sankaran S, Glavan T, Reay E, Dandekar S (2009). Heightened cytotoxic responses and impaired biogenesis contribute to early pathogenesis in the oral mucosa of simian immunodeficiency virus-infected rhesus macaques. *Clin Vaccine Immunol* 16:277-281.

George MD, Wehkamp J, Kays RJ, Leutenegger CM, Sabir S, Grishina I, *et al*. (2008). *In vivo* gene expression profiling of human intestinal epithelial cells: analysis by laser microdissection of formalin fixed tissues. *BMC Genomics* 9:209.

Giacaman RA, Asrani AC, Gebhard KH, Dietrich EA, Vacharaksa A, Ross KF, *et al*. (2008). Porphyromonas gingivalis induces CCR5-dependent transfer of infectious HIV-1 from oral keratinocytes to permissive cells. *Retrovirology* 5:29.

Giacaman RA, Nobbs AH, Ross KF, Herzberg MC (2007). Porphyromonas gingivalis selectively up-regulates the HIV-1 coreceptor CCR5 in oral keratinocytes. *J Immunol* 179:2542-2550.

Han Y, Ventura CL, Black KP, Cummins JE Jr, Hall SD, Jackson S (2000). Productive human immunodeficiency virus-1 infection of epithelial cell lines of salivary gland origin. *Oral Microbiol Immunol* 15:82-88.

Harada H, Goto Y, Ohno T, Suzu S, Okada S (2007). Proliferative activation up-regulates expression of CD4 and HIV-1 co-receptors on NK cells and induces their infection with HIV-1. *Eur J Immunol* 37:2148-2155.

Heggelund L, Muller F, Lien E, Yndestad A, Ueland T, Kristiansen KI, *et al*. (2004). Increased expression of toll-like receptor 2 on monocytes in HIV infection: possible roles in inflammation and viral replication. *Clin Infect Dis* 39:264-269.

Hladik F, Hope TJ (2009). HIV infection of the genital mucosa in women. *Curr HIV/AIDS Rep* 6:20-28.

Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, *et al*. (2007). Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* 26:257-270.

Hussain LA, Lehner T (1995). Comparative investigation of Langerhans' cells and potential receptors for HIV in oral, genitourinary and rectal epithelia. *Immunology* 85:475-484.

Imai K, Ochiai K, Okamoto T (2009). Reactivation of latent HIV-1 infection by the periodontopathic bacterium *Porphyromonas gingivalis* involves histone modification. *J Immunol* 182:3688-3695.

Jaspan HB, Robinson JE, Amedee AM, Van Dyke RB, Garry RF (2004). Amniotic fluid has higher relative levels of lentivirus-specific antibodies than plasma and can contain neutralizing antibodies. *J Clin Virol* 31:190-197.

Jotwani R, Muthukuru M, Cutler CW (2004). Increase in HIV receptors/co-receptors/alpha-defensins in inflamed human gingiva. *J Dent Res* 83:371-377.

Kazmi SH, Naglik JR, Sweet SP, Evans RW, O'Shea S, Banatvala JE, *et al*. (2006). Comparison of human immunodeficiency virus type 1-specific inhibitory activities in saliva and other human mucosal fluids. *Clin Vaccine Immunol* 13:1111-1118.

Kosub DA, Durudas A, Lehrman G, Milush JM, Cano CA, Jain MK, *et al*. (2008). Gamma/Delta T cell mRNA levels decrease at mucosal sites and increase at lymphoid sites following an oral SIV infection of macaques. *Curr HIV Res* 6:520-530.

Lane BR, Markovitz DM, Woodford NL, Rochford R, Strieter RM, Coffey MJ (1999). TNF-alpha inhibits HIV-1 replication in peripheral blood monocytes and alveolar macrophages by inducing the production of RANTES and decreasing C-C chemokine receptor 5 (CCR5) expression. *J Immunol* 163:3653-3661.

Lehner T, Mitchell E, Bergmeier L, Singh M, Spallek R, Cranage M, *et al*. (2000). The role of gammadelta T cells in generating antiviral factors and beta-chemokines in protection against mucosal simian immunodeficiency virus infection. *Eur J Immunol* 30:2245-2256.

Lester RT, Yao XD, Ball TB, McKinnon LR, Kaul R, Wachihi C, *et al*. (2008). Toll-like receptor expression and responsiveness are increased in viraemic HIV-1 infection. *AIDS* 22:685-694.

Lewis SH, Reynolds-Kohler C, Fox HE, Nelson JA (1990). HIV-1 in trophoblastic and villous Hofbauer cells, and haematological precursors in eight-week fetuses. *Lancet* 335:565-568.

Li D, Xu XN (2008). NKT cells in HIV-1 infection. *Cell Res* 18:817-822.

Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, *et al*. (2009). Glycerol monolaurate prevents mucosal SIV transmission. *Nature* 458:1034-1038.

Lilly EA, Leigh JE, Joseph SH, Fidel PL Jr (2006). *Candida*-induced oral epithelial cell responses. *Mycopathologia* 162:25-32.

Lilly EA, Yano J, Fidel PL Jr (2010). Annexin-A1 identified as the oral epithelial cell anti-*Candida* effector moiety. *Mol Oral Microbiol* 25:293-304.

Lin AL, Johnson DA, Stephan KT, Yeh CK (2003). Alteration in salivary function in early HIV infection. *J Dent Res* 82:719-724.

Liu L, Wang L, Jia HP, Zhao C, Heng HH, Schutte BC, *et al*. (1998). Structure and mapping of the human beta-defensin HBD-2 gene and its expression at sites of inflammation. *Gene* 222:237-244.

Liuzzi G, Chirianni A, Clementi M, Bagnarelli P, Valenza A, Cataldo PT, *et al*. (1996). Analysis of HIV-1 load in blood, semen and saliva: evidence for different viral compartments in a cross-sectional and longitudinal study. *AIDS* 10:F51-F56.

Meiller TF, Hube B, Schild L, Shirtliff ME, Scheper MA, Winkler R, *et al*. (2009). A novel immune evasion strategy of *Candida albicans*: proteolytic cleavage of a salivary antimicrobial peptide. *PLoS One* 4:e5039.

Milush JM, Kosub D, Marthas M, Schmidt K, Scott F, Wozniakowski A, *et al*. (2004). Rapid dissemination of SIV following oral inoculation. *AIDS* 18:2371-2380.

Moore JS, Rahemtulla F, Kent LW, Hall SD, Ikizler MR, Wright PF, *et al*. (2003). Oral epithelial cells are susceptible to cell-free and cell-associated HIV-1 infection *in vitro*. *Virology* 313:343-353.

Moutsopoulos NM, Nares S, Nikitakis N, Rangel Z, Wen J, Munson P, *et al*. (2007). Tonsil epithelial factors may influence oropharyngeal human immunodeficiency virus transmission. *Am J Pathol* 171:571-579.

Moyes DL, Runglall M, Murciano C, Shen C, Nayar D, Thavaraj S, *et al*. (2010). A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of *Candida albicans* in epithelial cells. *Cell Host Microbe* 8:225-235.

Mundy DC, Schinazi RF, Gerber AR, Nahmias AJ, Randall HW Jr (1987). Human immunodeficiency virus isolated from amniotic fluid. *Lancet* 2:459-460.

Myint M, Steinsvoll S, Yuan ZN, Johne B, Helgeland K, Schenck K (2002). Highly increased numbers of leukocytes in inflamed gingiva from patients with HIV infection. *AIDS* 16:235-243.

Neutra MR, Mantis NJ, Kraehenbuhl JP (2001). Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol* 2:1004-1009.

Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, *et al*. (2002). Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 347:1151-1160.

Page-Shafer K, Sweet S, Kassaye S, Ssali C (2006). (C2) Saliva, breast milk, and mucosal fluids in HIV transmission. *Adv Dent Res* 19:152-157.

Quinones-Mateu ME, Lederman MM, Feng Z, Chakraborty B, Weber J, Rangel HR, *et al*. (2003). Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *AIDS* 17:F39-F48.

Qureshi MN, Barr CE, Hewlitt I, Boorstein R, Kong F, Bagasra O, *et al*. (1997). Detection of HIV in oral mucosal cells. *Oral Dis* 3(suppl 1):73-78.

Rebbapragada A, Wachihi C, Pettengell C, Sunderji S, Huibner S, Jaoko W, *et al*. (2007). Negative mucosal synergy between Herpes simplex type 2 and HIV in the female genital tract. *AIDS* 21:589-598.

Rodriguez-Iñigo E, Jiménez E, Bartolomé J, Ortiz-Movilla N, Bartolomé Villar B, José Arrieta J, *et al*. (2005). Detection of human immunodeficiency virus type 1 RNA by *in situ* hybridization in oral mucosa epithelial cells from anti-HIV-1 positive patients. *J Med Virol* 77:17-22.

Schacker T, Zeh J, Hu H, Shaughnessy M, Corey L (2002). Changes in plasma human immunodeficiency virus type 1 RNA associated with herpes simplex virus reactivation and suppression. *J Infect Dis* 186:1718-1725.

Semba RD (2000). Mastitis and transmission of human immunodeficiency virus through breast milk. *Ann N Y Acad Sci* 918:156-162.

Shacklett BL, Critchfield JW, Ferre AL, Hayes TL (2009). Mucosal T-cell responses to HIV: responding at the front lines. *J Intern Med* 265:58-66.

Sheikh AU, Polliotti BM, Miller RK (2000). *In situ* PCR detection of HIV expression in the human placenta. *Methods Mol Biol* 137:75-86.

Shugars DC (1999). Endogenous mucosal antiviral factors of the oral cavity. *J Infect Dis* 179(suppl 3):431-435.

Shugars DC, Wahl SM (1998). The role of the oral environment in HIV-1 transmission. *J Am Dent Assoc* 129:851-858.

Soeiro R, Rubinstein A, Rashbaum WK, Lyman WD (1992). Maternofetal transmission of AIDS: frequency of human immunodeficiency virus type 1 nucleic acid sequences in human fetal DNA. *J Infect Dis* 166:699-703.

Sprecher S, Soumenkoff G, Puissant F, Degueldre M (1986). Vertical transmission of HIV in 15-week fetus. *Lancet* 2:288-289.

Steele C, Fidel PL Jr (2002). Cytokine and chemokine production by human oral and vaginal epithelial cells in response to *Candida albicans*. *Infect Immun* 70:577-583.

Steele C, Leigh J, Swoboda R, Fidel PL Jr (2000). Growth inhibition of *Candida* by human oral epithelial cells. *J Infect Dis* 182:1479-1485.

Stringer JS, Goldenberg RL (2000). Subclinical chorioamnionitis as a targetable risk factor for vertical transmission of HIV-1. *Ann N Y Acad Sci* 918:77-83.

Sun L, Finnegan CM, Kish-Catalone T, Blumenthal R, Garzino-Demo P, La Terra Maggiore GM, *et al*. (2005). Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection. *J Virol* 79:14318-14329.

Sweet SP, Cookson S, Challacombe SJ (1995). *Candida albicans* isolates from HIV-infected and AIDS patients exhibit enhanced adherence to epithelial cells. *J Med Microbiol* 43:452-457.

Taha TE, Gray RH (2000). Genital tract infections and perinatal transmission of HIV. *Ann N Y Acad Sci* 918:84-98.

Takeda K, Akira S (2005). Toll-like receptors in innate immunity. *Int Immunol* 17:1-14.

Tiemessen CT, Shalekoff S, Meddows-Taylor S, Schramm DB, Papathanasopoulos MA, Gray GE, *et al*. (2009). Cutting edge: unusual NK cell responses to HIV-1 peptides are associated with protection against maternal-infant transmission of HIV-1. *J Immunol* 182:5914-5918.

Torres SR, Garzino-Demo A, Meiller TF, Meeks V, Jabra-Rizk MA (2009). Salivary histatin-5 and oral fungal colonisation in HIV+ individuals. *Mycoses* 52:11-15.

Vacharaksa A, Asrani AC, Gebhard KH, Fasching CE, Giacaman RA, Janoff EN, *et al*. (2008). Oral keratinocytes support non-replicative infection and transfer of harbored HIV-1 to permissive cells. *Retrovirology* 5:66.

Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, *et al*. (1998). Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 280:427-431.

Veazey RS, Gauduin MC, Mansfield KG, Tham IC, Altman JD, Lifson JD, *et al*. (2001). Emergence and kinetics of simian immunodeficiency virus-specific CD8(+) T cells in the intestines of macaques during primary infection. *J Virol* 75:10515-10519.

Viscarello RR, Cullen MT, DeGennaro NJ, Hobbins JC (1992). Fetal blood sampling in human immunodeficiency virus: seropositive women before elective midtrimester termination of pregnancy. *Am J Obstet Gynecol* 167(4)(pt 1):1075-1079.

Volmink J, Mahlati U (2005). HIV: mother to child transmission. *Clin Evid* 14:818-827.

Weindl G, Naglik JR, Kaesler S, Biedermann T, Hube B, Korting HC, *et al*. (2007). Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. *J Clin Invest* 117:3664-3672.

Willumsen JF, Filteau SM, Coutsoudis A, Uebel KE, Newell ML, Tomkins AM (2000). Subclinical mastitis as a risk factor for mother-infant HIV transmission. *Adv Exp Med Biol* 478:211-223.