Plausibility of HIV-1 Infection of Oral Mucosal Epithelial Cells

M.C. Herzberg^{1*}, A. Vacharaksa^{1,2},
K.H. Gebhard¹, R.A. Giacaman^{1,3},
and K.F. Ross¹

¹Department of Diagnostic and Biological Sciences, School of Dentistry, University of Minnesota, Minneapolis, Minnesota, and the Mucosal and Vaccine Research Center, Minneapolis VA Medical Center, Minneapolis, Minnesota; ²current address: Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia; and ³current address: Departamento de Rehabilitación Buco-máxilofacial, Universidad de Talca, Talca, Chile; *corresponding author, mcherzb@umn.edu

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ABSTRACT

The AIDS pandemic continues. Little is understood about how HIV gains access to permissive cells across mucosal surfaces, yet such knowledge is crucial to the development of successful topical anti-HIV-1 agents and mucosal vaccines. HIV-1 rapidly internalizes and integrates into the mucosal keratinocyte genome, and integrated copies of HIV-1 persist upon cell passage. The virus does not appear to replicate, and the infection may become latent. Interactions between HIV-1 and oral keratinocytes have been modeled in the context of key environmental factors, including putative copathogens and saliva. In keratinocytes, HIV-1 internalizes within minutes; in saliva, an infectious fraction escapes inactivation and is harbored and transferable to permissive target cells for up to 48 hours. When incubated with the common oral pathogen *Porphyromonas gingivalis*, CCR5–oral keratinocytes signal through protease-activated receptors and Toll-like receptors to induce expression of CCR5, which increases selective uptake of infectious R5-tropic HIV-1 into oral keratinocytes and transfer to permissive cells. Hence, oral keratinocytes—like squamous keratinocytes of other tissues—may be targets for low-level HIV-1 internalization and subsequent dissemination by transfer to permissive cells.

In 2007, HIV/AIDS affected more than 33.2 million people worldwide, with approximately 2.5 million newly infected people and 2.1 million fatalities of AIDS-related illnesses (UNAIDS, 2008). Most primary infections with HIV-1 occur at mucosal surfaces. In adults, the mucosal surfaces are exposed during sexual activity (oral, vaginal, and anal). In infants, exposure

and infection are a result of breast-feeding; the oral and oropharyngeal mucous membranes are the initial sites exposed to virus. Heterosexual intercourse accounts for more than 80% of current infections, including a dramatic increase in worldwide prevalence among women during the last few years (UNAIDS and World Health Organization, 2006).

DEVELOPMENT OF SUCCESSFUL TOPICAL ANTI-HIV-1 AGENTS AND MUCOSAL VACCINES

Among the highest research priorities from the 2008 National Institute of Allergy and Infectious Diseases HIV Vaccine Summit is to “define the first events leading to HIV . . . entering the gut-associated lymphoid tissue” (Fauci *et al.*, 2008). When introduced into the mouth, infectious HIV-1 first encounters the oral and oropharyngeal tissues (Herzberg *et al.*, 2006), but the sites of primary infection are not known. Attempts at vaccine development have proven inadequate in clinical trials in part because the fate of the virus upon exposure to mucosal surfaces is largely unknown. In uninfected men, unprotected oral sex with infected men elicits a salivary IgA1-mediated response that neutralizes HIV-1 (Hasselrot *et al.*, 2009). Although an immune response can result from a direct encounter with virus during primary mucosal infection, whether the squamous epithelial cells serve as a latent HIV-1 reservoir is unclear (Richman *et al.*, 2009). The mobilization of an antiviral immune response can neutralize the expanding pool of infecting virus and thus represent a measure of successful resistance (Li *et al.*, 2009b). If the immune response prevents viral entry into cryptic sites and prevents latent infection, a successful vaccine might augment the induced salivary antiviral response. Recent successes with the development of topical anti-HIV-1 agents in nonhuman primates (Li *et al.*, 2009a) suggest that an anti-HIV-1 response at the mucosal epithelial surface can prevent transmucosal infection by HIV-1 during clinical exposures. There is a growing awareness that HIV-1 encounters with mucosal epithelium are critical to the clinical course of infection.

FATE OF HIV-1 IN SQUAMOUS EPITHELIA

A multicellular layer of squamous epithelial cells covers the oral and oropharyngeal mucosae and the urogenital mucosae. After primary clinical exposures, R5-tropic HIV-1 systemic infection

Key Words

HIV/AIDS, epithelia, oral epithelium, infectious disease, mucosal immunity, vaccines.

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Fig. 1. Nonproductive infection of oral keratinocytes by HIV-1. X4- or R5-tropic HIV-1 binds and internalizes into oral keratinocytes in a manner independent of the chemokine coreceptor specificity (CXCR4 or CCR5). A noncanonical HIV-1 receptor likely exists—perhaps, a surrogate for CD4. X4- and R5-tropic HIV-1 internalizes in the keratinocyte and uncoats, and viral RNA is reverse transcribed and integrates into the keratinocyte genome. Integration is stable, but new HIV-1 transcripts are not detected.

predominates (Hu *et al.*, 2000), and in HIV-1-seropositive individuals, oral mucosal keratinocytes are infected with HIV-1 on the basis of viral RNA detection using molecular techniques (Rodriguez-Inigo *et al.*, 2005). To study the fate of HIV-1 during encounters with squamous mucosal keratinocytes, we have studied oral keratinocytes that may model the epithelial cells from other squamous mucosae.

We have reported that X4 and R5 HIV-1 internalize and nonproductively infect oral (and oropharyngeal) keratinocytes, as marked by key viral life cycle events, including integration without apparent production of new viral transcripts or virions *in vitro* (Vacharaksa *et al.*, 2008) (Fig. 1). The receptor for HIV-1 on the surface of oral keratinocytes is unknown. The expression of X4-tropic HIV-1 selective CXCR4 coreceptor notwithstanding, X4- and R5-tropic HIV-1 are both internalized. Nonproductive infection represents one of several possible fates of HIV-1 in oral keratinocytes. Similarly, squamous epithelial cells from the genitourinary tract can be nonpermissively infected by X4- and R5-tropic HIV-1 (Hladik *et al.*, 2007). Like vaginal keratinocytes, HIV-1 internalizes into oral keratinocytes within 1 to 30 minutes (Dietrich *et al.*, manuscript in preparation) and integrates by 3 hours (Vacharaksa *et al.*, 2008). Yet, there is no substantive evidence that HIV-1 can replicate in oral epithelial cells, although earlier reports of the fate of HIV-1 in human oral keratinocytes present conflicting results (Acheampong *et al.*, 2005; Liu *et al.*, 2004). Oral keratinocytes harbor infectious virus, however, and transfer to or *trans*-infect permissive cells, including peripheral blood mononuclear cells, MOLT-4, and TZM-bl reporter cells (Giacaman *et al.*, 2008; Vacharaksa *et al.*, 2008).

Although oral epithelial cells can harbor and *trans*-infect infectious virions to permissive cells, what is the evidence that HIV exposure of oral epithelial cells results in systemic dissemination? Human *in vivo* data are not known. In adult and infant nonhuman primates, however, when cell-free SIV is exposed

atraumatically to the oral epithelium, infection of the oral epithelium and oropharyngeal tonsils is apparent within a day, with spread to the gastrointestinal mucosa several days later (Ruprecht *et al.*, 1998; Milush *et al.*, 2004). It is unclear whether the disseminating virus is residual from the original primary infecting inoculum or newly replicated SIV. In human tonsils studied *ex vivo*, HIV readily binds to the epithelium, but trafficking and infection of the organized lymphoid tissues is limited (Maher *et al.*, 2004). HIV-1 replicates in human tonsils but not as effectively as in the rectal mucosa, and it is associated with differential expression of HIV coreceptors and the proximity of CD4 cells (Grivel *et al.*, 2007). Whereas infection of oral keratinocytes is nonproductive, the association with infectious HIV-1 is suggested to be significant nonetheless when *trans*-infection results in a small population of infected CD4 T cells in the tissue.

PUTATIVE RESTRICTIONS AGAINST HIV-1 REPLICATION IN ORAL KERATINOCYTES

At this time, it is unclear why oral keratinocytes support only nonproductive infection by HIV-1. Potential restrictions against replication could include eukaryotic initiation factor 3 subunit f, which can specifically interfere with the 3' end processing of HIV-1 mRNAs (Valente *et al.*, 2009); non-Vpu-neutralized tetherin, which impedes release of HIV-1 (Mangeat *et al.*, 2009); TRIM and other capsid-targeting cellular proteins, which contribute to cell cycle-dependent inability to infect (Yamashita and Emerman, 2009); and APOBEC family proteins (Schumacher *et al.*, 2008; Ogawa *et al.*, 2009). How these and other restrictions prevent HIV-1 replication in oral keratinocytes is an important area for future investigation.

ORAL EPITHELIUM AS A MODEL FOR GENITOURINARY INFECTIONS

The genitourinary and oral mucosa are lined by squamous epithelia that are morphologically and phenotypically similar (Hladik and McElrath, 2008). Interspersed between the epithelial cells, Langerhans (immature dendritic) cells are generally localized in the basal and suprabasal layers. Although no quantitative assessment has been reported, Langerhans cells appear to populate the vaginal epithelium at a greater density than in oral epithelium (Cutler and Jotwani, 2006b), although quantitative assessments are not reported. Interestingly, the Langerhans cells of the vaginal mucosa are able to sample antigen at the mucosal surface, but antigen sampling at the surface of the oral mucosa has not been reported.

Although controversial, recent evidence in nonhuman primates (Li *et al.*, 2009a) suggests that HIV-1 translocates intact vaginal and endocervical epithelia HIV to cause a disseminated infection. Like oral keratinocytes (Vacharaksa *et al.*, 2008), ectocervical epithelial cells internalize and transfer intact viral particles to permissive cells (Wu *et al.*, 2003), and vaginal epithelial cells transcytose HIV-1 in a transwell system, albeit at a low rate (Bobardt *et al.*, 2007). Taken together, current evidence suggests that the oral and genitourinary squamous epithelia generally harbor infectious virus without becoming productively

infected. Harbored virions in keratinocytes may functionally contribute to dissemination of HIV-1 to permissive cells. In some conditions, perhaps under pressure from coinfecting microorganisms, it is plausible that these mucosal keratinocytes might become productively infected by HIV-1 and amplify virus for systemic spread.

BATHING FLUIDS

The mucosal secretions contain substances that can modify the infectivity and course of HIV-1 infection. The molecular constituents of these bathing fluids are generally similar, although the proportions might differ from tissue to tissue depending on the glandular and cellular sources. Among the mucosal fluids, whole and fractionated saliva, breast milk, colostrum, seminal plasma, and cervicovaginal secretions all show anti-HIV-1 activity associated with lactoferrin (Kazmi *et al.*, 2006). Seminal plasma and cervicovaginal fluids are less effective against HIV-1 than colostrum, whole milk, and whole saliva.

During primary clinical exposures, viable X4- and R5-tropic HIV-1 is introduced into the oral cavity and oropharynx in breast milk (Van de Perre *et al.*, 1991) or semen (Pasquier *et al.*, 2009). The bolus of HIV-1 is mixed and diluted, however imperfectly, in saliva. Salivary anti-HIV activity is likewise diluted and potentially neutralized given that breast milk and seminal plasma increase the titer of freshly harvested HIV-1 *in vitro* (Acheampong *et al.*, 2005; Southern and Southern, 2002). When primary HIV-1 infection is diagnosed, virions are no longer detectable in the oral cavity and oropharynx (Herzberg *et al.*, 2006), and shed virions in saliva are not infectious (Goto *et al.*, 1991). Virions are probably inactivated by salivary anti-HIV molecules (Kazmi *et al.*, 2006). In contrast to this conclusion, primary infection in the gastrointestinal mucosa is thought to result when the HIV-1 bolus passes unremarkably through the nonpermissive oral environment and is swallowed (Brenchley *et al.*, 2006).

The actual infectious titer (*e.g.*, TCID₅₀) of HIV-1 in breast milk and semen, containing cell-free and cell-associated virus, is difficult to estimate (Chan, 2005; Yamaguchi *et al.*, 2007). When incubated in fresh crude whole saliva, however, HIV-1 virions remain sufficiently infectious when harbored by oral keratinocytes to *trans*-infect permissive cells (Dietrich *et al.*, unpublished data). Although saliva is swallowed at an average rate of 0.5 mL per minute (Rudney *et al.*, 1995), the HIV–breast milk or HIV–semen bolus mixes imperfectly with saliva and is able to interface for several minutes or longer with the oral epithelium. In some anatomic sites, the HIV-1 bolus may encounter stasis, remaining in prolonged apposition to mucosal keratinocytes. Indeed, spermatozoa, a surrogate marker for HIV-1 virions in seminal fluid, were retained within tonsillar crypts in *ex vivo* organ culture experiments (Maher *et al.*, 2004). Hence, infectious inocula may show extended interactions with mucosal surfaces.

Coated with a salivary film, oral mucosal keratinocytes bind high-molecular-weight mucins (MG1; MUC5B) and other salivary molecules (Bradway *et al.*, 1992). Indeed, the salivary mucin glycoprotein MUC5B binds HIV-1 (Habte *et al.*, 2006), which likely docks with cell surface glycoproteins MUC1 and/or gp340 (DMBT1) on the keratinocyte (Stoddard *et al.*, 2007). The fate of HIV-1 in the oral cavity and oropharynx may depend

on the rates of inactivation by saliva, clearance by swallowing, and capture by mucosal keratinocytes.

Most of the estimated 300 to 1300 salivary proteins (Guo *et al.*, 2006) are in solution (sol phase) (Glantz *et al.*, 1996), whereas the high-molecular-weight mucin fraction (MG1; MUC5B; > 1000 kDa) self-assembles into a gel phase (Wickstrom *et al.*, 2000). Crude separation of these phases can be accomplished by centrifugation at $10,000 \times g$ for 10 minutes (Herzberg *et al.*, 1979). The partitioned clarified supernatant contains smaller mucin glycoproteins and gp340, sIgA, amylase and other proteins, glycoproteins, and lipids. The sedimented mucous gel phase contains MUC5B and complexed proteins, desquamated oral epithelial cells, bacteria, and viruses. Indeed, mucoid proteins are known to bind influenza virus (Stone, 1949), and saliva is hospitable and transmits many viruses, including members of the herpesvirus family (Miller *et al.*, 2006). As a bathing fluid for HIV-1, saliva maintains a pH of about 6.7 and is hypotonic relative to serum. Admixed breast milk or seminal fluid would increase osmolality to approach serum levels. As the flow rate increases to 1.5 mL per minute, the buffer capacity of saliva approaches that of plasma. HIV-1 should be stable in this fluid environment, except for the presence of antiviral molecules.

Putative salivary anti-HIV-1 molecules (Kazmi *et al.*, 2006) include secretory leukocyte protease inhibitor (McNeely *et al.*, 1995), β -defensin 2 (hBD-2) (Quinones-Mateu *et al.*, 2003), proline-rich proteins (Robinovitch *et al.*, 2001), thrombospondin (Crombie *et al.*, 1998), lysozyme (Lee-Huang *et al.*, 1999), salivary peroxidase and α -defensins (Kazmi *et al.*, 2006), lactoferrin (Saidi *et al.*, 2006), GP340 agglutinin (Wu *et al.*, 2006), and mucins, including MUC5B (Habte *et al.*, 2006). GP340 and MUC5B appear to agglutinate and sequester virus from CD4+ target cells *in vitro* (Habte *et al.*, 2006; Malamud *et al.*, 1997). In infected individuals, salivary anti-HIV-1 molecules apparently attenuate HIV-1 virions sufficiently enough to virtually eliminate risk of salivary transmission to uninfected individuals. The converse may not be true. The effectiveness of saliva as an anti-HIV-1 agent depends on the mode of action, the kinetics of inactivation, and the ability of the virus to become harbored or shielded from inhibitors. When a bolus of HIV enters the oral cavity of an uninfected individual, saliva may be insufficiently effective (Yeh *et al.*, 1992) to inhibit uptake and infection of mucosal epithelial cells (Dietrich *et al.*, unpublished data).

COINFECTION MUCOSAL PATHOGENS

During clinical presentation, a bolus of HIV-1 is invariably exposed to coinfecting commensal bacteria and endogenous pathogens resident in mucosal biofilms. Certainly when existing infection causes mucosal lesions, HIV-1 infection and dissemination are favored. Indeed, ulcerative and nonulcerative sexually transmitted diseases increase the risk for HIV-1 infection. In particular, nonulcerative sexually acquired infection caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis* (Laga *et al.*, 1993), and *Trichomonas vaginalis* (Buve *et al.*, 2001; Laga *et al.*, 1993) are associated with higher rates of HIV-1 infection. Given that mucosal infections acquired by sexual contact are more prevalent in groups at high risk for HIV-1 infection (Aral *et al.*, 2006), HIV-1 acquisition in association with coinfection is not

Fig. 2. R5-HIV and *Porphyromonas gingivalis* coinfection of oral epithelial cells: **(A)** *P. gingivalis* gingipains signal oral keratinocytes through PAR-1 and PAR-2, and LPS signals through TLR2 and TLR4. **(B)** Signaling of the oral epithelial cell leads to upregulation and expression of CCR5 on cell surface. **(C)** *P. gingivalis*-upregulated CCR5 promotes selective internalization of R5 HIV-1 and intracellular harboring. **(D)** Harbored infectious virions in oral keratinocytes are *trans*-infected to permissive cells. R5 HIV-1 is transferred and captured by CD4+ permissive cells, where virus replicates and dissemination can occur.

well explained by mucosal lesions. In general, the risk of acquisition of HIV-1 increases with concomitant sexually transmitted diseases, including gonorrhea (Schwarcz *et al.*, 2002) and HSV-2 (Lingappa and Celum, 2007).

Omnipresent viral infections and HIV-1 appear to exacerbate both infections, suggesting synergistic mechanisms. In HIV-positive individuals, several DNA viruses replicate in the mucosal tissues and shed into the mouth, including HSV-1 and HSV-2; cytomegalovirus; Epstein-Barr virus; human herpes virus 6, 7, and 8; and human papilloma virus (Mbopi-Keou *et al.*, 2002). The DNA viruses induce cytokine release by mucosal cells, which could activate HIV-1 replication and increase risk of HIV-1 infection.

Coinfection with other microorganisms also appears to promote infection by HIV-1. In the oral context, we have shown that *Porphyromonas gingivalis*, a ubiquitous periodontal pathogen, upregulates expression of CCR5 on oral keratinocytes (Giacaman *et al.*, 2007) (Fig. 2). Healthy oral and oropharyngeal keratinocytes are CD4[−] CXCR4⁺ CCR5[−] *in vitro* (Vacharaksa *et al.*, 2008) and *in vivo* (Cutler and Jotwani, 2006a; Kumar *et al.*, 2006). Given that CXCR4 is the coreceptor for infection by X4-tropic HIV-1 and CCR5 for R5-tropic virus, it is unlikely that selective primary clinical infections by R5-tropic HIV-1 could be explained at this level. At the mucosal surface, the receptor tropism of HIV-1 may be modified by endogenous coinfecting microflora, including *P. gingivalis*. As we report, *P. gingivalis* signaling through the protease-activated receptors and Toll-like receptors 2 and 4 (Fig. 2A) induces keratinocyte

CCR5 appearance as a surface receptor (Giacaman *et al.*, 2007) (Fig. 2B). Expression of CCR5 in response to *P. gingivalis* increases nonproductive *trans*-infection of harbored R5-tropic HIV-1 to permissive cells (Giacaman *et al.*, 2008) (Fig. 2C, 2D). This pathway of harboring infectious HIV-1 in oral keratinocytes and *trans*-infecting permissive cells represents another fate of HIV-1 in oral keratinocytes. Although harboring and *trans*-infection show no coreceptor tropism, the selective fate of R5-tropic HIV-1 is increased by *P. gingivalis*, which has been reported to reactivate latent infection of HIV-1 in mononuclear cell lines by chromatin modification through butyrate-specific inhibition of histone deacetylases (Imai *et al.*, 2009). Although numerous reports suggest that the clinical expression of periodontitis and other oral infection is altered in patients with HIV/AIDS, it remains unclear whether preexisting periodontitis increases the risk of acquisition or systemic dissemination of HIV-1. Nonetheless, potential copathogens can exploit alternative mechanisms to promote HIV-1 infection.

MUCOSAL EPITHELIAL CELL RECEPTORS FOR HIV-1

HIV-1-associated receptors are atypical on squamous keratinocytes when compared to T cells, macrophages, and dendritic cells. Unlike white blood cells, oral squamous epithelial cells are CD4[−] and CXCR4⁺ CCR5[−] in the absence of inflammation (Jotwani *et al.*, 2004; Kumar *et al.*, 2006; Vacharaksa *et al.*, 2008). Whereas oral and vaginal epithelial cells show similar phenotypes, gastrointestinal epithelial cells can express CXCR4

and CCR5. The CD4⁺ gastrointestinal epithelial cells transcytose HIV-1 in a CXCR4- or CCR5-dependent manner (Bomsel, 1997; Bobardt *et al.*, 2007), suggesting that these receptors might mediate HIV-1 entry by a CD4- and membrane fusion-independent mechanism. Although they do not generally express CCR5, oral keratinocytes take up and *trans*-infect HIV-1 without preference for a particular chemokine receptor-HIV-1 tropism (Vacharaksa *et al.*, 2008). On CD4⁺ cells, the V3 loop domain of gp120 has been suggested to interact with alternative receptors, including galactosylceramide (Cook *et al.*, 1994), whereas a gp120 envelope-independent mechanism of epithelial cell infection also occurs (Pang *et al.*, 2000). On CD4⁺ epithelial cells, alternative receptors may include heparan sulfate proteoglycans (Bobardt *et al.*, 2007), ICAM-1, LFA-1 (Fortin *et al.*, 1998), DC-SIGN (Geijtenbeek *et al.*, 2000), DC-SIGNR (Pohlmann *et al.*, 2001), langerin (de Witte *et al.*, 2007), mannose receptor (Liu *et al.*, 2004), and gp340 (Stoddard *et al.*, 2007). Hence, mucosal keratinocytes may be infected by HIV-1 through several routes, resulting in different intracellular pools of HIV-1.

Primary infection of oral epithelium by HIV-1 through a receptor-mediated mechanism depends on the distribution and density of receptors in proximity to the virus. Although various cells in the gingival tissues collectively express most of the putative HIV-1 receptors and coreceptors—including CXCR4, CCR5, langerin, DC-SIGN, mannose receptor, and galactosylceramide (Jotwani *et al.*, 2004)—the distribution is neither homogeneous nor prominent on the surface keratinocytes. Indeed, the receptor distribution and density changes with inflammation (Jotwani *et al.*, 2004). Inflamed gingival and tonsil epithelia show similar patterns, with variable numbers of CXCR4⁺ and CCR5⁺ keratinocytes stretching continuously across the stratified epithelium with greater density in the basal and suprabasal layers (Kumar *et al.*, 2006). Heparan sulfate proteoglycan-positive keratinocytes are prominent in all epithelial layers. Although not reported in the oral epithelium, CXCR4⁺ CCR5⁺ cells are identified as CD4⁺ lymphocytes infiltrating the tonsil epithelium (Kumar *et al.*, 2006). The low density of relevant known HIV-1 receptors in the superficial oral epithelium may represent a restriction to primary HIV-1 infection, whereas noncanonical (CD4⁺ CXCR4⁺ CCR5⁺ and alternative receptor-independent) uptake pathways may be functional (Vacharaksa *et al.*, 2008).

TRANSFER TO DENDRITIC CELLS

What is the postulated fate of HIV-1 harbored by oral keratinocytes? As we have modeled *in vitro* and as consistent with primate models, HIV-1 harbored by oral keratinocytes most likely *trans*-infects Langerhans cells *in vivo*. Langerhans or immature dendritic cells are resident in the epithelium proximal to basal and suprabasal keratinocytes (Cutler and Jotwani, 2006a), and they form a dendritic network (25 cells/mm³ in patients 14 to 39 years old and 7 cells/mm³ in patients 61 to 74 years old), maximizing the area of antigen capture (Zavala and Cavicchia, 2006). The volume density of keratinocytes is estimated to be at least 1000-fold greater than that of Langerhans cells. Hence, encounters between HIV and keratinocytes may be 1000-fold more likely than with Langerhans cells. Intraepithelial T cells

are exceedingly rare in the oral epithelium even during inflammation, whereas dendritic cell-T cell interactions occur in the lamina propria of the oral epithelium (Cutler and Jotwani, 2006b). As we have shown, Langerhans cells and T cells are comparatively more abundant among epithelial cells in tonsils (Kumar *et al.*, 2006). After internalization of HIV-1, immature dendritic cells activate and emigrate from the epithelium to dock with and transfer HIV-1 to CD4⁺ T cells in the connective tissues (Sugaya *et al.*, 2004). Emigrating DCs and HIV-1-infected T cells disseminate infection to organized mucosal-associated lymphoid tissues.

ORAL EPITHELIUM AS A “TROJAN HORSE”

The oral tissues are thought to be protected or nonpermissive to infection and unable to serve as foci of systemic infection. Despite these assumptions, we present cogent evidence from the literature and our own published and unpublished work to show the plausibility of primary R5-tropic HIV-1 infection of the oral epithelium and oropharynx. By extrapolation from atraumatic oral exposure to SIV in the macaque, any amount of infectious HIV-1 that infects and crosses the mucosal epithelial lining to infect a T cell must be considered clinically significant. As suggested for genital epithelia (Hladik and McElrath, 2008), primary exposure may result in HIV-1 reaching immature dendritic cells by a paracellular route, through frank breaches, or upon capture by Langerhans cells (immature dendritic cells) at the mucosal surface. Most likely, HIV-1 internalizes in superficial oral epithelial cells by a noncanonical mechanism, or it moves paracellularly to internalize in suprabasal and basal keratinocytes, which are modeled by cells cultured *in vitro*. In the presence of saliva and *P. gingivalis*, R5-tropic infectious virus is therefore likely to be selectively internalized and transferred from oral keratinocytes to immature dendritic cells, setting the stage for systemic dissemination. Hence, the oral environment has the potential to overcome salivary antiviral factors and favor primary clinical infections by R5-tropic HIV-1 resulting in systemic dissemination. Keratinocytes that contain integrated (or perhaps latent) copies of the HIV-1 genome or harbor infectious HIV-1 may also serve to reinfect the host during chronic disease. Furthermore, latent HIV-1 infection can be reactivated by *P. gingivalis*, suggesting that activation of latent integrated copies of HIV-1 in keratinocytes could be a cryptic source of virus able to reinfect the host. As cryptic sites of HIV-1 infection, oral epithelial keratinocytes may be a threat to current antiviral pharmacologic therapies and so challenge the development of successful vaccines.

The evidence presented in this review supports the plausibility of HIV-1 infection of oral mucosal epithelial cells. The fate of HIV-1 must now be determined more robustly in mucosal epithelial tissues *ex vivo* and *in vivo*. Armed with these data, we will better understand the mechanism of primary HIV-1 infection acquired across mucosal surfaces.

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