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# Oral Mucosal Expression of HIV-1 Receptors, Co-receptors, and α-defensins: Tableau of Resistance or Susceptibility to HIV Infection?

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**Keywords**

HIV-1 receptors; DC-SIGN; HIV-1-co-receptors; CCR5; α-defensin-1; gingiva; human

The basic premise of whether transmission of HIV-1 through the oral mucosa actually occurs, and through what route, is a topic of intense interest. Our work has focused on HIV-1 receptors/co-receptors and α-defensin-1 *in situ* in human gingiva. Regardless of HIV-1 infection, the role that C-type lectin receptors might play in periodontal pathogenesis is of great interest. We have shown that the gingival lamina propria, when inflamed, becomes increasingly infiltrated with DC-SIGN+MR+ dermal dendritic cells (DDCs), while the inflamed epithelium shows a decrease in Langerin+ Langerhans cells (LCs). Moreover, DDCs and LCs contribute to the mature CD83+ DC pool *in situ*, and form immune conjugates with CD4+ T-cells in the lamina propria (Jotwani and Cutler, 2003). This raises the intriguing possibility that oral mucosal DCs may be involved in HIV-1 transfer to T-cells *in situ*. However, this possibility is tendered by the challenges faced by the virus in gaining access to oral mucosal immune cells, including their ability to survive the salivary defenses, cross the mucosal barrier, resist inactivation by α-defensins, and overcome the paucity of co-receptor CCR5 in (healthy) oral mucosa (*i.e.*, required for productive infection [Jotwani *et al.*, 2004]). To date, there is little evidence of direct infection by HIV-1 of oral mucosal DCs/T cells and other cells *in situ*. Abbreviations used in this paper: CP, chronic periodontitis; CCR5, chemokine receptor 5; CXCR4, C-X-C receptor 4; DCs, dendritic cells; DC-SIGN, DC-specific ICAM-3 grabbing non-integrin; DDC, dermal dendritic cells; LCs, Langerhans cells; LP, lamina propria; MR, mannose receptor.

## Introduction, Results, and Discussion

Among the many significant questions that have arisen regarding the susceptibility of oral mucosa to HIV-1 infection are the following: (1) Is the oral mucosa a significant route of infection of HIV-1? (2) If so, how does the HIV-1 virus evade the innate defense mechanisms *(i.e.*, saliva) and penetrate the mucosa? (3) Which cells are primarily infected by HIV-1 in the oral mucosa? (4) If the oral cavity is not a significant route of infection, can it serve as a reservoir of HIV-1 infection, *i.e.*, inside-out infection from the blood? The literature and data presented in the recent HIV workshop on innate/specific mucosal

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immunity (see Workshop Reports, this issue), it is clear that answers to the above questions are wanting.

## Is the oral mucosa a significant route of infection?

The conventional wisdom is that HIV-1 transmission through oral mucosa and its secretions is uncommon (Rothenberg *et al.*, 1998; Cohen *et al.*, 2000), due to a combination of anatomical and biochemical factors (Baron *et al.*, 1999; Shugars *et al.*, 2002). However, there is evidence that blood and seminal proteins in saliva can protect HIV-1 from the killing effects of saliva (Baron *et al.*, 1999, 2000). Moreover, two-week-old CXCR4-positive keratinocytes are susceptible to low-grade infection by HIV-1 under specific conditions *in vitro*; furthermore, the HIV-1 is able to infect adjacent leukocytes (Liu *et al.*, 2003). Analysis of our data suggests that CXCR4 expression in oral mucosa is very limited (Jotwani *et al.*, 2004); moreover, other groups (Quiñones-Mateu *et al.*, 2003) have questioned the *in vitro* methods previously used (Liu *et al.*, 2003) to promote HIV-1 infection, and have not independently been able to demonstrate HIV-1 infection of keratinocytes. The main cell types in gingiva which can express HIV-1 receptors/co-receptors are dendritic cells (DCs), CD4+ T-lymphocytes, and macrophages. Studies performed in our laboratory have demonstrated that oral mucosa, like other mucosal surfaces, contains at least two subsets of DCs: Langerhans cells (LCs) and dermal DCs (DDCs) (Jotwani and Cutler, 2003). It is generally agreed that DCs capture HIV-1 at entry sites, migrate to lymph nodes, and transmit HIV-1 to CD4+ T-cells. Recent evidence suggests that HIV-1 uses a spectrum of receptors belonging to the mannose C-type lectin receptor (MCLR) family to attach to different DC subsets (Turville *et al.*, 2002). These include DC-specific ICAM-3 grabbing non-integrin (DC-SIGN) expressed by DDCs, macrophage mannose receptors (MR) expressed by DDCs, and macrophages and Langerin expressed by LCs. However, though these receptors can mediate virus attachment and transmission *in vitro*, their *in vivo* relevance in HIV-1 pathogenesis is uncertain.

In previously published studies, we used immunohistochemistry to analyze gingival samples from a population of chronic periodontitis (CP) subjects and healthy adult controls (Jotwani *et al.*, 2004). Our results established that CP is accompanied by a significant increase in the numbers of DC-SIGN+ DDCs and a trend for increased MR in the lamina propria. Both DC-SIGN and MR are significantly associated in a linear fashion with gingival inflammation. The expression of Langerin in inflammation appears to decrease. This is consistent with the efflux of LCs out of the epithelium to the underlying lamina propria, in response to inflammatory signals. This is particularly noteworthy in view of evidence, *via* double-immunofluorescence analysis, that DDCs and LCs contribute to the CD83+ mature DCs pool (Jotwani and Cutler, 2003), present in the T-cell-rich lamina propria (Jotwani *et al.*, 2001); moreover, CD83+ DCs form immune conjugates with CD4+ T-cells *in situ* (Jotwani and Cutler, 2003). Thus, the ‘stage is set’ for a productive HIV-1 infection of T-cells in the oral disease CP. Changes in expression of factors relevant to HIV-1 infection are outlined in the Table.

## Are all the players present?

Co-receptor expression is critical to HIV-1 infection. HIV-1 enters CD4+ T-lymphocytes and macrophages *via* CD4, in conjunction with chemokine receptors CCR5 and or CXCR4. Infection is mediated by binding of the surface subunit of HIV-1 envelope glycoprotein (Env), gp120, to CD4 on the target cell (Simmons *et al.*, 2000). This interaction results in a conformational change in gp120 that allows it to bind to the chemokine receptor (*e.g.*, CCR5). It has been observed that, during initial stages of HIV-1 transmission through sexual contact, non-syncytium-inducing (NSI) or macrophage (M)-tropic primary viruses predominate and use CCR5 (Connor *et al.*, 1997). In contrast, in the late stages of infection,

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syncytium-inducing (SI) T-cell line (T)-tropic viruses emerge which preferentially use CXCR4. SI viruses are more virulent and are associated with higher rates of $CD4^+$-T-cell decline. It is generally believed that expression and regulation of HIV-1 receptors/co-receptors at different anatomic sites (Zhang *et al.*, 1998; Jameson *et al.*, 2002) govern their susceptibility or resistance to infection with HIV-1. Vaginal mucosa is thought to be more resistant than rectal mucosa to HIV-1 infection, due in part to low expression of CCR5. In the rectum, numerous DC-SIGN + CCR5+ CD4+ DCs and CD4+ CCR5+ cells are expressed throughout the lamina propria, beneath the luminal epithelium, whereas in the vagina, subepithelial DCs in the lamina propria express moderate levels of DC-SIGN, and a small number of these cells co-express CCR5 (Jameson *et al.*, 2002). In the colonic epithelium, there is a predominant apical expression of CXCR4 and CCR5, suggesting that intestinal epithelial cells can serve as a target for entry of HIV-1 across the colonic mucosa (Dwinell *et al.*, 1999).

The importance of co-receptor CCR5 in HIV-1 pathogenesis is also supported by the finding that a genetic mutation in CCR5 delta 32 (which occurs naturally in a small percentage of individuals) makes them highly resistant to HIV-1 infection (Huang *et al.*, 1996). In view of these observations and the results of our published study documenting low expression of CCR5, and restricted expression of CXCR4 in oral mucosa (Jotwani *et al.*, 2004), it is reasonable to assume that these factors may play a distinct role in the resistance of gingiva to infection with HIV-1.

Resistance to HIV-1 infection in human gingiva can also be mediated by antimicrobial proteins, the defensins. Analysis of α-defensin-1 expression in human gingiva by conventional RT-PCR and quantitative real-time PCR demonstrates their expression during health (Jotwani *et al.*, 2004). Several studies have independently confirmed the anti-HIV potential of α-defensins-1–3 (Zhang *et al.*, 2002; Mackewicz *et al.*, 2003). Anti-HIV-1 activity of α-defensins has been shown to operate at least at two levels, including direct inactivation of virus particles and affecting the ability of target CD4+ T-cells to replicate the virus (Mackewicz *et al.*, 2003). Regarding the cells which produce α-defensin-1, –2, and –3, neutrophils are recognized as a principal source (Lehrer and Ganz, 2002). However additional sources have been described, including NK cells, γδ T-cells, B-cells, and monocytes/macrophages (Agerberth *et al.*, 2000). Recent evidence suggests that monocytes may be principal sources of α-defensins (Mackewicz *et al.*, 2003). Neutrophils increasingly infiltrate the gingival mucosa during CP and are likely to be the major sources of α-defensins (Dale, 2002), consistent with our evidence of significantly increased expression of α-defensin-1 during CP (Jotwani *et al.*, 2004). Analysis of these data underscores the need for the identification of the oral mucosal cells expressing α-defensins. The inflammatory infiltrate in CP contains many immune cell types (Jotwani *et al.*, 2001), which, in other studies, have been shown to contain α-defensin activity (Agerberth *et al.*, 2000; Lehrer and Ganz, 2002). Recently, it has been shown that human β-defensin (hBD)-2 and –3, produced by human oral epithelial cells, can also block HIV-1 replication *via* a direct interaction with virions and through modulation of the CXCR4 co-receptor *in vitro* (Quiñones-Mateu *et al.*, 2003). hBD-1 and hBD-2 are constitutively expressed on healthy gingiva. Constitutive expression of hBD-2 (10 μM *per* gram of tissue) is sufficient to inhibit replication of the HIV-1 X4 isolate (Sawaki *et al.*, 2002; Quiñones-Mateu *et al.*, 2003).

In conclusion, we have observed that, in gingival health, the low expression patterns of HIV-1 receptors/co-receptors by gingival cells suggest an unfavorable environment for infection with HIV-1. During CP, there is an increase in the number of cells co-expressing HIV-1 receptors/co-receptors; however, this is accompanied with ten-fold increases in α-defensin-1, known to have potent anti-HIV-1 activity. Further studies are required to determine whether oral mucosal DCs and T-cells are infected in $HIV\text{-}1^+$ subjects, and to

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clarify the role of defensins (both α and β) in oral mucosa, so that protective strategies for other mucosal surfaces can be developed.

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**TABLE**

Factors in Human Oral Epithelium Relevant to HIV-1 Infection

| Healthy Epithelium | Chronic Periodontitis |
|---|---|
| CXCR4 low expression | DC SIGN + DC increased |
| CCR5 low expression | Macrophage mannose receptor increased |
| α-defensin-1 expressed | Langerin expression decreased |
| β-defensins expressed | CD83-positive mature macrophages increased; α-defensins markedly increased |