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The Oral Mucosa Immune Environment and Oral Transmission of HIV/SIV

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Summary

The global spread of human immunodeficiency virus (HIV) is dependent on the ability of this virus to efficiently cross from one host to the next by traversing a mucosal membrane. Unraveling how mucosal exposure of HIV results in systemic infection is critical for the development of effective therapeutic strategies. This review focuses on understanding the immune events associated with the oral route of transmission (via breastfeeding or sexual oral intercourse), which occurs across the oral and/or gastrointestinal mucosa. Studies in both humans and simian immunodeficiency virus (SIV) monkey models have identified viral changes and immune events associated with oral HIV/SIV exposure. This review covers our current knowledge of HIV oral transmission in both infants and adults, the use of SIV models in understanding early immune events, oral immune factors that modulate HIV/SIV susceptibility (including mucosal inflammation), and interventions that may impact oral HIV transmission rates. Understanding the factors that influence oral HIV transmission will provide the foundation for developing immune therapeutic and vaccine strategies that can protect both infants and adults from oral HIV transmission.

Keywords

HIV; SIV; oral transmission; inflammation; SIV natural hosts; mucosal immunity

Introduction

The global spread of human immunodeficiency virus (HIV) is dependent on the ability of the virus to efficiently cross from one host to the next by traversing mucosal membranes. An effective vaccine or therapeutic agent that prevents HIV transmission most likely will need to function at or near the mucosal surface (1, 2). Therefore, it is critical that we thoroughly understand the multiple routes of virus exposure that can lead to HIV infection, including rectal, vaginal, penile, and oral HIV exposure. This review focuses on just one of these routes of exposure, oral transmission, when HIV infection occurs across the oral and/or

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gastrointestinal (GI) tract. Oral transmission of HIV primarily occurs in two distinct settings, breast milk consumption by infants born to HIV-infected women and oral-genital contact during adult sexual intercourse.

Access to antiretroviral treatment (ART) and bottle-feeding has dramatically reduced oral HIV transmission via breast milk in developed countries. However, in developing countries, over 300,000 new mother-to-child transmission (MTCT) events occur each year (3), and nearly 40% of these HIV infections are acquired through breastfeeding (4). Despite the risk of HIV acquisition via breastfeeding, exclusive breastfeeding of infants born to HIV-infected mothers is recommended by the World Health Organization (WHO), since, even in the presence of HIV, breastfeeding results in reduced infant mortality, partly due to immune benefits from passive transfer of antibodies and other bioactive products (5). Therefore, HIV transmission via breast milk continues to be a major route of infant HIV acquisition.

Oral transmission of HIV via oral-genital contact was initially difficult to quantify as it requires detailed and accurate knowledge of an individual's sexual practices, which often include multiple high-risk behaviors. In fact, initial reports indicated that oral intercourse was a low risk sexual activity for transmitting HIV (6–15). However, more recent epidemiologic studies have provided clear evidence that HIV can be transmitted via receptive oral intercourse (ROI) (16–20), and studies in the simian immunodeficiency virus (SIV)/macaque animal model provide further proof that oral transmission of HIV can occur (21–25).

To investigate oral transmission of HIV, we discuss studies in both humans and SIV monkey models to provide a comprehensive understanding of this route of infection. This review evaluates the immune factors that influence HIV/SIV susceptibility as well as interventions that might be utilized to inhibit HIV transmission via oral HIV exposure.

Infant oral transmission

Infants born to HIV-infected mothers, who do not acquire HIV *in utero* or perinatally, continue to be at risk of acquiring HIV through breastfeeding. This risk was first observed by Ziegler *et al.* (26) in the mid-1980s and subsequently quantified in a Kenyan trial that randomized women to breast or formula feeding (27). In this large, randomized study, the risk of HIV transmission attributable to breastfeeding was 44.1% (27). Since then, other studies have confirmed that, without intervention, between 20 and 40% of MTCTs occur during breastfeeding (4, 28). There are also a handful of case reports where postnatal MTCT of HIV occurred in the absence of breastfeeding where premastication of food by the mother may have been the route of transmission (29). However, this route of transmission appears to be far less common than breast milk transmission of HIV (30).

Because of the risk of HIV transmission through breastfeeding, formula feeding is universally recommended to HIV-infected mothers in developed countries. However, postnatal transmission of HIV remains complex and difficult to prevent in settings where formula feeding is neither safe nor affordable. In developing countries, breastfeeding is recommended even for HIV-infected women, due to increased morbidity and mortality from malnutrition and gastroenteritis in formula-fed infants (5, 31–33). Therefore, in developing countries, postnatal transmission continues to account for up to 39% of MTCT (4, 28, 34).

It is unclear if any particular period of time during lactation presents a greater risk for HIV-1 transmission. Some studies suggest that colostrum, the breast milk produced shortly after delivery, has a higher viral load than non-colostrum breast milk, and therefore, transmission may be higher early in life (35), while others studies have not observed this phenomenon

(36, 37). Regardless, the risk of MTCT transmission of HIV persists until the infant is fully weaned (4).

Factors associated with increased risk of breast milk HIV transmission include high maternal viral load, particularly breast milk viral load (38, 39), and duration of breastfeeding (4). Interestingly, low maternal $CD4^+$ T-cell count is also a major determinant of transmission independent of viral load (36). Clinical and subclinical mastitis (which are associated with increased milk viral load in the involved breast), breast abscesses or other lesions, and infant oral thrush have also been shown to increase the risk of transmission (38–43). One particularly important risk factor for breast milk transmission is the consumption of non-breast milk foods. In four large prospective studies conducted in Africa, exclusive breastfeeding reduced the risk of MTCT by up to 11-fold (31, 32, 36, 44), compared to women who supplemented breastfeeding with other foods (known as mixed feeding). This increased risk was independent of maternal breast milk viral load and mastitis (38, 43).

Multiple trials have demonstrated that mother and infant antiretroviral therapy during breastfeeding can dramatically reduce HIV breast milk transmission (28, 45–47). Implementation of these interventions has made breastfeeding remarkably safer for HIV-exposed infants around the world. In communities that have made these interventions widely available, rates of transmission during breastfeeding have decreased to 1–4% (46). However, over 300,000 infants are still infected with HIV each year (3). In addition, the prophylactic use of antiretroviral drugs can result in drug resistance in infants that become infected despite prophylaxis (48, 49). Although much progress has been made in reducing HIV transmission through breastfeeding, additional interventions are still necessary to eliminate infant HIV acquisition.

Adult oral transmission

In contrast to MTCT of HIV, sexual transmission of HIV following oral exposure is still somewhat controversial. Since the beginning of the HIV epidemic, case reports have been published of HIV-infected individuals who likely acquired HIV through sexual oral exposure. These reports have implicated oral contact with female (50–54) and male genital fluids (55–69), oral-anal contact (50), and oral-oral contact (70). Accuracy of sexual history reporting has been called into question, and oral exposure rarely occurred in the absence of other exposures (61, 71–73). However, one transmission pair, where both individuals independently reported only oral-genital contact, was confirmed by viral sequencing (69). Interestingly, the incident case in several of the reports may have acquired HIV while their oral mucosa was compromised by dental procedures (55, 65, 74), allergies (56), pharyngitis (64), chemotherapy (59), or periodontal disease (63, 65, 66, 70). Although case reports alone are unable to evaluate the contribution of sexual oral transmission to the HIV epidemic, the number of reports over the last three decades would suggest that sexual oral transmission of HIV can occur, particularly when the oral mucosa is compromised.

Early population studies of men who have sex with men (MSM) did not identify oral-genital contact as a risk factor for HIV seroconversion (6–15). However, these studies enrolled relatively small cohorts of very high-risk individuals, making them underpowered to detect transmission by lower risk sexual activity. One study in the 1980s evaluated oral-genital contact in serodiscordant, heterosexual couples and found a significantly higher amount of oral-genital contact among couples who transmitted HIV infection during the study (OR 7.29), especially in female partners of acquired immunodeficiency syndrome (AIDS) patients (75). Importantly, fewer sexual partners and less receptive anal intercourse (RAI), both well-established risk factors for sexual HIV transmission, were reported in this study compared to the MSM studies of the 1980s. The first report in MSM with no RAI showed a

significantly higher proportion of seroconverters among individuals that reported oral-genital contact (OR 3.0), although this did not consistently retain significance in multivariate models (76). Subsequently, a large, more conclusive study found that, after adjusting for RAI, each additional receptive oral intercourse (ROI) partner significantly increased the risk of HIV seroconversion by 1% (compared to 5% for RAI) (18). While a handful of small studies in the early 1990s suggested that heterosexual HIV transmission through oral-genital contact may be a relatively rare event (77, 78), the contribution of oral-genital contact, specifically ROI, to heterosexual transmission of HIV was confirmed by a large study in New York City sex workers. In this study, the risk of HIV acquisition associated with oral sex was increased in individuals that smoked crack cocaine, which is known to cause oral lesions (20). In addition to confirming the potential of HIV sexual transmission, following oral exposure, this study also suggested that inflammation at the oral mucosa may increase susceptibility to HIV.

HIV acquisition via sexual oral exposure occurs at a lower rate than RAI transmission of HIV. A single study in MSM estimated that the per-contact risk of HIV infection through unprotected oral sex with an HIV-infected partner or a partner of unknown HIV status is 0.04%, in comparison to 0.27% for unprotected RAI (19). However, there are no other estimates of per-contact or per-act risk of oral-genital transmission of HIV. These key epidemiologic studies were rapidly followed by the first report of successful HIV infection of adult rhesus macaques by oral administration of SIV, confirming that HIV infection can occur through oral exposure to HIV (see Animal Models of Oral Transmission section). Although the exact frequency of oral sexual transmission of HIV is difficult to assess, by the late 1990s, the evidence for sexual transmission of HIV across the oral mucosa, particularly via ROI, had been well established.

Despite the lower rate of transmission compared to RAI, oral-genital contact significantly contributes to the HIV pandemic. Surveillance data between 2001 and 2003 from UK MSM that do not participate in unprotected anal intercourse showed that 2.6 to 5.2% of MSM acquired HIV through oral transmission (16). Oral sex is practiced frequently both by heterosexual and homosexual couples (78), and use of barrier protection during oral-genital contact, even in HIV serodiscordant couples, is rare (6, 68, 79). Many studies have reported a dramatic decrease in high-risk sexual activity among MSM between the 1980s and the 2000s (18, 79, 80), resulting in a marked reduction in HIV incidence (79). However, sexual oral contact is generally considered a lower risk activity, and rates of unprotected ROI remain high (79, 80). Reduction in higher risk activities paired with continuation of lower-risk activities is consistent with an increased role for lower risk sexual oral transmission in the MSM HIV pandemic.

The contribution of oral exposure to heterosexual HIV transmission is less clear, since no incidence studies have been performed to date. It is likely that transmission by receptive fellatio in heterosexuals is similar to those observed in MSM. However, the contribution of oral-genital contact via cunnilingus and oral-anal contact is still unknown. However, the HIV transmission rate per sexual act for vaginal intercourse is 5% of that observed for RAI (81, 82), making most heterosexual transmissions of HIV low-risk transmissions in comparison to RAI transmission of HIV. This would suggest that the relative contribution of sexual oral contact to the heterosexual HIV pandemic exceeds that observed in MSM. Importantly, the majority of the current HIV epidemic occurs in developing countries, predominantly in Sub-Saharan Africa (3), and HIV incidence per sexual act significantly varies by socio-economic setting (82), making it critical to study oral-genital transmission of HIV in developing countries. The variability in sexual HIV transmission, following vaginal and rectal sexual exposure, is largely associated with differences in mucosal immune activation at the site of HIV acquisition (83). This is also likely the case in oral HIV

acquisition. However, studying oral HIV transmission is particularly challenging in human adults. Therefore, animal models are useful in determining the mechanisms underlying variation in oral mucosa susceptibility to HIV.

Animal models of oral transmission

To determine the immune events critical for protection against oral HIV exposure, we must understand the immune responses to HIV that occur prior to systemic virus dissemination and the establishment of latent reservoirs. In humans, identifying individuals within this time period is incredibly difficult due to the weeks to years before an individuals is diagnosed with HIV. Therefore, our understanding of early events, following oral transmission, comes largely from studies of simian immunodeficiency virus (SIV) infection in macaques (Table 1). The SIV strains utilized in these studies (generally SIVmac251) contain diverse quasispecies and can be used in macaques to mimic mucosal exposure to HIV (84–86). Initial studies of oral transmission in the SIV-macaque model by Baba *et al.* (22) determined that the infection was 6000 times more likely to occur during an oral exposure compared to a rectal exposure in adult macaques, although later studies have shown lower transmission rates with oral than rectal exposure, as predicted by human epidemiology studies (87). Subsequently, Van Rompay *et al.* (88) showed productive infection of infant macaques, following oral SIV exposure, and that transmission could be prevented by prophylactic treatment with ART. To evaluate the contribution of MALT to viral entry, Stahl-Hennig *et al.* (89) applied cell-free SIV directly to the tonsil. This study demonstrated that the tonsils are a potential site of transmission and an early site of explosive viral replication that plateaus 4–7 days post-inoculation. In a follow-up study, an attenuated SIV vaccine induced protection, following tonsillar administration of SIV in Rhesus macaques (90). Work in our laboratory has more comprehensively evaluated potential SIV entry sites, following non-traumatic application of high-dose SIV to the cheek pouch with subsequent swallowing, and has shown that in both infant and adult macaques SIV replication is concentrated in tissues proximal to the stomach, including the oral mucosa, esophagus, tonsils and draining lymph nodes, at days 1 and 2 post-exposure (24). Detailed evaluation of tissues from these macaques showed SIV-infected macrophages and $CD4^+$ T cells at lymphoid tissues by 4 days post-exposure, suggesting that these cells play key roles in early viral replication and dissemination (24). These findings provide evidence that oral transmission of SIV, and by analogy HIV, occurs before the virus reaches the stomach and that the virus is able to disseminate rapidly to draining and then distant lymph nodes.

Oral infection of macaques with SIV elicits an innate immune response that can be measured within days post-infection (23, 25, 91). A study by Abel *et al.* (91) evaluated the innate immune changes at different tissue sites in infant macaques 7 days after oral exposure. Elevations of both anti-viral (MX, IFN-α) and proinflammatory (IL6, IL12, CXCL10) genes, particularly in upper gastrointestinal tissues, suggests that early, innate immune responses to SIV may both inhibit and potentiate the establishment of systemic infection (91). Studies in our laboratory have shown a correlation between increased innate immune activation at mucosal sites (OAS, CXCL9 and CXCL10) in orally inoculated SIV infected macaques and slower disease progression (25). In contrast, an increase in the same immune modulators in lymphoid tissues and in blood was associated with a more rapid progression to simian AIDS (23). Also, 1–4 days after oral SIV infection we observed a migration of the innate immune T cell subset *gd* T cells from mucosal sites to draining lymph nodes (92). In addition to the high-dose SIV administration used in the studies described above, macaques can be successfully infected with SIV through repeated, low dose challenges, which better replicate viral exposure via semen and breast milk. Macaques orally exposed to low-dose SIV have a slightly slower rate of innate immune response

induction at both mucosa and lymph nodes (93). These findings indicate that following successful oral SIV infection, the innate immune system responds rapidly to the virus, which can be detected at both mucosal sites and lymph nodes within just a few days post-infection.

Comparison of oral SIV transmission with other mucosal transmission routes could provide clues for future HIV prevention strategies. Following high dose vaginal exposure to SIV, only a few clustered cells, predominantly $CD4^+$ T cells, are SIV-infected up to 3–4 days post-infection (94, 95). During this initial, local infection, it is believed that the founder SIV population expands, triggering a localized innate immune response and recruitment of additional target cells that fuel viral replication and facilitate further dissemination of the virus into lymph nodes and distal tissues (96, 97). These findings contrast with oral SIV challenge studies, where high-dose virus rapidly spread through the mucosa and into the lymphoid tissues by 1 to 2 days post-infection (24). The differential rate of viral spread in these two transmission models might be due to inherent differences in the oral/GI tract and vaginal mucosa, or could be due to sensitivity of the principle assays utilized for each study (nested SIV DNA PCR for oral transmission and *in situ* hybridization for vaginal transmission). Either way, the SIV-macaque model has provided key insights into the earliest events, following mucosal transmission, which will be important for the design of effective, prophylactic HIV interventions.

Establishment of HIV infection

Documented cases of HIV acquisition after oral exposure have occurred predominantly through ROI, where individuals are exposed to virus in semen or pre-ejaculatory fluid, and breastfeeding, when virus is consumed in maternal breast milk. All three fluids are well populated by leukocytes, particularly macrophages (98–100), that can harbor infectious virus, and contain detectable titers of cell-free virus, although the viral load in these fluids is generally lower than that observed in the blood (99, 101–106). However, the relative contribution of cell-free and cell-associated virus to HIV transmission is still unclear.

Multiple lines of evidence have suggested that cell-associated virus may be important in oral transmission of HIV. Cell-associated virus can withstand low pH environments, such as the stomach, better than cell-free virus (100, 107); HIV-infected macrophages can penetrate infant oral epithelium, allowing direct viral access to the HIV target cells of the lamina propria (108); and epithelial transcytosis of virus (see below) is most efficient with cell-associated virus (109–112). However, treatment of HIV-infected mothers with antiretrovirals, which dramatically reduces the risk of HIV acquisition in breastfed infants, decreases cell-free viral load in breast milk without reducing either the DNA or RNA load in HIV-infected cells (113–116). This would suggest that cell-free virus is more important than cell-associated virus in infant oral transmission of HIV. However, it is possible that ART may also reduce the infectivity of cell-associated virus. Interestingly, epidemiologic studies have shown that cell-associated virus titers predict breast milk HIV transmission during the first 9 months of life, where cell-free viral titers better predict HIV transmission in older infants (41, 117). This suggests that both cell-associated and cell-free virus can mediate oral transmission of HIV.

Once HIV enters the oral cavity there are a number of distinct tissue sites along the GI tract that may permit viral entry in to the host's tissue. These histologically distinct tissues include areas of stratified squamous epithelium with (e.g. gingiva) or without (e.g. esophagus and buccal mucosa) keratinization, mucosa-associated lymphoid tissue (MALT) (e.g. tonsils), and columnar epithelium (e.g. salivary glands, stomach and intestinal mucosa). Macaque models indicate that virus, following high-dose oral exposure, most likely initiates infection across the mucosa of the upper gastrointestinal tract, namely the mucosa of the oral

cavity, the tonsils and the esophagus (24, 91). The tonsils have a relatively high proportion of HIV target cells (118), and *ex vivo* and macaque studies have suggested that the tonsils may play a particularly important role in HIV oral transmission (119, 120). However, *ex vivo* studies of HIV replication in tonsil tissue have consistently used tissue obtained from therapeutic tonsillectomies, which are most commonly performed to remove tonsils that are enlarged due to infection or excessive immune response. Therefore, unlike most other mucosal tissues, tonsil tissues studied *ex vivo* are collected under inflammatory conditions (118), which is known to increase HIV susceptibility (see Inflammation and HIV/SIV Susceptibility section). Evaluating acute HIV infection with physiologically relevant, low-dose viral exposure (better representing natural exposure), is very difficult, even in the macaque model, making it difficult to determine the exact site of HIV/SIV acquisition within the upper gastrointestinal tract.

The predominant cell type in all mucosal tissues of the upper GI tract is epithelial cells, which do not express the classic HIV receptor CD4 and are not productively infected by HIV. However, cell-free and cell-associated HIV can be efficiently transcytosed across a wide range of epithelial tissues, allowing the virus to gain access to the HIV target cells of the lamina propria (108, 109, 112, 121–124). Virus transcytosed across the infant oral mucosa remains infectious (108). However, in adult oral mucosa, transcytosed HIV is not infectious, and successful penetration by cell-free virus only occurs when tight junctions are disrupted (112). Since epithelial cells are CD4 negative (118, 125), alternate receptors, namely galactosyl ceramide (GalCer) and heparin sulfate proteoglycans, are required for epithelial transcytosis (108, 109, 121–124, 126). The role of HIV coreceptors in transcytosis is still unclear. CXCR4 and CCR5 expression by mucosal epithelial cells varies, depending on the location of the mucosal tissue (118). For example, gingival epithelium expresses CXCR4 but little CCR5 (123, 127) whereas jejunal epithelium expresses substantial levels of CCR5 but no detectable CXCR4 (124). In tissues that express HIV co-receptors, blockade of the receptors by mAbs or inhibitors reduces transcytosis of the virus (123, 124), indicating that when present, HIV co-receptors can facilitate HIV transcytosis. However, both CXCR4- and CCR5-tropic viruses can be successfully transcytosed, regardless of the co-receptor expression of the mucosal tissue (108, 123). Interestingly, only CCR5-tropic virus has been observed to establish infection (see discussion below), suggesting that CCR5-dependent transcytosis of HIV is particularly important in HIV transmission or that selection of co-receptor tropism in transmitted HIV variants occurs after transcytosis. Although the transcytosis of HIV across mucosal epithelium has been well documented in *ex vivo* studies, its role in natural transmission is unknown.

HIV target cells, including $CD4^+$ T cells, macrophages and Langerhans cells, are present in adult and infant upper GI tissues (108, 112, 128–130). However, in healthy upper GI tissue, Langerhans cells are the predominant immune cell type in the epithelium with $CD4^+$ T cells and macrophages primarily observed in the *lamina propria* (108, 129, 131). Langerhans cells are tissue dendritic cells that express the HIV receptor CD4 (129) as well as the HIV co-receptor CCR5 (132). Although the cells are capable of internalizing HIV, they do not appear to produce many infectious virions, following viral integration (133, 134). However, like other dendritic cells, they can efficiently transfer virus to other HIV target cells of the *lamina propria*, including $CD4^+$ T cells and macrophages, via a mechanism known as trans-infection (135, 136). Interestingly, the density of Langerhans cells in oral mucosa is significantly lower than that observed in the vaginal, cervical and foreskin mucosa, which may result in a lower risk of HIV acquisition across the oral mucosa (129). If HIV can survive the inhospitable environment of the stomach, the intestinal mucosa also has numerous $CD4^+$ T cells present in the epithelium, making it possible for virus at this site to directly infect cells that have the capacity to spread HIV without the assistance of additional cell types.

Similar to HIV transmission through other mucosal routes, a narrow transmission bottleneck occurs during oral HIV transmission. One or two viral variants are responsible for establishing infection both in adult and infant oral transmission (137, 138), despite the diversity of HIV in the partner's semen or mother's breast milk. Higher SIV dose substantially increases the number of founder variants in macaque studies (93, 139–141), and a similar phenomenon has been observed in infants whose mothers seroconvert during breastfeeding (138, 142), likely due to high breast milk viral loads during acute infection. Also, similar to other routes of infection, only CCR5-tropic HIV viral variants establish infection *in vivo* (137, 142). Consistent with this observation, only macrophages infected with CCR5-tropic virus can migrate through infant oral mucosa to gain access to the HIV target cells of the lamina propria (108). Interestingly, HIV variants transmitted during breastfeeding have fewer glycosylation sites and shorter Env sequences compared to maternal viral variants (137), although this phenomenon was not recapitulated in the SIV/infant macaque model (138). There is some evidence that virus populations in both breast milk and semen can harbor unique viral variants relative to those observed in the blood (143). However, this compartmentalization is only observed in a subset of individuals, and the virus in breast milk and semen remains diverse and does not differ from blood viral variants in glycosylation sites or Env sequence length (40, 101, 105, 144–147). Inflammatory lesions at the mucosa can result in an increase in the number of variants that establish infection, suggesting that the viral bottleneck is greatest at the mucosa itself (148). However, it is also possible that an additional bottleneck may be present during dissemination of virus from mucosa to the systemic circulation. The narrow viral bottleneck in mucosal transmission of HIV despite the presence of cells that readily transcytose and replicate HIV suggests that anti-viral responses in the upper GI tract may serve a role in reducing, but not abolishing, HIV transmission following oral exposure.

Oral mucosa soluble, innate, and adaptive immunity to HIV

The ability of salivary immune components to inhibit HIV has been well studied. This has led to the discovery of a wide array of oral mucosa defenses against HIV, including both innate and adaptive immune responses (Table 2).

Saliva can rapidly kill HIV-infected leukocytes: preincubation of HIV with saliva reduces HIV infectivity, and saliva inhibits HIV replication in infected cells (149–158). Filtration of HIV-incubated saliva prior to application to target cells dramatically reduced the infectivity of the inoculum, a phenomenon not seen with filtered HIV alone (153, 155, 159). This phenomenon may be due to the action of salivary components that can aggregate HIV, including thrombospondin-1 (TSP-1) (160, 161) and mucins, particularly 5B, 7A, and 7B (162, 163), some of which are also present in breast milk.

In addition to HIV aggregation, there are also additional mechanisms by which salivary proteins protect against HIV. Mucin 1 from breast milk reduces DC-SIGN-dependent HIV infection by binding to specific carbohydrates on DC-SIGN (164). Mucin-containing components of human saliva can also strip gp120 from HIV viral particles, rendering them uninfectious (165). Salivary agglutinin, also known as gp340, inhibits CCR5- and CXCR4-tropic HIV infectivity by binding to gp120 (166, 167). Additionally, TSP-1 binds CD4 to block HIV entry into target cells (160). Lactoferrin, a protein present in high levels in both breast milk and saliva, is also an important component of salivary anti-HIV activity (168–170). Cystatin also shows anti-HIV activity at concentrations observed in saliva (154, 162). In addition, studies have also found a decreased risk of HIV transmission in mothers with elevated breast milk levels of sTLR2 (171), CCL4 (172), erythropoietin (173), IL15 (174) and specific long-chain fatty acids (175), indicating that there may be many additional host factors that can reduce HIV acquisition, following oral exposure.

Secretory leukocyte protease inhibitor (SLPI) has strong anti-HIV activity at concentrations found in human saliva (154) and is particularly effective at blocking HIV infection of monocytes (176, 177). Depletion of SLPI from whole saliva results in a substantial loss of salivary anti-HIV activity (154, 169, 178). *In vivo*, higher SLPI salivary levels in HIV-exposed infants reduce their susceptibility to HIV (179). However, breast milk levels of SLPI does not appear to correlate with reduced breastfeeding transmission of HIV (180). SLPI does not bind to HIV proteins or CD4, (154) but does bind Annexin II on macrophages (181). Blockade of Annexin II by SLPI or siRNA knockout of Annexin II reduces macrophage infection *in vitro*, suggesting a potential mechanism for SLPI's inhibition of HIV (181). In addition to expression in salivary glands, SLPI is expressed both intracellularly and extracellularly in infant and adult tonsils, and inhibition of SLPI expression in adult human tonsil tissue results in decreased inactivation of transcytosed HIV (112).

Another class of potent anti-microbial proteins that are abundant throughout the gastrointestinal tract (182–189), human β-defensins (hBD), has a similar role as SLPI in inactivating transcytosed HIV (112). HBD-2 and 3, and possibly hBD1, reduce HIV replication in infected cells, and can reduce the expression of CXCR4, but not CCR5 (190, 191). Alpha-defensins 1, 2 and 3, similar small proteins with anti-HIV activity (192, 193), are also expressed throughout the GI tract (183, 187), and breast milk levels of alpha-defensins correlate with reduced HIV acquisition in breastfed infants (194). Theta-defensins, also known as retrocyclins, a new class of circular defensins first identified in non-human primates, have anti-HIV activity in humans by binding CD4, GalCer and glycosylated HIV Env (195–197).

Interestingly, the gastrointestinal microbiome may also play an important role in protecting against HIV acquisition. Bacteria isolated from breast milk, particularly lactobacillus and pediococcal bacteria, common gastrointestinal microbiota, can inhibit HIV infection of target cells (198). One potential mechanism for how specific microbes may modulate HIV susceptibility is the ability of bacteria, like lactobacilli, to produce hydrogen peroxide (199). Saliva contains high levels of peroxidases, which utilize hydrogen peroxide to produce reactive oxygen species that can rapidly inhibit HIV (200).

It is important to note that most of these innate, anti-viral factors have been documented at other mucosal sites, including the vaginal and rectal mucosa. Although elevated mucosal expression of many of these same factors is correlated with reduced vaginal transmission of HIV (201, 202), HIV transmission still occurs even in the presence of innate anti-HIV factors. Although these factors may certainly be responsible for the low rate of HIV transmission per exposure, they are unable to entirely prevent HIV acquisition.

Anti-HIV adaptive immune responses can also provide protection against HIV acquisition, following oral exposure to HIV. Genetic variations in HLA genes modify the antigens targeted by adaptive immune responses, and infants that share HLA genotypes with their mother have increased susceptibility to breast milk transmission of HIV (179). In addition, particular infant HLA genotypes are associated with a much higher risk of breast milk HIV acquisition (203), highlighting the importance of adaptive immunity in reducing oral HIV transmission.

Antibody responses, both mucosal and systemic, appear to be particularly important in protecting against oral transmission of HIV. Strikingly, high dose intravenous administration of cocktails of neutralizing, anti-HIV antibodies to infant macaques protects against SHIV challenge (204, 205). However, it is important to note that only a subset of neutralizing antibody cocktails show full protection in neonatal macaques (204, 205). Studies of anti-

HIV responses in breast milk from HIV-infected mothers have shown that increased breast milk IgM responses (206) and antibody-dependent cellular cytotoxicity (ADCC) activity (207) decreases the risk of HIV infection in breastfed infants. Chronic exposure to HIV can induce anti-HIV antibody responses in the oral mucosa, namely IgA responses, as seen in HIV-uninfected partners of HIV-infected individuals (208). However, no studies have demonstrated protection by salivary anti-HIV antibodies *in vitro*, although these studies particularly focused on IgA antibodies (209), which are associated with increased risk of HIV transmission in breast milk (210). The ability of anti-HIV antibodies to block HIV transcytosis is somewhat unclear. Although some studies have observed effective inhibition of HIV transcytosis with breast milk-derived anti-HIV IgA and IgG (211, 212), other researchers have failed to observe this phenomenon (213). Interestingly, anti-HIV IgM and IgA applied to the basal side of epithelial cells can act within epithelial cells to block HIV transcytosis (214).

The role of T-cell responses in protecting against oral HIV transmission is less clear. Nearly 50% of HIV-exposed, uninfected infants in the absence of maternal art have detectable T cell responses (215, 216) and those responses are maintained for at least 6 months after birth (217). A recent analysis found that the breadth and magnitude of breast milk HIV-gag T cell responses correlate with reduced risk of breast milk MTCT (172). However, it is likely that both antibody and T-cell responses are important for full protection against HIV. Therefore, an HIV vaccine that could effectively block oral transmission of HIV will most likely elicit robust antibody and T cell responses at the site of virus exposure, the oral mucosa.

Inflammation and oral HIV/SIV susceptibility

Although infants are exposed to HIV-containing breast milk for many months, even in the absence of antiretroviral prophylaxis, only a relatively small number of infants born to HIV^+ mothers will become infected (218). Indeed, if we assume a 40% MTCT infection rate, then each infant born to an HIV^+ mother has a 16% chance of acquiring HIV during breastfeeding. This suggests that certain infants may be particularly susceptible to HIV. Understanding the factors that enhance oral HIV transmission will be critical for developing effective interventions to protect infants from postpartum transmission of HIV.

It is well documented that different types of mucosal inflammation at multiple sites impact sexual and vertical transmission of HIV (219–227). Oral mucosa inflammation is also a risk factor for oral HIV transmission. For example, infant oral candidiasis increases the risk of MTCT of HIV through breastfeeding (228). The risk of HIV acquisition associated with oral sex is increased in individuals that smoke crack cocaine, which is known to cause oral lesions (20), and oral sores are associated with HIV infection in crack cocaine users who performed ROI (OR 1.9) (229). In contrast, studies in HIV-uninfected, highly exposed individuals indicate that low levels of CD4+ T cell activation and quiescent $CD4^+$ T-cell phenotypes are associated with reduced HIV susceptibility (230–233). These studies suggest that inflammation at the oral mucosa increases susceptibility to HIV.

Two hypotheses offer a mechanism for the observed increase in HIV transmission, following inflammatory events at the oral mucosa. The first is that inflammation leads to a break in the mucosal barrier, permitting viral access to target cells that reside beneath the difficult-to-penetrate stratified squamous epithelium of the oral cavity. Second, inflammatory events can recruit activated HIV target cells to mucosal tissues, while the mucosal barrier remains intact. Indeed, inflammatory cytokines can directly increase HIV replication (234–237), indicating that an inflammatory mucosal environment alone may promote productive HIV infection at mucosal sites.

Due to its unique ability to provide detailed mucosal information both before and during early infection, our understanding of inflammatory events that influence oral transmission relies on the SIV-macaque model (Table 1). In one study, Chenine *et al.* (87) utilized 10% acetic acid to induce an inflammatory sore on the inside cheek pouch with subsequent exposure to low dose oral SHIV challenge. These investigators identified an increased risk in SHIV infection in treated macaques, which likely resulted in both increased access to underlying target cells, recruitment of additional target cells to the site of inflammation as well as an activation of the target cells themselves (87). In contrast, a study from our laboratory experimentally induced gingival inflammation in adult rhesus macaques that mimicked the mild to moderate gingivitis common in humans (238). This was accomplished by tying silk ligatures around the base of the teeth and softening the macaque's food with water. This treatment induced strong upregulation of multiple inflammatory markers in the oral tissue, including IL6, IL8, and IL18. Macaques were then orally exposed to repeated low dose challenge of SIVmac251 and, surprisingly, the rate of SIV transmission was similar in the gingivitis and control macaque groups, although an increase in the number of viral variants that establish the SIV infection was observed (238). One interesting distinction that may explain the different findings in these two studies is that while gingivitis does result in inflamed mucosa it does not necessarily compromised the mucosal barrier, which likely occurred with acetic acid treatment, potentially explaining the increased susceptibility in the acetic acid treated animals but not in the gingivitis animals.

In addition to inflammatory changes that are induced directly at the mucosa, activation of the systemic immune system may have an indirect impact on target cells at mucosal sites. Indeed, any infection, or even a vaccination, could potentially activate HIV target cells at both systemic and mucosal sites, potentially resulting in increased HIV transmission. This may have been the case in the STEP trial, where individuals who were previously exposed to the vaccine vector or were uncircumcised had an increased risk of acquiring HIV after vaccination (239). This suggests a complex interplay between vaccine-induced immune activation and mucosal tissues, which may have resulted in increased HIV susceptibility.

One perplexing observation worthy of additional study is the finding that mixed feeding, where infants receive breast milk and other foods, results in an increase in the rate of HIV breast milk transmission compared to exclusive breastfeeding. Indeed, in one study from KwaZulu-Natal, South Africa, infants who were mixed fed at any point after birth were almost 11 times more likely to acquire HIV than those who were received breast milk alone (36). In a second study, Kuhn and colleagues showed that the risk of postnatal HIV infection in the first four months of life was significantly lower among exclusively breastfed infants compared to mixed fed infants (31). To date, no difference in mastitis or maternal viral load, either in the systemic circulation or in breast milk, have been observed in mothers that mix feed, and mixed fed infants do not have an increase in GI permeability (38, 240, 241). However, mixed feeding is associated with an increased risk of a number of inflammatory conditions, including asthma, eczema, atopic dermatitis, food allergies, ear infections and diarrhea (242–244). We hypothesize that mucosal inflammation may be induced in mixed fed infants by exposure to novel antigens in food, exposure to food contaminants, including fungus-derived mycotoxins, physical disruption of the oral mucosa due to consumption of solid food or decreased exposure to the anti-inflammatory cytokines found in breast milk. The studies described here provide evidence that inflammation and activation of HIV target cells at the oral mucosa has the potential to increase the oral transmission of HIV.

Oral transmission in natural hosts

SIV natural hosts are African non-human primates who develop non-pathogenic SIV infection in the wild without progression to AIDS. These natural hosts include sooty

mangabeys, African green monkey, mandrill, and many others. Key features of SIV infection of natural hosts include: high viremia (245, 246), normal peripheral $CD4^+$ T-cell counts (246, 247), lack of microbial translocation despite significant loss of mucosal $CD4^+$ T cells (248, 249), and lack of immune activation during chronic infection (247, 249–251). These studies have led to a working hypothesis that the lack of disease progression in natural hosts is due to a lack of chronic immune activation (252, 253).

In natural hosts, MTCT of SIV, including oral transmission, is a rare occurrence both in the wild and captivity (254–257). Early studies in Ethiopian wild grivet monkeys, SIV natural hosts, showed very low incidence of SIV seropositivity before the onset of sexual activity (257). In a subsequent study, captive infant African green monkeys show progressive decline in anti-SIV antibodies, with no detectable anti-SIV antibodies by one year of age, consistent with progressive loss of transferred maternal antibodies without MTCT of SIV (255). More recently, efficiency of breastfeeding transmission in natural hosts was directly tested in female mandrills infected with SIV one day after delivery. Interestingly, despite high peak and set-point plasma viral loads in the mothers, none of the offspring had serological or virological evidence of infection by the end of breastfeeding (6 months) (256). This finding is in direct contrast to the breast milk transmission studies performed in the pathogenic host, rhesus macaques, as mentioned above, where high rates of infant infection were seen in similar study designs (258, 259). It is important to note that observation of captive breeding colonies have revealed similar suckling frequency and total duration of breastfeeding in both natural and non-natural hosts (J. Else, personal communication).

The sooty mangabey has been extensively studied as a model of nonpathogenic SIV infection in a natural host species. Similar to other natural hosts, MTCT of HIV in sooty mangabeys is rare. An unprecedented study of viral sequences in fecal samples from wild sooty mangabeys in the Tai Forest in Cote d'Ivoire identified only two likely MTCT events (where both mother and infant had nearly identical virus) in the context of 59% adult prevalence of SIV infection (260). In a recent study, we investigated MTCT of SIV in sooty mangabeys in a large colony of naturally SIV-infected sooty mangabeys, using serological and virological methods. Of 161 sooty mangabey infants born to SIV-infected mothers only 11 infants (6.8%) were defined as 'presumptively' vertically infected based on repeated serologic sampling with confirmatory viral loads in the first year of life (254). In comparison, 25–45% of human infants born to HIV-infected mothers acquire HIV from their mother in the absence of intervention (218). Interestingly, SIV-infected sooty mangabey infants do not have increased morbidity or mortality, indicating that the infection is nonpathogenic even when acquired early in life (254). Interestingly, the viral load of SIV-infected sooty mangabey infants was ~2-log lower than that observed in SIV-infected adult sooty mangabeys living in the same colony. These results confirm that vertical transmission, including breast milk transmission, is substantially less frequent in SIV-infected natural hosts than in HIV-infected humans or SIV-infected rhesus macaques.

We propose three non-mutually exclusive hypotheses to explain the restriction of breast milk SIV transmission in natural hosts: (i) lower levels of SIV in natural host breast milk than those observed in pathogenic infections, (ii) a relatively non-permissive breast milk and/or gastrointestinal microenvironment, with lower immune activation and the presence of innate and adaptive inhibitory factors, and (iii) insufficient target cells for establishment of infection in the natural host infant. To date, no definitive studies have yet been performed that test one hypothesis while controlling for all other potential confounding factors (and may be impossible to perform), but a preponderance of data suggests target cell restriction in the infant GI tract is a defining feature of natural hosts that limits MTCT.

In humans, breast milk viral load and breast milk cell-associated HIV correlates with the risk of MTCT (38, 39), but similar levels of cell-free SIV are seen in both SIV-infected African green monkeys and rhesus macaques (261). SIV RNA was measured in breast milk from mandrills infected with SIV postpartum, and high levels of breast milk RNA were found on days 7–14 post infection, but interestingly, the virus became undetectable in milk by day 21 (256). However, this phenomenon has not been observed in lactating African green monkeys and sooty mangabeys. Cell-associated SIV in chronically-infected African green monkeys is 1-log lower than in chronically SIV-infected rhesus macaques (261). This is likely due to the lower level of CD4+ T cells found in breast milk of SIV-infected African green monkeys, but it is unclear whether these lower levels of cell-associated SIV influence transmission rates. It should be noted that there was no difference in the level of cell-free and cell-associated SIV between blood and breast milk in African green monkeys.

Second, the maternal breast milk or infant oral microenvironment, including innate and adaptive immune responses, may be less favorable for transmission in natural hosts. Analysis of human milk and saliva has revealed numerous factors that inhibit HIV replication *in vitro*, including lactoferrin, SLPI, and mucins (see 'Innate and adaptive oral mucosa immunity to HIV' section). Other milk constituents, such as sTLR2 (171), CCL4 (172), and IL15 (174), correlate with reduced MTCT in epidemiologic studies. An exhaustive characterization of potential inhibitory factors in breast milk of natural hosts has not been performed, and, to date, there have been no studies of salivary inhibitory factors in natural hosts. However, we have analyzed the ability of breast milk collected from both sooty mangabeys and macaques to neutralize the infectivity of SIV and found a significant inhibition of infection with breast milk from both species. However, there was no difference between milk from the natural vs. non-natural hosts (unpublished). It has been proposed that African green monkeys have higher breast milk levels of neutralizing antibodies than rhesus macaques (261). This potential protective mechanism has not been tested in other natural hosts. However, given that cell-free virus levels are similar in the milk of natural and non-natural hosts, it seems unlikely that the milk of natural hosts contains potent inhibitors of SIV replication.

Successful HIV/SIV transmission via breast milk requires adequate target cells at infant GI mucosal tissues. Pandrea *et al.* (262) have demonstrated that the level of $CD4^+CCR5^+$ T cells in adult GI mucosa is extremely low in five different species of natural hosts, and that expression of CCR5 in adult sooty mangabeys is restricted to the effector memory populations (263). In infant mandrills, the percent of circulating $CD4^+CCR5^+$ T cells was less than 0.5% (256). We have extensively analyzed infant sooty mangabey target cells from multiple sites along the GI tract, including oral mucosa, esophagus and tonsils, and found a paucity of $CD4^+CCR5^+$ T cells (<10%) (authors' unpublished data). In recent work, both esophageal and jejunal $CD4^+CCR5^+$ T cells were remarkably few in young African green monkeys, but increased with age (264). In the same work, intrarectal SIV infection of African green monkeys was found to be dependent on the level of $CD4^+CCR5^+$ T cells at the site of exposure, with a significant positive correlation between the percentage of $CD4^+CCR5^+$ T cells and the number of transmitted founder viruses. These data suggest that a critical threshold for establishment of infection may not be reached in natural host infants exposed to SIV via the oral route.

In the rare sooty mangabeys who become infected via vertical transmission as well as experimentally infected neonatal African green monkeys, viral loads are 1–2 logs lower than those seen in animals who become infected later in life by the horizontal route (254, 265). This is particularly striking given that neonates and infants have a significantly higher percentage and absolute number of $CD4^+$ T cells compared to adults and further underlines

the important role of target cell restriction in determining the outcome of neonatal exposure to SIV.

By looking at both inflammatory conditions and natural hosts, it becomes clear that the mucosal immune environment, especially mucosal target cell levels, predicts HIV/SIV susceptibility. Increased mucosal inflammation in infant and adult oral mucosa facilitates the establishment of HIV and SIV infection. Evidence suggests that increased HIV target cells in inflamed oral mucosa paired with an increase expression of the HIV/SIV co-receptor, CCR5, results in increased infection of HIV target cells in the oral mucosa (Fig. 1, left). On the other hand, SIV natural hosts, whose infants have a dramatically lower risk of SIV acquisition, have lower levels of target cells in GI mucosa. Of particular importance is the paucity of $CD4^+CCR5^+$ T cells, suggesting that these cells are particularly important in facilitating HIV/SIV infection (Fig. 1, *right*).

Interventions

Antiretroviral therapy has significantly reduced the morbidity and mortality associated with HIV infection, but there is still neither a cure nor a vaccine for this disease. In this context, MTCT of HIV represents an enormous health care problem, with nearly 300,000 new pediatric HIV infections per year (3). Of these cases of HIV MTCT, almost half can be attributed to breastfeeding (27). In developing countries that are most affected by the AIDS epidemic, formula feeding, which can effectively eliminate HIV breast milk transmission, is neither safe nor affordable. Maternal and infant antiretroviral prophylaxis reduces the rate of transmission via breast milk to as low as 1–2% risk of transmission, but these therapies are associated with high cost, infant toxicity, viral resistance in both mothers and infants and unknown long-term consequences. Therefore, it is crucial that alternative interventions be developed to protect infants from HIV acquisition via breast milk (Table 3).

There have been a number of studies of extended infant postnatal prophylaxis to prevent breastfeeding transmission of HIV, testing the efficacy of a variety of different drug regimens (266). These studies have shown comparable efficacy to maternal antiretroviral prophylaxis during the breastfeeding period. Current WHO guidelines recommend daily nevirapine to breastfeeding infants until 1 week after breastfeeding has finished for infants born to mothers who are not on a triple antiretroviral regimen (Option A) or daily nevirapine or zidovudine for 4–6 weeks for infants born to mothers who are on a triple antiretroviral regimen (Option B) (5). An interesting question has been raised recently with the description of an infant ‘cured’ of HIV, following initiation of highly active antiretroviral therapy at birth (267). While this particular infant was not breastfed, one wonders whether the use of highly active combination antiretrovirals during breastfeeding may reduce reservoir establishment and viral latency in infants infected in the late postpartum or intrapartum periods.

Interventions to reduce mucosal immune activation in breastfed infants would be a welcome addition to the prophylactic antiretrovirals currently in use. WHO guidelines that encourage HIV-infected women to exclusively breastfeed their infants for 6 months, if replacement feeding is neither safe (due to contamination of water or food) nor readily available, likely result in a reduction in infant mucosal inflammation by delaying the introduction of solid foods (5). However, mixed feeding is a biological necessity, since all infants must at some point be weaned. Therefore, if we can identify specific exposures in mixed fed infants that result in increased mucosal immune activation, we may be able to reduce the risk of HIV acquisition associated with mixed feeding. Alternatively, by understanding the biological mechanisms underlying mucosal immune activation in the oral mucosa, immunomodulating

therapeutics can be developed to counteract the mucosal immune activation induced by mixed feeding.

Another approach that may be able to supplement current antiretroviral therapy is treatment of maternal breast milk to reduce its infectivity. Many sterilization and filtration processes have been devised that can effectively remove or reduce HIV viral infectivity (268–273). Unfortunately, most of these technologies require tools not commonly available in households in developing countries or are prohibitively expensive. In addition, some of the immunologic benefits of breast milk may be lost with aggressive treatment (274), potentially reducing the benefit of breastfeeding compared to replacement feeding.

An effective HIV vaccine for newborns ideally would be given at birth with extremely high safety and proven efficacy to reduce HIV acquisition and/or modulate disease progression should HIV infection occur. Indeed, passive immunization of neonatal rhesus macaques can provide protection against oral SIV infection (88, 204), highlighting the potential of vaccines to induce potent antibody responses for prevention of oral HIV infection in newborns (88, 205, 275). DNA vaccines that express specific viral antigens may be useful in the setting of neonatal immunization due to their generally lower safety concerns and ability to induce both humoral and cellular immunity. A study from Van Rompay *et al.* (276) demonstrated that systemic administration of a canarypox virus vector-based SIV vaccine (ALVAC-SIV) or modified vaccinia virus Ankara (MVA-SIV) expressing Gag, Pol, and Env did not show protection from infection but did result in reduced viremia, following oral SIV challenge in infant rhesus macaques. Other work has tested oral vaccination with a vesicular stomatitis virus based SIV vaccine (VSV-SIV) expressing Gag, Pol, and Env followed by a boost with intramuscular immunization of MVA-SIV, but this also failed to provide protection against oral SIV challenge despite robust antibody and cell-mediated immune responses at both systemic and mucosal sites (277, 278). Thus far, none of the potential vaccine candidates have shown high efficacy in preventing SIV infection of infant macaques.

The development of HIV vaccines to prevent oral transmission through breastfeeding in infants may be more difficult compared to other routes of transmission, due to the potential for frequent, high dose HIV exposure over months to years as well as the infant's immature immune system. More studies are needed to better understand infant immunity, the interaction between HIV and maternal immune modulators in newborns, and perhaps how to utilize our knowledge of the protection from oral transmission in natural hosts to impact the lives of children born to HIV-infected mothers.

Conclusions

The mucosal immune environment is a key determinant for a host's susceptibility to HIV. The studies presented here evaluate our current knowledge of HIV oral transmission in both infants and adults, the use of SIV models in understanding early immune events, oral immune factors that modulate HIV/SIV susceptibility (including mucosal inflammation), and interventions that may impact oral HIV transmission rates. One take-home message from these numerous studies is that understanding the mechanisms that underlie the recruitment of target cells to the GI tract will likely lead to a better understanding of immune modulations that can protect both infants and adults. It is likely that such studies of basic mechanisms of mucosal HIV transmission (both at the oral and other sites) will be necessary to provide a foundation for the development of immune therapeutic and vaccine strategies that can effectively protect both infants and adults from HIV transmission.

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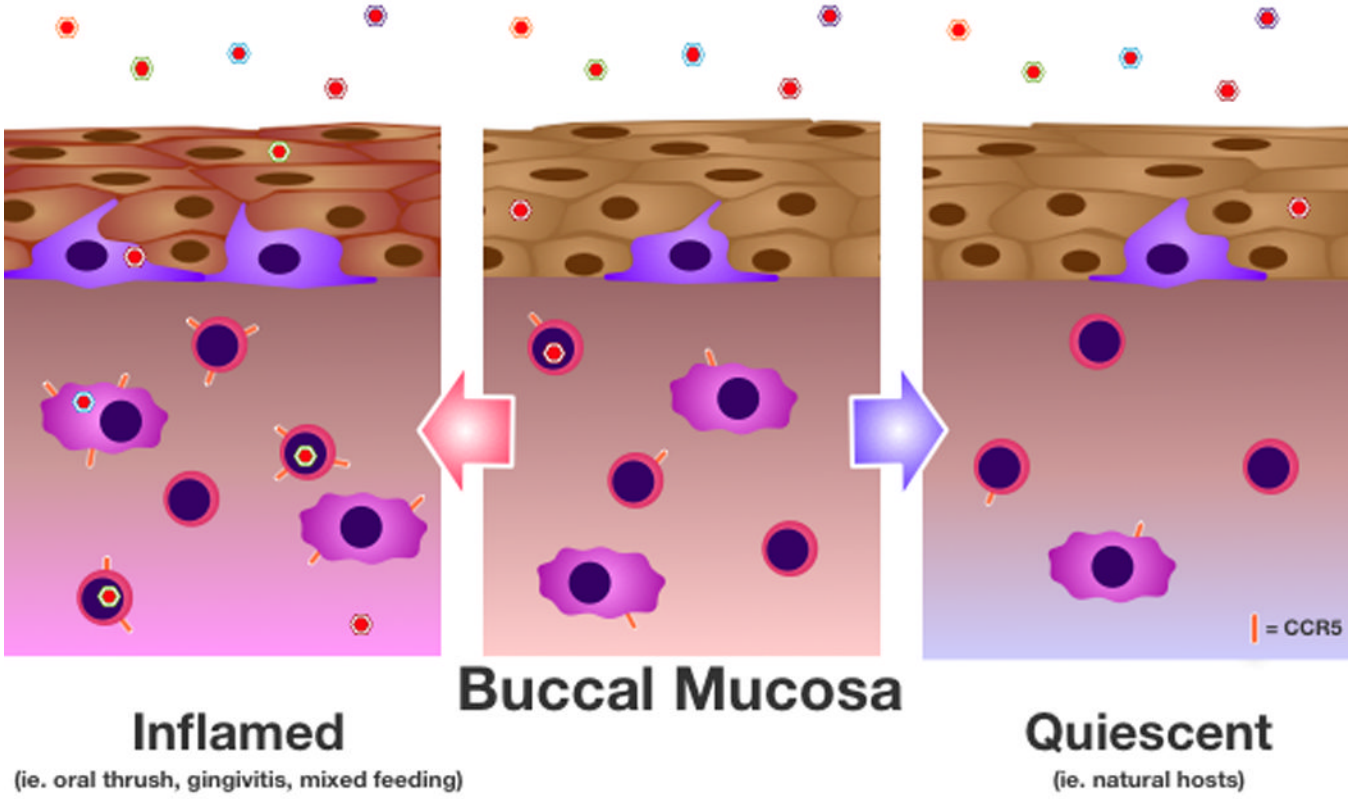

Fig. 1. Oral mucosa immune activation and HIV susceptibility

The buccal mucosa, non-keratinized stratified squamous epithelium that lines the inner cheek, is shown during exposure to an HIV/SIV quasispecies (variation in virus color reflects the variability in viral genetic sequence) under inflammatory (left side) and immune quiescent (right side) conditions. HIV/SIV can cross through the epithelium either through transcytosis or by migrating between disrupted cell-cell junctions, which can occur in response to inflammatory stimuli. This disruption of tight junctions results in an increase in the number of viral variants that successfully enter the *lamina propria*. Langerhans cells, which facilitate virus transfer to productively infected cells, reside in the basal layer of the epithelium and may increase in response to inflammatory stimuli. $CD4^+$ HIV/SIV target cells of the lamina propria, primarily macrophages and T cells, can express variable levels of the HIV/SIV co-receptor CCR5. Notably, CCR5 expression is enhanced, particularly on T cells, under inflammatory conditions, while CCR5-expressing cells are rare in quiescent mucosa. CD4+ cells with high CCR5 expression are most readily infected with HIV, resulting in more productively-infected cells in inflamed conditions and a paucity of infected cells in quiescent conditions. These infected cells then produce additional virus and facilitate dissemination of the virus throughout the systemic circulation, leading to the establishment of productive HIV/SIV infection.

Table 1

Macaque Studies of Oral SIV Transmission

Finding	Virus strain	Route of Virus Exposure	Publication Date	Reference
Adult macaques can be orally infected by cell-free virus alone	SIVΔB67	Drip with swallowing; variable dose	1996	(22)
Infants susceptible to oral SIV, and protected by prophylactic ART	SIVmac251	Drip with swallowing; high dose	1998	(88)
SIV can initiate infection via tonsils	SIVmac251	Direct application to tonsils; variable dose	1999	(89)
Attenuated SIV prevents SIV infection, implicating γδ T cells in protection	SIVmac251	Direct application to tonsils; high dose	2004	(90)
Oral SIV establishes infection in tissues proximal to the stomach	SIVmac251	Drip with swallowing; high dose	2004	(24)
Rapid induction of early innate genes in infants in tissues proximal to stomach	SIVmac251	Drip with swallowing; low dose	2006	(91)
Rapid induction of early innate genes in adults in tissues proximal to stomach	SIVmac251	Drip with swallowing; high dose	2007	(25)
Low dose challenge delays early innate gene activation	SIVmac251	Drip with swallowing; low dose	2011	(93)
Mucosal disruption (acetic acid) increases SHIV oral transmission	SHIV-R5 clade	Drip on site of acetic acid treatment; low dose	2010	(87)
Mucosal inflammation (gingivitis) does not increase oral SIV transmission, but does impact immune activation following SIV infection	SIVmac251	Drip on site of gingivitis; low dose	2013	(238)

Table 2

Anti-HIV Soluble Saliva Factors

Anti-HIV Factor	Mechanism of Action	Concentration in Saliva	Reference
Alpha-Defensins	Unknown	2715.2±1333.4 ug/mL	(183, 187, 192–194, 279)
Beta-Defensins	Inactivate transcytosed virus; Reduce CXCR4 expression	hBD2: 1.2–21 ng/mL; hBD3: 50–931ng/mL	(112, 190, 191,280)
CCL4	CCR5 binding	800ng/mL	(172, 281)
Cystatin	Unknown	1–2ug/mL	(154)
Erythropoietin	Unknown	Present	(173, 282)
IL15	Unknown	Present	(174, 283)
Lactoferrin	Bovine form blocks DC-SIGN binding; Human form unknown	6–30ug/mL	(154, 168–170)
Long Chain Fatty Acids	Unknown	Present	(175, 284)
Lysozyme	Unknown	8–16ug/mL	(154)
Mucins	HIV aggregation; Strip gp120 from virus; DC-SIGN binding (Muc1)	1.9mg/mL	(162–165, 285)
Salivary Agglutinin (gp340)	Unknown	Present	(166, 167)
Secretory Leukocyte Protease Inhibitor (SLPI)	Annexin II binding; Inactivates transcytosed virus	4–24ug/mL	(112, 154, 169,176–179, 181)
sTLR2	Unknown	Present	(171)
Theta-Defensins (retrocyclins)	CD4, GalCer and HIV Env binding	Unknown	(195–197)
Thrombospondin-a (TSP-1)	HIV aggregation; Binds CD4	1.1–12.8ug/mL	(160, 161)

Table 3

Current and Future HIV Interventions to Reduce Oral HIV Transmission

Intervention	Benefits	Challenges
Antiretroviral Prophylaxis	Proven efficacy Currently available for clinical use	Long duration of treatment Suboptimal adherence Drug resistance
Replacement Feeding	Proven efficacy Eliminates breast milk transmission	Increases infant mortality in developing settings Expensive Social acceptability
Exclusive Breastfeeding	Proven efficacy	Substantial HIV transmission Necessity to introduce solid food
Maternal Breast Milk Treatment	Effective at inactivating HIV	No clinical trials to date Expensive May remove beneficial components of breast milk
Infant Immune-Modulating Therapeutics	Permit safe use of multiple feeding methods	Target not yet identified Potential to modulate susceptibility to other pathogens
Vaccine	Block HIV transmission via multiple routes, including oral transmission	Only one trial in adults showed any efficacy Efficacy of current vaccine is very low