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THE MOUTH: A GATEWAY OR A TRAP FOR HIV?

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INTRODUCTION

Mucosal sites represent the primary routes of HIV transmission, yet the oral mucosa appears uniquely resistant to HIV-1 infection. Since the oral cavity is not conducive to either infection or transmission of HIV, characterizing the responsible resistance factors, particularly components of innate immunity, and their mechanisms of action may identify new opportunities for interference with viral acquisition at other mucosal sites. Although many of the innate immune factors described to date exhibit both antibacterial and antiviral activities, molecules that inhibit HIV-1 are largely HIV-1 specific. Nonetheless, if these oral innate factors can be identified, characterized, and harnessed, they may be useful in protection and/or intervention in acute HIV-1 infection. Endogenous molecules acting independently or synergistically, given their lack of substantive side effects, may represent an alternative novel approach for reducing HIV risk and suppressing infection. While HIV infection via breast milk does involve oral trafficking and is an important area of study, it will only be briefly discussed here. The major emphasis of this article is on the oral cavity and salivary substances that can influence HIV infection

Epidemiology of oral transmission of HIV

More than twenty-five years into the global HIV/AIDS epidemic, it is well established that in addition to blood, the reproductive and/or rectal mucosae represent the primary portals of HIV entry in adults. There is less definitive evidence regarding whether other mucosal sites serve as viral conduits and in particular, uncertainty remains as to the possibility of transmission of HIV-1 via the oral entrance to the gastrointestinal (GI) tract. This is not a trivial concern, since despite the successes of combination therapy for HIV/AIDS, infection rates remain high, compounded by persistence of latent viral reservoirs, antiretroviral drug resistance, and the lack of an effective vaccine or potential cure. Although it is often accepted that oral transmission is of little or no consequence, we have failed to capitalize on that information to identify the mechanism(s) of protection at the oral mucosal surface. These mechanisms could provide clues for novel strategies of prevention/intervention at other more vulnerable viral entry sites.

Early in the epidemic, evidence of the presence of HIV-1 in the oral cavity suggested the possibility of transmissibility via this route [1,2]. Whereas in some early studies [3–7], oral transmission could not be established, a few studies implicated this route as a potential, even likely mode of transmission (e.g., [8]). In larger cohorts of individuals and in well-designed studies to monitor oral transmission, the rarity of HIV-1 infection by this route was substantiated [9]. Nonetheless, potential shortcomings in these studies, including accurately documenting the definitive route of infection, underscore our lack of certainty regarding the relative risk of oral transmission. (Reviews: [10,11]).

The low frequency of documented oral transmission of HIV-1 could reflect low titers of infectious virus in saliva. While high levels of HIV-1 RNA and antigens can routinely be measured in oral fluids, infectious virus was not detected in early studies attempting to isolate infectious virus from saliva [12,13] or more recently in large cohort studies, despite plasma viremia [14,15].

Breast milk as a conduit for HIV transmission

In contrast to transmission of HIV in adults, the oral cavity does represent a documented gateway for infectious HIV in postnatal vertical transmission. The risk of oral HIV transmission from mother to infant through breastfeeding is high (~16%) in the absence of antiretroviral therapy and correlates with duration of breastfeeding and with milk HIV levels [16]. Even if breast milk contains relatively small amounts of HIV RNA, it is consumed in large quantities (up to a liter daily) [17] with repetitive exposure to the oral cavity and gastrointestinal mucosa.

Factors influencing HIV transmission via breast milk

A number of factors may contribute to infant susceptibility to infection through the oral route, including a potentially overwhelming viral inoculum, an inadequately developed innate defense response, and/or limited concentrations of inhibitors, although infant salivary levels of secretory leukocyte protease inhibitor (SLPI) can contribute to reduced transmission [18]. Timing is critical and although high levels of immunoglobulins and innate defense molecules are present in colostrum [19], the inhibitory mediators dissipate over time, and shortened intervals of breastfeeding have been recommended to reduce risk [16].

Lipids and other components of breast milk may also impact on virus transmissibility, as exemplified by members of the whey acidic protein family, such as ps20 encoded by the WFDC1 gene, that promote infection [20]. Breast milk contains both cell-free and cell-associated virus and whether one of these is preferentially transmitted is not clear, although recent evidence suggests that cell-associated HIV may be more important than previously appreciated [21]. Consequently, while the virus does enter through the infant's oral cavity, it still remains to be conclusively determined where transmission occurs, since oral mucosa, tonsils, upper gastrointestinal tract and the less acidic infant stomach have all been invoked as mucosal targets. The underlying pathways and resistance/susceptibility factors in vertical transmission are however, outside the scope of this review.

Epithelial resistance factors to oral HIV transmission

The indication that oral transmission was rare, albeit not risk-free, led to a search for underlying mechanisms of protection that might be inherent in oral mucosa and attempts to determine if such mechanisms were unique to the oral cavity. The epithelium, representing the initial barrier to pathogens such as HIV, is structurally heterogeneous, and the oral mucosa may represent a more formidable physical barrier as compared to vaginal, cervical, or rectal mucosa [22]. HIV-1 is thought to interact with surface receptors to transmit through an intact mucosal surface. Unique aspects of the intact oral mucosa that may contribute to reduced accessibility to HIV-1 includes the reduced numbers of Langerhan's (CD1⁺, HLA class II⁺) and dendritic cells (DC),

oral epithelial cell IgG Fc receptors and epithelial HLA class II antigens and/or the location of these cells further from the surface in oral as compared to vaginal, cervical and rectal epithelia [22].

Mechanisms of transcytosis involving GalCer, DC-SIGN, mannose receptor, heparin sulfate and gp340 that promote transport of virus across epithelium have yet to be fully explored in oral epithelium in contrast to their known involvement in intestinal and genital mucosa [23–25]. Uncertainties exist with respect to epithelial access, identity, location and numbers of binding receptors, viral transport mechanisms, and target cell availability across different mucosae. Nevertheless, recent efforts have begun to make inroads into defining differences that may influence the balance of resistance to infection versus susceptibility [26–28]. Disruption of the epithelium, whether due to trauma, infections, inflammation, oral ulcers or other oral disease manifestations may enable direct access of the virus to target cell populations, and also may serve to recruit new populations of susceptible CD4⁺ targets.

Mucosal target cell availability

CD4⁺ and coreceptor⁺ target cell availability is crucial to successful infection by HIV-1, but CD4⁺ and CD8⁺ T cells are also instrumental in generating an immune response against the virus. A cohort of inter-epithelial lymphocytes (IEL) reside at the basolateral surface of the oral epithelium and other epithelial tissues along with infiltrating Langerhans' cells and antigen presenting DC. In this regard, regional differences in distribution have been noted with these cells being fewer and more distal from the lumen in the oral epithelium [22]. Upon encountering HIV-1, patrolling DC can present viral antigens to T cells, particularly enriched in the lamina propria, and then traffic to draining lymph nodes (LN) to prime helper T lymphocytes (Th). Whether DC and lymphocyte trafficking from oral mucosa to LN parallels that observed in the intestine [29] is less well defined. Notably, expression of the activated mucosal homing receptor ($\alpha 4\beta 7$) on infected CD4⁺ T cells has been shown to bind HIV-1 gp120 [30], setting in motion signals for cell-cell viral dissemination. Also understudied is whether or not the dramatic and rapid depletion of lamina propria CD4⁺ T cells that occurs consequent to HIV-1 infection in the gut [31] is operative in the oral mucosa associated lymphoid tissues (MALT) [32]. If so, this could accentuate vulnerability to oral pathogens and co-infections. There has been no examination of oral mucosa in HIV-1 highly exposed, persistently seronegative individuals or elite controllers (HIV-1 RNA below detectable limits) to determine if their mucosal resources are more competent in resisting HIV infection.

Protective innate host defense pathways

Mucosal surfaces are defended by innate and adaptive response mechanisms, the former being an evolutionarily conserved host defense mechanism that occurs rapidly following exposure to a pathogen [33]. How, and if, the epithelial cells, DC, macrophages, NK cells, endothelial cells and other cells involved in the innate response differ between mucosal sites is in need of exploration as their ability to sense and respond to HIV-1 may be anatomically site-specific. Generation of innate effector molecules such as lysozyme, defensins, SLPI, cytokines, chemokines and type I IFNs that elicit and mediate antiviral responses, including enhancement of intracellular apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminases [34–36], may contribute to effective antiviral activity in the oral cavity. The salivary glands, a fount of secreted molecules with enzymatic and protective functions [37], may offer a selective, albeit not perfect advantage in this anti-HIV response. In this context, besides providing immune components, an adult produces up to a liter of saliva daily to sweep incoming pathogens downstream into a less hospitable acidic environment.

Not to be ignored, the multitude of microorganisms in the oral cavity, including bacteria, fungi, viruses, and protozoa likely influence the host response to HIV. First, mucosal permeability,

altered by bacteria and their products, along with inflammatory cell recruitment and activation, and protease and cytokine generation influence susceptibility to infection. In this regard, pattern recognition receptors (PRR), notably Toll-Like Receptors (TLR), but likely also non-TLRs including C-type lectin receptors, retinoic acid-inducible gene I (RIG-I)-like receptors and NOD-like receptors that recognize pathogen associated molecular patterns (PAMPS) and activate the innate immune response, represent the host's first line of molecular defense against many infectious agents. Engaging TLR2 on DCs increases HIV-1 virion production and transmission to CD4⁺ T cells, whereas TLR4 activation induces type 1 interferon (IFN) which suppresses HIV-1 production and transmission [38], via antiviral IFN-stimulated genes. Infection with other viruses, including human herpes virus (HHV8) and CMV also influences HIV-1 replication more directly [39,40]. The absence or reduced colonization of certain microbes, as shown for vaginal lactobacilli [41], portends enhanced HIV-1 transmission, underscoring the essential contributions of the local milieu to HIV-1 vulnerability.

Pathogen triggering of innate immune mechanisms results in the release of numerous soluble factors into oral mucosal secretions, which were initially hypothesized to have antiviral activity when whole saliva was first shown to block infection of PHA-blasted T lymphocytes *in vitro* [42,43]. These observations fueled the search for salivary anti-HIV molecules.

Overview of anti-HIV-1 activities in human saliva

Since the initial reports by Fultz [42] that incubation of HIV-1 with human or chimpanzee saliva led to inactivation of the virus, there have been hundreds of reports of saliva and/or its components blocking HIV-1 infectivity *in vitro*. Here, we survey publications in the field and focus on two molecules that have consistently demonstrated potent inhibition of HIV-1 activity and for which likely mechanisms of action have been identified.

Salivary molecules reported to have anti-HIV-1 activity *in vitro* include lactoferrin, lactoperoxidase, lysozyme, thrombospondin, mucins, proline-rich proteins, defensins, secretory leukocyte protease inhibitor (SLPI), and gp340 (aka salivary agglutinin/DMBT1) (Table 1). Most of these proteins also have anti-bacterial activity and while these mechanisms have been explored in detail, little information is available on the specific anti-HIV mechanism of action. Table 1 provides possible anti-viral mechanisms of action reported. Of interest, most of these proteins are components of the innate immune system. In addition, there are reports that molecules derived from oral microbes inhibit HIV-1 infectivity [44] and also the possibility that since saliva secreted into the oral cavity is hypotonic [45], it could lyse enveloped viruses and thus contribute to HIV-1 inhibition when whole saliva is tested.

It may seem surprising that human saliva contains such a diverse array of anti-HIV-1 activities. In this regard, there are also a large number of anti-bacterial proteins present that utilize diverse mechanisms of action, and likely evolved in response to the vast microbial flora that inhabits and attacks the body through the oral route. The same rationale might apply to anti-viral molecules, as the oral cavity is exposed to a spectrum of potential viral pathogens, although the published reports of antiviral activity in saliva are largely confined to HIV-1. However, many other viral pathogens are effectively transmitted via the oral cavity including HSV, EBV, rabies and flu. It is also noteworthy that most of the agents listed in Table 1 have both anti-bacterial and anti-viral activity.

Many of the molecules identified in saliva as demonstrating anti-HIV-1 activity have only been reported in a single publication, however there are numerous reports of anti-HIV-1 activity by defensins, and this topic has been extensively reviewed elsewhere [46,47]. We focus here on two molecules that have generated a significant body of information including activity against multiple strains of HIV-1 with identified mechanisms of action; gp340, which inhibits HIV-1

by binding to the virus, and SLPI that inhibits HIV-1 by interacting with target cell membrane proteins.

Anti-HIV-1 activity of SLPI

Secretory leukocyte protease inhibitor (SLPI) is an 11.7 kDa polypeptide found in mucosal secretions that antagonizes HIV-1 infection [48,49]. This cationic secretory polypeptide is a member of the whey acidic protein (WAP) family, possessing 2 cysteine-rich WAP motifs each containing 4 disulfide bonds [50] (Fig. 1A). SLPI has potent anti-protease activity against serine proteases, particularly neutrophil elastase and cathepsin G. Additionally, SLPI represents a pivotal contributor to innate host defense against viruses, bacteria and fungi (reviewed in [6,19,51]), in addition to its ability to protect against leukocyte protease-mediated tissue damage at sites of inflammation and to influence cell growth and tissue repair [52–56].

Constitutively generated in salivary gland epithelium [57], SLPI is abundant in saliva (μg levels), and is a constituent of other mucosal secretions including bronchial, nasal and cervical secretions as well as seminal fluid [58]. Although a major secretory product of leukocytes in rodents, its production is less defined in humans [59], where the predominant source of SLPI is the epithelium. Levels of mucosal SLPI are influenced by infectious agents, being decreased by HSV [60] but increased by CMV, *Candida albicans*, bacterial vaginosis [61–63], and HIV-1 [64]. In addition, regulation by hormonal [65] and inflammatory stimuli [66] may impact on resistance to HIV-1 infection. Although oral epithelial and salivary levels are considered sufficiently high to inhibit HIV-1 infectivity ($\geq 100 \text{ ng/ml}$) even in the absence of additional stimulation, less SLPI is expressed in tonsil epithelium, where HIV-1 may potentially gain access [26]. Importantly, depletion of SLPI from saliva attenuates the antiviral potential of this fluid [49] and levels of SLPI in saliva, breast milk and vaginal fluids have been positively correlated to transmission risk [18,67,68].

Mechanism of HIV-1 inhibition by SLPI

The ability of SLPI to block HIV-1 infection was first demonstrated in primary human peripheral blood monocyte-derived macrophages in which rSLPI blocked infection by R5 HIV-1 strains [48,49]. Interruption of infection occurred if the macrophages were transiently exposed to SLPI at physiologic concentrations prior to exposure to HIV, and SLPI was found to bind with high affinity to macrophages [48,49]. These findings implied a cellular target, rather than a direct effect on the virus. The blockage was shown to be downstream of viral gp120 binding to CD4 and interaction with CCR5, but prior to reverse transcription [48], likely targeting viral entry or arrest of fusion. In search of a potential SLPI binding molecule, the calcium and phospholipid binding protein annexin A2 (Anx2) was identified as a unique macrophage membrane receptor [69] (Fig. 2A) which may also link to actin, providing a potential clue to a trafficking mechanism. Involved in membrane organization and endocytic activities, Anx2 is also found on early endosomes where it guides biogenesis of multivesicular transport intermediates destined for late endosomes through Tyr-23 phosphorylation [70]. Since the membrane association of Anx2 depends on its N-terminus and also on membrane cholesterol, this observation is relevant in light of new findings that HIV-1 does not enter the cell via exclusive HIV-1 *env* fusion with the plasma membrane following interactions with its cognate receptors. Rather, viral entry can involve cholesterol-dependent endocytosis and a rate-limiting GTPase (dynamin) dependent fusion with endosomes leading to post-fusion uncoating and cytosolic liberation of viral contents [71]. The membrane and endosomal localization of these events is consistent with an involvement of membrane Anx2 in a post-CD4/CCR5 uptake step [69] and with evidence that down regulation of Anx2 or blockade of Anx2 by SLPI results in decreased viral replication. By co-opting permissive intracellular endocytic machinery, HIV-1 may remain below the radar of innate host defense, antibodies and fusion inhibitors.

In its replicative cycle, HIV-1 assembles, buds and exits from CD4⁺ chemokine coreceptor⁺ cells and in so doing, it hijacks macrophage membrane components including phosphatidylserine (PS) into its own membrane; PS can then be preferentially detected by Anx2-expressing recipient target cells [69,72]. The ability of SLPI to bind to Anx2 blocks the PS-Anx2 dependent early host cell-virus interaction (Fig. 2A). Additionally, in macrophages, the virus replicates and reassembles in intracellular vacuoles [73] or late endosomes where Anx2 resides and interacts with the HIV-1 Gag precursor, p55^{Gag} [74] to promote viral assembly. While not yet examined, since SLPI is acid stable, it could survive in endosomal pathways to potentially interfere with HIV-1 during assembly, budding and release, thus playing a role as HIV-1 is trafficking into and out of host cells.

HIV-1 infection and replication in CD4⁺ macrophages proceeds along parallel, but dissociable pathways from CD4⁺ T cells. These include mechanisms of entry, fusion, nuclear import, viral transcription, and sites of assembly and budding [75] due, in part, to differential expression of virally co-opted proteins. SLPI also blocks infection of T lymphocytes [76,77] that do not express membrane Anx2 [69]. This finding may be explained by recent studies that identified scramblase 1, a membrane protein with properties linked to bidirectional movement of phospholipids across the plasma membrane, as a T cell binding molecule for SLPI [78]. Scramblase interacts with the C-terminal cytoplasmic domain of CD4, and SLPI competitively binds to this same CD4-binding region of scramblase to inhibit X4 HIV-1 infection (Fig. 2B) [78]. Consequently, SLPI has the potential to blunt early steps in the HIV-1 infection cycle of both T cells and macrophages.

Another potential mechanism by which SLPI could suppress HIV-1 is through its effect on NFκB activation, necessary for HIV-1 replication [79]. In addition to acting at the cell surface [69], SLPI can cross cell membranes to exert certain functions intracellularly, including blocking NFκB activation [80] and TLR signaling [81]. While the nucleus may represent a site of interaction of SLPI with NFκB, the much broader functional importance of potential crosstalk between SLPI and NFκB may reveal expanded anti-inflammatory actions relevant to HIV pathogenesis. The ability of SLPI to attenuate NFκB activation, suppress inflammation and antagonize proteolytic activity, as well as its inhibitory effects on co-infections, may contribute to dampening the effects of HIV. Based on its prior therapeutic use for cystic fibrosis without evident toxicity [82], SLPI may be an ideal microbicide candidate to interrupt the interaction of virus with host cells and thus provide novel strategies to prevent HIV-1 acquisition.

Anti-HIV-1 activity of gp340 (SAG)

Salivary agglutinin (SAG), a product of the Deleted in Malignant Brain Tumors 1 (DMBT1) gene that is also referred to as gp340 (Fig. 1B), has been recognized for many years as being involved in microbial aggregation and adhesion. Isolated from human saliva, SAG in solution is capable of binding to many bacterial species resulting in bacterial aggregation and subsequent clearance from the oral cavity [83]. Several bacterial receptors for SAG have been identified and cloned, and these have been reported to bind both carbohydrate [84,85] and peptide epitopes [86] on SAG. Of interest, when SAG is immobilized, for example on the tooth surface, it can lead to bacterial adherence and, in this scenario, perhaps promote disease [87].

SAG also binds to HIV-1, and blocks HIV-1 infection *in vitro* [88]. Prakobphol *et al* [89] using a proteomic approach demonstrated that SAG was identical to the previously identified and cloned lung Scavenger Receptor Cysteine-Rich (SRCR) glycoprotein gp340, which is a product of the DMBT1 gene. There is an extensive literature relating to DMBT-1 functioning as a tumor suppressor in a variety of epithelial derived tumors [90–92].

Members of the SRCR family include a wide variety of proteins that contain the highly conserved SRCR cassette consisting of ~100 amino acids and either 6 (Group A) or 8 (Group B) cysteine residues [93]. Members of the SRCR family contain from 1–20 SRCR domains. The 14 SRCR domains of gp340 contain 8 conserved cysteine residues and are separated by SRCR interspersed domains (SIDS) (Fig. 1B). In addition, there are 2 CUB domains and a zona pellucida (ZP) domain near the C-terminus [94]. Members of the Group B SRCR family are expressed in a variety of tissues (gastrointestinal tract, pancreas, mammary gland, prostate, lung, salivary gland, trachea, eye), and can be found either on the cell surface, or secreted into the mucosal fluids; they can be detected in saliva, tears, bronchial and vaginal lavage. Hohenester et al., [95] published a crystal structure of a single SRCR domain for the Mac-2 binding protein (M2BP), a tumor-associated antigen and matrix protein, as a template for the entire SRCR protein superfamily. A more complete description of the SRCR superfamily can be found in a number of recent reviews [93,96]. The multifunctional mechanism(s) by which a molecule can be both a tumor suppressor and a member of the innate immune system remains an intriguing enigma [109].

Gp340 in the oral cavity

Studies on salivary-derived gp340 demonstrated that the protein was present in both parotid and submandibular secretions and the anti-HIV-1 activity appeared to be acting on the virus, rather than the host cell [97]. Upon incubation with saliva, gp120 was “displaced” from the virion, suggesting that the envelope of HIV-1 was the target for gp340 binding [88]. On purification by HPLC, the active anti-HIV-1 fractions of submandibular saliva were observed to contain two high molecular weight glycoproteins, SAG and MG2 [88]. The anti-viral effects of SAG appear to be restricted to HIV-1 with little or no activity against HSV, adenovirus, SIV, or HIV-2 [97], but displaying a broad reactivity against all tested HIV-1 R5 and X4 isolates [98–100]. SAG is present in the saliva of most individuals tested, albeit at different levels, and based on twin studies, the level of antibacterial activity appears to be genetically determined [101]. The secreted molecule is a high molecular weight glycoprotein containing both O- and N- linked carbohydrates. Immuno-electron microscopic studies revealed high reactivity localized in secretory granules at sites distinct from amylase localization suggesting separate pathways for secretion of SAG and amylase. Modest labeling was observed on the surface of the Golgi and on salivary gland ducts [102–104].

BiaCore analysis of SAG interaction with immobilized gp120 demonstrated that binding was Ca^{++} dependent, and the protein bound to gp120 derived from diverse HIV-1 strains with K_d values ranging from 10^{-7} to 10^{-10} M, comparable to the binding affinity of gp120 to CD4. The binding site for SAG was shown to be distinct from the CD4 binding site on gp120, and indeed, prebinding of CD4 to gp120 enhanced SAG binding [99]. Utilizing a set of overlapping 15 amino acid peptides derived from the gp120 sequence immobilized on a 96 well plate, the binding sites on env for gp340 were determined. The primary site was localized to a conserved amino acid sequence (CTRPNYNKRKR) at the stem of the V3 loop of gp120 [99] (Fig. 3A); this sequence is conserved in most HIV-1 strains, but is less conserved in HIV-2 and SIV. Utilizing a clone of DMBT1 (obtained from Professor Holmskov, University of Southern Denmark, Odense DK) the entire molecule was expressed, as well as the N-terminal SRCR domain and the truncated gp340 protein expressed in mammalian cells demonstrated anti-HIV-1 activity *in vitro* [105]. The N-terminal SRCR exhibited characteristics similar to the entire gp340; namely it bound to the same gp120 sequences in a Ca^{++} dependent manner, and was effective in blocking infection with both CCR5- and CXCR4-tropic viruses.

Membrane bound and soluble gp340

Although gp340 is found in cervical-vaginal lavage, the levels are $1/100^{\text{th}}$ of those seen in saliva [106]. However, in contrast to oral cells, histochemical and immunologic studies of cell

lines and primary tissue obtained from the female reproductive tract have documented surface bound gp340 [107]. Cell lines obtained from human ectocervix, endo-cervix, and vagina [108] were treated with monoclonal antibodies to gp340 and analyzed by flow cytometry (FACS) and immunohistochemistry and demonstrated gp340 localized to the cell surface [107]. In contrast, FACS analysis of cells derived from the oral cavity lacked membrane gp340 since in these cells the molecule is localized primarily in secretory granules. To analyze the function of surface-expressed gp340, HEK-293 cells were transfected with a plasmid expressing full-length gp340, and the molecule was expressed on the cell surface. Cells expressing gp340 on their surface bound HIV-1 and were able to transfer the virus to CD4⁺ cells up to 4 days later. These effects were observed with vaginal and cervical epithelial cells but not with pharyngeal cells [107]. In a subsequent study monocyte-derived macrophages were found to express gp340 on their surface, and HIV-1 infection of these cells was blocked by the gp120 derived peptides previously identified as the binding site for gp340 [109]. This study also demonstrated that transient expression of gp340 in several cell lines (U937, A301, and Sup-T1) led to enhanced HIV-1 infection that could be blocked by the gp120 peptide responsible for gp340 binding. We have recently shown that gp340 can also be involved in mediating direct HIV-1 transcytosis from the apical to basolateral surface both with genital tract epithelial cells in culture and with explants of endocervical tissue [23]. Based on these observations, one could speculate that in the female reproductive tract, cell surface gp340 could serve to enhance HIV-1 infection by sequestering virions for future transfer to CD4⁺ target cells. Moreover, it could facilitate HIV-1 entry through the mucosal barrier similar to the mechanisms described for heparin sulfate [110].

The accumulated data on gp340 suggests that soluble gp340 (as it occurs in saliva) can bind to conserved sites on gp120 of HIV-1 and as a result, block viral infection. In contrast, gp340 localized on cell membranes (as seen in the vagina and cervix) can bind HIV-1 and present viable virus to CD4⁺ host cells, and transcytose the virus across an intact cell layer in the female reproductive tract. Thus, along with SLPI, soluble gp340 or inhibitors of its cell surface interaction with HIV-1 represents potential microbicide products that could be used to prevent vaginal or rectal infection by HIV-1

A summary of the properties of soluble and cell surface gp340 is shown in Table 2. In this review of the two most studied oral HIV inhibitors, SLPI and gp340, we were struck by a number of both overlapping and discreet aspects of these mediators of innate defense, as well as the impact of their milieu on their functional repertoire. Although SLPI and gp340 coexist in oral and genital mucosal compartments, the microenvironment in each of these compartments is unique and likely contributes to HIV susceptibility and resistance. To understand potential differences in the possibility of infection via the oral vs. genital or rectal routes we have focused on several considerations:

Physiologic—In the oral cavity there is continuous fluid and mediator production, which effectively sweeps pathogens away into a hostile acidic environment in the GI. The continual flushing and retrograde accessibility of infectious pathogens, coupled with anatomic differences in epithelial structure and function deter oral mucosal accessibility. Moreover, oral pH is conducive to maximum enzymatic activity while the female reproductive tract is less so, since the oral pH is 6–7 while the genital pH is ~4, but increases rapidly to ~7 with the influx of semen.

Molecular—Levels of soluble gp340 and SLPI are 10–100 fold higher in the oral cavity than in the female reproductive tract, and gp340, in particular, may define the oral cavity as a trap for HIV, while SLPI affords mucosal resistance. Another key factor may be that gp340 in the female reproductive tract is localized to the cell surface where it can sequester and transcytose virus, in contrast to its entrapment and clearance of HIV orally. It appears that other innate

molecules display a similar spectrum of activities in the oral environment compared to the reproductive tract.

The similarities discussed between SLPI and gp340 are summarized in Table 3.

CONCLUSIONS

Defining the earliest events in mucosal protection (oral) and/or viral penetrance and infection (genital and rectal) is key to understanding how HIV-1 successfully subverts innate immunity and how mucosal portals of entry might be altered to deter transmission. The mucosa is the primary target for vaccines and microbicides, and the disappointing outcomes to date underscore our need to better understand mucosal immunity. Since adaptive immune responses may be delayed and/or ineffective, a re-emphasis on the potential of harnessing innate immunity may suggest alternative approaches to prevention and treatment. Short of eradication, suppression of HIV-1 in the infected host remains a major goal of antiretroviral therapy, but the innate host response offers strategic avenues for the challenging, but preferable outcome of prevention. In this regard, two innate mediators, gp340 and SLPI, one of which acts on the virus and the other on the host cell may provide complementary actions to deter HIV-1 acquisition and productive infection. The active domains of gp340 and SLPI, and their viral and host targets could provide strategies for blocking HIV-1 infection at mucosal entry points.

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Figure 1.
Schematic of SLPI (A) and gp340 (B) structures, indicating conserved cysteines.

A Macrophage

B. T cell

Figure 2.
Diagram of SLPI mechanism of action in macrophages (A) compared to T cells (B).

A. Oral **B. Female Reproductive Tract**

Figure 3.
Diagram of soluble gp340 blocking HIV-1 infectivity in the oral cavity compared to membrane-bound gp340 binding HIV-1 in the female reproductive tract.

Table 1

Anti-HIV-1 agents and their mode of action

ANTI-HIV-1 AGENT	PROPOSED MECHANISM OF ACTION	REFERENCE
Lactoferrin	Binds to gp120 and blocks entry	[111–114]
Lysozyme	Peptide-disrupts virus or alters signaling pathways	[115]
Thrombospondin	CSVTCG peptide binds to gp120	[116]
Mucin	Binds and clumps viruses	[88,117,118]
Proline-Rich Proteins	?	[119]
α , β , θ Defensins	Bind to HIV-1 and CD4, multiple mechanisms	[120–122]
Lactoperoxidase	Free radical oxidation	[123]
Secretory Leukocyte Protease Inhibitor (SLPI)	Binds to annexin A2 in host target cell	[48,49,69]
gp340 (DMBT1, SAG)	Binds N-terminal stem region of gp120 V3 loop	[99,105]

Table 2**Properties of soluble and cell surface gp340**

Soluble gp340
<ul style="list-style-type: none">• RT-PCR demonstrated that predominant synthesis of gp340 occurs in salivary glands, trachea, lung, small intestine and stomach; other sites of synthesis identified include brain, testis, uterus, pancreas, mammary gland and eye.• Identified in saliva, tears, intestinal epithelial secretions, bronchial/tracheal lavage; lower amounts in cervical/vaginal lavage and in epidermis and hair follicles• Soluble gp340 demonstrates both anti-bacterial and anti-viral activity• gp340 purified from saliva, as well as recombinant gp340 inhibit HIV-1 infection• Binds to conserved amino acid sequence at the stem of the V3 loop
Cell surface gp340
<ul style="list-style-type: none">• gp340 has been observed on the surface of macrophages in blood, lung, intestine, apical surface of intestinal epithelial cells, cervical and vaginal epithelial cells; lesser amounts on the corneal and conjunctival surfaces.• Cell surface gp340 binds HIV-1, and bound virus can be transferred to CD4+ cells• gp340 on genital epithelial cells appears to promote both HIV transmission and transcytosis

Table 3

Similarities between SLPI and gp340

	SLPI	gp340
Secreted by epithelial cells	+	+
Found on mucosal surfaces throughout the body	+	+
Conserved disulfide bonds	+	+
Require Ca++ for binding	+	+
Demonstrate both anti-bacterial and anti-viral activity	+	+
Demonstrate tumor suppressor and/or enhancer activity	+	+
Increased in response to inflammation	+	+