Infectious Human Immunodeficiency Virus Can Rapidly Penetrate a Tight Human Epithelial Barrier by Transcytosis in a Process Impaired by Mucosal Immunoglobulins

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Mucosal surfaces are the main natural site of entry into the body for human immunodeficiency virus (HIV). Herein, an alternative mechanism for virus spread is described. The mechanism, which involves transcytosis of endosome-internalized HIV-particles, was generated by contact of HIV-infected cells with the apical surface of an epithelial cell line. Transcytosed viruses rapidly (in 20–30 min) access the serosal side of the epithelial barrier without infecting the epithelium itself. In turn, transcytosed HIV could infect host submucosal mononucleated target cells, and thus the infection could spread. An investigation was done to determine whether mucosal antibodies could block HIV transcytosis. Both secretory IgA (S-IgA) and IgG that were purified from colostrum from HIV-seropositive women impaired HIV transcytosis, irrespective of the level of the recombinant HIV envelope anti-gp160–specific activities in an ELISA. However, specific S-IgAs were more efficient than IgG. Therefore, mucosal-specific S-IgA to HIV-1 could be relevant to reducing infectivity of HIV-1 in corporeal fluids.

The gastrointestinal, anorectal, and genitourinary tracts are considered to be the major routes of natural infection for human immunodeficiency virus (HIV) [1]; however, the initial cellular targets of HIV in the first hours of infection have not been identified. The mucosae of the gastrointestinal, anorectal, and genitourinary tracts have a covering of polarized epithelial cells. The surfaces of the vagina, exocervix, prepuce, and anus exhibit a pluristratified organization, whereas the rectum, endocervix, and intestine are covered by a simple epithelial monolayer [2]. Polarized simple epithelial cells have a plasma membrane that is separated into clearly distinct domains by tight junctions: the apical domain, which faces the tract lumen, and the basolateral domain, which faces the serosal side and the internal milieu [3]. To achieve their polarized barrier functions, epithelial cells have evolved transcytosis, a characteristic pathway of membrane trafficking that allows selective and rapid transcellular transport from the apical to the basolateral pole of the epithelium [3, 4]. “Cargo” that undergoes transcytosis across the epithelium is encapsulated in transcytotic vesicles that prevent any contact between the cargo and epithelial cell cytosol.

The various secretions that transmit HIV to the gastrointestinal, anorectal, and genitourinary tracts (i.e., semen, cervicovaginal secretions, and colostrum) contain cell-free HIV particles and numerous HIV-infected mononuclear cells [1]. Chronically infected mononuclear cells contact the apical epithelial cell surface, there is an efficient and rapid budding of HIV virions at the contact site. In contrast to the cell-free HIV particles, these freshly released viruses are internalized into epithelial endosome-like structures. After several days, epithelial cell infection may proceed from these structures [5]. However, in vivo, epithelial cells have not been found to be consistently infected [6]. In the macaque, the targets infected 1 day after simian immunodeficiency virus (SIV) intravaginal inoculation are dendritic cells in pluristratified squamous mucosa or mononucleated cells located under the simple endocervix epithelia [7]. In these studies, the very first steps of infection (i.e., from viral contact with the mucosal luminal surface to infection of the target cells) remain unclear.

An In Vitro Model to Study the First Step of HIV Mucosal Penetration

Using an in vitro model, we investigated the early steps of transmucosal penetration across a tight epithelial monolayer that mimics simple epithelium. Intestinal (HT29, CaCo2, or I407) and endometrial (HEC-1) epithelial cell lines were cultivated in a two-chamber system [4] as a tight and polarized monolayer, as found in situ. The culture conditions optimized cells for polarity development and offered experimental independent access to both apical and basolateral medium [4, 8]. The apical pole of the epithelial barrier was exposed to mononuclear or CD4+ T lymphoid cell lines chronically infected with different HIV type 1 (HIV-1) or type 2 (HIV-2) isolates [9, 10] or with HIV primary isolates derived from peripheral blood mononuclear cells (PBMC) of HIV-seropositive individuals. Under these experimental conditions, HIV undergoing transcy-
HIV Transcytosis Generated by Cell-to-Cell Contact

Using this experimental system, we demonstrated that HIV particles were internalized by the epithelium after contact of HIV-infected cells with the apical surface of an epithelial cell line [11]. Next, we showed that internalized viruses crossed the epithelial tight barrier using transcytosis, the transcellular pathway. We also showed that the epithelial barrier remained intact during this transport, thus preventing any pericellular translocation of viruses or HIV-infected cells. The monolayer was considered to have integrity if it had a stable transepithelial resistance (130 ± 3 Ω cm² for the I407 cell line barrier) and a functional transcytosis pathway. The latter was shown through the limited transepithelial transport (i.e., the constitutive transcytosis of 14C-Inulin, a small molecule unable to use the pericellular pathway). After a 2.5-h experiment, 1.5% ± 0.2% of apically added 14C-Inulin was present in the basolateral milieu. Furthermore, to definitively preclude any pericellular translocation of infected cells, we prelabeled HIV-positive cells with a fluorescent dye. At the end of the experiment, fluorescent cells were undetectable in the basolateral chamber and in the monolayer below the tight junctional complex.

HIV transcytosis was a rapid (~1 h) and efficient mechanism that applied to viruses generated from both HIV-1 and HIV-2-infected cells. Furthermore and in agreement with in vivo observations, transcytosis did not require patent epithelial cell infection [1, 7, 12, 13]. Transcytosis occurred equally with HIV generated from mononucleated cell lines or with HIV derived from PBMC of HIV-seropositive patients. Of note, HIV transcytosis is not a property restricted to cell lines chronically infected by HIV laboratory isolates; it can also occur with PBMC infected by HIV primary isolates. Indeed, it is well established that HIV passage in immortalized cell lines acquires biologic properties that differ from primary virus isolated from activated PBMC. In this regard, many studies using only cell line-passaged viruses are being reevaluated.

HIV-1 is genetically classified into various subtypes that initially appeared to be geographically distributed according to characteristics for the viruses found in different countries or subcontinents. HIV-1 subtypes A and D are mainly found in Africa, subtype B is found in Europe and the United States, and subtype E is found in Asia. However, the specific biologic properties of these different subtypes are not well understood. We found that HIV-1 subtype E transcytosed with a higher efficiency than other HIV-1 subtypes. These data correlate with infectious secretions like sperm or milk from seropositive patients [15].

Molecular Mechanisms of HIV Transcytosis

What are the molecular mechanisms that govern HIV transcellular transport? We investigated both the involvement of the viral envelope glycoprotein expressed at the virus surface and its potential target cell receptors in transcytosis [11]. Antirecombinant HIV envelope gp120 antibodies and anti-galactosyl ceramide antibodies, the alternative HIV envelope receptor, both inhibited HIV transcytosis. In contrast, antibodies directed against CD4 receptors, the principal HIV envelope receptor, had no effect on transcytosis. This lack of inhibition was not surprising since, as is the case in situ, the epithelial cell lines that we used did not express CD4+ cells [2, 16, 17]. These data suggest that HIV transcytosis occurs through a receptor-mediated mechanism utilizing either HIV envelope gp120 or its alternate receptor galactosyl ceramide (or both). Galactosyl ceramide is a lipid that is enriched at the apical pole of various epithelial cells and in human colon [18], where it may form microdomains through hydrogen bonding. One may suggest that the conserved serine and threonine amino acid residues of the HIV envelope gp120 may transiently form hydrogen bonding with epithelial galactosyl ceramide, thereby inducing a tight binding between HIV particles and the epithelial barrier. Subsequent transport therefore may occur by receptor-mediated endocytosis.

Transcytosis of HIV Results in Infectious Virus

We next addressed the infectious capacity of HIV that undergoes transcytosis. Could such virions infect target cells such as human macrophages or CD4+ T lymphocytes [11]? When placed at the basolateral side of the epithelia, each of these mononuclear cell types was permissive to transcytosed HIV, indicating that mononuclear cells from the submucosa may be downstream targets of transcytosed HIV in vivo. Infection of these cells could in turn convey the virus to lymph nodes and allow its systemic propagation.

Molecular comparison by heteroduplex mobility assay of the virus population undergoing transcytosis with the viral apical input revealed that transcytosis resulted in a selection of HIV-1 variants. This specificity or selection mechanism may be of great importance for natural spread and for the development of HIV infection.

Cell-Free Virus Transcytosis Is Inefficient

In addition to HIV-infected cells, cell-free HIV particles are also detected in secretions from HIV-seropositive patients. However, these HIV particles are found in various quantities, and their infectious potentials do not correlate with the stage
HIV May Also Cross Mucosae by Several Mechanisms Other Than Transcytosis

HIV may penetrate mucosae (tight or loose) by mechanisms other than transcytosis of the epithelial barrier. These non-mutually exclusive mechanisms include (1) penetration by transcytosis through M cells, a mechanism suggested by cell-free HIV-1 studies on HIV-infected nonpermissive rabbit M cells [21] but not confirmed by morphologic studies on intestinal biopsies from seropositive patients [1]; (2) penetration by transport through pluristratified loose epithelia by Langerhans’ cells [13] infected 2 days after cell-free SIV infection in the macaque model [7]; and (3) through trauma that gives direct access of infected cells or cell-free virus to the blood [22]. It is also clear that in situ the access and transmission of HIV-infected cells to the apical surface may be complicated by the specific luminal microenvironment (e.g., mucus, glycocalix) [23]; however,
mononuclear cells are well equipped with various cell adhesion molecules to bypass these obstacles [24].

Our working model is summarized in figure 1. HIV generated by cell-to-cell contact from either of the two major cell types that carry infectious HIV can cross an intact, polarized human epithelial monolayer. HIV transmission across a tight simple epithelial barrier by transcytosis may represent a rapid and efficient mechanism for sexual transmission of HIV.

Mucosal Secretory IgA (S-IgA) and IgG Antibodies to HIV Envelope gp160

Most immunoglobulins that bathe the mucosa at secretory sites consist of IgA polymers that have been actively transported across the epithelial barrier after binding with the transmembrane polyimmunoglobulin receptor [25]. S-IgA is released in the lumen, where it is involved in the immune barrier that protects against the entry of pathogens into the body [26]. S-IgA is not the sole mucosal immunoglobulin, since various proportions of IgG are also found in colostrum and in cervicovaginal secretions. IgG from these secretions is believed to be serum derived but could also originate from local synthesis [27, 28]. The role of IgG in mucosal defense remains unclear. Little is known about the humoral mucosal immune responses in immune deficiency states. The protective role of S-IgA and of IgG in the prevention of infections remains to be elucidated, as does their specific composition in vaccine immunization strategies. S-IgA and IgG antibodies to HIV have been reported in nearly all external secretions. Various results have been presented—some with unaltered and some with decreased S-IgA-specific responses in the secretions of HIV-infected subjects [29–31].

To compare the ability of S-IgA and IgG to interfere with HIV mucosal penetration, we first evaluated the antibody carbohydrates of *Streptococcus sobrinus* in 5 colostrum samples from HIV-seropositive women [32]. *S. sobrinus* is a cariogenic species that induces a widespread chronic stimulation of the mucosal-associated lymphoid tissue (table 1). The specific activities of both IgG and S-IgA varied greatly between the individual samples and the antigen, as evaluated by ELISA. The specific activity of IgG to recombinant HIV envelope gp160 was always greater than that of S-IgA. In contrast, the S-IgA activity to carbohydrates was 3- to 20-fold greater than the IgG activity in all colostrum samples tested. Similar results were obtained with paired S-IgA and IgG purified from cervicovaginal secretions of HIV-seropositive women [32].

### Table 1.

Activities and specific activities of secretory IgA (S-IgA) and IgG to gp160 and to *Streptococcus sobrinus* cell wall carbohydrates (CHO) in colostrum (Col) samples from HIV-1-infected women.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Anti-gp160</th>
<th>Anti-CHO</th>
<th>(SE) Inhibition of transcytosisb</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S-IgA IgG</td>
<td>S-IgA IgG</td>
<td>%</td>
</tr>
<tr>
<td>Col-20</td>
<td>3.3 (6) 100 (6)</td>
<td>ND ND</td>
<td>81.5 (6) 69 (9)</td>
</tr>
<tr>
<td>Col-48</td>
<td>36 (120) 1400 (1300)</td>
<td>112 (50) 19 (2)</td>
<td>69 (5) 47 (5)</td>
</tr>
<tr>
<td>Col-63</td>
<td>14 (14) 320 (120)</td>
<td>115 (20) 35 (2)</td>
<td>83 (9) 53 (3)</td>
</tr>
<tr>
<td>Col-64</td>
<td>139 (320) 654 (340)</td>
<td>776 (200) 211 (10)</td>
<td>82 (5) 54 (5)</td>
</tr>
<tr>
<td>Col-84</td>
<td>21 (20) 625 (1500)</td>
<td>368 (30) 18 (2)</td>
<td>67 (7) 51 (8)</td>
</tr>
<tr>
<td>Normal Col (pool)</td>
<td>0 0</td>
<td>772 (600) 35 (20)</td>
<td>0 (5) 0 (6)</td>
</tr>
</tbody>
</table>

All specimens except normal pooled Col were HIV positive.

*a Antibody data are specific activities expressed as antibodies in arbitrary units (AU)/total S-IgA or total IgG in mg; data in parentheses are antibodies in AU/mL. ND = not determined.

*b Inhibition of HIV-1 transcytosis by S-IgA and IgG in Col. Purified S-IgA or IgG (20 μg) were introduced to apical chamber 10 min before addition of HIV-infected lymphocytes. Transcytosis results are expressed as % of transcytosis inhibition compared with that of control, which consisted of nonspecific Col S-IgA and IgG. SE = standard error.
present in the apical medium. Thus, inhibition of transcytosis may occur at the mucosal surface prior to virus entry into the epithelium.

The clear difference between inhibition efficiency of S-IgA and IgG may also be explained by the dimeric structure of S-IgA. As a dimer of immunoglobulin molecules, S-IgA contains four antibody attachment sites that can confer to S-IgA a higher binding efficiency compared with that of the IgG molecule. Furthermore, the specific activities of the immunoglobulins were determined by ELISA, using a recombinant HIV envelope protein. This technique does not allow an analysis of conformational epitopes. In addition, in the present study, we did not map the epitopes of the colostrum immunoglobulins.

Some information, however, can be gained from our results. The patients that provided the colostrum samples were infected with HIV-1 subtype A. However, the immunoglobulins from the respective colostrum could neutralize transcytosis from the corresponding subtype as well as other HIV subtypes. This observation may suggest that the epitope(s) involved in HIV transcytosis neutralization are conserved throughout HIV subtypes. Such a hypothesis has been suggested for HIV cell infection neutralization, another type of HIV–target cell interaction [33].

Furthermore, the presence of anti-HIV S-IgA at mucosal sites has been correlated with protection from infection in HIV-exposed but uninfected individuals. Of note, the S-IgAs that protect against infection recognize HIV envelope epitopes located on the gp41 transmembrane subunit but not on the gp120 surface subunit or the gp160 precursor. In contrast, the S-IgAs of HIV-positive partners recognize mainly gp120 and gp160 molecules. These results point to a role for the gp41 HIV envelope subunit in mucosal protective immune responses [34]. Along these lines, preliminary experiments performed in our laboratory indicate that conserved epitopes on HIV envelope gp41 subunit located proximal to the transmembrane domain may participate in HIV transcytosis neutralization activity. In addition, the S-IgA displays a higher specific activity for this epitope in ELISA than do the paired IgG molecules. However, the S-IgA–recognized HIV-1 neutralization epitope(s) remains to be characterized. The fine mapping of these epitope(s) would provide additional information that is crucial to the design of an efficient local humoral immune vaccine against AIDS.

References