The interaction between human immunodeficiency virus type 1 (HIV-1) and primary mucosal cells isolated from normal human small intestine was investigated. Purified primary intestinal epithelial cells could transport cell-free HIV-1 to mononuclear cells, although the epithelial cells did not support viral replication. An unexpected finding was that primary intestinal macrophages were markedly less permissive to HIV-1 than were blood monocytes. The reduced permissiveness appeared to be due to the near absence of surface CCR5 on resident intestinal macrophages. Surface CCR5 could be up-regulated on the monocytes but not the intestinal macrophages by HIV-1 and gp120. Impaired permissiveness of intestinal macrophages to HIV-1 may play an important role in the low prevalence of HIV-1 mRNA-expressing macrophages in the lamina propria during HIV-1 infection in vivo. Characterization of the biologic properties of HIV-1 transport and infection in primary mucosal cells will be key to elucidating the pivotal role of mucosal surfaces in HIV-1 disease.

The mucosa is believed to play a fundamental role in the pathogenesis of human immunodeficiency virus (HIV-1) disease [1, 2]. Mucosal surfaces are the route by which HIV-1 enters the body in the vast majority of infected persons, excluding those who acquire the virus intravenously. The mucosa also may participate in the initial selection of the genotypic and phenotypic minor variants that are isolated from persons with acute HIV-1 infection. After the virus enters the lamina propria, either by transport across the epithelium or via circulating cells, the abundant numbers of resident mucosal macrophages and lymphocytes could serve as target cells for the virus. The presence of mucosal microorganisms or their products and the increased tissue levels of cytokines are potential sources of factors capable of activating HIV-1-infected mononuclear cells for virus expression. As local and systemic immunosuppression emerges, the mucosa becomes predisposed to a complex and diverse array of opportunistic pathogens. Herein, we review the local events involved in mucosal transmission, selection, and reservoir function in HIV-1 disease pathogenesis.

Mucosal Transmission of HIV-1

The gastrointestinal and genital tract mucosae are the surfaces through which HIV-1 is acquired in homosexual and heterosexual transmission, respectively. Less well appreciated is the role of the mucosa of the upper gastrointestinal tract in vertical transmission when the fetus or infant swallows HIV-1-infected amniotic fluid in utero, infected blood or cervical secretions intrapartum, or infected breast milk postpartum.

The mechanism(s) by which virus inoculated onto a mucosal surface enters the lamina propria is not known but may involve M cells, epithelial cells, or both. M cells are specialized epithelial cells that bind macromolecules and microorganisms, including certain viruses, to their apical surface and transport them by a nondegradative process to the basal surface for exocytotic delivery to interdigitating mononuclear cells. Murine and rabbit M cells can transport HIV-1 to underlying mononuclear cells [3], but whether human M cells are involved in the translocation of HIV-1 to the underlying organized lymphoid structures (Peyer’s patches) is not known.

Epithelial cell transport of virus is an alternative mechanism for HIV-1 translocation from the mucosal surface to the underlying lymphoid tissues, particularly in the genital tract mucosa, which does not contain M cells. The possible role of epithelial cells in the transport of virus to the diffuse lymphoid tissues in the lamina propria was recently suggested by elegant in vitro studies, in which HIV-1 released from peripheral blood leukocytes crossed an epithelial cell line barrier by a transcellular vesicular pathway [4]. The transcytosed virus productively infected mononuclear cells located below the barrier on the basolateral side of the epithelial cells. The ability of the cell lines to transport virus within 30 min suggests that the detection of epithelial cell transport of HIV-1 in vivo is unlikely.

Using our recently described technique for the isolation and purification of lamina propria macrophages [5], we isolated and purified primary intestinal epithelial cells and tested their ability to transport virus to mononuclear cells. Primary intestinal epithelial cells were exposed to HIV-1, washed free of virus, and...
then co-cultured with monocyte-derived macrophages, after which the culture supernatants were monitored for p24. The progressive increase in the levels of p24 in the macrophage culture supernatants suggested in these preliminary experiments that the macrophages had taken up and replicated virus that had been released by the epithelial cells (figure 1). The primary epithelial cells did not support HIV-1 replication, and control red blood cells and latex beads did not transport virus to the macrophages. Thus, primary intestinal epithelial cells also appear to be capable of transporting HIV-1.

**Mucosal Selection of HIV-1**

A high degree of genomic errors occur during viral replication in persons chronically infected with HIV-1, resulting in a mixture of virus species. Such variants display 90%–94% sequence homology, whereas HIV-1 isolated from acutely infected persons shows a nucleotide sequence homology of >99% [6]. The presence of such a high level of sequence homology in acutely infected persons suggests that a particular virus species among the population of variants in the chronically infected donor is selected by the recipient host after inoculation onto a mucosal surface. In one study, HIV-1 isolated from recent seroconverters with epidemiologically unrelated HIV-1 infections displayed complete sequence homology in the V3 loop region of env [7], which is associated with macrophage tropism in primary HIV-1 isolates [7, 8]. Similar selectivity appears to occur during vertical transmission [9].

In addition to genotypic selection, acutely infected persons show phenotypic selection, which is reflected in the homogeneity for the nonsyncytium-inducing (NSI), macrophage-tropic phenotype [6, 10]. The selection of genotypic- and phenotypic-(NSI, macrophage-tropic) restricted virus species during the interval between inoculation of virus onto a mucosal surface and seroconversion suggests that selection occurs in the mucosa. Moreover, since NSI, macrophage-tropic isolates use CCR5 as the co-receptor for virus entry, the above studies suggest that CCR5 may be involved in mucosal selection.

**Mucosa as a Reservoir for HIV-1**

After entry into the lamina propria, HIV-1 encounters an abundance of potential CD4+ target cells, including resident lymphocytes and macrophages and circulating lymphocytes and monocytes, which traffic through the mucosa. The subsequent redistribution of HIV-1–infected mononuclear cells by random or homing mechanisms likely accounts for the presence of infected cells in distant mucosal sites, such as the esophagus.

![Figure 1](image-url)  
**Figure 1.** Primary intestinal epithelial cells (referred to as epithelial cells on figure) transfer HIV-1 to target mononuclear cells. Primary intestinal epithelial cells, heat-killed epithelial cells, and epithelial cells treated with zidovudine (50 μg/mL) were exposed to HIV-1_{inu} (1000 TCID₅₀/mL) for 1 h, washed free of virus, and then added to cultures of primary blood monocytes. Every 4th day, monocyte culture supernatant was harvested for p24 determination. (Primary intestinal epithelial cells do not survive in culture beyond 24 h. In control experiments, red blood cells and latex beads did not transfer virus.) Results suggest that live but not dead epithelial cells and zidovudine-treated epithelial cells transfer HIV-1 to mononuclear cells.
As the largest lymphoid organ in the body, the intestinal lamina propria is also the largest repository of macrophages [12]. In this regard, we recently used in situ hybridization to identify HIV-1 mRNA–expressing mononuclear cells in esophageal mucosa [13]. Such cells were detected predominantly in HIV-1–infected persons with mucosal infections, appeared to be mainly macrophages, and had an overall prevalence of 0.06%.

Since macrophages play a fundamental role in HIV-1 disease pathogenesis [14], we investigated the biologic properties of HIV-1 infection in our purified primary intestinal macrophages [5], using well-characterized macrophage-tropic isolates of HIV-1 [15, 16]. The ability of lamina propria macrophages to support productive infection was demonstrated by the release of p24 antigen, the presence of proviral DNA in the cells, and the ability of zidovudine to inhibit infection. It was a surprise to find that the titer of peak p24 production was 2–3 logs higher in peripheral blood monocytes than in mucosal macrophages (figure 2). The low level of viral replication in the macrophages may contribute to the low prevalence of HIV-1 mRNA–expressing cells detected in the esophageal mucosa discussed above.

The marked reduction in the permissiveness of intestinal macrophages to HIV-1 was not due to the isolation procedure or differences in CD4 expression. Rather, the intestinal macrophages expressed almost no surface CCR5 compared with the monocytes [16] (figure 3). Both cell populations, however, contained comparable levels of CCR5 mRNA. Of interest, exposure of monocytes, but not intestinal macrophages, to HIV-1 (or gp120) led to increased surface expression of CCR5. Consistent with a previous report [17], exposure of the monocytes to bacterial lipopolysaccharide (LPS) resulted in diminished surface CCR5; however, exposure of the macrophages to LPS had no effect. Although the mechanism of the reduced CCR5 is under investigation, the above results suggest that the reduced HIV-1 infection by the intestinal macrophages is due to impaired permissiveness to HIV-1 entry associated with the near absence of cell surface CCR5.

Conclusion

The findings discussed above suggest the following sequence of mucosal events. Blood monocytes, which are CCR5+/CD14+, circulate through the mucosa where they encounter local inflammatory or chemotactic signals that direct their migration into the lamina propria. As they migrate, they en-

![Figure 2](image-url)
counter bacterial products, such as LPS, which activate the cells and cause down-modulation of CCR5 [16]. The migrating monocytes take up residence in the lamina propria and differentiate into macrophages, losing CD14 [5] by an unknown mechanism. LPS-induced reduction in surface CCR5 expression would reduce resident macrophage permissiveness to HIV-1 that had entered the lamina propria by transcytosis across the epithelium. In persons already infected with HIV-1, virus-infected CCR5<sup>-</sup>/CD14<sup>-</sup> monocytes migrating from the circulation into the lamina propria tissue would also encounter LPS, resulting in reduced HIV-1 expression, as previously reported for blood monocytes [18, 19], possibly through the release of C-C chemokines capable of suppressing HIV-1 infection [20]. Thus, LPS-induced down-modulation of CCR5 and HIV-1 expression could contribute to the relatively low prevalence (0.06%) of HIV-1 mRNA–expressing cells in the mucosal lamina propria, which could in turn contribute to the relatively low frequency (<1%) of virus transmission during mucosal exposure to virus.

References


