

may be related to the fact that lymphocytes are very sensitive to nutritional status because they do not store glycogen and rely on the import of extracellular glucose for their energy supply (La Cava and Matarese, 2004). From an evolutionary perspective, it may make survival sense to dampen immune responsiveness during periods of starvation in order to divert more energy to food gathering.

The new data provide a possible strategy for expanding regulatory T cells in vitro that retain suppressive activity. It will be of great interest to determine whether such cells can be used therapeutically in the treatment of autoimmune diseases. The results also open up new ways of thinking about therapies targeting leptin in vivo for the treatment of inflammatory and autoimmune diseases. However, in addition to the effects previously mentioned, leptin also influences hematopoiesis, angiogenesis, bone and lipid metabolism, insulin secretion, and the reproductive system. Consequently, in vivo treatments targeting such a broad-acting molecule present a high risk of unexpected and undesir-

able side effects, and considerable caution and more research are warranted. That said, it will be interesting, for example, to determine whether leptin might serve as a natural adjuvant in vaccinations. Used in a localized rather than systemic manner, leptin might simultaneously stimulate T helper 1 (Th1) responses while downmodulating regulatory T cells to produce uniquely potent T cell priming without producing substantial side effects. It might also be possible in the future to expand or contract antigen-specific subsets of regulatory T cells through the use of bivalent leptin-receptor agonists or antagonists. Another possible strategy to avoid side effects might be to use siRNA or other inhibitors to target downstream signal-transduction pathways in distinct cell types. Thus, the new findings on regulatory T cell and leptin biology reported by De Rosa et al. (2007) expand the context for discerning how regulatory T cell homeostasis might be perturbed in pathogenic responses and provide insights into how we might modulate responses therapeutically.

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## HIV's Vagina Travelogue

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Details of how HIV-1 is transmitted across mucosal barriers remain sparse. In this issue of *Immunity*, Hladik et al. (2007) describe an organ culture system for imaging HIV-1 interaction with vaginal epithelial T cells and Langerhans cells early after infection.

*Travelogue*: An (illustrated) lecture about places and experiences encountered in the course of travel; hence a film, broadcast, book, etc., about travel; a travel documentary.

(Oxford English Dictionary online: <http://www.oed.com/>)

The female genital tract is the primary route for heterosexual transmission of

the human immunodeficiency virus (HIV1, referred to in the text as HIV). On the basis of studies with macaques exposed to simian immunodeficiency virus (SIV), it is thought that during male-to-female transmission, virus in semen, either cell-free or cell-associated, penetrates the stratified squamous epithelium of the vagina or the columnar epithelium of the endocervix to infect cells within or below the epi-

thelium (reviewed in Pope and Haase, [2003]). This is followed by dissemination of the virus to lymphoid organs, particularly gut-associated lymphoid tissues (GALT), within the first few days after infection (Li et al., 2005). Despite intensive efforts to uncover mechanisms by which HIV traverses the mucosal barriers and establishes infection, our understanding of this process remains limited. It is unclear

which cells are the first to interact with and to be productively infected with the virus, whether there are environmental factors in the reproductive tract tissues that influence infection, and whether there are local immune-system responses directed at the virus. An understanding of these processes is critical for developing prophylactic microbicides and vaccines.

Some clues as to how HIV interacts with mucosal cells in the reproductive tract may come from studies on infection of GALT early after exposure of humans and macaques to HIV or SIV, respectively. Within days after vaginal exposure to SIV, there is massive viral replication and loss of CD4<sup>+</sup> memory T cells in the intestinal lamina propria of macaques (Li et al., 2005). Similarly, patients who had recently sero-converted were found to have profound depletion of lamina-propria CD4<sup>+</sup> T cells (Brenchley et al., 2004). Moreover, the intestinal lamina propria of patients treated successfully with highly active antiretroviral therapy (HAART) remained depleted of CD4<sup>+</sup> T cells despite recovery of circulating CD4<sup>+</sup> T cells to normal amounts (Gualdupe et al., 2003). There is as yet no explanation for this remarkable finding, although it may be due to irreversible damage incurred by the lamina propria during acute infection, such that T cells can no longer home to the intestine, or to persistence of virus in the face of HAART, perhaps because of unusual properties of the cells in the lamina propria, such as dendritic cells that can harbor virus in vacuolar compartments. Although the cervicovaginal mucosa does not have the density of lymphoid tissues that is found in the gastrointestinal mucosa, both contain an abundant number of effector and memory T cells that are likely to be important in providing protection from breaches in the mucosal barrier. In both tissues, the T cells are in close contact with numerous mucosal dendritic cells (DCs) that are involved in innate responses to commensal and pathogenic microorganisms and that may also have important roles in the dissemination of HIV and also in host responses against the virus. In the murine intestine, DCs are organized in a dense network and often extend

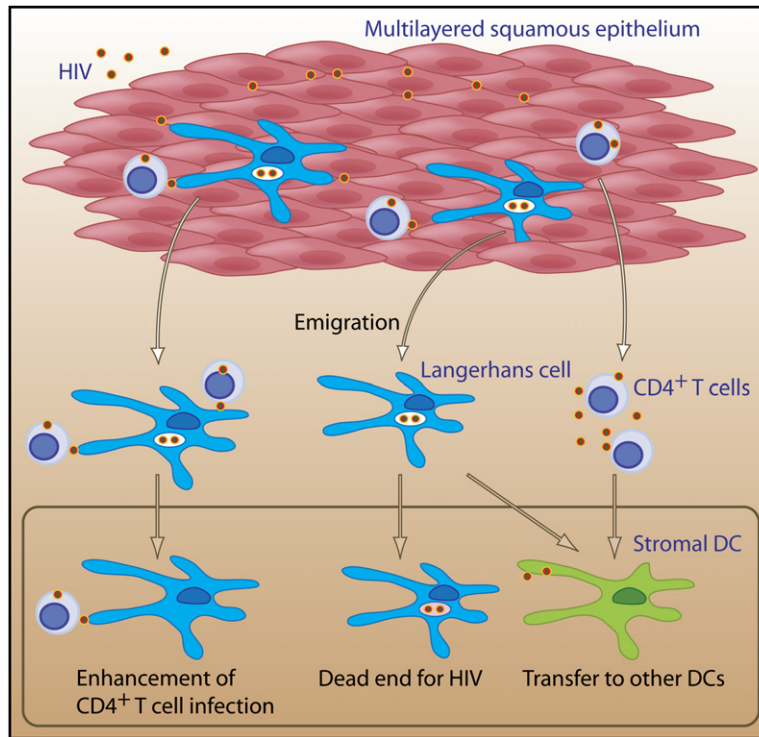
processes through epithelial tight junctions to sample the luminal environment. Langerhans cells (LCs), a specialized type of DC found in skin and vaginal epithelium, may function similarly within the stratified epithelium, and may thus provide access for HIV particles to the vaginal epithelium. Transcytosis or any breach in the epithelial barrier may also allow HIV particles to cross the epithelial barrier. The underlying stroma also contains cells known to interact with HIV, such as T cells, macrophages, and DCs.

Because it is not feasible to study acute events after heterosexual transmission of HIV in humans, research in this important area has relied on the simian model and also on *in vitro* infection of organ cultures established with cervicovaginal tissue from human surgical samples. In the vagina, the layers of stratified squamous epithelium and the mucus that they secrete form the outer component of the mucosa and a natural obstacle to infection. Analysis of intact human cervical-tissue organ cultures has shown that HIV can penetrate the stratified epithelium and infect memory CD4<sup>+</sup> T cells within several hours, but this process appears to be relatively inefficient, and some investigators have raised the possibility that LCs may have a key role early in infection. Because lymphocytes and LCs exit the epithelium within hours after establishment of organ cultures, it has been difficult to determine whether cells are infected within the epithelium proper or in the submucosal stromal tissue.

In this issue of *Immunity*, Hladik et al. (2007) describe a modified organ culture system that allowed for the separation of the epithelial sheets from the underlying stroma in human vaginal explants. This permitted analysis of cells that were infected exclusively within the epithelium, as well as examination of cells that migrated out of the epithelium into the culture medium. Most of the intraepithelial T cells expressed the surface markers CCR5 and CD45RO, denoting a memory phenotype. The intact epithelial sheets were exposed to HIV particles, whose interaction with resident immune cells was then examined. Through confocal microscopy to detect fluorescently la-

beled virions interacting with cells in the epithelium, approximately half of the CD4<sup>+</sup> T cells were found to have bound virions at 2 hr after infection. A majority of these cells had fluorescent signals on the cytoplasmic side of the plasma membrane, indicating that infection had occurred. Both binding and infection of CD4<sup>+</sup> T cells were dependent on CD4 and CCR5, the molecules that make up the viral receptor complex. HIV was also found associated with LCs at this early time point, but the virions were localized in a perinuclear intracellular compartment and replication was undetectable. The uptake of virus by LCs was only partially inhibited by antibodies specific for CD4 and CCR5, both of which are expressed at the surface of LCs. Inhibition of HIV binding to C type lectins by the use of mannan had very little effect, suggesting that molecules such as langerin, which are known to interact with glycans on the envelope glycoprotein of HIV, do not have a major or an exclusive role in viral internalization. This is consistent with the notion that HIV uptake into DCs can occur by multiple means. Virions within LC intracellular compartments were readily observed by electron microscopy for up to 3 days after infection. In contrast, intact virions were found only at the plasma membrane in T cells.

The authors did not observe obvious contact between LCs and CD4<sup>+</sup> T cells that were undergoing infection at 2 hr. The results therefore suggest that HIV directly infects T cells within the vaginal epithelium and that this process is not dependent on LC-mediated viral uptake or enhancement of infection *in trans*. This is an important point in light of a growing body of literature suggesting that numerous microorganisms are taken up by dendritic cells and may exploit these specialized antigen-presenting cells by hiding out in their intracellular vesicular compartments (van Kooyk and Geijtenbeek, 2003). It has been known for some time that DCs are relatively resistant to infection with HIV, yet they potently enhance infection of CD4<sup>+</sup> T lymphocytes *in trans* (Cameron et al., 1992). The significance of this process has yet to be demonstrated for HIV infection *in vivo*, and the mechanism for



**Figure 1. HIV Interactions within Vaginal Mucosa during Primary Infection**

The vaginal mucosal tissue includes a multilayered squamous epithelium and intercalated Langerhans cells (LCs) and intraepithelial T cells. Beneath the epithelium, the stroma contains dendritic cells (DCs), T cells, and macrophages. As described in Hladik et al. (2007), in organ cultures, HIV infected the intraepithelial CD4<sup>+</sup> T cells and was also detected in intracellular compartments of LCs. Upon emigration from the tissue, LCs and T cells formed complexes carrying HIV in the interface (left). The fate of the intracellular virions in the perinuclear vesicles of LCs is still unknown. They could be transmitted to T cells (bottom left), facilitating their infection, or they could be trapped and not transmitted (bottom center). Dendritic cells in the stroma could be infected by virus derived from either T cells or LCs (bottom right).

this enhancement has been the subject of considerable debate. Initial studies showed that virus-particle uptake by endocytic mechanisms is necessary for DCs to enhance infection of T cells, and this finding was supported by subsequent publications suggesting that virus is transmitted from multivesicular bodies in DCs to conjugated T cells across an “infectious synapse” (McDonald et al., 2003). Consistent with such an interpretation, fluorescently labeled HIV was found to localize preferentially toward the cell-cell interface in conjugates of T cells and LCs that had emigrated from vaginal explants at 60 hr of culture (Hladik et al., 2007). Thus, HIV that persists in vacuolar compartments in LCs may be involved in infecting CD4<sup>+</sup> T cells at later time points after exposure of the vaginal epithelium (Figure 1). However, this interpretation has been chal-

lenged by a recent finding that only virions bound to the surface of DCs are transmitted *in trans* to activated T cells, which suggested that viral particles within DC intracellular compartments are not competent to be released and infect T cells (Cavrois et al., 2007). If this is correct, then the virions observed by Hladik et al. in LCs after infection of vaginal epithelial explants may represent a dead-end by-product of infection (Figure 1). Recent studies have demonstrated that DCs have mechanisms that control acidification of endosomal compartments, ensuring that antigen taken up for cross-presentation to T cells is not degraded (Savina et al., 2006). The presence of intact HIV particles within membrane-delimited compartments may reflect this unique property of DCs, but additional studies are warranted to determine the fate and infectious compe-

tence of these virions, particularly *in vivo* in infected patients.

The elegant imaging studies of Hladik et al. highlight the potential importance of memory T cells and LCs as the first targets of HIV infection in the human vagina. The observation by electron microscopy of intracellular intact virions several days after infection in emigrated LCs, coupled with the finding of viral particles in LC:T cell conjugates, is consistent with the possibility that LCs catalyze CD4<sup>+</sup> T cell infection and/or promote transport of infectious particles to draining lymphoid organs. It therefore remains possible that the success of the virus in establishing a systemic infection depends on its early interaction with vaginal epithelial LCs, particularly when the amount of virus reaching the intraepithelial microenvironment is limited. Continued development of organ culture systems that closely mimic the *in vivo* environment will be essential for better understanding the earliest steps in HIV transmission.

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