

# Gastrointestinal Tract and the Mucosal Macrophage Reservoir in HIV Infection

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**The gastrointestinal tract (GIT) is a primary site for human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infection, replication, and dissemination. After an initial explosive phase of infection, HIV establishes latency. In addition to CD4 T cells, macrophages are readily infected, which can persist for long periods of time. Though macrophages at various systemic sites are infected, those present in the GIT constitute a major cellular reservoir due to the abundance of these cells at mucosal sites. Here, we review some of the important findings regarding what is known about the macrophage reservoir in the gut and explore potential approaches being pursued in the field to reduce this reservoir. The development of strategies that can lead to a functional cure will need to incorporate approaches that can eradicate the macrophage reservoir in the GIT.**

Macrophages are one of the most abundant immune cells in the gut (1, 2). Morphologically, gut macrophages are similar to most resident tissue macrophages, with a mononuclear shape and a granular cytoplasm, and they are highly phagocytic and microbicidal (3–5). They express major histocompatibility complex (MHC) class II, CD36, CD68, CD163 (6, 7), and CD209 (7) but have low levels of CD80, CD86, and CD40 (8–11). Unlike monocytes in peripheral blood that are largely CD14<sup>+</sup>, human mucosal macrophages in the gut have been shown to express a CD13<sup>+</sup> CD14<sup>−</sup> CD16<sup>−</sup> CD64<sup>−</sup> CD89<sup>−</sup> CD32<sup>−</sup> phenotype (12, 13). The expression of CD4 and CCR5/CXCR4 key receptors for human immunodeficiency virus (HIV) infection, on macrophage populations has been shown to differ based on the sites the cells reside in. Shen et al. (14) showed that vaginal macrophages expressed CD4 and CCR5/CXCR4 similarly to blood monocytes, whereas intestinal macrophages expressed little or no detectable CD4 and CCR5/CXCR4 (15–17).

Interestingly, under normal conditions or in response to Toll-like receptor (TLR) ligands, gut macrophages constitutively secrete anti-inflammatory cytokines, such as interleukin-10 (IL-10), rather than proinflammatory mediators, such as IL-12, IL-23, tumor necrosis factor alpha (TNF- $\alpha$ ), IL-1, IL-6, and interferon-inducible protein 10 (IP-10) (18–22), suggesting that intestinal macrophages may be critical for maintaining immune homeostasis in the gastrointestinal tract (6). Smythies et al. (23) reported that intestinal macrophages displayed significant inflammation anergy even though they had avid phagocytic and bactericidal activity. Depletion of mucosal macrophages was shown to be associated with increased susceptibility to colitis and graft-versus-host disease in mice (24–26). Likewise, altering the phenotype of macrophages to that of a proinflammatory phenotype as seen during HIV replication was associated with increased inflammation and tissue damage (27).

In addition to their role in maintaining mucosal immune homeostasis, gut macrophages, like dendritic cells (DC), have been shown to sample microbes directly from the intestinal lumen and transfer these antigens to DC for processing or transport to the draining mesenteric lymph nodes (28–32). Other studies have shown that intestinal macrophages could migrate to the lumen of the intestine and contribute to cell-mediated immunity against microbes in the gut (33).

These studies reveal an important role for gut macrophages in immunity and homeostasis in the intestinal mucosa. Given their relative abundance in the intestinal mucosa, susceptibility to HIV infection, and the extensive involvement of gut in HIV replication and dissemination, the role of gut macrophages as a viral reservoir in HIV persistence, like monocytes/macrophages in other sites (34–36), has been an area of intense study.

## THE GUT MACROPHAGE RESERVOIR IN HIV INFECTION

Macrophages, unlike CD4 T cells, are long-lived cells, resist the cytopathic effects of HIV infection/replication (37, 38), and are able to disseminate virus (39). Interestingly, unlike blood-derived macrophages, gut macrophages require about 2 to 3 log more HIV for infection (15). Likewise, Shen et al. (14) observed that gut macrophages were less permissive to HIV infection than vaginal macrophages, presumably because of lower levels of the CD4 and CCR5 receptors required for HIV entry and infection.

Interestingly, however, Zalar et al. (40) showed that both CD64<sup>+</sup> and CD68<sup>+</sup> gut macrophages in patients currently undergoing highly active antiretroviral therapy (HAART) had detectable levels of p24 and HIV DNA. Likewise, Josefsson et al. (41) found that myeloid cells isolated from the gut-associated lymphoid tissue (GALT) of HIV patients on antiretroviral therapy were positive for HIV. Yukl et al. (42) observed that infected myeloid cells in the gut accounted for ~4% of the total HIV DNA. Moore et al. (43) showed that mucosal macrophages isolated from the small intestinal mucosa of rhesus macaques either acutely or chronically infected with simian immunodeficiency virus (SIV) carried viral DNA, albeit at levels lower than memory CD4 T cells in the gut. These studies suggest that though gut macrophages were less permissive to HIV infection, they could readily support viral infection.

Surprisingly, studies have shown that though gut macrophages had detectable levels of HIV DNA, there was little or no detectable

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HIV RNA (14), raising the possibility that gut macrophages, unlike macrophages at other mucosal sites, such as the vaginal mucosa, likely support viral entry but not replication. In line with this argument, Shen et al. (17) showed that the inability of intestinal macrophages to support active viral replication was due not only to the low level of CD4/CCR5 receptor expression on gut macrophages but also the failure of these cells to activate NF- $\kappa$ B, which is an essential requirement for HIV transcription. Further evidence comes from studies that looked at the latent viral reservoir and rebound in plasma viremia after HAART was stopped. Chun et al. (44) reported that the rebounding plasma virus was genetically distinct from the cell-associated HIV RNA and the replication-competent virus within the detectable pool of latently infected CD4 T cells, suggesting that viral reservoirs other than CD4 T cells likely were a major source of viral rebound. Interestingly, however, Lerner et al. (45) showed that the GALT was not a major source of the plasma viral rebound seen after cessation of HAART, supporting the argument that infected non-CD4 T cells, such as the gut macrophages, likely were not productively infected.

In contrast to the studies cited above, Igarashi et al. (46), using rhesus macaques infected with the simian-human immunodeficiency virus (SHIV) mutant SHIV<sub>DH12R</sub>, showed that during late stages of viral infection, tissue macrophages exhibited significant productive viral infection, were resistant to the cytopathic effects of SHIV, and constituted the primary viral reservoir following the rapid and irreversible depletion of CD4<sup>+</sup> T cells. In another study, Matsuyama-Murata et al. (47) quantified proviral DNA and compared the mutation patterns of viruses in various secondary lymphoid tissues and peripheral blood from rhesus macaques that were infected with SHIV and developed an AIDS-like syndrome. They observed that the amounts of viral DNA were higher in tissues than in blood. Interestingly, the mutation patterns of viruses found in the plasma were most similar to those of viruses found in the jejunum and mesenteric lymph nodes, with >50% of SHIV-expressing cells being CD68<sup>+</sup> macrophages. These studies suggest that gut macrophages are not only capable of getting infected but may be an important site for productive viral replication.

The presence of viral DNA in gut macrophages but their apparent lack of permissiveness raises intriguing questions regarding how these cells get infected. A number of potential mechanisms have been proposed to explain these phenomena. Shen et al. (17) argued that the lack of permissiveness was likely due to the significantly lower levels of CD4 and CCR5 expression on gut macrophages and the presence of viral DNA within these cells was probably due to either low-level CD4/CCR5 receptor-mediated viral entry or endocytosis. Interestingly, however, Meng et al. (16) showed that macrophages in the lamina propria expressed detectable albeit low levels of CD4 but not CCR5 or CXCR4, suggesting that CD4-mediated endocytosis could potentially contribute to the infection of gut macrophages.

Others have suggested that gut macrophages might be infected prior to their migration into the gut. Smythies et al. (5) found that macrophages resident in the intestinal mucosa expressed high levels of IL-8 and transforming growth factor beta (TGF $\beta$ ) receptors but showed little or no chemotactic response to stroma-derived IL-8 and TGF $\beta$ . In contrast, blood monocyte-derived macrophages displayed similar levels of receptors but actively migrated in response to conditioned medium from the lamina propria extracellular matrix containing IL-8 and TGF $\beta$  produced by epithelial and mast cells. These observations suggest that intestinal mac-

rophages are predominantly derived from blood monocytes that migrate to the intestinal mucosa in response to chemotactic stimuli. Blood monocytes have been shown to express high levels of CD4 and CCR5 (14), are turned over at high rates (48), and support higher levels of HIV replication than differentiated macrophages (14).

Likewise, Allers et al. (6) found that macrophages were significantly enriched in the gut of untreated HIV patients. This was accompanied by a corresponding decrease in blood monocytes and increased expression of gut homing receptor  $\beta$ 7 on these cells, suggesting that mucosal homing blood monocytes may be a major source of macrophages that infiltrate the gut mucosa. Increased migration into the gut was accompanied by a 4- to 16-fold increase in the secretion of proinflammatory cytokines/chemokines like IL-1 $\beta$ , CCL5, CXCL9, and CXCL10. Jarry et al. (49) showed that the densities of CD68<sup>+</sup> and CD11c<sup>+</sup> macrophages were significantly increased in the duodenum of HIV-infected patients.

Interestingly, Maheshwari et al. (50) reported that intestinal stroma-conditioned medium suppressed the ability of macrophages to support HIV infection and p24 antigen expression; this suppression could be reversed by cytomegalovirus (CMV)-mediated induction of TNF- $\alpha$  that acted in *trans* to increase HIV infection. As such, gut macrophages from HIV-infected patients with CMV colitis were found to be highly supportive of HIV infection (51), raising the possibility that a change in the nature of gut macrophages to a proinflammatory phenotype may contribute to the increased permissiveness of these cells to HIV infection. Similarly, Allers et al. (6) suggested that increased infiltration of macrophages into the mucosa during HIV infection potentially promotes local inflammation that in turn could recruit more macrophages and contribute to the increased infection of these cells by HIV.

Once infected, a number of factors likely contribute to the persistence of the macrophage reservoir. Macrophages are long-lived cells that are resistant to the cytopathic effects of HIV (37). On the other hand, HIV-1 envelope glycoprotein has been shown to disrupt Fas and TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis and regulate the induction of the prosurvival cytokine macrophage colony-stimulating factor (M-CSF) (52), whereas HIV Nef has been shown to prevent apoptosis in macrophages by inactivating the proapoptotic Bad protein (53). Others have shown that HIV infection induces non-canonical telomerase activity in macrophages that protects them from oxidative stress and DNA damage (54). Another study showed that SIV-specific CD8<sup>+</sup> T cells were unable to suppress viral replication in SIV-infected macrophages (55).

#### GUT MACROPHAGE HIV RESERVOIR AND FUNCTIONAL CURE

The advent of HAART has had a significant effect on controlling HIV disease progression, leading to a better overall outcome. Though HAART has been able to suppress viral loads in the peripheral blood of HIV-infected individuals, it has not been effective in eradicating the viral reservoir. Some have suggested that the differential effects of HAART on macrophages and CD4 T cells may play a role (56). Given the abundance of the macrophage reservoir in the gut, functional cure strategies would need to target these cellular sources of infection for it to be effective. A number of novel strategies targeting macrophages have been proposed that may hold promise and are in the early stages of development.

One possible approach is the use of an erythrocyte-medi-

ated delivery of drugs like clodronate to macrophages. Liposome-encapsulated clodronate has previously been shown to deplete macrophages (57–59). Serafini et al. (60) showed that clodronate in encapsulated erythrocytes and combined with azidothymidine (AZT) and dideoxyinosine (DDI) was successful in depleting macrophages, lowering the level of viral DNA, and delaying viral rebound in the mouse model for AIDS. Though these results are exciting, successful uptake of clodronate depends on phagocytosis by macrophages, a function that has been shown to be impaired in gut macrophages during HIV infection (61). In a recent study, however, Burwitz et al. (62), using liposomal alendronate, an analog of clodronate, in cynomolgus macaques showed that there was a >50% transient decline in the proportions of circulating monocytes and tissue macrophages in the colon. Taken together, these studies suggest that by using clodronate or its analogs, the gut macrophage reservoir could potentially be reduced to decrease the viral burden in patients undergoing therapy.

Berre et al. (63) examined the inhibitory effect of an antibody against the scavenger receptor CD36 that is used by newly formed virions in virus-containing compartments in macrophages. Their results showed that silencing of CD36 in HIV-infected macrophages inhibited both the release of virions and the transmission of virus to CD4 T cells, suggesting that therapeutic blocking of CD36 could potentially prevent viral dissemination from infected macrophages.

In contrast to the results reported above, Sacha et al. (64) reported that SIV Gag- and Nef-specific CD4<sup>+</sup> T cells in SIV elite controller animals displayed direct effector function and eliminated SIV-infected macrophages. These T cells were found in elite controller macaques very early after infection, hinting that they may be essential in controlling the spread of the virus. If a mechanism for inducing this type of response could be identified, it could have significant implications for reducing the macrophage reservoir. Additional studies are urgently needed to explore these strategies in greater detail.

## CONCLUSION

Gut macrophages constitute a major cellular reservoir of HIV infection. There is considerable debate as to how these gut macrophages get infected in the absence of key cellular receptors required for HIV infection and whether or not HIV actively replicates in these cells once infected. It is difficult to determine at this point how easy it will be to eradicate the gut macrophage reservoir, and numerous challenges remain. Additional studies are needed to better clarify these issues. It is clear, however, that given their long life span, infected gut macrophages play a key role in HIV persistence. Developing strategies that can completely eradicate these cellular sources of infection is essential for obtaining a functional cure in HIV-infected patients.

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