Gastrointestinal Tract and the Mucosal Macrophage Reservoir in HIV Infection

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Abstract

The gastrointestinal tract (GIT) is a primary site for human immunodeficiency virus (HIV) 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 these 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.

Developing strategies that can lead to a functional cure will need to incorporate approaches that can eradicate the macrophage reservoir in the GIT.
Introduction

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 are highly phagocytic and microbicidal(3-5). They express 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+, human mucosal macrophages in the gut have been shown to express a CD13+CD14-CD16-CD64-CD89-CD32- phenotype(12, 13). The expression of CD4 and CCR5/CxCR4, key receptors for HIV infection, on macrophage populations have been shown to differ based on the sites they reside in. Shen R et al(14) showed that vaginal macrophages expressed CD4 and CCR5/CxCR4 similar to that of blood monocytes whereas intestinal macrophages expressed little or no detectable CD4 and CCR5/CxCR4(15-17).

Interestingly, under normal conditions or in response to TLR ligands gut macrophages constitutively secrete anti-inflammatory cytokines such as IL-10 rather than pro-inflammatory mediators such as IL-12, IL-23, TNF-α, IL-1, IL-6, and 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-vs-host disease in mice(24-26). Likewise, altering the phenotype of macrophages to that of a pro-
inflammatory phenotype as seen during HIV replication was associated with increased inflammation and tissue damage (27).

In addition to its role in maintaining mucosal immune homeostasis, gut macrophages, like dendritic cells, have been shown to sample microbes directly from the intestinal lumen and transfer these antigens to dendritic cells (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 monocyte/macrophage 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 2-3 logs more HIV for infection (15). Likewise, Shen et al (14) observed that gut macrophages were less permissive to HIV infection when compared to vaginal macrophages presumably because of lower levels of CD4 and CCR5 receptors required for HIV entry and infection.
Interestingly, however, Zalar et al (40) showed that both CD64+ and CD68+ gut macrophages in patients currently undergoing HAART had detectable levels of p24 and HIV DNA. Likewise, Josefsson et al (41) found that myeloid cells isolated from the 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 SIV carried viral DNA albeit at levels lower than memory CD4 T cells in gut. The above 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 have detectable levels of HIV-1 DNA there was little or no detectable 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 NFκB 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 highly active anti-retroviral therapy (HAART) was stopped. Chun TW (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 above studies, Igarashi et al (46) using the SHIVDH12R infected rhesus macaque model showed that during late stages of viral infection tissue macrophages exhibited significant productive viral infection, were resistant to cytopathic effects of SHIV, and constituted the primary viral reservoir following the rapid and irreversible depletion of CD4+ T cells. In another study, Matsuyama-Murata et al (47) quantified proviral DNA and compared 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 amount of viral DNA was higher in tissues as compared to blood. Interestingly, the mutation patterns of viruses found in the plasma was most similar to that of viruses found in the jejunum and mesenteric lymph nodes with >50% of SHIV expressing cells being CD68+ macrophages. These studies suggest that gut macrophages were 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 TGFβ receptors but showed little or
no chemotactic response to stromal derived IL-8 and TGFβ. In contrast, blood monocyte
derived macrophages displayed similar levels of receptors but actively migrated in
response to conditioned media from the lamina propria extracellular matrix containing
IL-8 and TGFβ produced by epithelial and mast cells. These observations suggest that
intestinal macrophages 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 as compared to 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 β7 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 - 16 fold increase in secretion of pro-inflammatory
cytokines/chemokines such as IL-1β, CCL5, CXCL9, and CXCL10. Jarry et al (49)
showed that the density of CD68+ and CD11c+ macrophages were significantly increased in the duodenum of HIV infected patients.

Interestingly, Maheshwari et al (50) reported that intestinal stroma conditioned media suppressed the ability of macrophages to support HIV infection and p24 antigen expression; this suppression could be reversed by Cytomegalovirus (CMV) mediated induction of TNFα 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 that of a pro-inflammatory 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 cytopathic effects of HIV(37). On the other hand, HIV-1 envelope glycoprotein has been shown to disrupt TRAIL (Fas, tumor necrosis factor-related apoptosis-inducing ligand) mediated apoptosis, and regulate the induction of the pro-survival cytokine, M-CSF(52), whereas HIV Nef has been shown to prevent apoptosis in macrophages by inactivating the pro-apoptotic Bad protein(53). Others have shown that that HIV infection induces non-canonical telomerase activity in macrophages that protected them from oxidative stress and DNA damage(54). Another study showed that SIV specific CD8+ T cells were unable to suppress viral replication in SIV infected macrophages(55).
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 peripheral blood of HIV infected individuals, it has not been successful in reducing the viral reservoir. Some have suggested that the differential effect of HAART on macrophages as compared to 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 that target macrophages have been proposed and are in the early stages of development that may hold promise.

One possible approach is the use of an erythrocyte-mediated delivery of drugs such as Clodronate to macrophages. Liposome encapsulated Clodronate has been previously shown to deplete macrophages (57-59). Serafini et al (60) showed that Clodronate in encapsulated erythrocytes when combined with Azidothymidine (AZT) and dideoxyinosine (DDI) was successful in depleting macrophages, lower the level of viral DNA, and delay viral rebound in the mouse model for AIDS. Though 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, a second generation 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 the gut
macrophage reservoir could be potentially reduced using Clodronate or its analogs 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+ 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 are essential for obtaining a functional cure in HIV infected patients.
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