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# Co-Infection with Opportunistic Pathogens Promotes Human Immunodeficiency Virus Type 1 Infection in Macrophages

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Human immunodeficiency virus type 1 (HIV-1) infection is dependent on susceptible host cells that express both CD4 and chemokine co-receptors. The co-receptor CCR5 is associated with primary infection by macrophage-tropic virus isolates, whereas CXCR4 is commonly associated with T cell- and dual-tropic viruses. Once infected, lymphocytes and macrophages may replicate HIV-1 or harbor latent virus, depending on environmental factors and cellular activation. Immune activation is often associated with viremia, which is consistent with enhanced infection and viral replication in activated cells harboring virus. In this regard, opportunistic infections activate the immune system with the detrimental sequelae of enhanced viral replication and viremia. Under these conditions, viral expansion extends beyond T cells to tissue macrophages, many of which are co-infected with opportunistic pathogens. The opportunistic infections promote macrophage susceptibility to HIV-1 through cytokine modulation and altered chemokine co-receptors, potential targets for intervention.

Human immunodeficiency virus type 1 (HIV-1) infection requires access to host cells that are susceptible to and capable of replicating the virus. Cellular susceptibility is dependent upon membrane expression of CD4 and the chemokine co-receptors CCR5 and CXCR4 and possibly others, including CCR3 and CCR2b (reviewed in [1]). Among the susceptible cellular hosts are CD4<sup>+</sup> T lymphocytes, dendritic cells, and mononuclear phagocytes. In blood and in lymphoid tissues, HIV-1 is primarily associated with CD4<sup>+</sup> T cells. In the tissues, dendritic cells trap HIV-1 for presentation to lymphocytes and arguably replicate virus [2, 3]. Circulating monocytes, on the other hand, are seldom HIV-1 positive, and in the tissues macrophages are infrequently recognized as viral hosts [4, 5]. However, this cellular distribution markedly changes in the face of immune activation, particularly during opportunistic infections (OIs) [4–7].

The ability of HIV-1 to infect and disable T cells renders the host susceptible to a variety of OIs. The relentless decline in T cells due to death or redistribution [8] and the apparent insufficiency of T cell-derived macrophage-activating agents, such as interferon (IFN)- $\gamma$ , leave macrophages impotent in microbicidal functions [4].

For example, unchecked intracellular replication of *Mycobacterium avium* complex (MAC) converts an otherwise non-pathogen into an opportunist capable of wreaking havoc on the host immune system [9]. After entering through the gastrointestinal mucosa, MAC, phagocytized by macrophages in the lamina propria, spreads throughout the submucosal tissues and is transported to lymph nodes. From the lymph nodes, the organisms enter the circulation, and as a result, disseminated disease occurs in immunocompromised hosts. The contribution of MAC to enhanced morbidity and mortality in AIDS is evidenced by the ability of antimycobacterial therapy to not only alleviate MAC pathology but also to inhibit viral replication [9, 10]. Recent evidence suggests that therapeutic intervention for other OIs, including *Mycobacterium tuberculosis* and *Pneumocystis carinii*, also dampens AIDS-based pathogenesis [7].

The intriguing evidence that OI therapy reduces viremia or progression of AIDS (or both) prompted further analysis of tissue deposits of MAC and HIV-1 for assessment of potential co-regulatory events. Initial analysis of lymphoid tissues co-infected with HIV-1 and OI revealed dramatic levels of HIV-1 expression. In some cases, the numbers of HIV-1-positive cells in tissues co-infected with OIs exceeded by >100-fold that seen in tissues infected with HIV-1 only (figure 1) [5, 6].

## Characterization of Cellular Hosts of HIV

On the basis of morphologic findings, many of the HIV-1-positive cells in lymphoid tissues that were infected by both MAC and HIV were phagocytic cells and multinucleated giant cells. Within the sites of infection, a plethora of macrophages engorged with acid-fast bacilli was readily apparent. Phagocytosis of the MAC was not impaired, but once within the

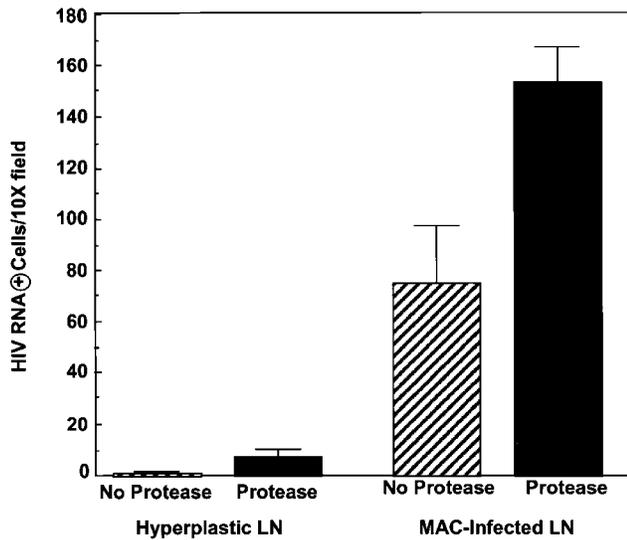
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**Figure 1.** Enhanced HIV-1 RNA-positive cells in lymph nodes (LN) co-infected with *Mycobacterium avium* complex (MAC). LN sections from patients infected with HIV-1 only or co-infected with HIV-1 and MAC were subjected to in situ hybridization with <sup>35</sup>S-labeled sense (not shown) and antisense probes for HIV-1 viral RNA before and after protease treatment, which unmasks complexed HIV-1 [5, 6] (Molecular Histology, Gaithersburg, MD). Protease pretreatment was necessary to reveal HIV-1 RNA in hyperplastic LN, whereas in MAC co-infected tissue, intensity of HIV-1 signal was pronounced even without protease and further increased after treatment. HIV-1-positive cell numbers were quantified by image analysis.

macrophages, the organisms apparently multiply unimpeded. Further analysis revealed that many of the cells with HIV-1 RNA, as detected by in situ hybridization, also contained intracellular MAC, which was identified by acid-fast staining and confirmed the myeloid lineage of the viral host cells [5, 6].

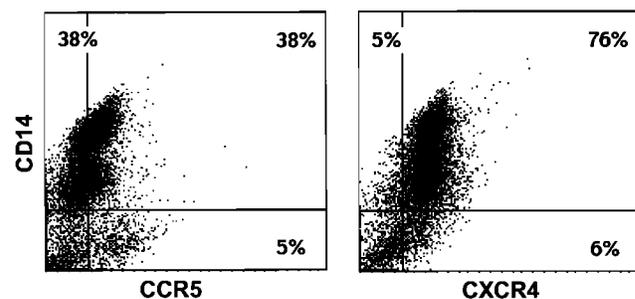
Dual-labeling with immunohistochemical markers revealed that HIV-1-positive cells often co-expressed CD68, a macrophage antigen, as well as the macrophage-specific HAM56 marker [5]. Moreover, the macrophage-associated markers, lysozyme,  $\alpha$ 1 antitrypsin, and  $\alpha$ 1 antichymotrypsin, were all displayed in these infected cells [6]. In addition, the cellular phenotype of the HIV-1-positive cells often included expression of S100 and p55, two markers generally considered representative of HIV-1-positive dendritic cells [11]. Because dendritic cells and myeloid cells share a common CD34<sup>+</sup> ancestry, the usual differential expression of these antigens may reflect their unique patterns of maturation or the influence of microenvironmental factors (or both). However, within the context of a lymphoid tissue bombarded with cytokines, microbial products, virus infection, and other factors, the usually distinct phenotypes may merge. As a consequence, the cellular phenotype expressed under the extreme conditions present in a lymph node infected with both HIV-1 and an OI may represent a phenotypic spectrum of cells with both dendritic cells and myeloid markers.

Regardless of these phenotypic parameters, the convergence of stimuli within this milieu enables the myeloid cells to become hosts and incubators for HIV-1.

### Mechanisms of Accelerated Viral Replication

Viral expansion in the co-infected tissues is not only augmented but also represents a shift in host cell repertoire. The transition from infected T cells to a predominance of HIV-1-positive macrophages may represent a change in susceptibility to infection or a change in the pace of viral replication. The shift in host cell is, in part, due to the depletion of T cells that typifies advanced HIV-1 disease [8]. In the absence of a detectable OI, the progression from follicular hyperplasia to follicular involution and lymphocyte depletion and the elevated viral replication or viremia may not be as evident. With the emergence of an OI, however, lymph node architecture and T cell numbers deteriorate and macrophages proportionally increase. To explore the potential mechanisms whereby these myeloid cells become a pivotal target of the virus, we evaluated the ability of the commonly encountered OI (MAC) to influence parameters contributing to virus internalization.

The chemokine receptors CCR5 and CXCR4, and possibly CCR3 and CCR2b, function in concert with CD4 to permit viral fusion and entry into cells [1]. Of these seven transmembrane domain, G protein-coupled co-receptors, CCR5 is used preferentially by many primary or macrophage-tropic HIV-1 isolates, CXCR4 functions in the entry of T cell or dual-tropic virus, and CCR3 and CCR2b appear more restricted and less critical [1]. Monocytes freshly isolated from peripheral blood and identified by the CD14 marker express both CCR5 and CXCR4 (figure 2). Since both CD4 and CCR5 are essential to



**Figure 2.** Monocytes express CCR5 and CXCR4. Peripheral blood monocytes were isolated by elutriation and stained with phycoerythrin-conjugated anti-CD14 (a monocyte antigen) and polyclonal anti-CCR5 generated against synthetic peptide derived from N-terminal region of CCR5 at 10  $\mu$ g/mL followed by goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC) or anti-CXCR4 (PharMingen, San Diego) and FITC-conjugated goat anti-mouse IgG (Jackson Immuno-Research Laboratories, West Grove, PA) [6]. Cells were analyzed by flow cytometry (FACSscan; Becton Dickinson, San Jose, CA). Nonadherent, nonactivated CD14<sup>+</sup> monocytes express both CCR5 and CXCR4.

macrophage infection, their levels of expression were monitored after exposure to MAC antigens. Peripheral blood monocytes were cultured in suspension or for a week as adherent monolayers and then stimulated with MAC antigens. Not only did MAC antigens enhance CCR5 expression at the mRNA level, but cell surface CCR5 was increased as determined by flow cytometry using a polyclonal anti-CCR5 antibody [6]. CD4 levels measured in parallel were not enhanced by MAC antigens, nor were CXCR4 receptors. Similarly, infection of adherent monocyte/macrophages with viable *M. avium* augmented CCR5 on these cells, increasing both the number of CCR5<sup>+</sup> cells and the intensity of staining on individual cells. These data suggest that OI, by modulating one of the requisite HIV-1 entry points on macrophages, can enhance susceptibility to virus infection. Moreover, these in vitro observations reflect the substantial increase in CCR5-expressing cells detected within lymphoid tissues that are co-infected with HIV-1 and MAC compared with those infected with HIV-1 only [6].

In addition to influencing the initial stages of infection, OI appear to up-regulate cytokines, which may, in turn, contribute to the regulation of viral replication in an autocrine or paracrine fashion. In this regard, MAC stimulates tumor necrosis factor (TNF)- $\alpha$  expression, which under appropriate conditions, can enhance viral replication [12–13]. During disseminated MAC infection, elevated TNF- $\alpha$  levels are also reported in the circulation of patients [14]. Besides influencing viral replication, TNF- $\alpha$  may also contribute to enhanced cofactor expression. Characterization of the promoter region of CCR5 revealed consensus sequences for NF- $\kappa$ B binding sites [15], which suggests that MAC may either directly influence receptor expression or, by up-regulating TNF- $\alpha$ , indirectly augment CCR5 transcription through NF- $\kappa$ B. The presence of three IFN-stimulated response elements in the CCR5 promoter [15] also implicates a role for IFN- $\gamma$  in the regulation of this pathway (Peng et al., unpublished observations). Thus, multiple factors in the complex milieu of co-infection may be critical in the dramatically elevated levels of viral expression, and identification of these factors may suggest pathways to stop or block entry and replication.

### Inflammation and Virus Production

The presence of cytokines and other cellular, soluble, and matrix-associated factors that may impact on viral replication are not limited either to the lymphoid tissue or to sites of OIs. In fact, tissues characterized by ulceration or other inflammatory pathologies without an infectious component are commonly sites with augmented viral replication [16] (Wahl et al., unpublished observations). The enhanced numbers of cells possessing HIV-1 in these lesional sites may also reflect inflammation-induced synthesis and secretion of chemokines and other recruitment factors in addition to regulatory cytokines. Enhanced expression of chemokine receptors is not only of

significance for virus entry but also for recruitment of potential new target cells. For example, CCR5 recognizes the CC chemokines (MIP1 $\alpha$ , MIP1 $\beta$ , and RANTES), which are increased in response to MAC infection (Hale-Donze et al., unpublished data) and at sites of inflammation, and they play a role in the inflammatory process and in the recognition of chemotactic signals. The presence of these CC chemokines may also bias host cell selection since T cells are more sensitive to chemokine inhibition of HIV-1 infection than are macrophages [17].

Collectively, the plethora of inflammatory mediators localized to sites of infection and inflammation appear to have a profound impact not only on shifting HIV-1 into cells of myeloid lineage but also on the extent of local viral replication. Localized viral production may also be reflected by increased viremia or declining levels of circulating CD4. Dramatic reversals of high virus loads and dwindling CD4 levels have been associated with inflammation control [16].

### Summary

The evidence for a reciprocal relationship between OIs and HIV-1, in which each favors the other, offers insight into the overlapping benefits of anti-OI and antiviral therapy. In this regard, not only does antimycobacterial therapy reduce progression of HIV-1 disease, but highly active antiretroviral therapy can promote immune responsiveness to mycobacteria [18]. New evidence indicates that the augmentation of HIV-1 is not restricted to infectious microorganisms but extends to sites of inflammatory disease. The shared constellation of interacting events in infection and inflammation may reveal common inhibitory targets, and further characterization of the contributing factors involved in the initiation and maintenance of viral reservoirs represents a key step in the goal of controlling re-surgent viral replication and its consequences.

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### References

- Berger EA. HIV entry and tropism: the chemokine receptor connection. *AIDS* 1997;11:S3–16.
- Reinhardt TA, Rogan MJ, Viglianti GA, Rausch DM, Eiden LE, Haase AT. A new approach to investigating the relationship between productive infection and cytopathicity in vivo. *Nat Med* 1997;3:218–21.
- Pope M, Frankel SS, Mascola JR, et al. Human immunodeficiency virus type 1 strains from subtypes B and E replicate in cutaneous dendritic cell–T cell mixtures without displaying subtype-specific tropism. *J Virol* 1997;71:8001–7.
- Wahl SM, Orenstein JM, Smith PD. Macrophage functions in HIV-1 infection: In: Gupta S, ed. *Immunology of HIV infection*. New York City: Plenum Press, 1996:303–36.
- Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections. *Science* 1997;276:1857–61.

6. Wahl SM, Wild T, Peng G, Hale-Donze H, Doherty M, Mizel D, Orenstein JM. *Mycobacterium avium* complex (MAC) augments macrophage HIV-1 production and increases CCR5 expression. *Proc Natl Acad Sci* **1998**; 95:12574–9.
7. Wahl SM, Orenstein JM. Immune stimulation and HIV-1 replication. *J Leukoc Biol* **1997**; 62:67–71.
8. Rosenberg Y, Anderson AO, Pabst R. HIV-induced decline in blood CD4/CD8 ratios: viral killing or altered lymphocyte trafficking? *Immunol Today* **1998**; 19:10–6.
9. Horsburgh CR, Metchock B, Gordon SM, Havlik JA, McGowan JE, Thompson SE. Predictors of survival in patients with AIDS and disseminated *Mycobacterium avium* complex disease. *J Infect Dis* **1994**; 170:573–7.
10. Chin DP, Reingold AR, Stone EN, et al. The impact of *Mycobacterium avium* complex bacteremia and its treatment on survival of AIDS patients—a prospective study. *J Infect Dis* **1994**; 170:578–84.
11. Frankel SS, Tenner-Racz K, Racz P, et al. Active replication of HIV-1 at the lymphoepithelial surface of the tonsil. *Am J Pathol* **1997**; 151:89–96.
12. Poli G, Kinter A, Justement JS, et al. Tumor necrosis factor- $\alpha$  functions in an autocrine manner in the induction of human immunodeficiency virus expression. *Proc Natl Acad Sci USA* **1990**; 87:782–5.
13. Osborn L, Kunkel S, Nabel GJ. Tumor necrosis factor  $\alpha$  and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of nuclear factor  $\kappa$ B. *Proc Natl Acad Sci USA* **1989**; 86:2336–40.
14. Haug CJ, Aukrust P, Lien E, Muller F, Espevik T, Froland SS. Disseminated *Mycobacterium avium* complex infection in AIDS: immunopathogenic significance of an activated tumor necrosis factor system and depressed serum levels of 1,25 dihydroxyvitamin D. *J Infect Dis* **1996**; 173:259–62.
15. Guignard F, Combadiere C, Tiffany HL, Murphy PM. Gene organization and promoter function for CC chemokine receptor 5 (CCR5). *J Immunol* **1998**; 160:985–92.
16. Silver S, Wahl SM, Orkin B, Orenstein JM. Impact of ulcerative colitis on CD4 T cells and on tissue and circulating HIV: a case report. *J Hum Virol* (in press).
17. Schmidtayerova H, Sherry B, Bukrinsky M. Chemokines and HIV replication [letter]. *Nature* **1996**; 382:767.
18. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4<sup>+</sup> T cell homeostasis and function in advanced HIV disease. *Science* **1997**; 277:112–6.