

Chemoattractants Attract HIV Researchers

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In this issue of the *Journal of Experimental Medicine*, Loetscher et al. show that receptors for β -chemokines are up-regulated when T lymphocytes are activated by interleukin-2 (1). This paper will be of considerable interest not just to those who have worked for some years to identify conditions whereby chemokines could attract T cells, but also to HIV researchers and conceivably to retrovirologists in general. It is not often that two seemingly unrelated fields, chemotaxis and retroviral infection, meld to create an entire new area of research, but that is exactly what has happened over the past six months.

For several years, it has been known that CD8⁺ T cells secrete factors that suppress HIV-1 replication in CD4⁺ T cells (2-4). The nature of these factors remained elusive, however, until December of last year when Cocchi et al. showed that the β -chemokines MIP-1 α , MIP-1 β , and RANTES contributed to the CD8⁺-cell suppressive effect (5). It is probable that these β -chemokines are not the only components of CD8⁺ cell conditioned medium that have an anti-viral effect against HIV-1, but it is certain that they are important components of the cocktail.

It has also been known, for over a decade in this case, that HIV-1 needs a species-specific (but not cell lineage-specific) accessory fusion factor (second receptor) to enter CD4⁺ cells (6-8). The expression of CD4 is necessary, but not sufficient, for efficient HIV-1 replication. In the absence of the second receptor, HIV-1 can bind to its target cells (via CD4), but the fusion process is not initiated (6). The identity of the second receptor remained unknown despite much searching by many research groups. The log-jam in this field was finally broken in April of this year by the publication of a paper from Ed Berger's group at the National Institutes of Health showing that the second receptor for at least some strains of HIV-1 was LESTR (fusin), a member of the same receptor superfamily as the β -chemokine receptors (9).

The inhibitory effects of MIP-1 α , MIP-1 β , and RANTES are largely restricted to primary, NSI strains of HIV-1 (5, 10). However, LESTR was clearly shown to be the second receptor for strains of HIV-1 adapted to growth in permanent cell lines (TCLA strains) and primary viruses of the more aggressive, SI phenotype (9). Furthermore, LESTR is not known to be a β -chemokine receptor; indeed, its physiological ligand is presently unknown (9, 11). These discrepancies notwithstanding, the potential connection between Berger's paper and that of Cocchi et al. did not go unappreciated by many research groups (5, 9).

The β -chemokine receptors are from the seven transmembrane-spanning, G-protein-coupled superfamily (12-16). Dozens of these receptors are encoded by the human genome, and they bind a range of ligands, including peptide hormones, neuropeptides and the α - and β -chemokines. Other members of the receptor superfamily are involved in vision, taste, and smell perception. As the superfamily name suggests, the receptors span the plasma membrane seven times, so that about half of the protein is buried in the membrane. The extracellular domains, especially the NH₂ terminus, are involved in ligand binding, the intracellular regions in coupling to the effector systems of signal transduction pathways, via G-proteins (12-16).

Several groups commenced a search for a β -chemokine receptor that could serve as the second receptor for primary, NSI strains of HIV-1. It did not take long for the recently published CKR-5 receptor (17) to be identified as an HIV-1 second receptor (18, 19). CKR-5 appears to be the counterpart of LESTR for NSI primary viruses, and its second receptor functions are inhibited by β -chemokines. Thus, β -chemokines inhibit HIV-1 replication by blocking the fusion of the virus with its target cell, perhaps by a competitive interaction with the receptor (18). In addition, it has been shown that CD4⁺ T cells from some persons who have been multiply exposed to HIV-1 yet remain uninfected (EU individuals) are incompetent at fusing with NSI HIV-1 strains (18). The defect in the EU cells may lie at the level of the CKR-5 receptor, either because this is nonfunctional for HIV-1 entry or because it is ligated endogenously by the β -chemokines that are over-secreted from the EU T cells.

Many questions on the relationship between the β -chemokines and HIV-1 replication in vitro and in vivo remain unanswered, but the present paper of Loetscher et al. addresses a significant issue (1). The principal finding in the paper is that CKR-1 and CKR-2 are upregulated in response to IL-2 stimulation. IL-4, IL-10, and IL-12 are partial activators, whereas several other cytokines are ineffective. Triggering of the cells via CD3 or CD28 (or non-specifically by PHA) does not upregulate CKR-1 and CKR-2; indeed, anti-CD3 or anti-CD28 activation of CKR-1- and CKR-2-expressing cells actually downregulates receptor expression (1). CKR-1, like CKR-5, is a MIP-1 α and RANTES receptor (unlike CKR-5, CKR-1 does not bind MIP-1 β avidly) (12, 13), and it can function, albeit to a very limited extent, as an HIV-1 second receptor (18). CKR-2 is a receptor for MCP-1 and MCP-3, β -chemo-

kines that are most active on monocytes and macrophages, and does not bind MIP-1 α , MIP-1 β , and RANTES (20). CKR-5 expression was not studied in the present paper, (1) but it is reasonable to speculate that its regulation in response to cytokines and mitogens might be broadly similar to that of CKR-1 and CKR-2. Whether and how LESTR expression is affected by exogenous stimuli is also unknown at present.

From the perspective of HIV research, it will be important to define the relationship between the expression of CKR-5 and LESTR and the activation state of CD4⁺ T cells. The dynamics of HIV-1 replication are such that at most 1% of virions come from latently infected cells (21–23). It has been suggested that some virus is present in cells that became infected while activated but then became quiescent (24). It is unclear, however, if HIV-1 can actually infect a resting cell in vivo. Are second receptors for HIV-1 even expressed on quiescent cells to allow HIV-1 entry? The β -chemokine receptors CKR-1, CKR-2, and CKR-3 are IL-2-induced genes that are minimally expressed on quiescent CD4⁺ T cells (1). Is this true of CKR-5 and LESTR? The SI strain LAI has been reported to enter quiescent cells quite well (25), so an interesting theoretical scenario is that LESTR might be constitutively expressed on quiescent cells, permitting entry of SI strains of HIV-1, whereas CKR-5 expression requires cell activation. If this were the case, SI viruses might have a larger pool of CD4⁺ T cells in which to replicate under in vivo conditions. Furthermore, although factors such as nuclear membrane dissolution during cell division and upregulation of transcription factors such as NF κ B are important (26, 27), the

upregulation of second receptors could also contribute significantly to the increased ability of activated CD4⁺ T cells to replicate HIV-1.

A second implication of the results of Loetscher et al. covers T cell subsets. It has been known for some time that CD45RO⁺ memory T cells are more sensitive than CD45RA⁺ naive T cells to the chemoattractant properties of the β -chemokines, and memory cells also secrete more β -chemokines than naive cells (28, 29). It has not, however, been clear why. Furthermore, several contradictory results on this issue have been reported in the literature. Loetscher et al. discuss several explanations of conflicting findings, and clarify the situation greatly (1). Their observations that the chemotactic responsiveness of CD4⁺ T cells depends upon the activation state of the cells, and hence on the degree of β -chemokine receptor expression, should be important contributions to researchers working on lymphocyte chemotaxis. And there may also be an impact of these findings on HIV-1 pathogenesis studies, as it is possible that CD4⁺ T cell subsets may vary in their patterns of β -chemokine receptor expression. Hence different CD4⁺ subsets may be differentially susceptible to different HIV-1 strains, and this could, in principle, contribute to (or even account for) the evolution of the HIV-1 phenotype that occurs during disease progression (30). It is noteworthy that memory T cells are preferentially lost during HIV-1 infection in vivo (31, 32).

In summary, the new study from Loetscher et al. will be significant to established chemokine researchers, and to those now attracted towards these molecules by their involvement in HIV-1 pathogenesis.

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Note added in proof: Three additional groups have also reported findings similar to those in references 18 and 19 (33–35).

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