

A new entry route for HIV

Identification of HIV-1 variants capable of entering T cells via the CD8 receptor suggests a new mode of viral pathogenesis. But are these variants rare, aberrant viruses or a real problem? (pages 65–72)

Over the last few years, a consensus has emerged as to the identity of the cell surface molecules required for infection by HIV-1 (ref. 1). The viral envelope protein first binds to CD4, a protein found on the surface of a limited number of cell types, including T-helper cells and macrophages. The HIV-1 envelope protein then undergoes a conformational shift that allows binding to a second cell surface molecule, termed a co-receptor. The majority of primary HIV-1 isolates use the chemokine receptor CCR5 as the co-receptor while a minority, observed particularly in late stage patients, utilize CXCR4 (Fig. 1). At this point, the envelope undergoes a second conformational shift that triggers the entry of the HIV-1 virion into the target cell.

Though a small number of HIV-1 isolates seem able to infect cells using yet other co-receptor in conjunction with CD4, and an even smaller number are able to infect directly via CCR5 or CXCR4, without any requirement for CD4, such variations on the major pathway seem to be quite rare. In this issue, Saha *et al.*² present data characterizing two novel primary isolates of HIV-1 that infect cells via a completely different mechanism. Specifically, these authors have identified two HIV-1 isolates that are not only able to infect cells through the cell surface CD8 molecule, rather than CD4, but can do so without the presence of either the CCR5 or the CXCR4 co-receptor. This observation emphasizes the remarkable functional plasticity of the HIV-1 envelope protein and suggests that the accelerated decline observed in some late stage AIDS patients may in part reflect the development of viral variants able to attack cells, such as CD8⁺ cytotoxic T-lymphocytes (CTL), which are not targeted at earlier stages.

Several groups have previously reported the existence of infected CD8⁺/CD4⁺ T-cells in both AIDS patients and SIV infected monkeys^{3–5}. In fact, one group reported that CD8⁺/CD4⁺ T cells encompassed the majority of infected cells in the peripheral blood of several late stage AIDS patients (ref. 3). In contrast, infected CD8 cells were found to be very rare, and frequently undetectable, in asymptomatic patients

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with more than 200 CD4 cells/ μ l (ref. 3). However, no molecular analysis of the HIV-1 proviruses present in CD8⁺ cells has been reported and it was generally assumed that these infected CD8⁺ cells arose by infection of the immature, dual-positive population of CD4⁺/CD8⁺ lymphocytes that serve as precursors for both populations of single positive T cells. In contrast, Saha *et al.*² chose to directly analyze the cellular tropism of viruses obtained from immortalized, HIV-1 infected CD8⁺/CD4⁺ T-cells derived from two AIDS patients. Surprisingly, the two viruses they isolated proved capable of replicating in not only CD4⁺ peripheral blood lymphocytes (PBLs) but also in highly purified CD8⁺ PBLs that

contained no trace of CD4 mRNA (Fig. 1).

While these data argue against a requirement for CD4, they do not answer the question of whether infection by these two novel isolates of HIV-1 requires CD8 or is instead mediated by other cell surface molecules, such as the CCR5 or CXCR4 co-receptor. To address this issue, Saha *et al.*² showed that these HIV-1 isolates could infect not only a human CD8⁺/CD4⁺ T cell line (KRCD8) but also both a human (HeLa) and a simian (COS) cell line that had been engineered to express CD8 (Fig. 1). The authors did not observe HIV-1 infection of the parental, non-transfected cell lines. Finally, the authors showed that monoclonal antibodies against CD8 were able to block infection of both KRCD8 and CD8⁺ HeLa cells by these two viral isolates. Antibodies against CD4, which are effective in blocking infection of CD4⁺ T cells by standard HIV-1 isolates, did not block infection by these viral isolates.

Based on these well-controlled experiments, infection by these two novel HIV-1 variants can apparently be effectively mediated by CD8. We should bear in mind that these viral variants retain the ability to infect cells via CD4, and the authors state that this mode of infection also requires the CXCR4 co-receptor². So does CXCR4 function as a co-receptor during CD8-mediated infection? Surprisingly, the answer appears to be no. The authors present fluorescent-activated cell sorting (FACS) data to suggest that KRCD8 are negative for both CXCR4 and CCR5 whereas COS cells, which the authors show are susceptible to infection by these novel virus isolates upon expression of CD8, lack both CXCR4 and CCR5. This result is truly remarkable in that the co-receptor interaction, even more than binding to CD4, has been thought to be crucial to the conformational shift in envelope that triggers HIV-1 infection¹. It remains possible, however, that these viruses use an as yet unidentified co-receptor during the infection process (Fig. 1).

The discovery of HIV-1 variants that can infect cells via CD8, rather than CD4, in the absence of either CCR5 or CXCR4 is a startling result that will require confirmation and further investi-

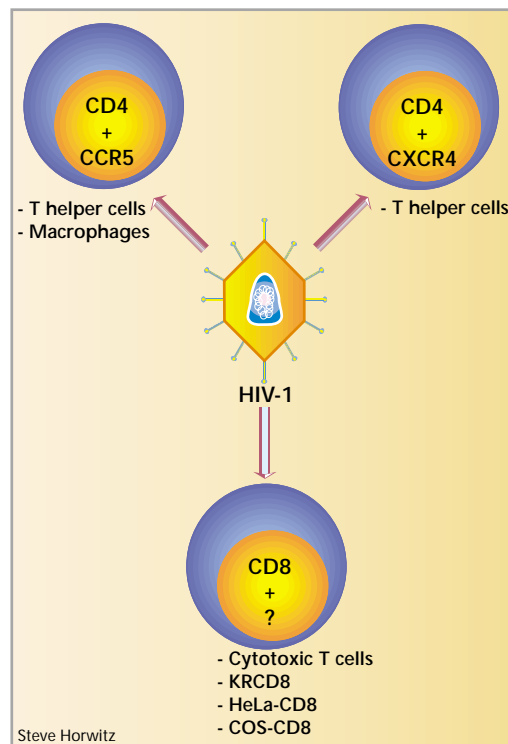


Fig. 1 HIV-1 is known to infect cells that express CD4 and CCR5, such as T helper cells and macrophages. During the later stages of disease progression, HIV-1 variants that infect CD4 and CXCR4-expressing T helper cells may also evolve. Saha *et al.*² present evidence to suggest that some HIV-1 variants infect CD8⁺ cells, in the absence of either CCR5 or CXCR4. The authors show that these viral variants can infect HeLa and COS cells engineered to express CD8, as well as cytotoxic T cells and CD8⁺ T-cells.



gation by the HIV research community. But, assuming that these findings are indeed confirmed, what do they mean for the treatment and pathogenesis of HIV? Though it has not yet been demonstrated, it seems likely that the highly unusual tropism of these HIV-1 isolates is mediated entirely by the viral envelope. Therefore, the widely used HIV-1 reverse transcriptase or protease inhibitors should remain fully efficacious in patients infected by similar virus variants. It remains possible, however, that novel drugs¹ that target HIV-1 envelope mediated fusion, or that act by blocking co-receptor function, would not be effective against CD8-tropic strains of HIV-1.

It will also be important to determine how frequently CD8-tropic HIV-1 variants occur in patients. Based on the unusual sequence of the *env* genes of these HIV-1 variants², it seems probable that they are, in fact, rare. This would explain why such CD8-tropic virus variants have not been reported previously, although this could also reflect the fact that few scientists would be likely to try growing a novel HIV-1 isolate on CD8⁺/CD4⁻ cells.

As noted above, HIV-1 infected CD8⁺/CD4⁻ cells are rarely observed in early stage patients but they can become much more common as disease progresses³. It is possible that CD8-tropic viruses may evolve in

certain patients during the late disease stage and may then contribute to an acceleration in pathogenesis. For example, these viruses might replicate in, and kill, CD8⁺ CTL directed against HIV-1 and hence further undermine the host's ability to control viral infection. In this context, it is interesting to note that CD8⁺ cells, unlike CD4⁺ cells, actually increase in number during the long asymptomatic phase of HIV-1 infection but can decline substantially once AIDS is diagnosed^{6,7}. Though this decline has generally been thought to reflect an indirect killing mechanism, such as apoptosis⁸, it is possible that direct infection of CD8⁺ cells by HIV-1 could contribute to their decline in some patients. The *in vivo* evolution of CD8-tropic HIV-1 variants may bear some similarity to the appearance of CD4-tropic variants that utilize CXCR4, rather than CCR5, as a co-receptor. Such CXCR4-tropic variants arise late in disease, in only a portion of HIV-1 infected patients, and may accelerate disease progression once they appear.

The findings of Saha *et al.*² suggest the existence of HIV-1 variants that are able to infect cells through CD8, in the absence of either CCR5 or CXCR4. It remains to be determined whether these are highly unusual isolates or, less probably, if acquisition of CD8 tropism is instead a fairly common

development in advanced AIDS patients. In either case, it will be important to determine whether CD8-tropic HIV-1 can indeed promote disease progression and, most importantly, whether such variants are capable of transmission.

1. Doms, R.W. Beyond receptor expression: The influence of receptor conformation, density, and affinity in HIV-1 infection. *Virology* **276**, 229-237 (2000).
2. Saha, K. *et al.* Isolation of primary (HIV-1) that target CD8⁺ T lymphocytes using CD8 as a receptor. *Nature Med.* **7**, 65-72 (2000).
3. Livingstone, W.J. *et al.* Frequent infection of peripheral blood CD8-positive T-lymphocytes with HIV-1. *The Lancet* **348**, 649-654 (1996).
4. Semenzato, G. *et al.* CD8⁺ T lymphocytes in the lung of acquired immunodeficiency syndrome patients harbor human immunodeficiency virus type 1. *Blood* **85**, 2308-2314 (1995).
5. Tsubota, H. *et al.* CD8⁺ CD4⁻ lymphocyte lines can harbor the AIDS virus *in vitro*. *J. Immunol.* **143**, 858-863 (1989).
6. Margolick, J.B. *et al.* Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. *Nature Med.* **1**, 674-680 (1995).
7. Roederer, M. *et al.* CD8 naive T cell counts decrease progressively in HIV-infected adults. *J. Clin. Invest.* **95**, 2061-2066 (1995).
8. Herbein, G. *et al.* Apoptosis of CD8⁺ T-cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* **395**, 189-194 (1998).

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Yersinia lead SUMO attack

Little is known about the mechanism by which Yops, proteins that *Yersinia* inject into the cytosol of macrophage, cause downregulation of the inflammatory response and diseases such as the plague. Now it appears that Yops are the first bacterial member of a new family of ubiquitin-like proteases.

Until recently, the mechanisms of host invasion by pathogenic bacteria were a black box, with most concern focused on their sensitivity to antibiotics. This perspective has changed, however and the interaction between pathogen and host can now be described in molecular terms. An article recently published in *Science* by Orth *et al.*¹ revealed the strategy employed by bacteria of the genus *Yersinia* to sabotage cell signaling.

Yersinia pestis, which was responsible for the 'Black Death' of the Middle Ages, has an impressive ability to overcome host defense mechanisms and replicate rapidly. The closely related food-borne pathogens *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* cross the intestinal barrier and multiply in the gut-associated lymphoid tissues. Although these bacteria cause infections that are generally self-limited, they

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share their major pathogenic mechanism with *Y. pestis*. This mechanism is a 'type-III secretion system' that allows an extracellular bacterium docked at the surface of a host cell to inject into the cell's cytosol specialized proteins that paralyze cell function and sabotage its communicative abilities². The type-III secretion system has been identified in more than fifteen major animal or plant pathogens. In the case of *Yersinia*, the injected effectors, called 'Yops', target cells of the immune system (especially macrophages), block phagocytosis and downregulate the inflammatory response.

In the mouse model of *Yersinia* infection, expression of interferon gamma (IFN- γ)

and tumor necrosis factor alpha (TNF- α) increases just before death. In contrast, infection with avirulent strains causes immediate production of these cytokines, indicating that one mechanism of pathogenic *Yersinia* may be to slow down the onset of the inflammatory response³. *In vitro* studies show that a particular Yop, called YopJ (YopP in *Y. enterocolitica*), can suppress TNF- α release by macrophages⁴, IL-8 release by epithelial cells⁵ and splenocyte proliferation⁶. All these events result from the ability of YopJ/P to interrupt two major cell signaling cascades: the mitogen-activated protein kinase (MAPK)^{4,7} and the NF- κ B pathways^{5,8} (Fig. 1).

NF- κ B is a transcription factor whose activation initiates the onset of inflammation. It is normally sequestered in the cytosol by an inhibitory factor called I κ B, which is degraded after its phosphoryla-