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Battling AIDS

The human immunodeficiency virus (HIV-1) is a particularly insidious retrovirus. It infects helper T cells that normally respond to viral infections and manages to escape cytotoxic T cells that normally destroy virus-infected cells. This leads to immune system impairment, with subsequent opportunistic infection, progression to AIDS (acquired immune deficiency syndrome), and death. Thus, much effort has been focused on identifying and developing compounds that suppress viral production once HIV has entered the cell or, more recently, that block HIV from entering the target cell — as described, for example, on pages 906 and 953 of this issue of *Nature Structural Biology*.

Using reverse transcriptase, HIV converts its RNA genome into DNA that can be incorporated into a host cell's chromosome. The integrated DNA (called a provirus) can remain latent for many years and then become activated to produce large amounts of viral proteins and RNA from which viral particles can be made. Assembly of these viral particles depends on the HIV protease that processes and activates the viral proteins. Currently approved therapies for HIV infection inhibit one of two viral specific enzymes — the reverse transcriptase or the protease (Fig. 1). A third class, integrase inhibitors, is under development. Therapies using a combination of drugs from different classes, including at least one protease inhibitor, are the most effective treatments available and offer real hope for people living with HIV/AIDS of having a longer and better life.

Problems with current drug therapies

The principal problems with these drugs is their limited potency, their toxicity, and their time-limited benefit — largely due to the development of drug resistance. It is now clear that the sustainability of the therapeutic response to anti-retroviral therapies depends on the level of suppression of viral replication. Therapies that only partially suppress replication may ultimately promote the emergence of resistance. However, complete suppression is difficult to achieve because of pre-existing drug-resistant viral strains, suboptimal use of HIV drugs by physicians, poor adherence to complicated and toxic regimens by patients, and high cost (~$15,000 a year).

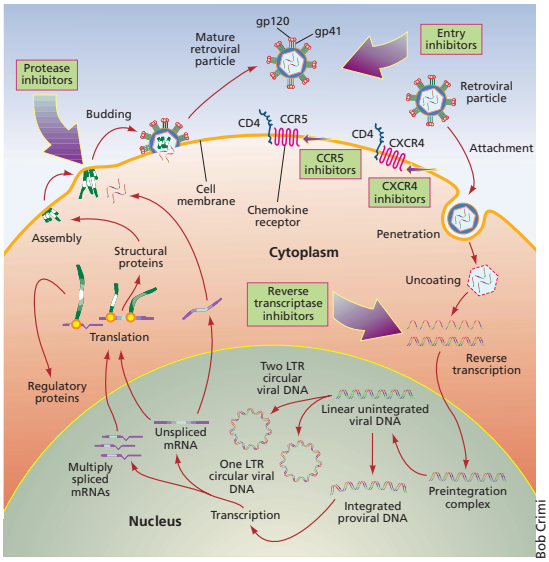

Fig. 1 The complex viral life cycle of HIV-1 points to a number of potential targets for drug intervention.

Another problem is that current anti-viral drugs apparently do not eradicate HIV from the body even when it is undetectable in the blood. This failure has been suggested to result from a pool of chronically HIV-infected, long-lived, dormant T cells[1–4]. The provirus in these resting lymphocytes remains hidden from the immune system and inaccessible to the drugs that act on the virus as an infectious RNA species. Thus, a cure for HIV is not likely to be achieved using the current anti-retroviral therapies. One way to deplete these viral reservoirs may be to combine potent anti-retroviral therapy with immune therapy[5]. The idea is to lure latent HIV out of hiding using immunostimulatory cytokines, such as interleukin-2, and then prevent viral replication using anti-retroviral drugs as HIV emerges from the reservoirs.

New ways to attack HIV

While immune therapy together with potent anti-retroviral therapy may represent a promising approach, eradication of HIV clearly remains a difficult problem. Thus, researchers are actively seeking ways to develop new classes of

drugs with different modes of action. To this end, the focus has shifted to identifying reagents that block HIV entry into the cell rather than inhibiting viral replication.

HIV-1 is an enveloped retrovirus and its envelope protein mediates the early binding and entry steps in infection (Fig. 1). The HIV-1 envelope protein is initially made as the precursor gp160. This protein is heavily glycosylated and then proteolytically cleaved into gp120 (the surface segment) and gp41 (the transmembrane segment), which remain non-covalently associated and oligomerize, most likely as trimers, on the surface of the viral particle. The gp120 protein binds to CD4 and a chemokine coreceptor (CXCR4 or CCR5), which are present on susceptible cells such as T lymphocytes and macrophages. Upon binding of gp120 to the target cell receptor, gp41 undergoes a conformational change to a fusion-active state that includes exposure of the 'fusion' peptide at the N-terminus, which can interact with the target membrane.

Given the number of steps involved, the process of viral entry has many potential targets for inhibitors that could impede the virus at a very early stage of the infection cycle. These include soluble CD4, chemokines, and small molecules including peptides and organic compounds[6,7]. Soluble versions of CD4 have been found to be effective against laboratory-adapted HIV strains but not primary HIV isolates and thus do not hold much promise as therapeutic tools. The targeting of chemokine receptors has focused on using the chemokines themselves or small molecule inhibitors of the chemokine receptors. Chemokine receptor ligands have been shown to block HIV infection either by down-regulating the expression of chemokine receptor in the target cells and thus reducing the number of possible HIV entry points or by competitively blocking the interaction between gp120 and the receptor. Likewise, small molecule inhibitors seem to work by binding to the chemokine receptor and preventing its interaction with gp120.

Another approach is to make use of the known high-resolution structures of gp41 and use a structure-based approach to design inhibitors that block HIV infection by preventing gp41 activation. The gp41[8–10] protein in its activated conformation is a six helix bundle. The N peptides form the three central helices and the C peptides form the three outer helices. Synthetic C peptides, corresponding to the C helix, have been shown to specifically inhibit HIV-1 membrane fusion by blocking formation of this activated structure. While clinical trials show that C peptide results in a significant decrease in viral load that is equivalent to that observed with existing drugs[11], the peptide has to be delivered twice daily by injection. Such treatment would be considered only for patients who have exhausted other more convenient options. To increase the inhibitory potency of such compounds and to develop ones with more desirable pharmacologic characteristics, such as oral bioavailability and central nervous system penetration, researchers are turning to other techniques such as mirror-image phage display[12] and combinatorial chemistry[13].

Based on the structure of gp41, a deep hydrophobic pocket on the N peptide was identified as a potential binding site for small molecules that could block the conformational change of gp41, and thus prevent cell fusion[8,14]. As reported in this issue of *Nature Structural Biology* (page 953), and discussed in an accompanying *News & Views* on page 906, Ferrer and coworkers described the use of the C peptide as a lead compound. They removed the residues that insert into the hydrophobic pocket and substituted them with non-natural elements that were combinatorially added to the end of the truncated peptide. Using a combinatorial library of 61,275 potential ligands covalently linked to the truncated C peptide, they identified a combination that binds well to the N peptide and thus inhibits gp41-mediated cell fusion. This new strategy represents a promising step towards finding new drugs that target HIV entry using structure-based drug design together with combinatorial chemistry. However, it should be pointed out that the ultimate goal of this study is to identify a small molecule with no peptide attached that blocks HIV entry. Clearly that goal is at least several 'design steps' away.

The continued need for prevention

While progress has been made to develop new lines of attack against HIV, the need for an HIV vaccine grows more urgent. This is because >90% of the ~33 million people predicted to be infected with HIV by the end of 1999 are in developing countries where triple therapy is prohibitively expensive and where the related services required to ensure their safe and effective use are complex[15–17]. There are two main targets in the development of a safe and effective vaccine: the induction of neutralizing antibodies and of protective cytotoxic T-lymphocyte responses. Both of these aims have met with limited success and the challenge remains formidable. Thus, as is often the case, the best way to combat HIV is quite simple — prevention. So it is particularly discouraging that the recent conference on HIV in Atlanta, Georgia (August 29–September 1, 1999) sponsored by the Center for Disease Control and Prevention reported that there is a record rate of infection among women and minorities. According to health officials there is a growing complacency about HIV among some people most at risk. They argue that while much of the effort to fight the epidemic is devoted to care and treatment, more needs to be directed toward prevention.

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