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New developments in the biology and treatment of HIV

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The last 2 years have seen tremendous advances in the ability to treat HIV type 1 (HIV-1) infection, fueled in part by new discoveries on the biology of the virus. Many patients have gained years of life thanks to the introduction of new treatment regimens. Nonetheless, much remains to be accomplished. The newer anti-HIV-1 regimens do not fully eliminate the virus infection, thereby requiring lifelong treatment. Although the new therapies have had considerable impact in the developed world, they are difficult to apply in the developing world where HIV-1 infection is the most devastating. In addition, the emergence of viral variants resistant to the available treatments drives the need to discover new therapeutic agents. Here we describe the advances that have led to the dramatically improved therapy and discuss recent findings in HIV-1 biology that may lead to new anti-viral agents.

HIV-1 Infection and AIDS

Until about 2 years ago, medical science could offer little to alter the course of HIV-1 infection. After initial transmission of HIV, viral particles accumulate in blood to high levels within a few weeks, but levels then fall concomitant with the onset of the host immune response. Thereafter, the disease usually remains quiescent for a prolonged period, often for years or even decades, a phase termed clinical latency.

During this period, the number of cells bearing the CD4 protein on their surfaces ($CD4^+$ cells) declines at a slow rate because of killing by HIV. The CD4 protein itself is an essential element of signaling pathways regulating immune responses to infection. $CD4^+$ cells are important components of the immune system. Many $CD4^+$ cells circulate in blood and are normally present at about 1,000 per microliter of blood plasma. HIV replicates in $CD4^+$ cells, killing them in the process. Over time the number of $CD4^+$ cells declines as the body's ability to replenish them becomes exhausted. The resulting failure of the immune system is accompanied by an increase in the amount of HIV in blood.

The end result of HIV-1 infection is AIDS, a condition defined by the presence of circulating antibodies against HIV and counts of $CD4^+$ cells below 200 per microliter. Toward the end of the disease course, the loss of $CD4^+$ cells permits increasingly severe infections to take hold. Immunocompromised patients fail to fight off infections from agents not normally hazardous to humans, such as microbes carried by cats or sheep. These opportunistic infections and other pathologies eventually result in death.

Problems with Earlier Therapies

Early anti-HIV-1 therapy had little success, due in large measure to the development of viral variants resistant to the antiviral agents. Recent studies have revealed that the development of resistance is a consequence of the highly dynamic nature of HIV replication (1–3). During the period of clinical latency, new virions are synthesized at a very high rate, with as many as 10^{10} virions produced and destroyed per day. Productively infected $CD4^+$ cells survive only 2.2 days and are rapidly replaced from the bone marrow so as to maintain a near-constant population (4).

Coupled with this, the small HIV genome (10^4 bp) is copied by error-prone enzymes, the cellular RNA polymerase and the viral reverse transcriptase (RT). RT makes roughly one error per 10^4 bases copied, so that each viral genome bears on average one mutation. Because of the very large population of viruses *in vivo*, many drug-insensitive mutant viruses are present in patients before treatment even begins (3).

Protease Inhibitors and Triple Combination Therapy

The recent introduction of more effective drugs has changed this bleak picture. If treatment can suppress viral replication to a low enough level, then drug-insensitive mutants have a greatly reduced probability of arising. The development of a new class of inhibitors, the protease inhibitors, has brought this goal within reach for many patients.

Late during viral replication, the HIV proteins must undergo precise cleavage at several sites to complete formation of infectious viral particles. The virus encodes an enzyme that performs this function, called the protease. Inhibitors of the protease were introduced in 1995 after an intensive development effort (for review see ref. 5).

For some patients, treatment with the more potent protease inhibitors alone succeeded in driving virus levels below the limits of detection in blood, though in many cases low levels of replication were likely still present. For some treated patients, viral levels have stayed low for prolonged periods. However, mutants of HIV resistant to protease inhibitors can arise, and many patients have suffered relapses concomitant with the development of such mutant strains (6).

Most effective of all have been mixtures of antiviral drugs, particularly cocktails of two RT inhibitors and a protease inhibitor (triple combination therapy). Such cocktails demand the development of several mutations for viruses to become insensitive. Resistance to *in vivo* levels of protease inhibitors requires roughly four mutations in HIV, plus two or more to confer resistance to the RT inhibitors (for review see ref. 7). This "genetic barrier" has proven to be a formidable obstacle to viral replication, in that multiply mutant viruses resistant to the combination therapy are unlikely to be present before initiation of treatment. Hence resistant viruses can only arise as a consequence of *de novo* mutation during replication in the presence of the inhibitors. The likelihood of these multiple mutations appearing is greatly diminished with triple combination therapy because the population of replicating virus is greatly reduced by treatment. At present patients have been on triple combination therapy for as long as 2 years, with only a low rate of relapse due to the development of triply resistant viruses. How long this benefit will persist is an open question, but there is no doubt that triple combination therapy represents a major advance in treating HIV infection (7).

Triple combination therapy does not represent a cure, however. Three recent studies reported that patients who have maintained very low viral loads for up to 30 months nevertheless harbor replication-competent virus in a small number of $CD4^+$ cells (resting T cells) (8–10). This discovery of

long-lived reservoirs of virus-infected cells has caused hopes for a cure to recede. However, one aspect of these new findings is encouraging. Sequence analysis of DNA from viruses present in patients after prolonged treatment did not reveal any of the changes characteristic of drug resistance. Evidently replication of the virus in these latently infected cells is tightly suppressed, suggesting that virus in this compartment may not be evolving to drug resistance at a high rate.

Nevertheless, the development of drug-resistant variants of HIV represents a long-term threat to the success of triple combination therapy. Many patients starting triple combination therapy already harbor drug-resistant HIV variants due to previous treatments, leaving combination therapy in many cases no better than monotherapy. It is estimated that 30–40% of HIV-infected people in the United States may already have viruses resistant to protease inhibitors. For the case of AZT, the antiretroviral agent in use the longest, new infections by AZT-resistant viral strains have been documented. Furthermore, many patients cannot tolerate the side effects of triple combination therapy and so cannot undergo treatment at all. These concerns focus intense interest on the development of new anti-HIV therapies.

An Attractive New Target for Antiviral Agents: The HIV-1 Coreceptors

A particularly exciting discovery in the last 2 years has been the recognition of a new host factor for infection that explains a lingering mystery. Some people are known to have been exposed to HIV-1 many times but did not become infected, and blood cells from some of these people could not support replication of HIV after experimental infection (11). Stunningly, some of these people were found to be mutant in the newly discovered cofactor, explaining their resistance (ref. 12; for review and references see ref. 13).

It has been known for about 10 years that the receptor for HIV is the CD4 protein present on the surface of cells killed by HIV-1. However, it was suspected that CD4 was not the only molecule involved in mediating virus entry into cells because some cells bearing CD4 on the surface could not be infected. After long effort, Berger and coworkers (14) developed a functional assay that allowed the first example of such a "coreceptor" protein to be identified, a molecule now called CXCR4. The CXCR4 protein turned out to be a cell-surface receptors for chemokines, molecules that promote accumulation of lymphocytes at sites of infection or wounding.

Exciting though this work was, it was also clear that other coreceptors likely mediated entry by different HIV-1 variants. Some strains of HIV-1 favored infection of T cells, while others favored infection of a class of immune system cells called macrophages. The coreceptor for infection of macrophages was also implicated as a chemokine receptor, because certain chemokines could block infection of cultured macrophages by HIV-1 (15). After a heated race, several groups isolated the macrophage coreceptor at about the same time, a protein now called CCR5 (16–20).

Strikingly, those people who had been multiply exposed to HIV but not infected harbored inactivated copies of the gene for CCR5 (12). Evidently a lack of CCR5 blocks establishment of infection by HIV-1. Later molecular epidemiology surveys confirmed this conclusion and greatly increased the numbers of CCR5-mutant people studied.

More recently, a small number of exceptional cases have been found in which people mutant for the CCR5 gene have become infected, raising the question of whether the lack of CCR5 blocks infection by some routes but not others. HIV is known to be transmissible by three routes: transfer of blood products, birth, and sexual contact. Possibly the lack of CCR5 is protective only against transmission across mucous membranes, which likely involves initial infection of macrophages or related cells. Ongoing studies should soon clarify this issue.

Other Prospects for HIV Therapy

Another promising and unexploited target for inhibitor development is the virus-encoded integrase protein. After infection of a sensitive cell, retroviruses synthesize a DNA copy of the viral RNA genome (reverse transcription), and integrate that copy into a chromosome of the host (integration). The integrase protein carries out the initial covalent steps of DNA integration. At present, integrase is the only viral enzyme for which clinically useful inhibitors are not available. There are no known proteins required in human cells that resemble the integrase in function or sequence, raising the hope that selective integrase inhibitors might be relatively nontoxic. Many compounds have been reported that can inhibit integrase protein *in vitro*, and intense efforts are underway to adapt these inhibitors as clinically useful drugs (for review see ref. 21). In addition, a cellular protein seems to be selectively important in integration, the HMG I protein (22). Efforts are underway to determine whether HMG I or other viral and cellular proteins may also be useful as targets for antiretroviral agents.

AIDS Worldwide

Many HIV-infected people in the developed world can now be treated with therapy likely to improve the length and quality of life, but in the developing world, AIDS continues to spread untreated and unchecked. The United Nations AIDS program estimates that 20 million people are infected in the sub-Saharan Africa alone. Zimbabwe, Uganda, and Botswana may lose a quarter of their adult populations to AIDS. Worldwide, over 30 million adults may be infected and 2.3 million people are expected to die of AIDS this year.

Reducing the burden of AIDS in the developing world may require a radically different approach, such as use of an anti-HIV vaccine. The obstacles, however, are daunting. Ethical testing of any vaccine will require large experimental and control populations, making trials difficult and expensive. In the United States, a long-term initiative to develop a vaccine has been launched, but this effort will not soon yield a usable vaccine. The AIDS pandemic likely will remain devastating for a long time to come. However, the new developments reviewed here do offer some sound reasons for long-term hope.

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