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PATHOGENESIS AND TREATMENT OF HIV-1 INFECTION: RECENT DEVELOPMENTS (Y2K UPDATE)

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1. ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). The pathogenesis of HIV-1-induced disease is complex and characterized by the interplay of both viral and host factors, which together determine the outcome of infection. An improved understanding of the pathogenic mechanisms of AIDS, combined with recent insights into the dynamics of viral infection may provide powerful new opportunities for therapeutic intervention against this virus.

2. INTRODUCTION

HIV-1 is estimated to infect at least 33.4 million people worldwide, based on World Health Organization estimates (http://www.unaids.org). This updated review describes recent advances in our understanding of the pathogenesis and viral dynamics of AIDS. In the approximately two and a half years since our original review, important new insights into the biology and lifecycle of HIV-1 have been brought to light following advances in therapy which have resulted in prolonged suppression of viral replication. These advances, as well as progress in vaccine development and future directions for therapeutic management of HIV-1 infection are discussed.

3. PATHOGENESIS OF HIV-1 INFECTION

The pathogenesis of HIV-1 infection reflects the complex interplay between virus replication, virally-induced lymphocyte killing, and the immune response of the host. The latter includes both beneficial responses, which suppress viral replication, and harmful responses which enhance replication and exacerbate cell killing. Each of these aspects of the pathogenesis of HIV-1 infection will be considered separately; HIV-1 neuropathogenesis is not discussed here but has been reviewed elsewhere (1).

3.1. Disease progression in HIV-1 infected persons

The typical pattern of HIV-1 infection *in vivo* is shown in figure 1. It should be noted, however, that virus infection does not always conform to this representative scenario. For example, in about 1-5% of virus-positive individuals, infection may be non-progressive with no decline

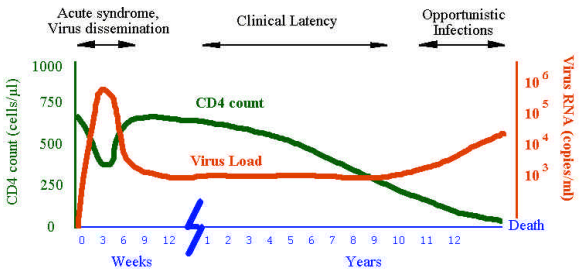

Figure 1: Schematic representation of the course of HIV-1 infection *in vivo*. *Note: All units (CD4 count, virus RNA, timeline) are very approximate.*

in $CD4^+$ lymphocyte counts and very low levels of viral RNA (2-5). This nonprogressive state may in some cases reflect viral factors, such as infection by naturally-occuring, partially attenuated, strains of HIV-1 (6-8). However, it is likely that host factors (especially genetics) may play an even more important role in (1) determining the likelihood that an HIV-1 exposed person may become infected by the virus, and (2) influencing the rate of disease progression in a virally-infected individuals.

Host factors which may influence the rate of HIV-1 disease progression include polymorphisms in genes that regulate viral replication or antiviral responses, and/or polymorphisms in the regulatory elements associated with these genes (i.e., promoter mutations). Careful genetic analysis of human populations has revealed an association between the rate of disease progression and specific polymorphisms in genes encoding viral coreceptor molecules (CCR5, CXCR4), chemokines (SDF1, RANTES) or other immune response molecules (e.g., HLA molecules) (9-19). The rate of disease progression also appears to be MHC class I diversity, with heterozygotes exhibiting a slower rate of progression than homozygotes (9). Furthermore, the disease-modifying effects of certain genetic traits are themselves subject to modification by racial/ethnic factors (20, 21). Thus, host genetic factors which influence susceptibility to HIV-1 are complex and multifactorial. This topic is discussed in greater detail in a number of recent review articles (22-24).

3.2. Viral replication and viral dynamics

The viral dynamics of HIV-1 infection have been well studied, and are the subject of excellent reviews (25, 26). In most cases, high level viremia occurs during all phases of infection, including the period of clinical latency (27, 28), and average daily production of HIV-1 has been estimated at approximately 1×10^{10} virions (29). As noted by Coffin, these findings are consistent with a simple steady-state model of HIV-1 viral dynamics, in which virus production and virus elimination are in equilibrium (30, 31).

The prolonged, high level output of HIV-1 *in vivo* reflects an active, ongoing, process in which CD4 lymphocytes are being infected and killed in large numbers. Indeed, estimated total $CD4^+$ cell destruction rates are on the order of $1\text{-}2 \times 10^9$ cells per day (27, 28). Thus, roughly 1% of the body's total complement of $CD4^+$ lymphocytes (2×10^{11} cells) are eliminated, and replaced, each day over a period of many years. It has been suggested that this level of $CD4^+$ T lymphocyte production may place a high, and ultimately non-sustainable, burden on the replacement system – eventually resulting in an immune collapse. However, more recent studies have challenged this somewhat simplistic paradigm (see section 4).

Nonetheless, HIV-1 replication and virus load are clearly the driving forces behind viral pathogenesis. This has been convincingly demonstrated by several studies. Perhaps the most compelling data are those reported by Mellors and colleagues (32). These investigators found that, among persons with equivalent baseline $CD4^+$ T cell counts, individuals with high baseline plasma HIV-1 RNA loads (>10,190 molecules/ml) died more rapidly (mean of 6.8 years) than individuals with low (< 10,190 molecules/ml) baseline plasma HIV-1 RNA loads (time to death > 10 years). As a result of these and other studies, plasma HIV-1 RNA determinations, which can be performed by three commercially available assays -- branched DNA (bDNA), reverse transcriptase-polymerase chain reaction (RT-PCR) and nucleic acid sequence-based amplification (NASBA) -- are now a fundamental part of clinical practice (33).

Attempts to better understand the relationship between virus load and disease progression have revealed that the relationship between average viral load and the time from seroconversion to death is remarkably constant (34). This suggests that survival time may be determined not only by a patient's present virus load, but also by that individual's history with the virus. This view is consistent with the idea that HIV-1 infection may be progressively lymphodegenerative, and it predicts that following seroconversion, any given person can withstand only a finite amount of virus replication (34).

3.3. Viral reservoirs

Although the level of HIV-1 RNA load in peripheral blood can be readily measured, less information has been garnered concerning viral replication in lymphoid tissues, principally due to the relative inaccessibility of these compartments. Haase and colleagues have applied a quantitative image analysis technique to analyze the viral burden in lymphoid tissues (35). A strikingly large and stable pool of extracellular virions was detected within lymphoid tissue, trapped on the surfaces of follicular dendritic cells (FDC). This FDC virus pool was estimated to be 10 to 40 times larger than the productive virus pool, and it may therefore accounts for most of the total body burden of HIV-1 RNA (roughly 10^{11} copies of HIV-1 RNA) (35). The FDC virus pool may be important for the perpetuation and spread of viral infection within lymphoid tissue. However, the amount of virus trapped on FDCs decreases very rapidly following successful antiviral therapy, which suggests that the binding and dissociation of virions from FDCs is in equilibrium with production and clearance of virus from the blood (36).

Quantitative measurements of viral decay kinetics following initiation of effective antiviral regimens have provided additional insights into the reservoirs of

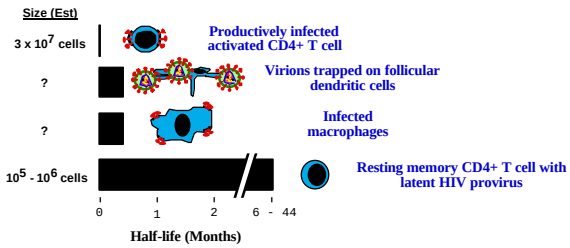

Figure 2. Cellular reservoirs of HIV-1 infection.

HIV-1 infection of the host. Initial studies revealed that plasma levels of HIV-1 RNA decreased by roughly 100-fold within the first two weeks following initiation of potent antiviral therapy (27, 28). This massive reduction in virus load reflects the rapid decay and elimination of plasma virions and virus-producing $CD4^+$ T cells. Based on these experiments, the half-life of extracellular HIV-1 virions was calculated to be < 6 hours, while the half-life of virus-producing $CD4^+$ T cells was found to be approximately 1 day (27-29, 37). The pool of virus producing cells is surprisingly small, and has been estimated to be around 3×10^7 cells (38). The *effective* size of the HIV-producing cell population may be even smaller (on the order of 5×10^5 cells), since not all RNA-producing cells may generate virus that reach a target cell (39).

The relatively small population of productively infected $CD4^+$ T lymphocytes accounts for the great majority of virus generation during the steady state of infection. However, additional cellular reservoirs are also important. This was first revealed in a study reported by Perelson and coworkers. In this study, Perelson *et al.* showed that, after the rapid first phase of decay during the initial 1-2 weeks of antiretroviral treatment, plasma virus levels declined at a considerably slower rate (37). This second phase of viral decay was attributed to the turnover of a longer-lived virus reservoir or infected cell population, which was determined to have a half-life of 1-4 weeks. This reservoir accounts for only a small fraction of total virus production in an untreated person (1% or less), and it therefore becomes evident only when the productively infected $CD4^+$ T cell pool has decayed away. Perelson and colleagues have suggested that this second reservoir of HIV-1 infection may represent virus-infected macrophages (37). This would be consistent with current estimates of the turnover rate of macrophages in uninfected persons.

Continued follow-up of persons who have remained on HAART for extended periods of time has provided strong evidence for the existence of an additional, latent virus reservoir in long-lived $CD4^+$ memory T lymphocytes (figure 2). As a result, replication-competent HIV can be recovered from the $CD4^+$ lymphocytes of persons who have been successfully treated with HAART for as long as 2 years or more (40-42). This latent reservoir accounts for a third phase of HIV-1 decay during HAART. In this case, the kinetics of decay are extremely slow, and the half-life of the latent reservoir has been estimated at between 6 and 44 months (43, 44). Estimates of the size of this latent reservoir suggest that it may comprise at least 1×10^5 cells (41), and possibly as many as 10^6 cells (38). As a consequence, the predicted time required for effective anti-retroviral therapy to fully eradicate HIV from the body ranges anywhere from 10 to 60 years. This would suggest that a true virologic cure will be unattainable using conventional antiretroviral regimens.

Understanding of the viral dynamics of HIV-1 infection has been complicated by the realization that HIV-1 continues to replicate at low levels throughout HAART (44-47). Grossman and colleagues have suggested that the very slow rates of viral decline which occur following initiation of HAART can be best explained if most of the virus is produced by cells infected *after* the commencement of treatment (45). Therefore, these authors have postulated that the slow decline of virus levels is not due to the very slow turnover of a stable latent reservoir of HIV-1 infection, but rather it is due to ongoing HIV-1 replication cycles of progressively decreasing amplitude. This alternative explanation for HIV-1 decay kinetics is far more compatible with the notion that HIV-1 can be completely eradicated from the infected host – provided that antiretroviral drugs with improved pharmacologic and antiviral properties can be developed.

3.4. Viral dynamics of acute HIV-1 infection

HIV-1 RNA load is relatively stable throughout much of the course of the viral infection (at least, until the onset of frank AIDS). Indeed, it appears that the steady-state, or equilibrium, level of HIV-1 RNA load is established within the first several months following virus infection. Thus, in early HIV-1 infection, a high virus burden is predictive of rapid disease progression (48, 49). This suggests that the first few weeks and months following the initial HIV-1 infection may represent a key phase in the pathogenesis of AIDS, in which the virus is able to replicate to very high levels, to "seed" lymphoid organs, establish a latent reservoir (50), and generate a state of equilibrium with its host. As a result, there is considerable interest in understanding the viral dynamics and pathogenesis of the acute phase of HIV-1 infection.

Recent evaluation of viremia during acute HIV-1 infection has revealed that the mean initial viral doubling time was very rapid (10 hours) and that the peak of viremia occurred at approximately 21 days after infection (51). The virus doubling time in two individuals who discontinued antiretroviral therapy was roughly five-times slower, persumably because of the presence of acquired host immunity in these individuals (51).

3.5. Viral genetic variation

The rapid emergence of viral quasispecies (closely related but genetically distinct viral variants) is one of the hallmarks of lentiviral infections (52), both in humans and in primates. This property is also characteristic of RNA viruses as a whole, in large part due to the high mutation rate of RNA polymerases (53). In considering the genetic variation of HIV-1, it is instructive to note that the average viral generation time during the steady state of infection (defined as the interval between the release of a

viral particle and the infection of a new host cell and release of a new burst of progeny virions) has been estimated at 2.6 days (29). Thus, HIV-1 replicates at a rate of 140 generations per year, for a period of ten or more years. It can be readily appreciated that this will lead to rapid and explosive genetic variation (30), since the mutation rate of HIV-1 is roughly 3×10^{-5} per base per replication cycle, and the viral genome size is 10^4 base pairs. If 10^{10} new viral genomes are produced each day, then on average, every mutation at each position in the viral genome will occur several times -- in just a single day. This unprecedented genetic diversification has important implications for the evolution of viral variants with resistance to antiviral drugs. One can predict that such mutations will occur inevitably and rapidly -- except, perhaps, when multiple antiviral drugs are used in combination and when therapy is initiated early during the course of virus infection.

Viral genetic variation may also be driven, to some extent, by the host immune responses (53, 54). As a result, viral genetic diversity is greatest in clinically healthy individuals and much less in persons with AIDS (where the immune response has become severely impaired) (53, 55, 56). Thus, the extent of intrahost HIV-1 evolution is to some degree related to the length of the immunocompetent period (54). It is less clear whether HIV-1 genetic diversity plays any direct role in the pathogenesis of AIDS. Nowak and colleagues have proposed the existence of an "antigenic diversity threshold", in which the ever-expanding genetic diversity of HIV-1 eventually exhausts the capacity of the immune system to respond, resulting in an immune collapse (57). However, direct support for this theory has been elusive (53, 54).

3.6. Protective host immune responses

In all likelihood, the pathogenesis of AIDS reflects a balance between viral replication and the immune response. Available evidence suggests that humoral immunity is probably not the dominant mode by which HIV-1 replication is controlled, under normal circumstances. Primary HIV-1 isolates are relatively resistant to neutralization by patient sera, unlike T-cell line-adapted strains (58-60). In addition, although HIV-1 infected long-term non-progressors produce vigorous serum antibody responses, virus strains isolated from these individuals were not neutralized by autologous sera (61). The weak nature of the neutralizing antibody response in HIV-infected persons (and in vaccine recipients) suggests that the molecular structure of HIV-1, and of its major surface envelope glycoprotein (gp120), may play a role in preventing the generation of neutralizing antibodies. It is certainly not the case that primary HIV-1 isolates are inherently resistant to neutralization *per se*, since antisera capable of neutralizing primary virus isolates can be generated in mice, using experimentally modified forms of gp120 (so called "fusion-competent" immunogens that capture the transient gp120-CD4-coreceptor structures which arise during HIV binding and fusion (62)).

In contrast to humoral immune responses, cellular responses to HIV-1 are widely believed to be critical to the control of natural virus infection. Several studies have shown that there is a temporal association between the generation of HIV-specific cytotoxic T cell (CTL) responses and the decline in viremia that occurs during primary HIV-1 infection (63, 64). Furthermore, the level of HIV-specific CTL responses has been shown to correlate inversely with plasma virus load and disease progression (65, 66).

If CTL responses are, in fact, able to suppress viral replication, why does viral replication continue at high rates in many patients with eventual failure of the immune system? One theory is that CTL escape mutants are generated. Consistent with this, Borrow *et al.* showed that primary virus infection was associated with the rapid clearance of the transmitted strain, followed by selection for a virus population comprised of strains bearing a mutation in the major immunodominant CTL epitope (67). CTL escape mutants have also been identified late in infection and may contribute to disease progression (68). However, one patient was described in which the dominant virus variant continued to be recognized by CTLs throughout disease progression. Absence of broadening of CTL responses and lack of T cell proliferative responses in this patient indicate that ineffective CD4 help may have been the basis for the lack of functional CTLs (69).

Pantaleo and colleagues have made the interesting observation that mobilization of a broad T cell receptor (TCR) repertoire during acute HIV-1 infection is associated with a relatively stable clinical course, while mobilization of a restricted subset of $CD8^+$ T cells is associated with more rapid disease progression (70). If, as these authors suggest, the $CD8^+$ T cell families which are expanded during acute infection are rapidly deleted, then it is relatively easy to understand how a broad TCR repertoire could be important in controlling the early stages of HIV-1 infection, and thereby establishing a relatively low steady-state level of HIV-1 RNA load. It has also been noted that CTLs directed against p24 have a stronger inverse correlation with viral loads than those directed against gp120 (71, 72). This suggests that not all HIV-specific CTL are of equal importance in terms of their ability to control virus replication *in vivo*.

Several recent reports underscore the importance of strong Th1 cell function in controlling viral replication (69, 71, 73). In the absence of $CD4^+$ help, antiviral $CD8^+$ CTLs may be non-functional (74). Other protective aspects of the immune response may include the production of high levels of soluble inhibitors of virus infection and replication -- as first noted by Levy and colleagues (75). The most well characterized examples are the beta-chemokines, macrophage inflammatory proteins 1 (MIP-1) alpha and beta and RANTES (regulated on activation, normally T-cell expressed and secreted), which are shown in figure 3. A correlation between high levels of beta-chemokines and more favorable clinical status in HIV-1 infected patients has been reported (76, 77), suggesting that these chemokines may contribute to the control of virus replication in infected individuals. Furthermore, elevated levels of these chemokines have been found in exposed-uninfected person (76, 78), and thus may also contribute to resistance against infection.

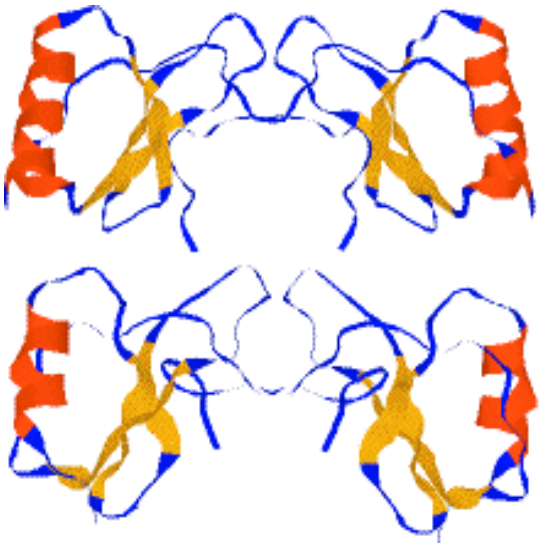

Figure 3. Structural representation of the beta-chemokines, MIP-1 beta (top) and RANTES (bottom). Red coils represent alpha-helices and orange sheets represent beta-sheets. Both chemokines are homodimers composed of identical subunits. At the amino acid level, RANTES and MIP-1 beta exhibit 49% identity, but structurally the two molecules are even more closely related (see above), and both bind to the same receptor, CCR5. The above molecular representations were generated with RasMol (http://science.glaxowellcome.com/science/rasmol/rasmol/predef.htm) using the PDB database (http://www.rcsb.org/pdb/) files 1HRJ.PDB (RANTES) and 1HUM.PDB (MIP-1 beta).

The beta-chemokines can interfere with the ability of M-tropic HIV-1 strains to infect T cells (79), by blocking the binding of HIV-1 gp120 to the CCR5 entry cofactor, as illustrated in figure 4 (80-85). Infection of monocyte/macrophages by these same HIV-1 strains is not blocked (86, 87), which is somewhat surprising, since macrophages express CCR5 (86). This suggests that the process of HIV-1 entry in these cells may be different than for T cells (88, 89). This might also explain why macrophages cannot be infected by T-tropic HIV-1 strains even though they express CXCR4 (86). Furthermore, primary T cells express both CCR5 and CXCR4, and are thus susceptible to both M- and T- tropic viruses (90). Thus, the role of chemokine receptors in cell tropism is probably much more complex than first appreciated. This notion is reinforced by the fact that CCR5 is differentially expressed on distinct Th subsets. High level CCR5 expression on Th1 cells may be important in impairing protective immune responses (91).

3.7. Immunopathogenic mechanisms

The last two years have yielded much new information which has contributed to a refinement of original models of AIDS pathogenesis. Some areas remain unresolved, however. One such controversy concerns the predominance of CCR5-utilizing (R5) variants in the early stages of HIV-1 infection. It has been postulated that R5 viruses predominate because they efficiently infect macrophages and dendritic cells, and are therefore capable of initiating infection at mucosal surfaces. However, this hypothesis fails to adequately explain all available data. In SIV-infected macaques, macrophages, dendritic cells (DC) and T cells have all been reported to become infected at very early time points following mucosal virus transmission (26, 92, 93). Immature dendritic cells in the skin and at mucosal surfaces, are known to be highly efficient at transmitting HIV-1 to $CD4^+$ T cells and macrophages (94-96), and could therefore play a role in determing which HIV-1 become transmitted across mucosal surfaces. However, recent studies have also shown that the majority of T cells derived from human cervicovaginal mucosa express CCR5 as well as CXCR4, and are susceptible to infection with both R5 and X4 viruses (97). Furthermore, most DC from these tissues were not found to express CCR5 (97). Thus, the differential expression of viral coreceptors may not be sufficient to explain why R5 viruses are transmitted more efficiently via the mucosal routes than are X4 viruses.

The mechanism for $CD4^+$ T cell depletion in HIV-1 infection is also incompletely understood. Labeling studies evaluating Ki67 expression (which detects proliferating cells) or telomere length have concluded that the turnover of T cells is not markedly increased in HIV infection, supporting the concept that T cell depletion occurs due to diminished renewal of $CD4^+$ cells (98-100). Other studies support an "open drain/open tap" model, wherein T cells are proliferating and being killed at an increased rate, which eventually exhausts the capacity of $CD4^+$ T cells to be replenished (27, 101, 102). However, this hypothesis has recently been questioned (see section 4).

Overall, this is a controversial area of research. Careful analyses of the rates of T cell production have provided no evidence to support the existence of a defect in the generation of $CD4^+$ T cells in HIV-1 infected persons (98, 103). Furthermore, overall rates of $CD4^+$ T lymphocyte production have been shown to increase dramatically following highly active antiretroviral therapy (HAART) -- indicating that the CD4 replacement machinery in HIV-positive persons is not irreversibly damaged (103, 104). Rather, present evidence suggests that HIV-1 infection may lead to a significant reduction in the half-life of circulating $CD4^+$ and $CD8^+$ T cells, and that this is not adequately compensated by a commensurate increase in the production of $CD4^+$ T cells (103).

One additional outcome of recent experiments on the generation of T lymphocytes in HIV-infected individuals has been the realization that the human thymus remains surprisingly active even into late adulthood (104, 105). The role of thymocyte infection in AIDS pathogenesis is still not clear. HIV enters immature ($CD4^+CD8^+$) thymocytes, but does not replicate until the thymocytes interact with the thymic epithelium, thereby eliciting the production of cytokines that trigger viral replication (106, 107). This may contribute to thymic damage during HIV-1 replication. However, virally-induced thymic damage does not lead to irreversible abrogation of thymic function, since recent thymic emigrants have been detected at normal levels in HIV-positive individuals who initiated HAART therapy (104, 105). Thus, the pathogenic significance of HIV-1's effects on the thymus remain uncertain.

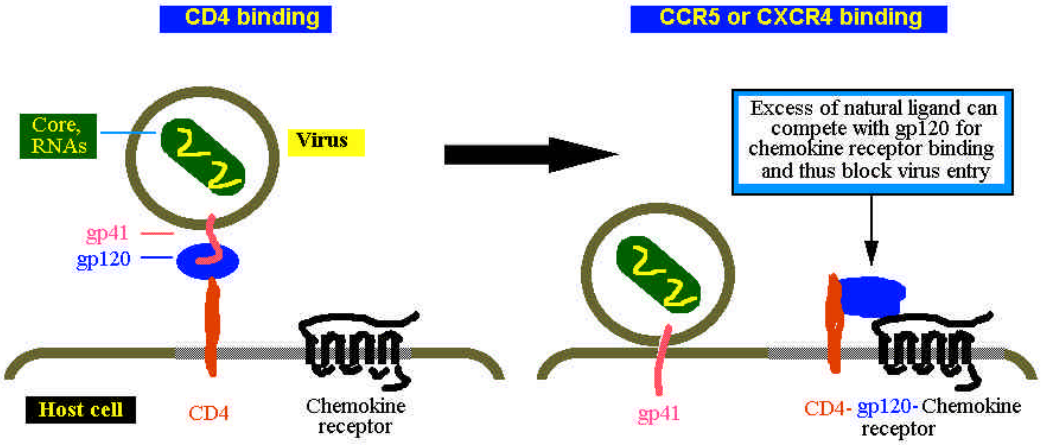

Figure 4. Model for HIV-1 entry in T cells. HIV-1 gp120 first binds to cellular CD4. This results in a conformational change in gp120, allowing it to bind to the chemokine receptors CCR5 or CXCR4, thereby forming a trimolecular complex (CD4-gp120-CCR5/CXCR4). An excess of the natural ligands for CCR5 or CXCR4 can competitively inhibit this step of infection (as noted). After binding to the chemokine receptor, gp120 is thought to become stripped off the virion, thereby exposing a hydrophobic domain at the N-terminus of gp41, which mediates fusion of the host cell and virus membranes, thereby allowing the virus core to enter the host cell cytoplasm.

In addition to its ability to kill thymocytes, HIV-1 has also been shown to induce apoptosis of both virally infected $CD4^+$ cells and bystander cells, including $CD8^+$ cells (108-110). While there has been some debate as to the basis for the observed increase in apoptosis in uninfected bystander T cells (109, 111), the increase in T cell apoptosis during HIV-1 infection may be related to a general state of immune activation which may be driven by persistent antigenic stimulation from virions trapped on lymphoid follicles (102) or by release of soluble gp120 and Tat (112-116). Once again, the pathogenic significance of HIV-1 induced T cell apoptosis remains contentious -- although the absence of virally-induced apoptosis during nonpathogenic infections of chimpanzees has been interpreted as being consistent with the notion that apoptosis may play some role in virally-mediated disease induction (117, 118).

T cell activation during HIV-1 infection is also subject to regulation by endogenous proinflammatory cytokines such as tumor necrosis factor (TNF) alpha and interleukin-6 (IL-6) (119, 120). Levels of these proinflammatory cytokines are often high during the acute phase of HIV-1 infection (121-123), particularly in individuals who experience a severe or symptomatic acute primary HIV-1 syndrome (123, 124). This may in part explain why extensive T cell activation during the early phases of HIV-1 infection is associated with more rapid disease progression (49, 124).

T cell activation may also contribute to the replication of HIV-1 during the post-acute phase of virus infection. For example, HIV-1 infection of cultured $CD4^+$ T cells is greatly enhanced in the setting of antigen-specific immune activation (49, 125), and virus replication increases dramatically *in vivo* after vaccination of HIV-1 infected persons with a variety of immunogens (126-128). The increased susceptibility of T cells from HIV-1 infected persons to activation-induced cell death may be important to the pathogenesis of immune deficiency and may help to explain the accelerated course of HIV-1 induced disease in areas where immune activation may be persistent or chronic due to endemic parasites and other pathogens (129).

An important corollary of the relationship between immune activation and HIV-1 replication is the prediction that immune suppression should lead to a reduction in the viral burden and perhaps even to an improvement in clinical status (130, 131). Immune suppressive therapy has been associated with a rise in $CD4^+$ cell counts in asymptomatic HIV-1 infected persons (the median CD4 increase in a cohort of 44 persons who received oral prednisolone for one year was 119 cells per microliter (132)). In addition, cyclosporin A treatment of rhesus macaques that were experimentally infected with SIV resulted in a decrease in the duration of viral antigenemia during acute infection (133).

A state of generalized immune activation may result not only in an increase in T cell apoptosis, and a rise in viral replication, but also may exert effects on the production of beta-chemokines and other chemotactic factors. This may have complex effects on viral pathogenesis. For example, Nef production within HIV-infected macrophages has been shown to result in the secretion of chemokines which recruit and activate T cells, thereby facilitating virus replication and transmission (134). Beta-chemokines can also enhance HIV-1 replication in cultured cells, under certain conditions (135, 136). On the other hand, cells may be protected from HIV-1 infection through the action of beta-chemokines (137), and natural resistance to HIV has been associated with elevated levels of beta-chemokines in hemophiliacs and infants born to HIV-infected mothers (138, 139). Thus, the role of beta-chemokines in HIV-1 pathogenesis remains to some extent unresolved (140).

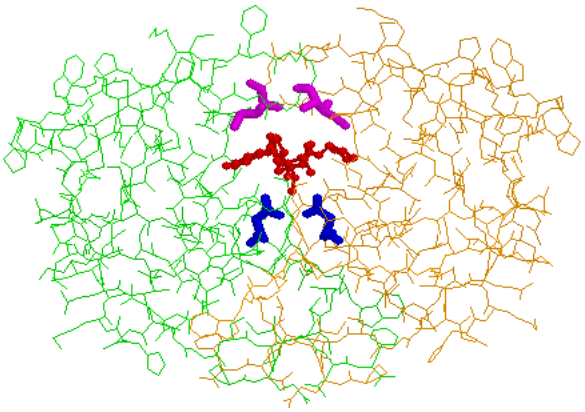

Figure 5. Structure of HIV-1 protease, complexed with the enzyme inhibitor VX-478. The individual protease monomers are colored orange and green. VX-478 has replaced the substrate, and is shown in red. The paired active site Asp residues (Asp25) are shown in blue, and the key residues involved in viral genetic resistance to VX-478 are shown in blue (Ile 50). This image was created with RasMol (http://science.glaxowellcome.com/science/rasmol/rasmol/predef.htm) using the PDB database (http://www.rcsb.org/pdb/) file 1HPV.PDB; protease residues are shown in the "wireframe" format. A 3-D version of this figure is available (http://www.bioscience.org/1997/v2/d/dewhurs1/htmls/prvxfram.htm).

4. RECENT DEVELOPMENTS IN ANTIRETROVIRAL THERAPY

4.1. Protease inhibitors and combination therapy

The HIV-1 Gag and Pol proteins are encoded in the form of large polypeptide precursors which must be proteolytically processed into mature proteins. The proteolytic cleavage of Gag and Pol precursor polyproteins is carried out by a virally-encoded aspartyl protease that is required for virus replication, and which has been structurally examined at the atomic level (reviewed in (141)). Using this information, enzyme inhibitors were designed (142), and their pharmocologic properties (e.g., oral bioavailability) modified so as to arrive at biologically effective antiviral drugs. The first to receive Food and Drug Administration (FDA) approval, in 1995, was saquinavir (invirase), followed swiftly by ritonavir (norvir) and indinavir (crixivan). Additional protease inhibitors that have been approved by the FDA include nelfinavir (viracept) and amprenavir (agenerase), the first new protease inhibitor to be approved in two years (http://www.vpharm.com/disease_targ/hiv.html).

HIV-1 protease is a homodimeric protein composed of two identical subunits of 99 amino acids each, and the protease inhibitors are transition state analogs enzyme much more tightly than does the natural substrate (since the substrate must be distorted to assume its transition state configuration). Thus, the presently available protease inhibitors function as competitive enzyme inhibitors. The structure of HIV-1 protease, complexed with one of its inhibitors (VX-478) is presented in figure 5.

The availability of HIV-1 protease inhibitors has permitted the development of sophisticated combination treatments for virus infection, many of which use triple drug combinations that combine protease inhibitors with nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (retrovir or AZT) and lamivudine (epivir or 3TC) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine (viramune). Therapeutic regimens which employ such drug combinations are commonly refered to as highly active antiretroviral therapy or HAART (see next section).

While protease inhibitors have emerged as an extremely powerful therapeutic tool, these drugs have also revealed unexpected side effects on lipid metabolism and storage. These side effects include peripheral lipodystrophy, hyperlipidaemia, and possible insulin resistance (143, 144). It has been suggested that this may be due to homology between the protease active site, and regions within proteins that regulate lipid metabolism, including low density lipoprotein-receptor-related protein (LRP) (143).

4.2. Advances in the prevention of maternal-infant transmission of HIV-1

Ever since the completion of the ACTG 076 drug trial in 1994, it has been known that antiretroviral therapy can exert a very profound effect on the incidence of mother-to-infant transmission of HIV-1 (145). Indeed, the ACTG 076 zidovudine monotherapy protocol resulted in a decline in the rate of vertical HIV-1 transmission from 25.5% to 8.3% (145). This in turn has resulted in a dramatic (67%) decrease in the number of perinatal AIDS cases in the U.S. from 1992 through 1997 (146). This decline was even more pronounced (80%) in infants under one year of age, and could not be explained either by a reduction in the number of births to HIV-positive women, or by a delay effects of therapy in delaying AIDS (146).

Attempts to improve therapeutic approaches to the prevention of maternal-infant transmission are ongoing. Regardless of the outcome of these studies, it has become apparent that developing nations (where the burden of HIV-1 infection is disproportionately high) lack the financial resources to provide extensive zidovudine treatment to pregnant women or their infants. Consequently, there has been considerable interest in determining whether lower cost strategies may also prove effective in preventing the vertical spread of HIV-1.

Initial studies showed that short-course zidovudine could also reduce maternal-infant transmission, at a much reduced cost compared to the regimen used in ACTG 076 (147). However, the real breakthrough has come in the form of studies on the NNRTI, nevirapine. This drug is much cheaper than zidovudine, and it was shown to lower the risk of HIV-1 transmission during the first 14-16 weeks of life by nearly 50% in a breastfeeding population in Uganda (148). Projections of the expected cost effectiveness of this intervention suggest universal treatment of all pregnant women in an area of high seroprevalence would result in a cost of $138 per case averted (149). This remarkable level of cost-effectiveness

has in turn has led to a call to buy nevirapine for developing nations (150).

4.3. Highly active anti-retroviral therapy (HAART)

The last few years have brought a roller-coaster ride of optimism, followed by disappointment, as the impact of HAART has become apparent. HAART's ability to cause a dramatic and sustained suppression of viral replication, and its effect on the course of HIV-1 associated disease gave rise to the hope that a true cure for infected persons might be feasible. Unfortunately, it has subsequently become apparent that this is unlikely to be the case – at least with current therapeutic modalities – since viremia can be re-activated following cessation of therapy. This section discusses a number of important findings relevant to HIV-1 pathogenesis that have surfaced through the monitoring of increasing numbers of patients on HAART, as well as some of the more recent developments in therapy.

4.4. Immune recovery following HAART

Largely because of the profound effect of HAART on survival of AIDS patients (151, 152), HIV-1 infection dropped from the 8th leading cause of death in the U.S. in 1996, to the 14th leading cause in 1997, with a 47.6% decrease in deaths between 1996 and 1997 (153). There has also been a significant decline in the incidence of Kaposi's sarcoma since the advent of HAART. Similar decreases in AIDS-related non-Hodgkin's Lymphoma were not reported, suggesting that it may be necessary to treat earlier in infection in order to prevent some other AIDS-related tumors (154, 155).

Following successful HAART, there is a prompt, dramatic rise in circulating memory CD4+ T cells. The mechanism for this early rise probably involves redistribution of cells following their sequestration in lymphoid tissues during the period of active viral replication (98) and it is followed by a slower increase in circulating naïve T cells (102, 156-158) -- although restoration of these cells may not occur if the patient is depleted of circulating naïve T cells prior to therapy (159). Within 6 months following the initiation of HAART, T cell reactivity to recall antigens is restored in many patients (156), but there may be residual perturbations in the CD4+ cell repertoire for 6 months or more after treatment (98, 160). Pathological changes in the lymph nodes are also reversed following HAART, taking up to 2.5 years to return to normal (161).

In terms of virus-specific immune responses, both activated and memory CTL populations decline progressively following the initiation of HAART (71, 162). The half-life of the decay in HIV-specific effector CTL has been estimated at 45 days (162), and is presumed to reflect the loss of ongoing antigenic stimulation during successful HAART. This may have important implications for the immunologic control of HIV-1 infection, following cessation or discontinuation of HAART therapy (see next section).

5. FUTURE DIRECTIONS

5.1. New antiviral drugs

Improvements in therapies for HIV-1 infection may come both from improved or novel antiviral drugs and from a better understanding of host immune responses to the virus. Several new classes of antiviral drugs are being developed, including inhibitors of the HIV-1 integrase (163, 164), as well as compounds targeted against the highly conserved HIV-1 nucleocapsid protein zinc fingers involved in genome packaging and virus assembly (165, 166). Compounds that block chemokine receptors are also being actively pursued; these include small molecules such as TAK-779 for CCR5, and T22, AMD3100 or ALX40-4C for CXCR4 (167-170).

Other molecules which inhibit virus entry may also prove to be effective antivirals. T-20 (previously known as DP178) is a synthetic peptide corresponding to a region of the transmembrane subunit of the HIV-1 envelope glycoprotein (gp41), that has been shown to block virus fusion and entry *in vitro* at nanomolar concentrations (171). In a phase I clinical trial in HIV-1 infected patients, the compound also exerted a potent antiviral effect (1.96 $\log_{10}$ median decline in virus load at the end of 14 days of monotherapy with T-20) (172). However, very large amounts of T-20 (~200 mg/day) were required to observe an antiviral effect *in vivo*, raising some concerns with respect to bioavailability issues and/or proteolytic degradation. In an effort to circumvent these concerns, Eckert and colleagues have developed D-peptide inhibitors that also target the gp41 coiled-coil pocket. These compounds and which are also thought to bind to the transiently exposed prehairpin intermediate within gp41, which plays an essential role in fusion of the virus with the host cell membrane, and they also inhibit HIV-1 replication, albeit at micromolar concentrations (173).

An adjunctive approach to antiretroviral therapy may be the use of immune modulators. Prevention of deleterious responses is one possibility, and this may be achievable through targeted blockade of specific inflammatory mediators, such as TNF-alpha. Specific inhibitors of this cytokine, such as thalidomide and pentoxifylline, have been investigated for their therapeutic potential in HIV-1 infected persons. To date, the results of these trials have been disappointing (174-176) although thalidomide has proven to be useful for the treatment of esophageal ulcers in AIDS patients (177).

It may also be possible to facilitate or even to partially restore immune function in persons with HIV-1 infection. Examples of such approaches include the use of interleukin-2 treatment, which has been shown to significantly elevate $CD4^+$ T cell levels (178) and the *ex vivo* expansion of $CD4^+$ cells using CD28 costimulation (179). The latter approach may also facilitate gene therapeutic approaches to HIV-1 infection, by allowing *ex vivo* transduction of $CD4^+$ T cells with retrovirus vectors, followed by selection and expansion of transduced cells (179).

5.2. Strategies for virus eradication

The dramatic early successes with HAART led to the hope that prolonged treatment would lead to the elimination of all HIV-1 within the body, and thus result in a true cure. As noted in section 3, currently available data

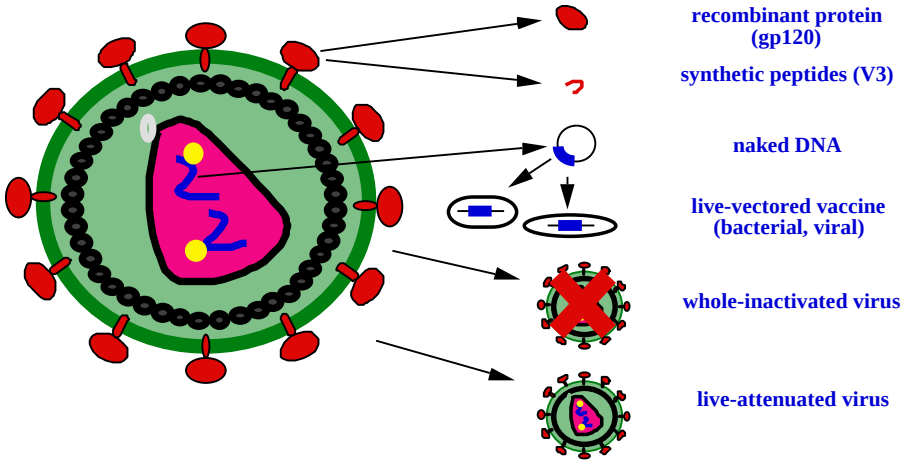

Figure 6. Current strategies in HIV vaccine development. Strategies which are not shown in this figure include the use of virus-like particles (pseudovirions) and the use of combination vaccine approaches (e.g., a DNA prime, live-vectored boost strategy). Pseudovirions can be generated by the co-expression of recombinant proteins such as Gag and Env, using either recombinant protein, DNA-based or live-vectored vaccine approaches (183).

strongly suggest this is unlikely to prove feasible, at least with current antiretroviral regimens.

It has been suggested that it may be possible to force the latent HIV-1 reservoir into a fully productive state, and to thereby bring about its eradication. This may be achievable through the use of immune-activating strategies, such as the administration of interleukin-2 (180, 181). Alternatively, cytoreductive strategies such as cyclophosphamide treatment may reduce the size of the latent HIV-1 reservoir (182), and thereby facilitate its elimination.

Additional improvements in antiretroviral therapy are likely to come as a result of a better understanding of the pharmacokinetics of antiretroviral drugs, their relative penetration into different cells and tissues, and patient adherence with complex and often toxic drug combinations. This is an especially important area in light of the fact that residual HIV-1 replication continues even in the face of successful HAART – i.e., HAART appears to be incapable of fully suppressing the replication of wild-type HIV-1 (44-47).

Finally, HIV-specific antigenic stimulation, either through immunizations or by brief interruptions in treatment (auto-vaccination), may also enhance the effectiveness of antiviral therapy, by preventing the decline in HIV-specific CTL responses that occurs in persons on HAART (71, 162). Current guidelines for the treatment of HIV are available online at: http://www.hivatis.org.

6. HIV VACCINES

Various approaches are being explored for the generation of HIV vaccines, and the U.S. has committed itself to developing such a vaccine by the year 2007. For reasons of space, HIV vaccines cannot not be discussed at great length in the present review. The reader is therefore refered to the NIAID AIDS vaccine website (http://www.niaid.nih.gov/daids/vaccine) and the International AIDS Vaccine Initiative (IAVI) website (http://www.iavi.org/newpage/menu.html) for updated information.

Since 1987, more than 40 different preventive HIV vaccines have been studied in clinical trials worldwide. These have a variety of different strategies, which are represented schematically in figure 6.

Among the current approaches to HIV vaccine development, a number of strategies merit special comment. These include the following:

6.1. Recombinant gp120

This is probably the most well studied candidate HIV-1 vaccine, but one which fails to generate measurable CTL responses. VaxGen, a San Francisco-based company, initiated the first Phase 3 efficacy trial of an AIDS vaccine in 1998 using its gp120 subunit vaccine known as AIDSVAX. The vaccine is safe, and it elicits a strong serologic response, with measurable levels of homologous virus-neutralizing antibodies (184). 5,000 volunteers will be enrolled in this Phase 3 study (184). A second Phase 3 trial with AIDSVAX was initiated in Thailand in 1999. It is expected that these efficacy trials will provide a definitive test for the hypothesis that gp120 subunit vaccines can elicit protective serologic immune responses against HIV-1.

6.2. Nucleic acid vaccines

Nucleic acid vaccine strategies have, to date, taken the form of DNA expression plasmids encoding HIV-1 gene products, which are usually injected either intradermally or intramuscularly, using a Gene Gun or similar device. Animal studies have shown that DNA

vaccines can elicit CTL responses and neutralizing antibodies against HIV and SIV antigens (185-188).

DNA vaccines have also been successful in protecting chimpanzees against a nonpathogenic HIV-1 challenge (185). HIV DNA vaccines can also elicit measurable, though somewhat weak, CTL responses in humans (189). Based on these data, human trials of DNA vaccines have been initiated in the U.S. The first such trial was supported by a U.S. biotechnology company, Apollon (recently acquired by Wyeth Lederle Vaccines) (190). Phase I clinical trials of two additional DNA vaccine candidates (one encoding HIV-1 Env and Rev, the other Gag-Pol) are being supported by NIAID, and plans for additional trials are underway. In addition to Wyeth/Apollon, other companies with HIV DNA vaccine programs at earlier stages of development include Merck, Chiron and Pasteur Merieux Connaught. Approaches to improve the efficacy of DNA vaccines include the use of "codon-optimized" expression constructs (191), as well as co-injection with plasmids encoding cytokine/immunostimulatory genes.

6.3. Multivalent vaccines

A major concern with HIV-1 vaccination efforts is the extreme genetic and antigenic diversity among HIV-1 strains, and among human populations. As a result of this diversity, two major issues have arisen. First, there is concern that candidate HIV-1 vaccines may elicit immune responses which may be protective against the immunizing strain and members of the same virus clade (e.g., other clade B viruses), but not against heterologous viruses or members of other clades (e.g., clades C and E). Second, there is concern that the diversity of human HLA molecules may make it difficult to develop an immunogen capable of eliciting strong class I-restricted T cell responses in all human population groups. In an effort to address these issues, some investigators are in the process of developing multivalent or multimeric HIV-1 vaccine strategies. Approaches include the use of multiple DNA or vaccinia virus (VV) vectored gp120 immunogens (192, 193), as well as the development of multiepitope "universal" CTL immunogens capable of class-I restricted presentation by 90% of more of the human population (194-196). The International AIDS Vaccine Initiative (IAVI) is currently sponsoring studies which are ultimately intended to assess the safety and immunogenicity of this approach in humans. In addition, human clinical trials of a multivalent vaccinia virus vector are being planned at St. Jude's Hospital (Memphis, TN).

6.4. Live-vectored vaccines

An array of novel different vector systems for HIV-1 vaccine delivery are currently in various stages of development. These include live-attenuated bacterial vectors, such as Bacille Calmette-Guerin (BCG) and Salmonella (197-199). These vectors are particularly intriguing since they are safe and can establish infection via a mucosal route (197, 198). Thus, they may elicit strong mucosal immune responses; testing of a recombinant Salmonella-HIV gp120 candidate vaccine is presently in a phase I trial (AVEG 029). New virus vector systems include improved poxvirus vectors such as canarypox vectors and modified vaccinia Ankara (MVA), as well as other vector systems, such as herpesviruses and Venezuelan equine encephalitis virus (VEE) (200, 201). IAVI is presently sponsoring studies on recombinant VEE replicon particles developed by AlphaVax; these studies will culminate in a Phase I human clinical trial within the next several years.

Canarypox and MVA vectors are important because they offer improved safety over conventional vaccinia virus vectors, due to their inability to undergo productive replication in human cells. Furthermore, studies with MVA-vectored vaccines in nonhuman primates have shown that this system can elicit potent CTL responses that may slow the progression of immunodeficiency disease in infected hosts (202, 203); CTL responses are enhanced when MVA-vectored vaccines are used to boost immune responses primed with a DNA vaccine (195, 204). To date, MVA-vectored vaccines have not entered human trials (although IAVI is currently sponsoring studies which are intended to result in such a trial). In contrast, a number of canarypox-vectored vaccines are presently in human clinical trials, including the recombinant vector vCP205, which expresses HIV-1 Gag, Env and protease, and which also elicits the efficient formation of HIV-1 pseudovirions (183).

6.5. Combination approaches

As noted above, it has become apparent that combination vaccine approaches elicit the most potent immune responses in nonhuman primates and in humans. As a result, several Phase I/II clinical trials of such approaches are now underway. These include Phase I/II trials of vCP205, followed by (or simultaneously with) a gp120 subunit boost, a p24 subunit boost or GMCSF. Other combinations include a vaccinia virus-prime plus protein (gp120)-boost strategy, a salmonella recombinant-prime plus protein boost strategy and a DNA-prime plus protein or canarypox-boost.

6.6. Plant based vaccines

Genetic engineering of plants has made it possible to explore the use of plant-based immunogens. This approach has resulted in the production of measurable immune responses to bacterial and viral antigens (including HIV-1) in both experimental animals (205-207), and in humans (208). It is possible that plant-derived products, including orally-delivered edible vaccines (208), may have future potential in the context of AIDS vaccine development.

6.7. Live-attenuated vaccines

This is perhaps the most controversial area in HIV-1 vaccine development. Initial studies with Nef-deleted strains of simian immunodeficiency viruses (SIV) showed that live-attenuated viruses can elicit immune responses that result in protection from challenge with infectious SIV (209). Subsequent findings with triply deleted SIV mutants have confirmed this (210, 211), although it has also become apparent that (1) there is variable level of vaccine protection by live attenuated SIV against heterologous challenge (210), and (2) that the

degree of immune protection by live attenuated SIV is inversely correlated with the extent of viral attenuation (212). Thus, strains which immunize most effectively also appear to be those which are capable of causing AIDS-like disease in some experimentally inoculated hosts (213, 214). The notion that live-attenuated vaccines for HIV may possess a significant risk for causing AIDS is further reinforced by recent studies on the Sydney Blood Bank Cohort. These individuals were all infected with a Nef-deleted and partially attenuated strain of HIV-1 (6), and while they have shown a delay in the development of clinical disease, several of those individuals now appear to be showing signs of progressive $CD4^+$ T cell depletion and immune damage (8). Nevertheless, the live-attenuated vaccine approach is a powerful and potentially low-cost strategy that is too important to overlook within the portfolio of AIDS vaccine approaches. As a result, both IAVI and NIH are presently funding efforts that are designed to further explore the safety and immunogenicity of this approach, using experimental animal models (rhesus macaques).

7. PERSPECTIVE AND SUMMARY

HAART has emerged as a major success story within the past 3 years, and it has exerted a profound influence on the survival and well-being of HIV-infected persons in the United States and Europe. It has, however, been of little value to the great majority of HIV-positive persons in the world, most of whom live in developing nations that lack the financial resources to afford expensive antiviral drugs. A major challenge for the next several years will be to develop low-cost approaches to the treatment of HIV-1 infection, which may be of value to persons living in these nations. The recent finding that short-course nevaripine therapy can successfully prevent maternal-infant transmission of HIV-1 may represent an important step in this direction.

Improvements in our basic understanding of the pathogenesis and viral dynamics of HIV-1 infection have resulted in a deeper comprehension of the issues which influence disease progression, survival, and the effectiveness of antiviral therapies. As a consequence, investigators are now poised to develop improved antiretroviral therapies. These will be further enhanced by the development of new antiviral drugs, and by an increasing understanding of the pharmacologic properties of currently used drugs.

Recent advances in HIV-1 vaccine development have also brought us to the brink of fundamental new discoveries. Studies which are presently ongoing, including Phase 3 vaccine trials, are likely to result in major insights within the next several years, and will yield data on the effectiveness of subunit vaccine approaches and the safety and immunogenicity of novel approaches, including combination strategies, DNA vaccines and multimeric/multiepitope vaccines. Such vaccines may have the potential to protect against initial HIV-1 infection, or to reduce viral load following infection. This in turn may slow disease progression and possibly reduce virus transmission rates. Use of vaccines in HIV-positive individuals may also represent an important adjunct to effective antiretroviral therapy. The next several years therefore represent an important and exciting time in HIV-1 research.

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