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Pathogenesis of Human Immunodeficiency Virus Infection and Prospects for Control

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In just six years after the initial description of the acquired immunodeficiency syndrome, much has been learned about the etiologic agent, the human immunodeficiency virus. The pathogenic mechanisms utilized by this virus to infect selectively and persistently T4+ lymphocytes and monocyte/macrophages, leading to immunodeficiency and neurologic dysfunction, are slowly becoming clear. Better understanding of the pathogenesis of human immunodeficiency virus infection is essential for the rational design of therapeutic and preventive strategies to combat this deadly virus.

The etiologic agent of the acquired immunodeficiency syndrome (AIDS) is now known as the human immunodeficiency virus (HIV). This virus has a diameter of approximately 100 nm, a lipid envelope, and a dense core consisting of core proteins, RNA-dependent DNA polymerase (reverse transcriptase), and genomic RNA. In addition to the standard retroviral *gag, pol,* and *env* genes, HIV encodes for at least five other genes. Two multi-exon genes, *tat* and *trs/art,* are important transcriptional or translational regulators of HIV synthesis. The functional roles of three additional genes—*sor, 3'orf* and R—have not been clearly established.

HIV INFECTION OF T4+ LYMPHOCYTES

The hallmark of the immunodeficiency in AIDS is a depletion of T4+ helper-inducer lymphocytes [1]. This defect is primarily the result of the selective tropism of HIV. HIV selectively replicates in T4+ lymphocytes, but not in T8+ lymphocytes [2]. In addition, HIV infection of T4+ cells *in vitro* can be blocked by monoclonal antibodies directed against specific epitopes on the T4 molecule [3–5]. In binding experiments of HIV to T4+ cells, McDougal et al. [6] found that immunoprecipitation of the T4 antigen resulted in the co-precipitation of gp120, the major envelope glycoprotein of HIV. Conversely, immunoprecipitation of gp120 co-precipitated the T4 molecule. Moreover, intracellular complexing of T4 and gp120 has also been demonstrated [7]. Recent studies by Maddon et al. [8] also support this idea. Certain human epithelial cells do not express the T4 antigen and are resistant to HIV infection.

Abbreviations: ADCC: antibody-dependent cell-mediated cytotoxicity ARC: AIDS-related complex AIDS: acquired immunodeficiency syndrome ddC: 2'-3'-dideoxycytidine HIV: human immunodeficiency virus PFA: trisodium phosphonoformate

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When the T4 gene is inserted into these cells, however, they become susceptible to HIV. Productive infection occurs and the infected cells fuse to form multinucleated giant cells and have shortened survival. The studies cited clearly establish the T4 molecule as the receptor for HIV.

After specific binding to the target cell, HIV enters the cell and is uncoated, although the mechanism of this process has not been clearly defined. One study has suggested that HIV entry occurs via receptor-mediated endocytosis [8]. A more extensive analysis by Stein et al., however, demonstrated pH-independent direct fusion of the virus envelope to the plasma membrane [9]. Following penetration, the genomic RNA is then transcribed into DNA by the reverse transcriptase. Subsequently, the DNA is circularized and integrated into the host chromosome by a virus-encoded enzyme (integrase, a product of the *pol* gene) during cell division. Interestingly, much of the DNA of HIV remains unintegrated in the cytoplasm. The HIV replication cycle is restricted at this stage until the infected cell is activated. *In vitro,* this is achieved by mitogenic, antigenic, or allogeneic stimulation [5,10] or by the addition of cytokines. Upon activation, transcription occurs, followed by protein synthesis with post-translational processing, including protein cleavage and glycosylation. Viral proteins and genomic RNA are then assembled at the cell surface, and mature virions are formed by budding.

With HIV replication, the T4+ cell is killed by an as yet unclear mechanism. Could one of the five novel genes of HIV be involved in cell killing? *Tat* and *trs/art* are essential regulators of viral synthesis and are unlikely to be the direct cause of cell death [11–15]. Base changes or deletions have been introduced in *sor* and *3'orf,* but these mutants retained their cytopathic effect [16–19], although *3'orf* mutations resulted in higher levels of virus replication, suggesting that this gene has a negative regulatory role in HIV expression [17]. There is currently no evidence to implicate *sor* or *3'orf* in cell killing. The role of the "R" gene in this process is unknown [20].

It seems likely that HIV envelope glycoproteins play an important role in killing T4+ cells, probably through cell-cell fusion. Fusion is observed when viral particles bud from the cell membrane of infected T4+ cells. This process results in the formation of syncytia (multinucleated giant cells), which then develop ballooning cytoplasm and promptly die. Lifson et al. have shown that these syncytia are composed of both infected and uninfected T4+ cells [21]. Uninfected T4+ cells are recruited into the syncytia because the gp120 on the budding virions specifically binds the T4 molecules on uninfected cells. Once bound, the fusion process is probably mediated by a different domain on the HIV envelope, possibly the transmembrane protein (gp41) since mutations in this region of *env* abolished the fusogenic property of HIV [19]. Furthermore, Sodroski et al. [22] and Lifson et al. [23] demonstrated syncytia formation by inserting only *env* into T4+ cells. Insertion of *env* into T4− cells did not induce syncytia. It appears that not only the glycosylated HIV envelope but also the T4 molecule is necessary for the fusion process, which provides a mechanism for killing infected, as well as uninfected, T4+ cells. This process is not likely to be the only mechanism, however, as normal peripheral blood lymphocytes are killed *in vitro* by HIV with little or no formation of syncytia. One would need to postulate that fusion can also involve different parts of the plasma membrane of a single HIV-infected cell. This process of autofusion would then lead to membrane permeability changes and cell death.

Additional mechanisms for T4+ cell depletion *in vivo* should be considered. Infected cells expressing HIV on the surface would be recognized and removed by

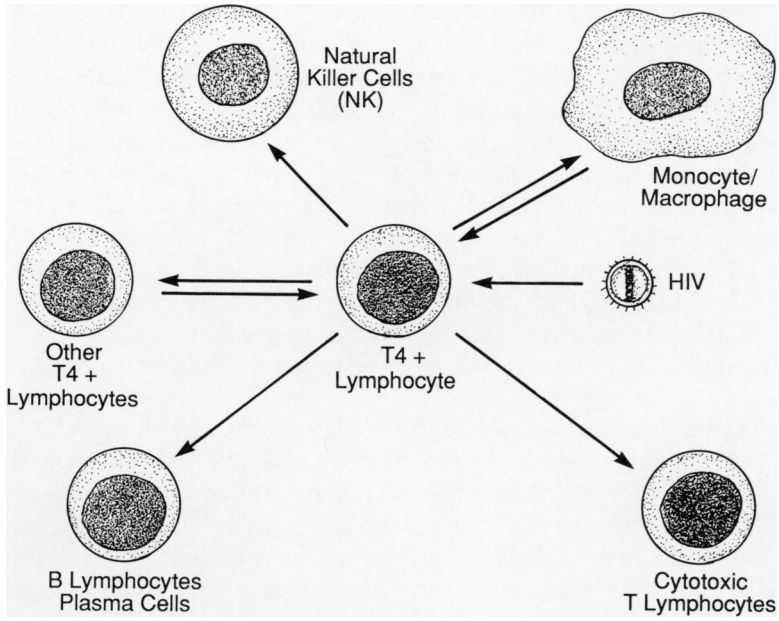

FIG. 1. Central role of the T4+ lymphocytes in the immune system.

immune surveillance mechanisms. In addition, uninfected T4+ lymphocytes may be coated by free gp120, which would also be recognized as foreign and then cleared by the immune system [24]. HIV-infected lymphocytes may also become more susceptible to superinfection by other pathogens, such as cytomegalovirus, herpes simplex, or hepatitis B virus. This type of enhancement, perhaps due to the transactivating property of the *tat* gene, may result in faster depletion of the T4+ lymphocyte population.

The T4+ helper-inducer lymphocyte is the orchestrator [1] of the immune response. It interacts, directly or indirectly via lymphokines, with monocyte/macrophages, cytotoxic T cells, natural killer cells, and B cells (Fig. 1). Therefore, even a selective depletion of the T4+ cell population can result in a multitude of immunologic deficits leading to the life-threatening opportunistic infections characteristic of AIDS.

HIV INFECTION OF MONOCYTE/MACROPHAGES

Monocyte/macrophages may also express the T4 molecule on the cell surface [25], and several studies have shown that this population can be infected by HIV [26–29]. Ho et al. found that normal blood monocyte/macrophages were infectable by HIV *in vitro,* and monocytes from infected persons can harbor the virus *in vivo* [26]. Similar findings were obtained by others using monocytes/macrophages derived from blood, bone marrow, brain, and lung [27–29]. Three groups did not observe cytopathic changes or cell death in infected monocyte/macrophages [26–28]. In contrast, Gartner et al. reported syncytia formation, although it was not as prominent as that seen in infected lymphocytes [29]. The relative refractoriness of infected monocyte/macrophages to syncytia formation and cell killing is probably due to a lower surface density of T4 molecules. This relative resistance to HIV cytotoxicity raises the possibility that monocyte/macrophages may serve as a reservoir for virus persistence in the host. In addition, because monocyte/macrophages are often the initial responders to an

infection, they may be the first type of cell to be infected by HIV. The infected monocyte/macrophages may then transmit the virus to susceptible lymphocytes.

HIV infection of monocyte/macrophages may cause a defect in chemotaxis, which has been described for the monocytes of AIDS patients [30]. The infection of alveolar macrophages may explain the higher incidence of pneumocystis pneumonia in AIDS patients compared with other immunosuppressed hosts. In addition, it is possible that monokine release is altered by HIV infection. Enhanced release of interleukin-1 or tumor necrosis factor could explain chronic fevers in AIDs, since both are endogenous pyrogens produced by monocytes [31,32]. Tumor necrosis factor is also a potent catabolic factor [32] and may be important in the pathogenesis of AIDS cachexia, known as slim disease in Africa [33]. The infected monocyte may also serve as a vehicle for transporting HIV to the central nervous system, leading to neurologic dysfunction.

Subacute encephalitis, also referred to as AIDS encephalopathy or AIDS dementia complex, is the most common neurologic problem in AIDS [34]. Substantial evidence is now available to support a direct etiologic role for HIV in this neurologic syndrome. Shaw et al. first reported the detection of HIV DNA and RNA in a few affected brains by Southern hybridization and *in situ* hybridization, respectively [35]. Subsequently, Ho and co-workers [36] were often able to isolate HIV from brain or cerebrospinal fluid of patients with subacute encephalitis. The amount of HIV detected in the neural tissues frequently exceeded that of blood or other tissues [36]. Intrathecal production of HIV-specific immunoglobulins in patients with subacute encephalitis [37] has also been demonstrated, thus supporting the presence of HIV in the central nervous system. These data, together with the similarities between HIV and lentiviruses capable of inducing encephalitis, strongly support HIV as the causative agent of subacute encephalitis. This hypothesis in turn suggests that HIV is neurotropic and that the central nervous system may serve as a sanctuary site for the virus [36].

The predominant cell population in the brain that is infected by HIV appears to be the monocyte/macrophage. Gabuzda et al. detected HIV antigens in mononuclear cells in affected brains, and these cells were then morphologically identified as monocyte/macrophages [38]. Koenig and co-workers also found the monocyte/macrophage as the cell type in the brain infected by HIV [39]. In addition, they showed that the multinucleated giant cells seen in subacute encephalitis contained HIV RNA and expressed monocyte markers as demonstrated by *in situ* hybridization and immunohistochemical staining, respectively. Similar results were reported by Wiley et al. [40], although they also noted HIV infection of cerebral endothelial cells and rare involvement of neurons and glial cells. These findings, along with previous demonstration of HIV infection of monocyte/macrophages *in vitro* [26–29], suggest that the infected monocyte/macrophage plays a central role in the pathogenesis of the neurologic disease associated with AIDS.

HIV PERSISTENCE

All HIV-infected persons should be considered infected and infectious for life unless an effective therapy is developed. Several viral properties contribute to this prolonged persistence. Similar to other retroviruses, HIV DNA is integrated into the host genome following infection. Therefore, it is difficult to eradicate HIV without also eliminating the infected T4+ cell. *In vivo* restriction of viral expression is also seen in HIV infection. Very little cell-free virus is found in infected persons and less than 1 in

10,000 circulating lymphocytes express detectable HIV mRNA [41]. Therefore, much of HIV appears to be restricted (or possibly latent) and not susceptible to immune clearance mechanisms.

Infected monocyte/macrophages contribute in part to HIV persistence because of their relative refractoriness to the cytolytic effect of HIV [26–28]. In addition, the infected monocyte/macrophages in the brain may be protected from many immune effector cells. These characteristics would allow monocyte/macrophages to serve as an important reservoir for HIV. A small fraction of infected helper-inducer lymphocytes can also survive HIV infection [42] and further contribute to virus persistence.

Considerable genomic diversity exists among HIV isolates [43–49] and the variability is most prominent in certain "hypervariable" regions of the external envelope glycoprotein [49]. It is widely believed without substantiation that immune selection is responsible for the heterogeneity. Variant viruses may evade immune recognition and contribute to viral persistence. This possibility has raised concerns about the efficacy of one vaccine preparation in protecting against many diverse HIV isolates [50]. There is to date, however, no evidence of HIV variants that are not recognized by the immune system of infected persons. Indeed, molecular studies of serial isolates from infected individuals do not support immune selection [51]. Variability of HIV may be better explained by errors of reverse transcription coupled with functional selection. Reverse transcriptase has an error rate several orders of magnitude greater than that of cellular DNA polymerases [48]. These errors are then amplified by the highly cytolytic nature of HIV, which results in multiple rounds of infection requiring many reverse-transcription steps. Other retroviruses, particularly transforming ones, require fewer rounds of reverse transcription and thus show less diversity [48]. HIV variants are viable if the mutations do not disturb the functional capacity of the virus. This finding is consistent with the observation that the hypervariable regions are primarily located on the external envelope glycoprotein, portions of which may not have functional importance. This finding also implies that the conserved regions of the envelope must have essential functional roles and should be considered strategic sites in the design of a vaccine for AIDS.

VACCINE DEVELOPMENT

No safe and effective vaccine for AIDS is currently available, and several major obstacles in the course of vaccine development must first be overcome. The observed genomic diversity among HIV isolates and the possibility that HIV transmission may occur via infected cells (instead of free virus) are the principal reasons for the prevailing pessimism regarding our ability to develop a vaccine for AIDS eventually. In addition to these scientific considerations, we are faced with the lack of a satisfactory animal model for evaluating HIV infection. The chimpanzee is the only animal infectable by HIV [52]; however, chimpanzees are an endangered species and are therefore in short supply and prohibitively expensive. Rhesus macaques are susceptible to infection by related primate retroviruses, STLV-III [53] and HIV-2 [54], and may represent a good surrogate model system for vaccine testing. The logistical and time constraints on clinical trials of candidate AIDS vaccines, as well as ethical and legal issues (e.g., product liability), represent additional difficulties anticipated in the vaccine effort [55].

There are, of course, also findings which can be viewed optimistically for the development of AIDS vaccine. HIV neutralizing antibodies and antibody-dependent

cell-mediated cytotoxicity (ADCC)—two important immune parameters in predicting vaccine efficacy—have been detected in AIDS patients and seropositive individuals [56–60]. Both neutralizing antibodies and the ADCC response have been found to be directed against envelope glycoproteins [59,61–64]. Studies to map the precise envelope domains important in eliciting HIV neutralizing antibodies have shown that several fall within well-conserved regions [59]. This result suggests that the heterogeneity among isolates may not be a major obstacle in vaccine development and that a single broadly protective vaccine may be possible.

Given the urgency of the AIDS epidemic, several candidate subunit vaccines have already reached testing in chimpanzees and, in one case, clinical trials in humans, despite the lack of full understanding of the important viral components to include or exclude in the vaccine preparation. Several purified or recombinant gp120 preparations have been used to immunize chimpanzees. Although specific anti-gp120 antibody response developed, none has protected the animals from HIV infection when challenged [55]. Zagury et al. have begun evaluating in human subjects (Zairians and the principal investigator) a recombinant vaccine composed of vaccinia plus the HIV *env* gene [65]. Furthermore, despite the lackluster results in chimpanzees, clinical trials with various candidate subunit vaccines are expected to start in the United States within a year [55].

It is possible that the "shotgun" candidate vaccines will not be protective and that a rational and stepwise approach to vaccine design will be necessary. An ideal AIDS vaccine should elicit immune effector responses that are significantly greater than those induced by the native virus, because the natural responses in HIV-infected persons are often inadequate. To this end, it will be important to define precisely the most vulnerable portions of HIV in terms of antibody neutralization and ADCC. The "vulnerability" of a region of the virus suggests functional importance; therefore, that particular domain is likely to fall within invariant regions of HIV. Those critical "soft spots" which are common to diverse isolates should then be dissected out and properly packaged for optimal presentation to the immune system of vaccinees.

ANTI-HIV CHEMOTHERAPY

Approximately five million persons worldwide are already infected with HIV, and a majority of them are expected to progress to AIDS or AIDS-related complex (ARC) in seven to eight years. The development of effective chemotherapy for HIV is therefore of paramount importance. This difficult task will undoubtedly require a rational and organized approach to antiviral design, production, and testing. Several features of HIV pathogenesis should be taken into consideration. First, HIV is a persistent virus and is likely to require prolonged (possibly life-long) treatment. Therefore, a drug should ideally be orally bioavailable and reasonably affordable. Second, HIV is neurotropic, which necessitates adequate drug penetration into the central nervous system. Third, better understanding of the HIV replication cycle has revealed several critical virus-specific steps, which are prime targets for antiviral chemotherapy (Table 1). Intense investigative efforts over a short period of time have resulted in the identification of many potentially useful compounds (Table 1), which have been recently reviewed elsewhere [66,67].

Antiviral agents with activity against HIV reverse transcriptase include azidothymidine (AZT, also known as zidovudine or Retrovir®) a 3′-azido-3′-deoxy analog of thymidine. AZT is phosphorylated to a triphosphate form by cellular kinases and

TABLE 1
Virus-Specific Targets for Anti-HIV Chemotherapy

Stage	Potential Intervention	Examples	References
Binding	Interference with gp120-T4 interaction	Anti-gp120 antibodies	[5]
		Leu3A, OKT4A antibodies	[3–5]
		Peptide T	[91]
		Free T4 molecules	—
Penetration	Alteration in target cell membrane fluidity	AL721	[92]
	Inhibition of fusogenic domain of HIV *env*	None	—
Reverse Transcription	Inhibition of reverse transcriptase	Azidothymidine	[66–71]
		Dideoxynucleosides	[74]
		Suramin	[81,82]
		HPA-23	[80]
		Phosphonoformate	[77]
Integration	Inhibition of integrase	None	—
Transcription/ Translation	Inhibition of *tat* function	None	—
	Inhibition of *trs/art* function	None	—
	Interference with HIV mRNA	Anti-sense RNA	[93]
Post-Translational Processing	Inhibition of glycosylation	Castanospermine	[94]
	Unknown	Ribavirin (?)	[84]
	Inhibition of myristylation	None	—
	Inhibition of HIV protease	None	—
Assembly/Release	Inhibition of assembly or release	Interferon-alpha	[87]
		Interferon-gamma	[89]
		Ampligen	[95]

incorporated into growing DNA chains, thereby preventing chain elongation [68]. It inhibits HIV replication *in vitro* at 1–5 μM [69], a concentration achievable *in vivo*. AZT has an oral bioavailability of 60 percent and adequate penetration into the central nervous system [70,71]. These properties led in 1985 to a phase 1 study in AIDS patients, which showed that drug recipients had partial reconstitution of immune responses [70]. This result then prompted a phase 2 multi-center, collaborative, placebo-controlled trial of AZT in 282 patients with AIDS (those following an initial episode of pneumocystis pneumonia) or ARC. The study was prematurely terminated in September 1986 because of significant differences observed in survival rates [72]. Nineteen patients had died in the placebo group, while only one died among drug recipients. In addition, there were significantly fewer opportunistic infections in AZT-treated patients, as well as improvement in their performance scores, skin test reactivity, and T4 lymphocyte numbers. Suggestions of *in vivo* antiviral effect with lower serum antigen (p24) were also evident. Six months after this study, AZT was licensed for use in this country for adults with AIDS or advanced ARC with established pneumocystis pneumonia or a blood T4 lymphocyte count of less than

200/mm^3. A preliminary study of AZT in patients with neurologic syndromes associated with AIDS has demonstrated some benefit [71] and forms the basis for more extensive investigations in the future.

AZT treatment is associated with considerable toxicity. The development of megaloblastic anemia is common, and more than 20 percent of the patients on AZT required blood transfusions [73]. In addition, neutropenia is another frequent (16 percent) complication, as well as headaches. Furthermore, the long-term side-effects of this drug are unknown. Another negative feature of AZT is its current cost of approximately $10,000 per patient per year, which is an enormous financial burden for patients or third-party payers.

Another nucleotide analog under active investigation is 2'-3'-dideoxycytidine (ddC), which has also demonstrated considerable *in vitro* anti-HIV activity [74,75]. It is phosphorylated by cellular kinases to a triphosphate form that inhibits HIV reverse transcriptase and its effect can be reversed by deoxycytidine [76]. Phase 1 clinical trials with ddC are currently in progress in patients with AIDS or ARC [67].

Trisodium phosphonoformate (PFA) is a pyrophosphate analog that also inhibits HIV reverse transcriptase *in vitro* [77]. Although the potential clinical use of this compound has been limited by the lack of an orally bioavailable form, PFA crosses the blood-brain barrier and has acceptable toxicity. Phase 1 clinical trials are now in progress in Sweden, using intravenous infusion of PFA in AIDS and ARC patients [78,79].

Other inhibitors of HIV reverse transcriptase include HPA-23 [80] and suramin [81,82]; however, they have not been found to be beneficial *in vivo*. In fact, in clinical studies it was concluded that suramin may actually be harmful [82].

Ribavirin is in a synthetic guanosine analog with broad-spectrum antiviral activity against both DNA and RNA viruses [83] and has demonstrated variable activity against HIV-1 replication *in vitro* [84]. Although its mechanism of action has not been established, it is converted to ribavirin-5'-triphosphate by cellular enzymes and may interfere with post-transcriptional processing [83]. A number of clinical trials have been conducted with ribavirin but have yielded contradictory or controversial results [85,86].

Interferons appear to act late in the HIV replication cycle and interferons alpha and beta inhibit HIV replication *in vitro* in a dose-dependent manner [87,88]. Recombinant human interferon gamma also has some demonstrable effect *in vitro* [89]. Although the penetration into the central nervous system is poor [67], interferons may be clinically useful in combination with other anti-HIV agents which act at different sites of the replication cycle.

Combinations of the antiviral agents that act by different mechanisms at various sites may reduce toxicity by lowering the effective concentration of an individual drug. Advantages include enhanced efficacy related to potential additive or synergistic activity as described for the following drug combinations *in vitro* [67]: AZT and interferon-alpha, phosphonoformate and interferon, phosphonoformate and ribavirin, and ddC and interferon-alpha. Of interest is the finding that one combination, AZT plus ribavirin, demonstrated antagonism *in vitro* [90]. Ribavirin appears to inhibit phosphorylation of AZT to its active triphosphate form. Clinical trials using synergistic combinations of these drugs are under way in patients with HIV infections.

Better understanding of the biology of HIV and the development or discovery of the antiviral agents listed in Table 1 represent major achievements in medical science, and

yet much remains to be done. Search for other anti-HIV compounds must continue, including those which will inhibit other virus-specific sites (e.g., the integrase, protease, or products of *tat* or *trs/art*). Drugs with promising *in vitro* characteristics, following appropriate preliminary toxicity and efficacy studies, should be quickly brought to clinical trials, which must be properly designed and executed. Governmental regulatory agencies must also facilitate the process of review and licensure while maintaining high scientific standards. These and other efforts will be necessary to confront this formidable foe, HIV.

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