

IMMUNOPATHOGENESIS OF HIV INFECTION

The immunopathogenesis of HIV infection was discussed at both the Atlanta and Los Angeles meetings by H. Clifford Lane, MD, from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

As noted by Dr Lane, the primary clinical problems associated with HIV infection are the opportunistic infections and neoplasms that occur as a result of the severe immunodeficiency that develops during the course of disease. The hallmark of this immunodeficiency is the decrease in quantity and function of CD4+ lymphocytes. As related by Dr Lane, the relative immune function in an HIV-infected individual is the net result of three competing forces: (1) destruction of CD4+ lymphocytes by the virus; (2) the ability of the patient to mount an immune response in an attempt to contain infection; and (3) the ability of the immune system to regenerate and to replenish CD4+ cells. Dr Lane's presentation focussed on the latter two components.

Immunologic Effector Mechanisms

Immunologic effector mechanisms important in the control of HIV infection include: (1) production of neutralizing antibody; (2) lysis of antibody coated cells; (3) induction of cytotoxic CD8+ T lymphocytes; (4) lysis of HIV-infected cells by natural killer (NK) cells; and (5) the activity of the helper/inducer CD4+ lymphocytes. The functioning of CD4+ lymphocytes is crucial for coordinating immune function and response. These cells also

elaborate cytokines that facilitate much of the complex communication and interaction among the different cells of the immune system.

As explained by Dr Lane, compared with healthy controls, alterations in patterns of cytokine production have been described in HIV-infected individuals and appear to be secondary to the destruction of CD4+ lymphocytes rather than a primary cause of HIV-related immunodeficiency.

The effectiveness of the immune response in controlling HIV replication is evidenced clinically by the long time between infection and disease and histologically by the containment of viral particles in the lymph nodes.

CD4+ lymphocytes may function in host defense against HIV infection by regulating CD8+ cell responses, antibody production, macrophage function, and NK cell function, much of which is mediated by cytokine production. Nonspecific immune responses to HIV infection may include two primitive elements of the immune system: complement, proteins that facilitate lysis of cell membranes and mediate leukocyte chemotaxis, and NK cells, which recognize and kill such altered cells as tumor cells and virus-infected cells. These immune mechanisms have relatively weak effects against HIV. CD8+ cells may also nonspecifically

inhibit HIV replication through both cytotoxic and noncytotoxic effects.

HIV-Specific Immune Responses

Specific immune responses to HIV infection include HIV-specific cytotoxic (CD8+) T cells and HIV-specific antibody responses. That the immune system is at least partially effective in controlling HIV replication following primary infection is evidenced clinically by the long time between infection and disease and histologically by the containment of viral particles in the germinal centers of lymph nodes. It remains unclear which of the HIV-specific immune responses play the major role in controlling infection.

In general, the cytotoxic CD8+ cells play an important role in controlling viral infections and tumors. HIV-specific cytotoxic T cells recognize, bind to, and lyse HIV-infected cells. These functions are enhanced by interaction with CD4+ lymphocytes. Cytotoxic T cells also elaborate several cytokines important in HIV-specific immunity, such as tumor necrosis factor-alpha (TNF- α) and interferon-gamma (INF- γ). As related by Dr Lane, studies have shown that there are high levels of CD8+ cells in healthy homosexual men and in individuals with relatively early HIV infection compared with controls and lower levels of CD8+ cells in

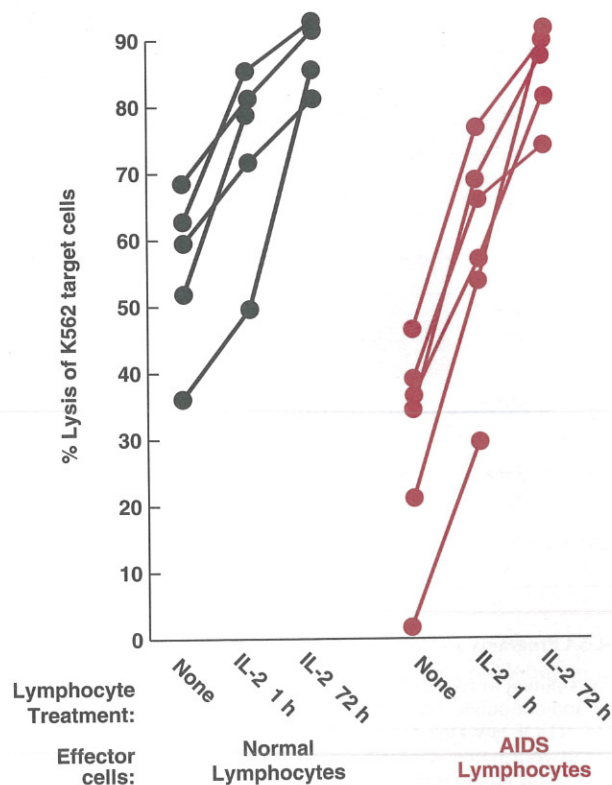
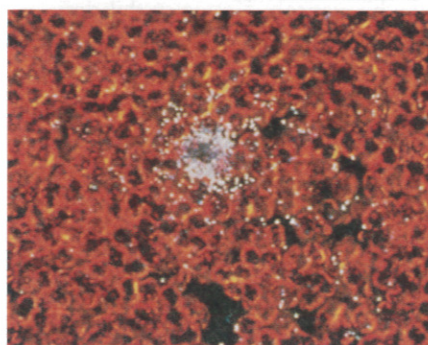
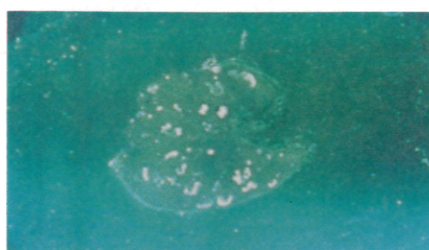


Figure 1. Percentage lysis of K562 target cells in vitro by lymphocytes from subjects without HIV infection or subjects with AIDS in the presence of interleukin-2. Adapted from Rook AH, et al. *J Clin Invest* 1983.

Early Stage



Intermediate Stage



Advanced Stage

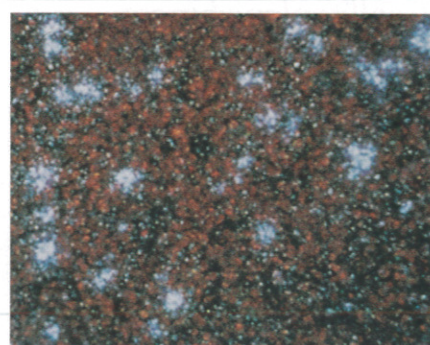


Figure 2. Changes in HIV distribution in lymph node tissue associated with disease progression. Virus was detected by in situ hybridization. The top panels show trapped virus. The bottom panels show infected cells. Figure courtesy of Drs. Pantaleo and Fauci.

individuals with much later stage infection (eg, those with opportunistic infections). Dr Lane presented data showing that T-cell specific killing of cytomegalovirus (CMV), as well as NK activity, are markedly reduced in patients with AIDS. In a comparison between six bone marrow transplant recipients and seven AIDS patients with CMV infection, NK killing of CMV was reduced from 61.1% to 34.1%; more striking was the comparative reduction in T-cell specific killing of CMV, which was 26.6% in the controls and 0.5% in the AIDS patients. As explained by Dr Lane, the lytic activity of the cytotoxic T cells from both healthy individuals and AIDS patients is enhanced by in vitro incubation of cells with interleukin 2 (Figure 1). The ability to increase this activity in cells from AIDS patients indicates the absence of an intrinsic defect in the cells and suggests that the diminished activity may be due to a defect in the ability of the host to induce cells to perform their normal effector functions. This induction would appear to occur through CD4+ lymphocyte-regulated cytokine elaboration. Thus, it may be the loss of the CD4+ cells that ultimately comprises the crucial defect in HIV-specific immunity.

HIV-specific antibodies, the other element of the specific immune response, bind to HIV and facilitate trapping of virus by follicular dendritic cells in the lymph nodes. These antibodies may directly neutralize virus and they also mediate antibody-dependent cellular cytotoxicity (ADCC), in which they bind virus or virally-infected cells that are subsequently killed by cells expressing Fc receptors.

As sketched by Dr Lane, the current understanding of the immune response to HIV infection is as follows: After acute primary infection, there is brisk viral replication, prompting induction of cytotoxic T cells and antibody production. With mounting of this response, the level of circulating virus decreases. This decrease is associated with killing of HIV-infected cells by cyto-

toxic T cells and antibody binding and trapping of virus by the follicular dendritic cells of the germinal centers of the lymph nodes. In early infection, there is a heavy concentration of trapped virus. As viral replication continues, lymph node architecture begins to be lost and trapping of virus is decreased. Very little virus trapping is evident in late-stage disease (Figure 2). Concomitantly, in initial infection, there are very few HIV-infected cells in the lymph node. The concentration increases with progression of infection; this is also seen with regard to the proportion of infected cells in the peripheral circulation. Genotypic analysis of lymph node proviral DNA has shown that the sequences are much more closely related to sequences in plasma

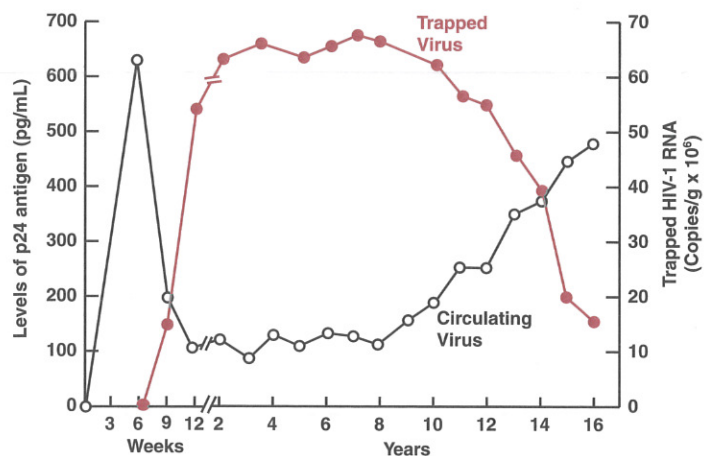


Figure 3. Relationship between levels of trapped virus (trapped HIV RNA copies/g x 10⁶) and circulating virus (p24 antigen concentration) over duration of infection.

than to the proviral DNA in circulating peripheral blood lymphocytes. This suggests that the lymph nodes are the site of ongoing active virus replication and the source of virus that eventually becomes evident in large quantities in the circulation (Figure 3).

CD4+ Lymphocyte Deficiencies

As stated by Dr Lane, it is the lack of ability to contain infection that appears to lead to the progressive decline in CD4+ lymphocyte count and progressive immunosuppression in HIV infection. Precisely how HIV destroys CD4+ lymphocytes continues to be investigated. The primary factor appears to be cell death as a consequence of productive infection. Potential explanations for how this occurs include intracellular accumulation of nonintegrated

viral DNA, toxic effects of viral proteins, and alterations in cell activation related, for example, to abnormalities in biochemical pathways that control the cell cycle. There is an increasing body of evidence that HIV infection causes cell death by triggering programmed cell death (apoptosis). The process can be understood as a suicide cascade of biochemical events leading to disintegration of the cell nucleus. This is a normal physiologic cellular pathway; it is used by the immune system in ontogeny to eliminate self-reactive clones and is important in tissue remodeling. According to Dr Lane, the evidence that HIV invokes this pathway upon direct infection of CD4+ lymphocytes is much stronger than evidence that it can trigger the process without infection of the cell.

T cell replenishment appears to occur through division of mature T cells rather than through stem cell differentiation.

Functional Characteristics of CD4+ Lymphocytes

As explained by Dr Lane, a number of interesting findings have been made regarding the functional capacities and the source of CD4+ lymphocytes in HIV-infected individuals. Normal functional characteristics of CD4+ helper/inducer T-lymphocytes include: (1) proliferation in response to mitogen, alloantigen, or protein antigen stimulation; (2) providing help to B lymphocytes; and (3) providing inductive signals for a variety of immunologic functions. Studies of the proliferative responses of T cells to mitogenic and antigenic stimulation have shown decreased activity in AIDS patients compared with controls. However, this deficiency is not an intrinsic deficiency of the T cells; see Figure 4. Studies with pokeweed mitogen stimulation have shown that the decreased proliferative response in unfractionated samples from AIDS patients is associated with an alteration in the ratio of CD4+ to CD8+ cells in these patients. Fractionated CD4+ and CD8+ cells respond to mitogen stimulation in a manner more comparable to those from control patients. Similarly, it was found that although response to alloantigen stimulation was decreased in unfractionated samples from AIDS patients, responses were actually comparable to control samples when correction was made for the relative decrease in CD4+ cell number. The decreased response to soluble protein or recall antigens, however, appears to be profoundly diminished and is not attributable to numerical or proportional CD4+ cell abnormalities alone. As shown in Figure 4, the deficient response to tetanus toxoid is present even when purified CD4+ lymphocytes are tested, thus indicating that the ability to recognize and respond to recall antigen is indeed lost in HIV infection. It appears that response to antigens that are present in relatively low frequency, such as tetanus toxoid, are lost relatively early in infection; responses to Pneumocystis or CMV antigens, however, to which a greater proportion

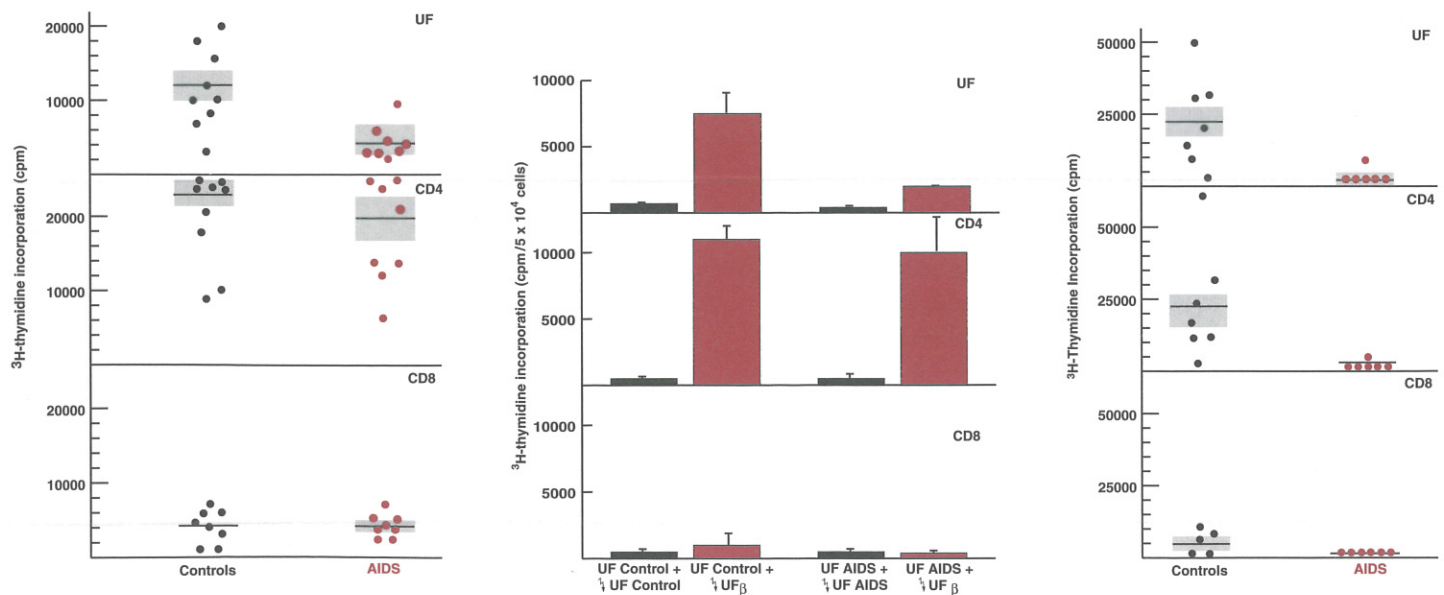


Figure 4. Proliferation responses of normal and AIDS lymphocytes to (left) pokeweed mitogen, (center) alloantigen, and (right) tetanus toxoid when samples were unfractionated (UF) or fractionated into CD4 and CD8 subsets. Adapted from Lane HC, et al. *N Engl J Med* 1985.

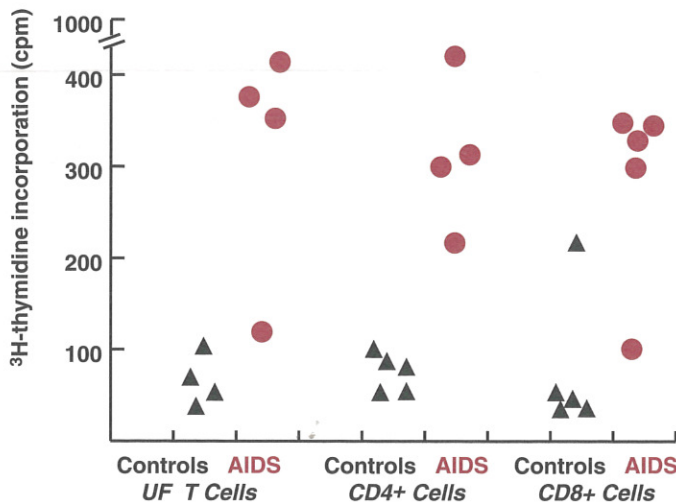


Figure 5. Spontaneous DNA synthesis for unfractionated (UF) T cells, CD4+ cells, and CD8+ cells from controls without HIV infection (▲) and from subjects with AIDS (●). Adapted from Lane HC, et al. *N Engl J Med* 1985.

of the T cell repertoire is devoted, are evident even in later stage disease.

Source of CD4+ Cell Replenishment

Investigations of the survival patterns of CD4+ cells and of the loss of antigen responsiveness by Dr Lane and colleagues have yielded notable results. There is always turnover of T cells of the immune system. Studies of the rates of spontaneous T lymphocyte division have shown that rates for both CD4+ and CD8+ cells fractionated from peripheral blood are substantially higher in AIDS patients than in HIV-negative controls (Figure 5). This is due in part to the fact that the body is attempting to replace the CD4+ cells destroyed through HIV infection.

In a series of studies in identical twins discordant for HIV infection, Dr Lane and colleagues have found that the source of the T cell replenishment appears to be division of existing mature T cells rather than stem cell differentiation—a finding that has implications for whether losses in T cell repertoire are reversible. In

Gains in CD4+ count with therapy may not be accompanied by reconstitution of elements lost from the antigen-specific CD4+ cell repertoire.

these studies, lymphocytes from the twin without HIV infection were tagged with a genetic marker and infused in the HIV-positive twin; consecutive samples were taken from the HIV-positive twin and analyzed by polymerase chain reaction (PCR) for the marker. It was expected that the proportion of marked cells

would rapidly decrease with time as cells were destroyed and cell replacement occurred via stem cells differentiating through the thymus or a comparable extrathymic environment. However, it was found that responses were variable, with proportions of marked cells declining slowly in some individuals and remaining

stable in others. The persistence of marked cells was observed for both CD4+ and CD8+ cells. Study of lymph node biopsies showed that the proportion of marked CD4+ cells in the peripheral circulation was similar to that in the lymph nodes in most cases. According to Dr Lane, these findings indicate both that the T cells that are turning over are primarily the existing mature cells, with very little contribution from stem cell differentiation, and that there is a remarkable distribution of CD4+ cells between the peripheral blood and the lymphoid tissue. Dr Lane stated that the findings raise some question regarding whether there is any significant stem cell differentiation to T cells in adults in general, regardless of HIV disease status.

A potential implication of these recent findings is that antigen-specific responses are lost from the T cell repertoire, they may not be able to be recovered. In the normal situation, there are different proportions of the T cells specific for particular antigens with small populations of antigens encountered relatively infrequently, such as tetanus, and greater populations for more ubiquitous antigens, such as *Pneumocystis* or CMV. Replenishment of the T cell pool as part of natural remodeling occurs through somatic cell division and perhaps through a thymic pathway, as well, with the continued division of the mature cells ensuring persistence of antigen-specific responses. In the case of HIV infection, with the increased rate of cell death, antigen-specific T cells present in low frequency may disappear entirely from the T cell pool. In the absence of entry of truly new cells through stem cell differentiation, numeric replenishment from somatic cell division will not keep pace with T cell destruction and the ability to mount certain antigen-specific responses may thus be lost. As stated by Dr Lane, it may be that the gains in CD4+ cell count that occur with antiretroviral or immunologic therapy may not be accompanied by reconstitution of lost elements of the host's antigen-specific repertoire. Whether such reconstitution can or does occur is the subject of ongoing investigation.

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