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The immunological and infectious sequelae of the acquired immune deficiency syndrome

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Appendix

THE IMMUNOLOGICAL AND INFECTIOUS SEQUELAE OF THE ACQUIRED IMMUNE DEFICIENCY SYNDROME

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On 17–18 May, 1988, a meeting at The Rockefeller University Conference Center in Mount Kisco, New York, brought together a small group of experts in the field of AIDS and related disciplines. Their purpose was to consider central issues in the pathogenesis, immunology, and therapy of HIV infections in a format that encouraged free discussion. There were no formal presentations. Session chairmen, who were largely responsible for the success of the meeting, posed a series of questions, organized discussants, and maintained an active and focused dialogue. Since the meeting was restricted in size, we thought it appropriate to review the four sessions for a wider audience.

Session I: Pathogenesis. Chairmen: Malcolm A. Martin and Bernard Fields

A. Modes of Entry

1. *State of Transmissable Virus.* A central question concerning the transmissibility of HIV infection is whether the virus is free and/or intracellular. While ~30% of serum samples contain low titers of virus, the cellular elements of blood contain levels that exceed those of serum 100-fold. The types of cells that are infected in blood are not known. If the virus is free, does it exist in the form of immune complexes in seropositive individuals who demonstrate a broad range of anti-viral antibodies? Would not such immune complexes be rapidly cleared from the circulation? Such considerations suggest that free virus may not be a significant factor in transmission. Nevertheless, the infectivity of plasma products such as Factor VIII no doubt stems from the presence of free virus.

Similar considerations pertain to the infectivity of other body fluids such as semen, saliva, and vaginal secretions. In the case of semen, which contains appreciable amounts of virus, virus can be detected in 0.01–5% of cells, which seem to be mononuclear leukocytes. Similarly, the examination of the testis by in situ hybridization reveals the presence of viral mRNA primarily in infiltrating leukocytes. It is not known whether mature spermatozoa contain virus nor what the contribution of prostatic cells and secretions are to the total virus load of semen. Both vaginal fluids and saliva contain low titers of virus and this is predominantly cell associated—most likely with hematogenously derived white cells. Only in the cerebrospinal fluid are there relatively high titers of free virus.

Investigators analyzing these questions have been hampered by the lack of suitable, sensitive bioassays for infectious virus. Most agree that live virus constitutes only a small fraction of total virions present in blood and body fluids. This may, in part, be the result of such antiviral agents in body fluids as cell-derived hydrogen peroxide, peroxidases, and halide ions.

2. *Transport across Mucosal Barriers.* The deposition of virus in the genitourinary or gastrointestinal tracts in either the free, complexed, or intracellular state may lead to transmission. The likelihood of transmission is enhanced if preexisting lesions are present or if trauma is associated with the sexual act. Epidemiologists now consider that preexisting breaks in surface barriers and the lack of circumcision facilitate viral passage. The lesions may be in the form of genital ulcers secondary to sexually transmitted diseases such as syphilis, chancroid, or herpes. The high incidence of heterosexual transmission in Central Africa may be the result of such factors. Promiscuity and receptive anal intercourse enhance the incidence of transmission.

The interaction of free virus or HIV-infected cells with epithelial cells is poorly understood. Whether or not there are epithelial receptors that recognize virus surface glycoproteins, and/or infected T cells and monocytes, is unknown. IgA antibodies against viral components, which might be present on the surface of respiratory and gastrointestinal epithelia could play a role in either neutralizing or enhancing infectivity.

In addition to epithelial cells there are other cellular elements with membranous processes that extend to or near the lumen of colon, vagina, or respiratory tract. Results with newer immunocytochemical reagents indicate that cells with the surface phenotype of mononuclear phagocytes are present in large numbers. Strongly MHC class II-positive cells of the dendritic and Langerhans series are also widely distributed, and both cell lineages are thought to be susceptible to viral entry and replication. There is in situ hybridization evidence that enterochromaffin cells of the intestinal crypts of seropositive men with chronic diarrhea are productively infected.

The low efficiency of viral transmission via sexual acts, calculated as less than 1 in 60, makes it difficult to study the factors involved in human disease except for retrospective analysis. For this reason investigators have been striving to develop animal models of HIV infection. The use of chimpanzees for transmission and vaccine trials is not ideal. This primate develops antibodies and is permissive for viral replication, but has failed to develop an immunodeficiency syndrome to date. In contrast, the rhesus monkey does develop an AIDS-like syndrome, succumbing to similar secondary microbial invaders when infected with the related SIV agent. Whereas the chimpanzee is now an endangered species and present in only small numbers, large breeding colonies of rhesus monkeys are now maintained in U. S. primate centers and appear to represent the species of choice.

3. *Initial Targets of Virus Replication.* At least three bone marrow-derived, blood-borne cell types are considered to harbor HIV. These are T lymphocytes, mononuclear phagocytes (monocytes, macrophages), and dendritic-Langerhans cells. The loss of helper type, $CD4^+$ T cells is associated with severe immunological deficiency, but a puzzling aspect of pathogenesis is the very low incidence of $CD4^+$ cells that are productively infected. This is usually <0.01% of $CD4^+$ cells in the peripheral blood, as shown by in situ hybridization techniques. Infection by HIV is best evaluated by the culture of blood leukocytes in the presence of the plant lectin PHA. Reverse-transcriptase activity and $ELISA^+$ glycoprotein antigens, such as p24, appear after a week or two in vitro. The recent development of the polymerase chain reactions (PCR) should provide a more sensitive and rapid technique for virus identification. The combined use of carefully separated cell populations, along with the PCR, should

yield important information on the types of cells that carry HIV and disease pathogeneasis.

Considerable interest has focused recently on blood monocytes and tissue macrophages as cells that both harbor and release infectious virions. In most instances, one has to co-culture blood-adherent monocytes with lectin-stimulated T blasts from uninfected controls to detect virus. However, monocyte cultures can be contaminated with small numbers of $CD4^+$ T cells and dendritic cells, and this complicates analysis of viral origin. Recent evidence suggests that monocytes cultured with growth factors contain large numbers of intracellular virions (within membrane-bound intracellular vacuoles), but release few to the environment. Whether these particles are infectious and what precludes their passage across the plasma membrane are questions for the future. It is the general experience that as the disease progresses to symptomatic AIDS, virus is more consistently identified in cultures of blood mononuclear cells.

More recent reports implicate dendritic cells and Langerhans' cells as being primary cells in the infectious process. We await more detailed analysis with highly purified populations, obtained throughout the course of the disease. These potent accessory cells for T cell replication represent an important element of cell-mediated immunity, and their infection by virus could compromise T cell function and/or represent a pathway for bringing HIV to T cells. Interesting new data from mouse transfectants, carrying a 5'-LTR-CAT construct, demonstrate very high levels of CAT expression in Langerhans' cells of tail skin. Similarly, Langerhans-like cells obtained from the bronchoalveolar lavage of patients with the pulmonary form of eosinophilic granuloma can express very high levels of reverse transcriptase after infection with exogenous HIV.

Spread Within the Host

1. *The Syndrome of Primary Infection and Early Replication.* Recent evidence from world-wide sources indicates that the early, extensive replication with HIV virus leads to a defined syndrome. Within 1–12 wk after transmission patients demonstrate fever, malaise, skin rash, lymphadenopathy, aseptic meningitis, and occasionally symptoms of encephalopathy. There is an initial fall in the T4/T8 ratio, and atypical lymphocytes are common. This syndrome in many ways resembles infection with EBV and CMV. Virus can be isolated from the blood for 2–3 wk, during which period antiviral antibodies are absent. After this time an immune response to the virus occurs, and both cytotoxic lymphocytes specific for virus-infected targets and specific antibodies appear. Initially, antibodies of the IgM class predominate, followed by IgG.

It is possible that infection occurs within the central nervous system during the early phases of the disease. Some believe that infected T blasts may enter the central nervous system (CNS), even passing across the blood brain barrier. Brain endothelial cells contain viral products, although no CD4 antigen can be demonstrated on their surface membranes. Considerable evidence suggests that macrophages, presumably derived from infected monocytes, are an important source of CNS virus. These elongate, stellate microglia have recently been shown to be widely distributed in the brain and they can express CD4 virus receptors. The wide secretory repertoire of these cells may contribute to normal brain function, while macrophage infection could

lead to functional abnormalities, perhaps of a reversible nature. This would be in keeping with the ability of AZT to reverse the servere symptoms of AIDS encephalopathy.

2. *Bone Marrow Involvement.* Little is known about the ability of HIV to enter and replicate within bone marrow cells, including multipotential and committed precursor populations and the more mature elements. One would expect that bone marrow depression is the cause of the pancytopenia seen in AIDS patients, but factors other than direct HIV infection of precursor cells may be involved. These would include parasitization by secondary invaders such as *Mycobacterium intracellulare*, which take up residence within bone marrow macrophages and thereby alter the formation of growth factors.

3. *T Cells and Lymphoid Organs.* See next section.

Session II: Human T Cell Dynamics. Chairmen: A. Fauci and I. Weissman

A. Frequency of HIV-infected T Cells

The difficulty in identifying productively infected T cells by in situ hybridization of bulk leukocytes from blood and lymphoid tissues is well known. Data with the polymerase chain reaction to quantify levels of HIV DNA are eagerly awaited. It remains unclear if there are latent or persistent states of HIV infection in primary human T cells, i.e., integrated or unintegrated proviral DNA that is not expressed or expressed at very low levels.

T cell and monocyte cell *lines* have been isolated that carry a latent form of HIV. Activation occurs with IUdR or with certain cytokines (TNF/cachectin, lymphotoxin, granulocyte/macrophage colony-stimulating factor [GM-CSF]). The possibility was raised that latent HIV in cell lines may represent defective provirus.

B. Mechanisms for $CD4^+$ T Cell Depletion

The earliest view of mechanism was the following sequence: (*a*) many T cells carry latent HIV, (*b*) both T cell and HIV are activated upon antigen encounter, and (*c*) T cell death ensues because of the cytopathic infection. Nevertheless, since so few T cells may be infected, there could be other important mechanisms whereby extensive, prolonged, and often irreversible $CD4^+$ lymphocyte depletion occurs. The following mechanisms were considered.

1. *Syncytium Formation.* Infrequent, infected cells express gp120 upon activation. These fuse to large numbers of $CD4^+$ uninfected cells, and in 3–24 h the syncytia die. It has been difficult to identify syncytia in situ, with the exception of macrophage giant cells in the brain, and syncytia are not readily detected in productively infected primary cells in vitro. Many of the studies on syncytium formation have used $CD4^+$ cell lines rather than primary, resting $CD4^+$ lymphocytes.

2. *Direct Cytotoxicity.* Individual cells may swell and die in cultures of blood mononuclear cells, or in cell lines carrying HIV mutants that are genetically unable to form syncytia. Toxicity may require gp120, but as core particles.

3. *Autoimmunity.* Antibodies to p18, a protein found on the surface of activated $CD4^+$ lymphocytes, may mediate cell death by antibody-dependent cell-mediated cytotoxicity (ADCC). Arguing against the generality of this mechanism are patients, who when given AZT or other chain terminators, show a marked increase in $CD4^+$ T cells and function within 2 wk of therapy.

4. *Loss of a $CD8^+$ Regulator Cell.* The existence of such cells was suggested by studies of patients who expressed HIV in culture only if the $CD8^+$ subset was substantially depleted. When $CD8^+$ and $CD4^+$ subsets have been separated by a 0.45-μm filter, the $CD8^+$ cell still reduced production of HIV from the $CD4^+$ subset. However, $CD8^+$ cell supernatants do not suppress infection, suggesting a labile factor. The $CD8^+$ subset does not reduce $CD4^+$ cell numbers or proliferative function, and does not seem to require matching at HLA. The $CD8^+$ cells suppress HIV production from macrophages as well.

5. *Increased Virulence of the HIV Isolate with Time.* This has been reported in a small sample of patients. This may represent mutation of an initial isolate, or outgrowth of a new isolate.

6. *Transfer of HIV from Infected Dendritic Cells to T Cells.* Dendritic cells are specialized to migrate from nonlymphoid tissues to the T areas of lymphoid organs, and to form stable aggregates with immunologically active lymphocytes. These properties may provide a physiologic pathway for T cell sensitization to antigens that have been deposited in tissues. Given the evidence that dendritic cells may be infected with HIV (Session I), these cells might serve as a reservoir to infect T cells whenever called upon to present antigen in situ.

C. Thymus Function

Beyond the possible pathways for T cell depletion in AIDS (above), there is the added unknown concerning the capacity of HIV patients to produce new T cells. It has been difficult to study thymus function in man and in AIDS, but the pathway for thympoiesis is being outlined in rodents.

The CD4 antigen is expressed relatively late in T cell development. Mouse bone marrow contains clonogenic thymocyte precursors at a frequency of 1/36,000. The majority have the phenotype Thy-1^{low} lineage ^{neg} (negative for CD4, CD8, CD11b, and a granulocyte antigen). 25–30 of these cells, isolated on a FACS, reconstitute all cell lineages in irradiated mice. New "stem cell antigens" are being identified. One mAb, SCA-1, can be used to further enrich the bone marrow progenitor. In rats exposed to 300 rad of ionizing irradiation, most regenerating thymocytes pass through a short-lived (<1 d) $CD4^-CD8^+$ stage. At least 50% of normal $CD4^-CD8^+OX44^-$ thymocytes also can be pulse labeled with BUdR, and within 16 h most became $CD4^+CD8^+$ in vitro.

SIV infection in primates might provide an opportunity to study thymus function in immunodeficiency. A novel mouse model was also described. Mice with severe combined immunodeficiency (SCID mice) lack B and T lymphocytes, and interestingly, succumb to *Pneumocystis carinii* pneumonia. The health of SCID mice can be improved with grafts of human fetal liver and thymus, and the mice can be returned to a conventional animal facility. The human thymus develops beneath the kidney capsule and contains human thymocytes, both human and mouse dendritic cells, as well as human class II-positive cortical epithelium. Human T cells are also found in the blood, but mysteriously, few are noted in peripheral lymphoid organs.

Session III: Vaccines and Protective Immunity. Chairmen: H. Koprowski and R. Lerner

The development of vaccines towards many infectious agents has depended upon the availability of animal models, and the induction of neutralizing antibodies. However, there are examples of vaccines that are effective in laboratory animals but not

in the relevant animal in the wild, and there have been vaccines to rabies and Herpes simplex that do not seem to elicit much neutralizing antibody. Possibly, protective T cells are induced in the latter situations. Also it is not clear why some vaccines, e.g., yellow fever, induce such long-lasting immunity.

The design of vaccines depends upon information on pathogenesis (Session I). For HIV, one unknown is the extent to which cell-free virus is needed to establish infection. If cell-associated virus is critical, the demands of an antibody-based vaccine increase since one must design neutralizing antibodies that block cell–cell transmission and not the penetration of free virus into cells. Another unknown is whether there are T cell–dependent resistance mechanisms. Both proliferative and cytotoxic T cells are being detected as part of the response to HIV in man and experimental animals. Their role in eliminating infected macrophages and dendritic cells for instance is not clear and will be difficult to evaluate without animal models.

Many aspects of vaccination were discussed in and outside of the sessions. Three topics are expanded here.

A. T Cell Epitopes

The predictive value of the Berzofsky/DeLisi model, in which sequences capable of forming amphipathic double helices act as helper T cell epitopes, was summarized for the malaria circumsporozoite protein. There were five major predicted sites, corresponding to about one-third of the protein. Four were found to stimulate blood leukocytes from donors in the endemic regions of Gambia. 24 other peptides were tested and were not T cell stimulatory. A candidate T cell stimulatory epitope occurs on a polymorphic region of the circumsporozoite protein.

Two peptides were selected from the conserved regions of HIV gp120. Both could form amphipathic helices and were nonglycosylated. The peptides would immunize mice, as assessed by T cell proliferative responses, and there were clear MHC-restricted responder and nonresponder mouse strains. One peptide also stimulated proliferation in cells from patients who had been immunized in Zaire with vaccinia-HIV *env* recombinants.

CTL epitopes in the HIV-env protein also are being analyzed in mice. Mice have been immunized with vaccinia-env recombinants, boosted in vitro with cell lines expressing VAC-env, and then tested for CTL activity against a panel of peptides on 3T3 (H-2^d) or L cell (H-2^k) targets. H-2^d mice were high responders to vaccinia-gp 160, while H-2^k were low. A large number of peptides, 29 from gp120 and additional ones from gp41, were tested. Only one was active in H-2^d mice (D^d restricted). This epitope was 308–322 in gp120, which lies in a major neutralizing site for antibodies (see below). The peptide, which has a central β turn, exhibits high variability when different HIV isolates were examined. If anti-env CTL provide protection against HIV, this result would imply that there is high mutation in the very region capable of eliciting B cell and CTL immunity. It will be important to follow this site longitudinally in HIV isolates. A second feature of the CTL work in mice is that only one of the five mouse class I molecules that were tested could present HIV gp120, but other HIV gene products need to be evaluated.

The tight restriction imposed by the MHC on antigen presentation was illustrated as well by data on murine lymphocytic choriomeningitis virus (LCMV). Inbred mice were immunized with vaccinia-glycoprotein or vaccinia-nucleoprotein. H-2^d and H-2^q had high levels of CTL to nucleoprotein, and to the same NP peptide, but

only H-2^b made CTL to glycoprotein. Mice could be protected against a lethal challenge by NP immunization. No neutralizing antibody was detected, just CTL.

Several groups have detected CTL from HIV-infected patients, most readily in patients who were not in the AIDS phase of the disease. The cells are capable of lysing syngeneic targets. HIV-specific and MHC-restricted activities have been detected. These CTL have been found in fresh blood, suggesting a significant persistent antigen load in situ.

B. Neutralizing Antibodies

HIV infection is notable for the very low ratio of neutralizing to total antigen-binding antibodies. There seem to be two sites on the gp120 molecule to which antibodies might be neutralizing, especially for free HIV virions. One is at 400–434, a highly conserved (HIV-1 and -2, SIV) region in gp120 that is involved in *binding* of gp120 to CD4 and is glycosylated. The other site is at 300–330 and is highly variable (see above). Antibodies to this latter site can neutralize *after* HIV binding and seem able to block fusion as well as syncytium formation. Seropositive individuals form little antibody to this site, but four experimental mAbs have now been raised.

Studies are in progress of the antibodies that are developing in a laboratory worker who was infected with HIV. The worker formed type-specific neutralizing antibodies to the 300–330 site. These antibodies could be blocked with a 24-amino acid peptide from this region. A year later, more crossreactive antibodies were formed to other gp120 sites. These antibodies could block gp120–CD4 binding and so presumably reacted with the 400–434 site. The HIV isolates from the worker are now showing variation in the 300–330 site, which might allow escape from type-specific neutralizing antibodies. It has not been determined if one clone of HIV is generating variants, or if there is selection of a different initial isolate.

C. Chimpanzee-HIV Studies

Chimps were passively immunized with a pool of hyperimmune human Ig containing broadly crossreactive neutralizing antibodies. These blocked binding, or the gp120/CD4 interaction, but not fusion. The chimps were immediately challenged with a cloned isolate of HIV intramuscularly. Circulating antibodies were evident at day 3, but only low titers at day 7. The short life span of the antibodies might reflect aggregation of the initial IgG inoculum. Also, it is not clear if antibodies were present at the intramuscular injection site. In any case, the passive immunization did *not* reduce infection with HIV. One unknown in the HIV-Chimp model is that while the leukocyte of these primates can be infected in vitro with HIV, for unknown reasons chimps are protected from acquiring AIDS in situ.

D. Summary

In spite of much ongoing experimentation, the consensus view is that a vaccine will be difficult to achieve. New strategies of chemo- and immunotherapy may bear more rapid results.

Session IV: Therapeutic Consideration. Chairmen: Martin Hirsch and Barry Bloom

A. Antiviral Chemotherapy

Infection with HIV involves several discrete steps, some of which are being evaluated as possible targets for antiviral chemotherapy. These include:

Binding of virus to target cell (soluble CD4, dextran sulfate, antibodies)
Fusion of viral membrane to host cell membrane
Viral penetration and uncoating
Reverse transcriptase (AZT, ddC, ddA, adenellene, cytallene)
Degradation of RNA by RNase
Integration of DNA into host genome
Transcription and post-transcriptional enhancers (tat, art)
Ribosomal frameshifting and proteolysis of polyprotein precursors
Viral component production and assembly (castanospermine).

A review of the current National Institutes of Health chemotherapy program indicates that ~30 drugs that have in vitro anti-HIV activity are under test in vivo. There are 35 funded Institutional programs, 27 protocol trials using 17 agents of which 9 are antiviral, 4 are biological response modifiers, and 5 are against opportunistic disease. The program is being carried out in an atmosphere of public pressure and in which there is extensive availability of selected agents in a black market.

AZT is the most widely used agent and it has resulted in 75% survival at 21 mo. Its use is complicated by severe bone marrow depression and transfusion requirements in some patients. 2'-dideoxycytidine also appears to be virustatic without significant bone marrow depression, but its use may be complicated by severe peripheral neuropathy. Significant reduction in p24 antigen occurs in approximately half the patients receiving the drug over a 30-wk course.

It is of interest that the dementia of AIDS appears to be reversible if patients are treated early with AZT. After a 6-wk course of therapy, CAT scans reveal a reduction in ventricular size; brain glucose utilization is increased, and psychometrics are improved. AZT is known to enter the CSF, with an accompanying reduction in the amount of p24 antigen in the CSF. Chemoprophylaxis is now being evaluated in asymptomatic seropositive patients.

Newer nucleoside analogs, which have cyclic 4 carbon chains instead of sugars (adenollene and cytallene) show promise in their ability to protect tissue culture cells (the ATH8 cell line) at 0.5–50 μm. Synthetic oligonucleotides with sulfur vs. oxygen groups could represent useful, long-lived antisense reagents. The general consensus is that chemotherapeutic breakthroughs will not occur in the near future.

B. Immunotherapeutics

The newer recombinant cytokines and lymphokines are under study alone or in combination with AZT. These agents such as IL-2, IFN-α, IFN-γ, and GM-CSF are administered by the parenteral route in an attempt to replace the products manufactured by the defieient $CD4^+$ T cells. The use of GM-CSF with AZT is also an attempt to protect the bone marrow. One target of these agents is the parasitized macrophage. These cells, in the absence of helper T cells, fail to become activated and are permissive hosts for a variety of opportunistic invaders that ultimately lead to the death of AIDS patients. The results of clinical trials should become available within the year.

Soluble CD4 molecules can inhibit viral infection in vitro at levels of 5 μg/ml. Current studies are using various segments of the CD4 molecule attached to cytotoxins and antibodies to seek out gp120-expressing targets. Primates are able to tolerate CD4 at levels of 10–25 μg/ml, but the ability of the molecule to protect primates from infection is unknown at this time.

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