The Science Base Underlying Research on Acquired Immune Deficiency Syndrome

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CURES FOR OR PREVENTION OF a particular disease entity rarely arise from a concerted effort aimed only at that disease. Such advances inevitably are built on the solid foundation of previous and ongoing biomedical research. Technological breakthroughs provide new opportunities to address questions that could not have been tackled previously; the research then proceeds at a more rapid pace. It is this sizable research effort, particularly in the fields of immunology and virology, that has provided both the knowledge and the technology to attack the problem of acquired immune deficiency syndrome (AIDS).

AIDS is a new disease of unknown etiology. In June 1981, the Centers for Disease Control first reported on patients who were succumbing to opportunistic infections such as *Pneumocystis carinii* pneumonia (PCP) in the absence of other underlying disease or induced immunosuppression. A month later, Kaposi's sarcoma, normally a slow-growing tumor seen in older men of Jewish or Mediterranean descent, was reported in young men—and in a considerably more virulent form. Previously, cases of PCP, other opportunistic diseases, and the virulent form of Kaposi's sarcoma had been seen only in patients whose immune systems had been suppressed by long-term illnesses such as cancer or by drug therapy used to treat cancer or prevent rejection of transplanted organs.

A relationship among all these cases was sought. Careful examination revealed that the single common characteristic seen in all patients was a severe and apparently irreversible suppression of the immune system.

All AIDS patients have shown evidence of severe immunodeficiency that is acquired and not congenital. While there is no definitive diagnostic profile, most patients develop an abnormality of T-cells (thymus-derived lymphocytes) when they are severely ill. AIDS victims characteristically have a smaller than normal number of helper T-cells (cells involved in inducing a wide variety of immune responses), while their suppressor T-cell subpopulation (cells involved in inhibiting immune function) is normal. These patients also manifest high levels of serum immunoglobulin because their antibody-secreting B-cells (bone-marrow-derived lymphocytes) are abnormally active (*1*).

The concept of subsets of immune cells (lymphocytes), each with a different specific function, is crucial to an understanding of AIDS. Although this concept and the ability to identify these subsets are relatively recent developments, they resulted from considerable research aimed at understanding the immune system.

Research in Immunology

The seeds of our current concepts of immune function were planted in the 1950s, when Niels K. Jerne developed his natural selection theory. This theory postulated that each cell with antibody-producing potential was programmed to make a single antibody with a given specificity; the cell was competent even in the absence of the particular antigen that would elicit the antibody response (*2*).

MacFarlane Burnet refined Jerne's hypothesis into the clonal selection theory. This theory states that the progenitors of antibody-producing cells are genetically programmed to make antibodies of one or several types; antigen interaction with receptors on the cell surface signals the cell to divide and commits it and its progeny to the production of antibodies directed against only that antigen (*3*).

Scientists next asked, What are the origins of antibody-producing cells? A series of experiments per-

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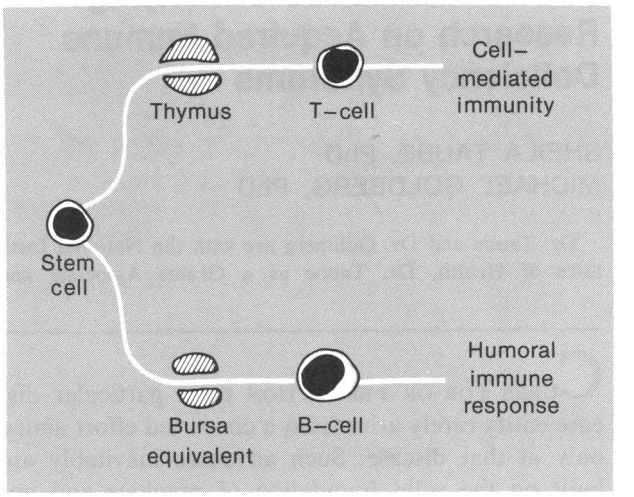
formed in mice demonstrated that, in an animal whose hematopoietic (blood cell forming) system had been depleted by irradiation, the system could be restored by injection of bone marrow or spleen cells from a compatible donor. These studies indicated that all the cells of the blood, including those of the immune system, are derived from a common precursor cell called a "stem cell." Differentiation of these stem cells occurs in the different organs of the hematopoietic system—the thymus and the spleen, for example (*4,5*)—and is determined by various factors in these organs.

Among the differentiated cells are cells that are capable of producing antibodies. In chickens, antibody production requires maturation of antibody-producing cells in the bursa of Fabricius, a lymphoid organ (*6*). In mammals, stem cells are formed in the bone marrow, and differentiation of antibody-producing cells occurs in an equivalent of the bursa. (Although no discrete anatomical site has been identified, the bone marrow appears to be part of the bursa equivalent.)

The antibody-producing cells (now known as B-lymphocytes, or B-cells) constitute one of two major branches of the lymphoid system (see figure) and provide the humoral immune response (antibody formation). The second branch is formed by passage of stem cells through the thymus. These cells are called thymus-derived lymphocytes, or T-cells, and are now known to be involved in tissue reactions (cell-mediated immunity). Cells from both branches of the lymphoid system are further modified when they reach peripheral lymphoid organs such as the spleen and lymph nodes.

The question for researchers in the early 1960s was, Which lymphocytes—those from bone marrow (bursa equivalent) or those from the thymus—are responsible for antibody production? It was while scientists were attempting to answer this question that a major discovery was made: cooperation between the two classes of lymphocytes is necessary to generate an antibody response.

Immune response has two major aspects: antibody formation, provided by lymphocytes derived from the bursa equivalent, and tissue reactions, mediated by thymus-derived lymphocytes. Both B-cells and T-cells arise from a common precursor



At that time, Robert A. Good, J. F. A. P. Miller, and Byron Waksman, working independently on different aspects of thymus function in animals, found that surgical removal of the thymus shortly after birth severely compromised the immune system. It was subsequently demonstrated that the ability to generate an antibody response could be restored by repopulating the thymus-deficient animals with thymus cells taken from compatible donors (*7–9*).

A series of experiments performed by H. N. Claman and his associates showed that both thymus and bone marrow cells are necessary for antibody production. In these experiments, mice were irradiated so that their immune systems were totally destroyed. Repopulation with either thymus cells or bone marrow cells alone was insufficient to restore the ability of the mice to respond to a challenge with antigen. Only when a mixture of thymus and bone marrow cells was injected did the mice produce antibodies in response to antigen challenge (*10*). A. J. Davies and his colleagues in London followed genetically marked thymus cells used in repopulation of irradiated mice to show that, although these cells proliferated in response to antigen challenge, they did not make antibodies, and that bone marrow cells, injected in the absence of thymus cells, neither proliferated nor produced antibodies (*11*).

Once it had been established that some type of cooperation between the two cell types was necessary, investigators began to dissect the immune system carefully in an attempt to characterize each cell type and define its precise role in the immune

response. They still had no answer to the question, Which cell type actually produces the antibodies?

Research in mouse genetics that had been going on since the 1930s provided the basis for the experimental approach used to solve this problem. Peter Gorer in England, and later George Snell at the Jackson Laboratory in Bar Harbor, Maine, used inbred strains of mice to define a system of allelic genes that is responsible for graft rejection (*12,13*). This system of alleles, known as the major histocompatibility complex, determines proteins on the surface of cells and the levels of some serum proteins. Using inbred strains of mice, scientists were able to elicit antibodies in one mouse against a single different cell surface protein from another mouse.

In 1968, J. F. A. P. Miller and his colleague, G. F. Mitchell, used the histocompatibility antigens in an experiment to demonstrate that thymus cells were necessary to stimulate antibody production by other lymphocytes (*14*). They removed the thymus at birth from a mouse of one histocompatibility type (mouse 1), then repopulated the animal with thymus cells from a mouse of another histocompatibility type (mouse 2). Mouse 1 was stimulated with an antigen several weeks later. Following antigen stimulation, spleen cells were removed from mouse 1. (Both T-cells and B-cells eventually migrate to and settle in the spleen.) The spleen cells were then treated with antiserum directed against cells from mouse 2. Spleen cells from a control animal of the same strain as mouse 1 were treated with antiserum directed against mouse 1 cells. The experiment was designed so that cells that reacted with antiserum were destroyed.

Following antiserum treatment, the spleen cell preparations were tested for ability to produce antibodies against the antigen originally used as stimulus. Antiserum against mouse 2 cells had no effect on antibody production by mouse 1 spleen cells; however, antiserum against mouse 1 cells significantly reduced antibody production by spleen cells of the control animal. In the case of mouse 1 spleen cells, the antibody against mouse 2 cells destroyed the T-cells that had come from mouse 2 but did not affect the B-cells of mouse 1. In the case of the control mouse spleen cells, the antiserum against mouse 1 cells destroyed both B-cells and T-cells. Thus, the cells responsible for antibody production must have come from mouse 1 and were not derived from the thymus.

This experiment formed the basis for an approach to the identification of lymphocyte subpopulations and their functional interactions. The mouse model

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was used to refine further the genetics of the histocompatibility complex, and the inbred strains were used to produce antisera to characterize the cells of the lymphoid system. In the 1970s, cooperation among lymphocyte subsets became a major focus of immunological research.

It has become clear that the variety of cell-mediated responses that have been observed represent different aspects of T-cell function and are carried out by different subsets of T-cells. There is T-cell : T-cell cooperation in these responses analogous to the T-cell : B-cell interaction seen in antibody production. The experiments establishing these lymphocyte subsets and their cooperative interactions were basically variations of the types of experiments used to establish the T-cell : B-cell interactions. Cell-surface antigens were identified and used to eliminate a particular cell type from a mixture of cells. By eliminating one cell or another, it was possible to characterize the interactions.

Further study of the major histocompatibility complex (MHC) indicated that these antigenic surface proteins had significant functions in both the humoral and the cell-mediated immune responses. Zinkernagel and Doherty demonstrated that cooperation between cells involved in response to viral infection requires presentation and recognition of MHC antigens along with the viral antigen (*15*). Shearer, at the National Cancer Institute, made the same observation for other antigens (*16*).

These findings raised further questions about regulation of the interactions among cells involved in the immune response. As the system was probed more deeply, greater complexity was discovered. In the early 1970s, Richard Gershon suggested that T-cells could actually inhibit an antibody response (*17*). This idea was vehemently resisted until the weight of evidence was overwhelming; however, it is now clear that there is a class of T-cells that regulates the immune response by actually suppressing it. In fact, the immune response is the expression of a delicate balance between the positive influences of helper T-cells and the negative influences of suppressor T-cells. Attempts are being made to

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restore the proper balance of T-cell populations in bone marrow recipients, and similar experiments are being conducted in AIDS patients on the basis of information obtained from the transplant studies.

Precise identification of the subsets of lymphocytes was made possible by the availability of monoclonal antibodies that could identify unique cell-surface antigens. These monoclonal antibodies were made possible by the development of hybridoma technology. In accordance with Burnet's clonal selection theory (described earlier) a given lymphocyte is stimulated to produce antibodies with a single specificity. All the antibody molecules from this cell and its progeny are identical. The cell and its progeny are called a "clone," and the antibodies produced are referred to as "monoclonal." Lymphocytes have a limited lifespan in culture that limits the quantities of antibody obtainable. In 1975, however, Köhler and Milstein fused antigen-stimulated lymphocytes with cells derived from a myeloma. This fusion resulted in a population of cells that continued to produce large quantities of the specific monoclonal antibodies and could be cultured indefinitely—in effect becoming immortal (*18*). Cells fused in this way are called "hybridomas."

The ability to form hybridomas allowed scientists to create a library of specific reagents that could be used to identify and analyze cellular components. These reagents have been critical to the identification of T-cell antigens. (In particular, the human major histocompatibility complex could not have been examined without hybridomas, since scientists do not have the luxury of inbred strains to work with, as in the mouse system.) Considerable research, supported by most of the components of the National Institutes of Health, is being performed to improve hybridoma production, to use monoclonal antibodies to dissect molecular interactions, to probe the genetics of antibody specificity, and to apply hybridoma technology to clinical studies. Monoclonal antibodies were used to characterize the altered T-cell ratios in AIDS victims.

The great complexity of the immune system has provided an enormous challenge for scientists and clinicians. There are intricate networks of checks and balances, and a variety of conditions can disturb the system. Efforts to understand the system and breakthroughs in our knowledge about the system's controls provide insights into other syndromes as well as the particular syndrome under study. Because understanding the immune system is important for understanding so many disease entities, and because the system is intrinsically fascinating, there is tremendous research activity in this field (see table).

Current research supported by the National Institute of Allergy and Infectious Diseases in the area of regulation of the immune response is aimed at further elucidation of the mechanisms of interaction among subsets of lymphocytes and between lymphocytes and macrophages (another type of lymphoid cell). The cooperative functions of T-lymphocytes have been found to correlate well with the presence of a specific cell surface antigen. T-cells have also been shown to secrete a soluble factor, called interleukin 2 (IL2), that is important in amplifying cell-mediated immune responses. Patients with AIDS have a deficiency of IL2 because of a defect in the numbers and function of the T-cell subset that produces the substance. Currently, NIAID is conducting limited trials of the effectiveness of administering IL2 to AIDS patients in the hope of reconstituting their defective T-cell immunity. In addition, investigators are working to define the origin, pathways of differentiation, and roles of the various lymphocyte subpopulations. Although much of this research was initiated before the characterization of AIDS, the results of these studies will further scientists' understanding of and ability to cope with AIDS.

The focus of studies supported by the National Cancer Institute is on the interaction of certain lymphocytes that kill tumor cells. Studies of the restriction of the cell-killing response by major histocompatibility complex antigens, as well as of the role of these antigens in lymphocyte subset interactions, are also supported by NCI.

Because cell-mediated immunological responses are significant components of various forms of arthritis, the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases supports considerable research effort in this area. In particular, NIADDK has supported development of a new way to measure the activity of certain T-lymphocytes.

There is considerable interest in the immune system because it appears to be intimately involved with a variety of major disease entities—chronic infections, cancer, arthritis and related inflammatory diseases, allergies, and chronic neurological dis-

orders, to name a few. All these syndromes represent examples of cell-mediated immune system dysfunction. Since they together affect millions of people, there is an enormous impetus to study cell-mediated immune function and its regulation.

Research in Virology

Virology is another area of research that is important to an understanding of AIDS. Most of the theories about the etiology of AIDS implicate an infectious agent, most likely a virus.

The era of modern virology began in 1949, when John Enders and his colleagues successfully grew poliovirus in cell cultures (*19*). This achievement led to the isolation and characterization of hundreds of previously unknown viruses and cleared the way for development of the field of diagnostic virology. It also was the first step in the development of many new viral vaccines. Moreover, it allowed scientists to study the interactions between viruses and their host cells.

Viral infections proceed in two fundamentally different ways. In an acute infection, the virus grows in the cell, produces progeny, and ultimately kills the infected cell. This type of infection occurs in influenza and many other common viral illnesses. The infection is normally stopped by the body's defense system (which includes lymphocytes and interferon).

The second type of infection results in a chronic interaction; the virus remains in the cell, sometimes in a "dormant" state and sometimes releasing small numbers of progeny virus particles. The host cell is not killed, and the body does not eliminate the virus. Herpes simplex virus "cold sores" typify this relationship; here, the viral nucleic acid remains associated with nerve cells and periodically produces localized infections of the nearby tissue. In healthy individuals this relationship can continue throughout a normal lifetime and generally creates only a periodic nuisance. However, when the immune system fails, the virus may become disseminated, and the infections are severe and frequently fatal. This is the case in AIDS patients.

Another example of chronic, persistent viral infection is the "slow infection." This term designates certain severe diseases of viral etiology that develop over a long period of time, often decades. One group of these diseases is caused by unconventional viruses and particularly affects brain tissue. D. C. Gajdusek, of the National Institute of Neurological and Communicative Disorders and Stroke, first demonstrated that kuru, a chronic degenerative central nervous system disease in man, was caused by a virus (*20*). This discovery led to demonstrations of viral etiology in other progressive degenerative diseases. The virus that causes kuru and the related unconventional viruses lead to diseases characterized by a noninflammatory pathology. Conventional viruses, on the other hand, induce progressive inflammatory diseases. Examples of persistent viral infections caused by conventional viruses include progressive multifocal leukoencephalopathy, cytomegalovirus (CMV) brain infection, and chronic meningoencephalitis in immunodeficient patients.

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It is not yet known why common viruses persist in some individuals and proceed to cause slow destruction of tissue. A fairly high percentage of normal adults have measurable circulating antibody against CMV, indicating prior exposure to the virus, but the virus itself can be isolated from less than 1 percent of the population. CMV appears to be reactivated under conditions of altered immunological status; it is a serious problem in transplant patients and cancer patients receiving chemotherapy. CMV has been associated with AIDS and Kaposi's sarcoma, and an understanding of the reactivation of CMV in patients with these diseases and in other immunosuppressed patients is of great importance. Several National Institutes of Health components are supporting research on CMV, including the basic biology of the virus, vaccine development, and development and testing of antiviral therapeutic agents (see table).

The advent of recombinant DNA technology particularly facilitated investigations of persistent and latent viral infections. Work by David Baltimore and Howard Temin on reverse transcriptase provided the initial tool. This enzyme allows scientists to copy RNA molecules and produce reagents that can be used to probe tissues for the presence of related nucleic acid sequences. Using this technique, it has been possible to demonstrate unequivocally the

presence of viral nucleic acid in cells. Recent research has shown that CMV nucleic acid sequences are present in Kaposi's sarcoma biopsy material. The discovery of human T-cell leukemia virus (HTLV) in AIDS patients was also made using recombinant probes (*21–23*). HTLV is a candidate as an etiologic agent for AIDS because it specifically attacks helper T-cells, which are the defective subpopulation in AIDS.

Research leading to the development of recombinant DNA technology was part of an ongoing effort to understand basic questions about cellular control mechanisms. Scientists have used recombinant DNA techniques to examine gene structure and function, to detect chromosomal abnormalities, and to detect viral sequences in tumors. Moreover, recombinant DNA technology now has been developed to the point where specific genes can be isolated, transferred via a variety of well-characterized vectors to new hosts, and turned on to produce the proteins encoded by the transferred genes. Such molecules as human growth hormone, human insulin, interferon, and viral proteins have now been synthesized by genetically manipulated bacteria, yeasts, and mammalian cells.

One protein that now can be synthesized by recombinant DNA techniques, and that may be important in the treatment of AIDS, is interferon. An observation that growth of one virus in a cell interfered with growth of a second virus led to the discovery of interferon by Isaacs and Lindenmann in 1957.

Interferon was initially defined as a protein produced in response to viral infections; this protein, when added to uninfected cultures, would protect them from infection by added virus. Intensive study of interferon has revealed that it is, in fact, a class of proteins that demonstrate a variety of potentially beneficial effects.

Three major types of interferon have been isolated and studied. One is the now classic antiviral interferon. The other two have been shown to affect the activity of certain T-lymphocytes. Each type is produced by different cells and appears to function somewhat differently.

Considerable research to probe the interactions between interferon and cells of the immune system is being supported by the National Cancer Institute and the National Institute of Allergy and Infectious Diseases. Interferons from different cell types are also being tested as antiviral and antitumor agents in clinical trials. Research on the relationship between serum interferon levels and the status of autoimmune disorders is supported by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. Other National Institutes of Health components support continuing efforts to understand control of interferon synthesis, mechanisms of action, and correlation with disease states (see table).

Interferon is also being tested in the treatment of AIDS because of its possible interaction with the immune system. The National Cancer Institute is testing human lymphoblastoid interferon in the treatment of Kaposi's sarcoma, and the National Institute of Allergy and Infectious Diseases is conducting clinical trials of the effectiveness of immune interferon in AIDS patients.

Summary

In order to define the clinical syndrome of AIDS and begin to deal with it effectively, scientists needed

Expenditures by the National Institutes of Health, FY 1982, for science base research related to acquired immune deficiency syndrome (dollars in thousands)

NIH components	Research areas		
	Cellular immunity [1]	Interferon [2]	Key viruses [3]
National Cancer Institute	$ 63,826	$ 8,316	$13,575
National Heart, Lung, and Blood Institute	3,273	97	1,250
National Institute of Dental Research	...	...	25
National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases	8,980	703	660
National Institute of Neurological and Communicative Disorders and Stroke	4,605	544	1,290
National Institute of Allergy and Infectious Diseases	17,477	3,398	7,569
National Institute of Child Health and Human Development	1,387	221	1,202
National Eye Institute	4,451	610	2,913
National Institute on Aging	407	...	136
Division of Research Resources	6,907	630	1,553
Total, NIH	$111,313	$14,519	$30,173

[1] Category includes research on immunoregulation; functions of suppressor, helper, and other T-cells; cellular and congenital immunodeficiency; and immunosuppression.

[2] Category includes research on interferons and lymphokines such as interleukin 2 but does not include expenditures for production of interferons.

[3] Category includes hepatitis B and non-A, non-B hepatitis viruses, cytomegalovirus, herpesvirus, and Epstein-Barr virus.

NOTE: Other significant science base research that will contribute to understanding of AIDS is being conducted on sexually transmitted diseases (FY 1982 expenditures, $2.99 million) and opportunistic infections (FY 1982 expenditures, $7.89 million).

to understand how the immune system works. Fortunately, considerable knowledge was available: research in immunology over the last two decades had provided the technological advances and basic information about cell-mediated immunity that were necessary for identification of the syndrome. Without this knowledge base, immune suppression would not have been recognized as the common link among AIDS patients manifesting a variety of infections and unusual neoplasms.

Similarly, research on infectious diseases, and in particular on the role of viruses as etiologic agents, has had an important bearing on understanding of AIDS. The epidemiologic data to date indicate that an infectious agent most likely is involved and that transmission of the disease requires intimate contact and perhaps some passage of blood. Among the candidates for viral agents are Epstein-Barr virus, cytomegalovirus, and human T-cell leukemia virus. All have been isolated from the cells of AIDS victims, but whether they are etiologic agents or opportunistic pathogens remains unresolved. Knowledge gained from the study of any of these viruses will contribute to understanding of AIDS, and vice versa.

In this paper, we have attempted to show the integral relationship between specific research on AIDS and the ongoing research effort in related disciplines. It is important to recognize that effective research is the result of careful consideration of which questions can and should be addressed and the development of innovative approaches to gain answers to those questions. Research on AIDS is proceeding as rapidly as it is only because of the solid foundations that have been developed in the areas of immunology and virology. It is this base of research that ultimately will provide the rationale and the tools for solving new problems.

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