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SEARCH

THE ROCKEFELLER UNIVERSITY MAGAZINE

Special Feature: The Many Facets of AIDS Research at Rockefeller



*HIV, the virus that causes
AIDS, magnified 640,000 times. →*

SEARCH

THE ROCKEFELLER UNIVERSITY MAGAZINE



Special Feature: The Many Facets of AIDS Research at Rockefeller

Twelve years after the start of the AIDS epidemic, the plague remains unconquered. Researchers at The Rockefeller University are among the thousands of scientists worldwide who are seeking new insights into the disease and its causative agent, the human immunodeficiency virus (HIV). In this issue's special feature, senior science writer Susan Blum surveys AIDS research at the university and shows how the roots of this research often reach back to pioneering studies at Rockefeller that predate the AIDS epidemic, but that have proved remarkably relevant to it.

COVER NOTE—This cover of *Search* features a rare, close-up look at HIV, the virus that causes AIDS. The electron micrograph shows a mature HIV particle—magnified about 640,000 times—attached to the “pit” in the surface membrane. This state represents the first step in the receptor-mediated uptake of HIV.

Despite the abundance of printed matter on HIV and AIDS, it was surprisingly difficult to locate a clear picture of the virus. Our search took us to the Aaron Diamond AIDS Research Center in New York, where we are indebted to its director, David Ho, to the *Seminars in Virology* editorial offices in London, and then to the Robert Koch Institut in Berlin, where Hans Gelderblom was extremely helpful. Gelderblom provided this micrograph, which originally accompanied an article by R.I. Connor and Ho, “Pathogenesis of Human Immunodeficiency Virus” that appeared in *Seminars in Virology* (volume 3, 1992).
—The Editor

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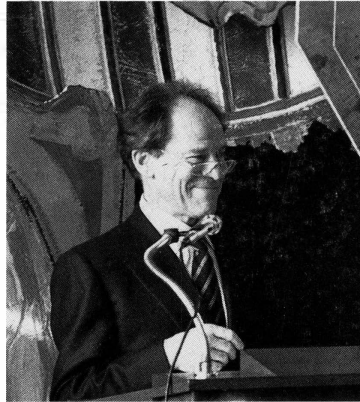
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The Rockefeller University is an equal opportunity employer and has an affirmative action program to increase the employment of women and members of protected groups at all job levels.

In 1969, the Surgeon General of the United States declared that the war against infectious diseases had been won. But clearly this proclamation of victory was premature. With the appearance of HIV and the AIDS pandemic, and the emergence of antibiotic-resistant strains of pathogenic bacteria across the world, infectious diseases remain a fundamental threat to all of humankind. This issue of *Search* features a comprehensive review of AIDS research at The Rockefeller University, covering the many facets of the university's effort to better understand HIV and design new methods to combat it.

The Rockefeller University was founded in 1901 as The Rockefeller Institute for Medical Research with the central mission of understanding the lethal secrets of disease-causing microbes and using this knowledge to design specific anti-microbial therapy. Since that time, this university has helped launch the antibiotic era and pioneer basic and clinical research into the mechanisms of microbial pathogenesis. As the review of AIDS research at The Rockefeller University demonstrates, this historic commitment continues here today.

From the university's beginning, many of its research programs have stemmed directly from the urgent public health needs of New York City and other urban centers. This issue of *Search* begins with a report delivered at the university this past spring by New York City's top public health official, Margaret Hamburg, on the interlocking challenges of AIDS, tuberculosis, and drug addiction. In addition, on July 21, 1993, the university sponsored a workshop on a growing crisis that has been exacerbated by the AIDS pandemic: the global emergence of antibiotic-resistant strains of deadly bacteria. The meeting, which included some of the nation's leading experts on public health and infectious diseases, was the first step in a larger university program focused on the problem of drug-resistant microbes. The Rockefeller University intends to continue to play a leading role in research into the mechanisms of antibiotic resistance, the development of new and more specific antimicrobial therapies, and the dissemination of information to physicians and scientists working at other institutions regarding this growing medical crisis. With our strengths in microbiology and molecular biology, the clinical research capabilities of The Rockefeller University Hospital, and our contacts with hospitals and research institutions around the world, The Rockefeller University is uniquely positioned to continue its historic mission into the twenty-first century.



A handwritten signature in black ink that reads "Torsten Wiesel". The signature is written in a cursive, flowing style.

Torsten Wiesel
President

Triple-Headed Disease Threat

tuberculosis
aids
drug addiction

New York City
Health Commissioner
Addresses Three Related
Epidemics at Rockefeller
University Meeting

In May 1993 the worlds of scientific research and public policy came together for an unusual meeting convened at The Rockefeller University. Sponsored by The Rockefeller University Council—a group of 160 friends of the university who promote public awareness of the importance of basic research in disease prevention and treatment—the meeting addressed the increasingly interconnected urban health crises of AIDS, tuberculosis, and drug addiction. For The Rockefeller University, founded in 1901 as the nation’s first biomedical research center, the gathering reflected its long-standing commitment to understanding and conquering infectious diseases. The panel of speakers included three leading Rockefeller researchers—Dr. Vincent Dole, Dr. Mary Jeanne Kreek, and the late Dr. Zanvil A. Cohn—as well as New York City’s Health Commissioner, Dr. Margaret A. Hamburg. The work of the three Rockefeller investigators is covered in the article that begins on page 8 (see The Battle Against AIDS). Following is a discussion of the remarks made by Hamburg.

You cannot live in a city like New York today without being in some way affected by the triple-headed disease threat of drug addiction, tuberculosis, and AIDS,” Hamburg began by telling the audience.

These three diseases are closely interrelated—primarily because many of the same factors underlie the incidence and spread of each: poverty, unemployment, inadequate access to

where, Hamburg explained, the reemergence of TB is connected to the AIDS epidemic because people whose immune function is seriously compromised are more susceptible to the disease. Hamburg estimated that as many as 40 percent of the city’s TB patients are also infected with HIV. With as many as a quarter of a million New Yorkers already HIV positive—and hundreds of thousands still at risk of AIDS—the num-



In her talk, Commissioner Margaret Hamburg stressed that society can no longer afford to neglect the burgeoning and interconnected urban health crises of AIDS, tuberculosis, and drug addiction.

health care, substandard housing, and homelessness. And this connection is not unique to New York City. “Poverty is a primary force fueling the crisis in public health throughout the country,” Hamburg said.

Three Overlapping Epidemics

The United States as a whole has experienced a 143 percent increase in the incidence of tuberculosis since 1980, and New York’s case load is disproportionately high, accounting for about 15 percent of the nation’s cases. Poverty alone cannot account for this resurgence. Here, as else-

ber of people susceptible to TB infection is significant.

To make matters worse, TB is much harder and more complex to treat in people with AIDS. “Even more alarming,” according to Hamburg, “the recently documented outbreaks of multidrug-resistant TB in New York City have disproportionately involved HIV-infected individuals.”

Even without the additional burden of TB, Hamburg said, AIDS will continue to take an enormous toll. It is already the leading cause of death among both men and women aged

twenty-five to forty-four in the city. The combination of AIDS and TB is especially deadly.

Fueling both of these overlapping epidemics is the problem of drug addiction. The use of shared intravenous needles is a major mode of transmission of HIV; in addition, drug addicts often engage in unsafe sexual practices. The result is that addicts, their partners, and their unborn children are placed at increased risk for AIDS and TB.

In New York, with approximately 200,000 intravenous drug users, IV drug use is linked either directly or through a sex partner's IV drug use to the majority of new HIV infections in men and women and is responsible for almost 80 percent of pediatric AIDS cases, Hamburg asserted. Many

of the homeless are also HIV positive and addicted to drugs.

These factors make it more difficult to treat and cure TB infections. They have also contributed to the rise in drug-resistant strains.

"Partial or non-completion of the six to nine months normally required to treat and cure TB fosters recurrence of infectious TB and the emergence of drug-resistant strains. Those individuals can then transmit drug-resistant TB to others and will require additional and costly treatment themselves," the commissioner explained.

Furthermore, many of these individuals obtain temporary housing in one of the city's networks of shelters and periodically serve time in the city's criminal justice system. Many of the shelters and jails are "poorly ventilated, overcrowded congregate settings—ideal breeding grounds for the spread of an airborne disease like TB," she said.

Hamburg was blunt in her assessment. "There is a substantial and

growing population which is either already suffering from a combination of TB, AIDS, and drug addiction, or is at increased risk for disease because of social circumstances, weakened health status, and heightened risk of exposure."

Strains in the System

These three epidemics place an "almost unbearable strain on an already overburdened health care system," said Hamburg, who oversees a department with a \$300 million budget and more than 4,000 employees. Countless New Yorkers, many without health insurance or access to routine care, many homeless, drug addicted, and suffering from AIDS and TB, must use the emergency rooms as their provider of first and last resort. This leads to sporadic treatment, inadequate medical care, and "terribly high burnout rates" for medical staff.

In addition, the commissioner said, our failure to recognize the vital role of public health and our focus on treatment rather than prevention has exacerbated these problems: "The infrastructure of public health has been terribly undermined over the past decade as a result of continuous budgetary cuts and thoughtless consolidation of agencies and programs." As a result, many core functions of public health, such as disease surveillance and epidemiology, outreach, prevention, education, infectious disease control, protection against environmental threats to health, and quality assurance have been compromised.

This failure has contributed to the reemergence of TB and other diseases once deemed eradicated or under control. Once effective anti-TB drugs were introduced, there was no further effort to limit nosocomial spread or to monitor TB patients after discharge. TB treatment programs, like case management and supervised therapy, never received enough support. In addition, awareness and training about TB, along with its appropriate management and treatment, diminished. This ham-

pered the ability to rapidly and effectively diagnose and treat TB, and resulted in fewer facilities for TB control.

"And so," Hamburg concluded, "despite full knowledge of how to prevent, control—and even cure—TB, we are struggling to cope with resurgent disease."

Prevention and Public Health

Hamburg emphasized that we can no longer afford the costs of neglecting medical problems until they pose an immediate threat to public health. "We spend billions of dollars on medical care that could have been averted had we been willing to invest in prevention. For instance, drug addiction costs this city countless dollars in lost productivity, law enforcement efforts, and health care costs—not to mention the terrible cost paid in wasted lives. Yet there are fewer than 44,000 drug treatment slots available to serve well over one half million New Yorkers who may need drug treatment."

In the face of these challenges, Hamburg said we must reaffirm our commitment to the goals of prevention and to public health as well as being more creative in devising methods to tackle today's public health threats. To combat these diseases effectively, we must acknowledge economic realities. In certain instances this may mean providing stable housing or supportive social services—counseling, entitlement assistance, life skills training, and linkage with substance abuse and alcohol treatment programs.

Both AIDS and drug addiction are illnesses rooted in individual behaviors, Hamburg said, and the development and treatment of active TB are directly influenced by an individual's ability to comply with prescribed treatment.

"Clearly, better understanding of how to reduce dangerous behaviors like intravenous drug use and unsafe sex, and how to increase treatment compliance are critical to our efforts to contain these diseases," she said. "Yet our investment in behavioral

"There is a substantial and growing population which is either already suffering from a combination of TB, AIDS, and drug addiction, or is at increased risk for disease."

research has been woefully inadequate.”

Need for Basic Research

The commissioner emphasized that research is an essential component of public health. She said: “It is to research that we turn for new drugs, better delivery systems, new diagnostics that are faster, cheaper, and easier to use, improved therapeutic interventions, and, if we are lucky, vaccines.”

In particular, more basic research into the nature and mechanisms of these diseases is paramount. “We need to increase our knowledge of the nature of the tuberculosis organism and the body’s immune response to it so we can employ the best strategies for diagnosis and treat-

ment. We desperately need to develop new drugs and new intervention strategies.

“Simultaneously, our efforts to better understand AIDS must continue and expand. We have not yet grasped the full nature of the AIDS virus or the immune response to infection with this virus; nor have we identified drugs that can safely and effectively kill the virus or interrupt its replication. And of course, we must continue to support efforts to create an effective vaccine.”

Research into the nature and treatment of addiction has perhaps been the most woefully lacking of all, Hamburg added, and concerted efforts must move forward on all these fronts.

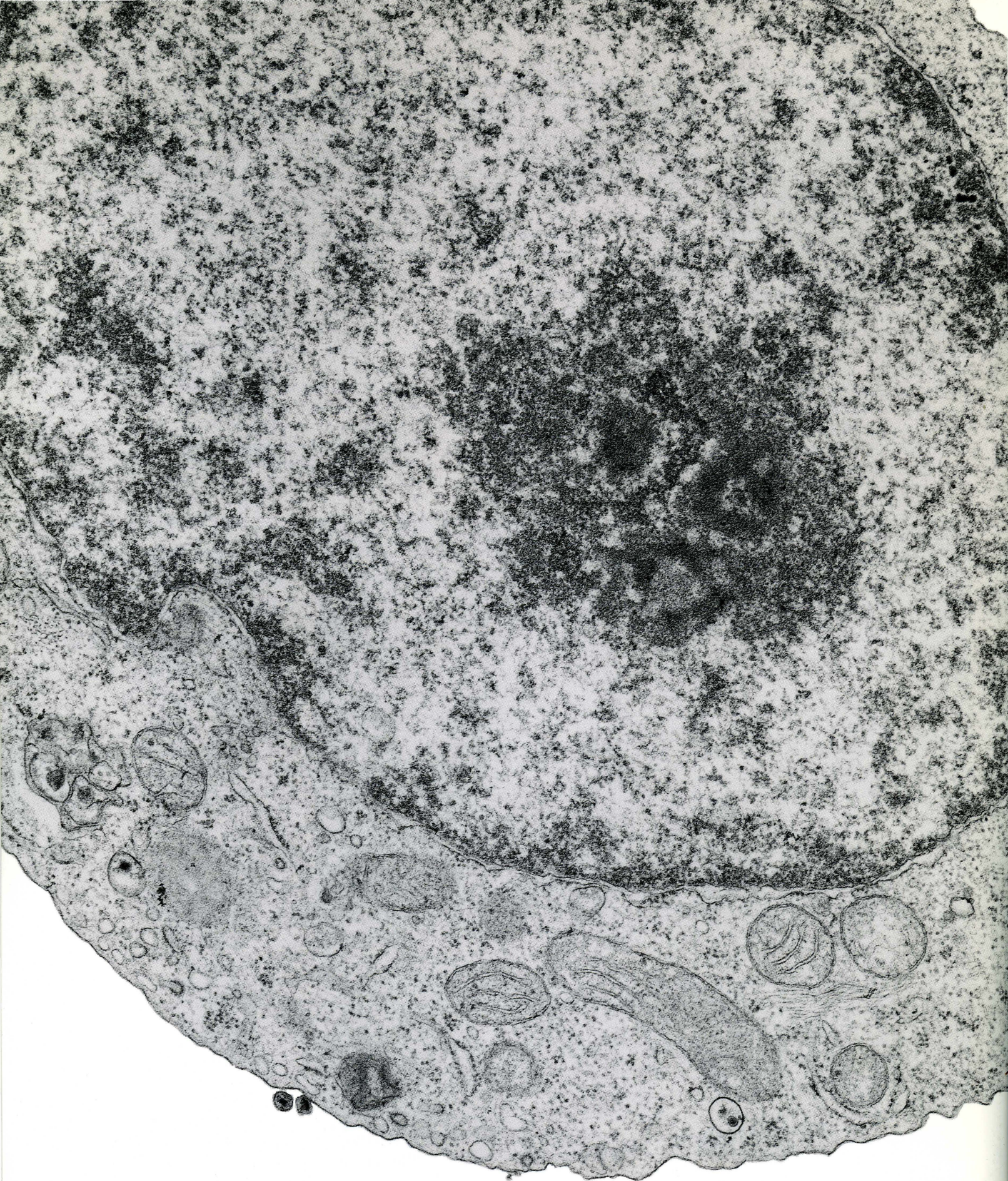
Hamburg reiterated that research

must be part of a much broader effort. “We must make certain that we continue to advocate forcefully for a robust public health system. We must strengthen our commitment to prevention efforts. And we must remain fully cognizant of the social forces that propel these deadly epidemics. As a city and as a nation, we can no longer afford the consequences of neglecting these concerns.”

“We have not yet grasped the full nature of the AIDS virus or the immune response to infection with this virus; nor have we identified drugs that can safely and effectively kill the virus or interrupt its replication.”



Speakers at a panel on urban health crises held at The Rockefeller University this spring included (from left to right) Professor Emeritus Vincent Dole, New York City Commissioner of Health Margaret Hamburg, Associate Professor Mary Jeanne Kræek, and the late Professor and Vice President for Medical Affairs Zanvil Cohn.



THE BATTLE AGAINST

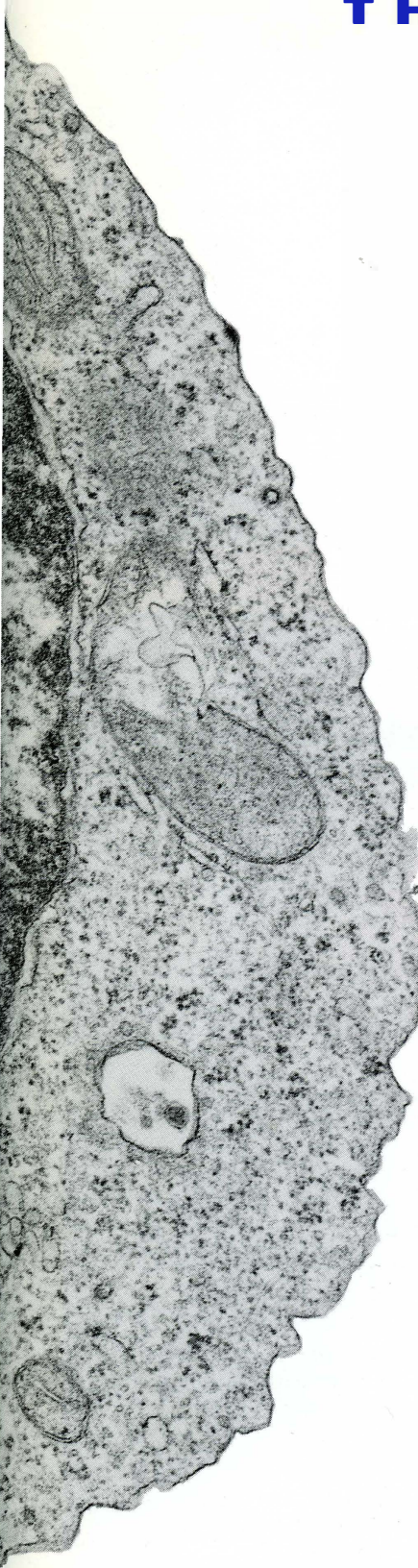
AIDS

by Susan Blum

Viruses are nature's canniest pathogens. Extraordinarily small, they must of necessity travel light, packing only their genes wrapped up in a protein coat. Most of the genetic information they carry is devoted to accomplishing their modest goals: reproducing within a cell and introducing themselves into other cells. To do their dirty work, viruses mobilize the machinery of the cells they infect to produce all the proteins they need to survive, thrive, and propagate.

Of all these efficient intracellular parasites, none is more clever than the human immunodeficiency virus (HIV)—the virus that causes AIDS. Not only does HIV commandeer the body's protein-production equipment, but it disarms its defenses, as well. HIV's prime targets are cells central to immune-system functioning—the helper T cells that turn on and amplify many immune-system responses, and the macrophages that help rid the body of pathogens and the cells they infect. By disrupting immune-system functions in numerous ways, infection with HIV sets the stage for the diseases that eventually claim the lives of people with AIDS, including opportunistic infections (such as *Pneumocystis carinii*), rare cancers (such as Kaposi's sarcoma), and tuberculosis.

The devastation caused by HIV is overwhelming, with no end in sight. The World Health Organization (WHO) estimates that more than one million people are infected with HIV in the United States, and another fourteen million are infected elsewhere around



The human immunodeficiency virus (HIV) turns infected cells into factories for new viral particles. This electron micrograph gives a dramatic view of new viruses (*small black dots*) assembling at the surface of a cell and budding from it. (Photo courtesy of Associate Professor Gilla Kaplan.)

Susan Blum is senior science writer in The Rockefeller University Public Affairs Office.

the globe. More than three-quarters of all current HIV infections are in Africa, but the epidemic is now growing most rapidly in Asia where, it is predicted, the number of new infections will soon outpace those in Africa. WHO estimates that by century's end, the total number of HIV infections will reach forty million.

The world desperately waits for an end to this plague, but as yet there is neither a cure for those already infected with HIV, nor a vaccine to protect those who are not. As researchers strive to devise better strategies to treat and prevent AIDS, they face many unanswered questions.

How, for instance, does the virus wreak its havoc on the immune sys-

tem? Can the body's defenses be bolstered once infection occurs? Why do some people succumb to the virus sooner than others? What types of immune responses must be elicited by a successful vaccine or immune therapy? At what stages might the viral life cycle be blocked? What functions are served by the various HIV genes? What measures can be taken to prevent the spread of infection with HIV?

Researchers at The Rockefeller University are among the thousands of scientists worldwide who are asking these questions—and others—in an attempt to conquer AIDS. They bring to their quest a unique combination of perspectives and scientific

talents to attack HIV from all quarters, including molecular biology, virology, infectious diseases, immunology, and patient-oriented studies. Often, the roots of their research reach back to pioneering studies begun decades ago in Rockefeller labs—studies that predate the AIDS epidemic, but that have proved remarkably relevant to it.

In the pages that follow, *Search* chronicles some of the AIDS research being conducted on campus. The scope and variety of these studies reflect the many challenges scientists face as they try to outsmart one of the most savvy pathogens they have ever had to confront.

Confronting HIV: The Immune System versus the Virus

Researchers in the Cohn-Steinman laboratory explore how the immune system responds to HIV and how that response might be bolstered

Despite more than a decade of research, scientists studying AIDS still do not know in detail how the immune system responds—and how it is impaired—when confronted with HIV. Underlying this mystery is the extraordinary complexity of the immune system itself. Countless details remain to be learned.

Many features that are known so far about the immune system come from the pioneering research of scientists in the Rockefeller laboratory headed by Ralph Steinman and, until his recent death, Zanvil Cohn. There, scientists are combining basic lab research with medical studies of specific diseases conducted at The Rockefeller University Hospital. Among the diseases under investigation are AIDS and tuberculosis (TB), a disease often associated with AIDS and exacerbated by it.

The laboratory's clinical and basic studies cover a wide range of questions relevant to AIDS, including the function of specific immune-system cells, their regulation, the interac-

tions among them, their beneficial and harmful effects, and the ways those effects might be manipulated to treat disease.

The researchers focus on a type of immune-system response known as cell-mediated immunity. As its name implies, this arm of the body's

One aim of the researchers is to boost beneficial immune-system responses to HIV. Another is to diminish responses that might be harmful.

defense repertoire pits immune-system cells directly against cells in the body that have been infected with bacteria or viruses (*see illustration, page 13*). The other arm of the immune system—known as humoral immunity—mobilizes proteins called

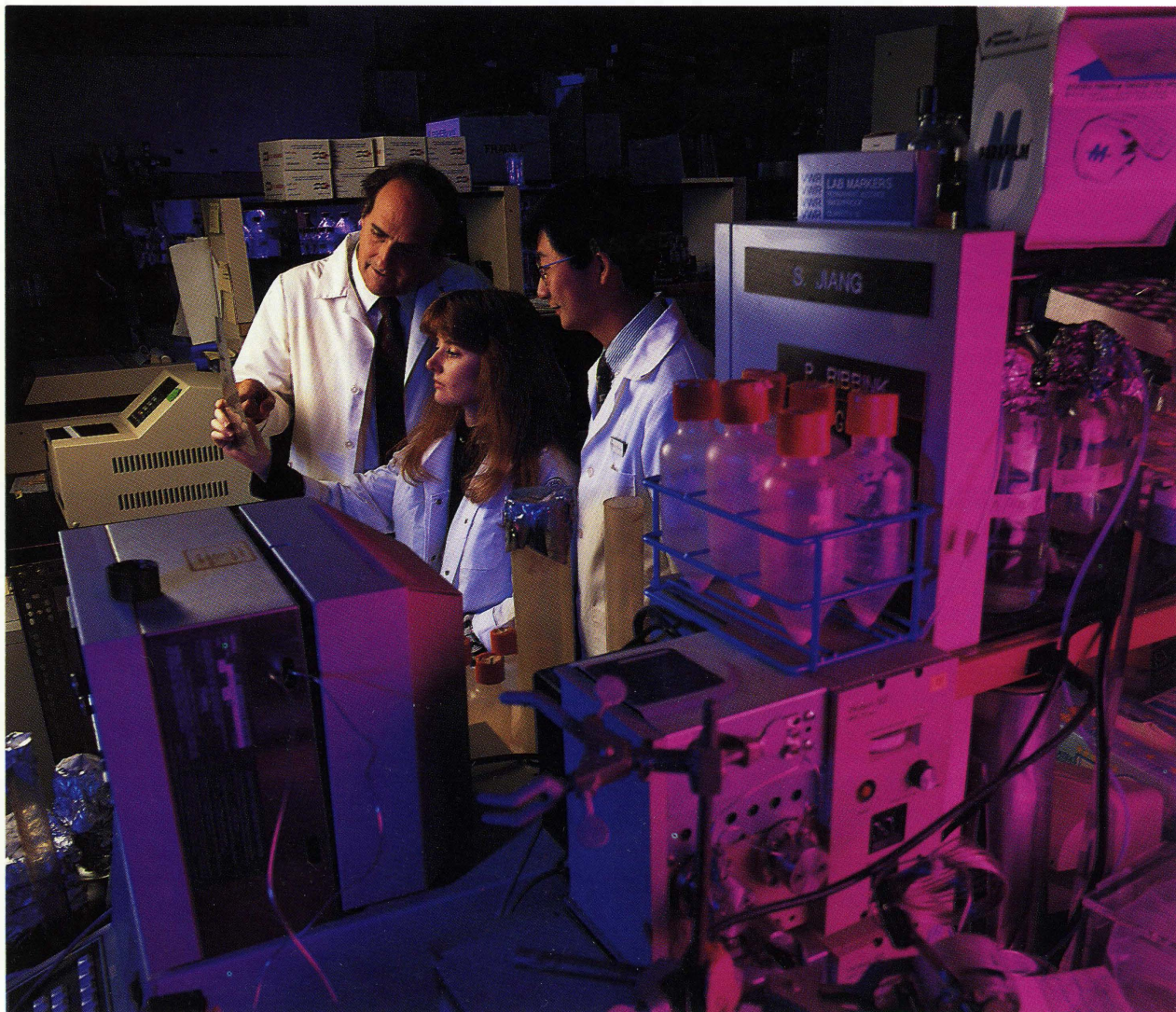
antibodies, which travel through the blood and the lymph system and neutralize pathogens before they can infect cells.

One type of immune-system cell is the macrophage, a cell that was a special research interest of Cohn's. In fact, his work was so central to the understanding of macrophages that Cohn was widely acknowledged to be the "dean" of macrophage studies. Macrophages kill in a number of ways, but their primary modus operandi is phagocytosis—a process of engulfment that sequesters and kills both free-floating microbes and the cells they infect.

T cells, or lymphocytes, are another class of immune-system cells, whose various names reflect the specialized proteins they sport on their surfaces. "Cytotoxic" CD8 T cells, for instance, engage in cell killing. CD4 T cells, or "helper" T cells, orchestrate a wide range of defensive responses through their effects on many other immune-system cells, including macrophages, CD8 T cells, and the cells that make



Above, Associate Professor Gilla Kaplan and Postdoctoral Associate André Moreira study how the drug thalidomide inhibits the activation of HIV. *Below*, Professor Ralph Steinman conducts research with Postdoctoral Associate Melissa Pope on dendritic cell-CD8 T cell interactions, and with Clinical Scholar Ian Tang on the effect of interleukin-2 on CD8 T cells.



antibodies. The catastrophic effects of AIDS are largely due to the fact that HIV infects these CD4 T cells, which are so vital to immune-system functioning.

Dendritic cells, whose existence was discovered by Steinman and Cohn, are yet another type of immune-system cell. Dendritic cells serve as sentinels that scout out "foreigners" such as invading pathogens, and present fragments of these foreigners (called antigens) to CD4 and CD8 T cells, which can then respond. Natural killer cells, or NKs, (many of whose functions were elucidated in the Cohn-Steinman lab) and lymphokine-activated killer cells, or LAKs, are additional types of immune-system cells that can also destroy infected cells in the body.

Goading the various immune-system cells into action are a wide range of proteins called cytokines. These hormone-like substances are produced by certain immune-system cells (notably CD4 T cells) and, in complicated biochemical feedback loops, induce immune cells to develop, proliferate, and become activated. Numerous cytokines have been under investigation in the lab for years, with particular emphasis on patient-oriented studies exploring how cytokines may modulate the immune response and affect disease states.

For example, a pioneering series of AIDS studies began in 1990, using a cytokine named interleukin-2 (IL-2). IL-2, produced by CD4 T cells, stimulates a wide range of other immune cells, such as CD8 T cells, NKs, LAKs, and CD4 T cells themselves. Ever since genetic engineering techniques first made it possible

to manufacture "recombinant" IL-2, the Rockefeller researchers have been using the engineered product to amplify immune response in a number of diseases, first with leprosy and now also with TB and AIDS.

which might help eliminate HIV-infected cells, cells infected with opportunistic pathogens, or both. Longer-term studies of the effects of low doses of IL-2 in patients with AIDS and/or TB on CD4 T cells and nonspecific killer cells are now getting under way directed by Kaplan.

In a related line of patient-oriented AIDS research, studies of IL-2's effects on another component of the cellular immune system—CD8 T cells—are under way under Steinman's direction. He and his colleagues have long been exploring the function of these cells. Unlike NKs and LAKs, which respond to a wide range of antigens, each "clone," or group, of cytotoxic CD8 T cells is genetically programmed to respond to just one of a pathogen's antigens.

Steinman explains, "HIV mutates very rapidly, allowing it to outfox the antibody limb of the immune system. But it is much harder for HIV to escape killer CD8 T cells, since they recognize parts of the virus that do not mutate." Thus, he and his colleagues are eager to see whether boosting CD8 T cells' activity might improve the body's ability to rid itself of HIV-infected cells.

Steinman also plans to explore whether interactions with dendritic cells might boost CD8 T cell activity. Dendritic cells normally present antigen to both CD8 and CD4 T cells; in so doing, they stimulate those cells into action.

Over a year ago, Steinman and his colleagues showed how dendritic cells can be key players in the loss of CD4 T cells that is a hallmark of AIDS. They found that intact HIV "hitchhikes" on dendritic cells that carry the antigens of other pathogens

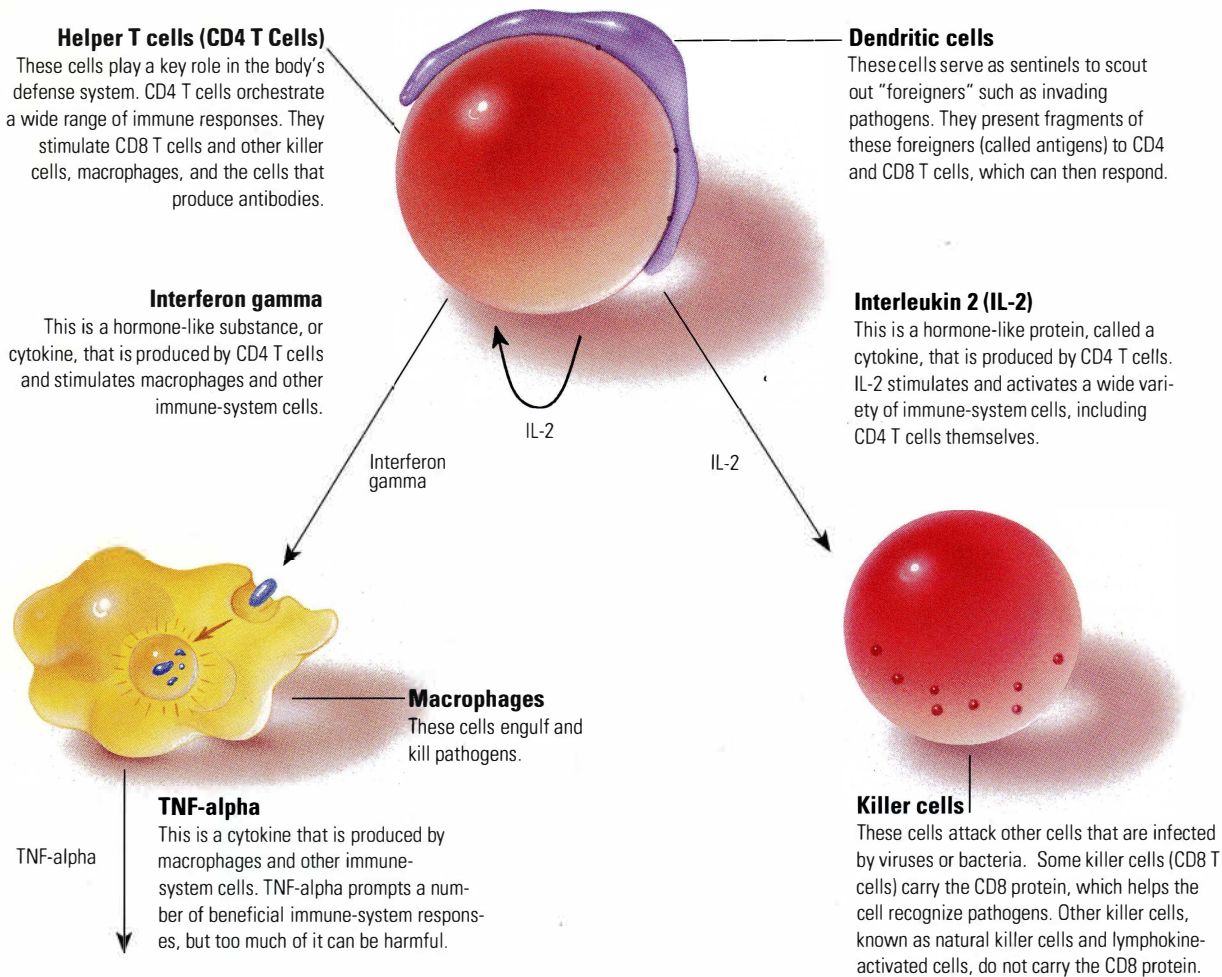


"Given that AIDS is characterized by a depletion of CD4 T cells—the very cells that produce IL-2—we reasoned that IL-2 therapy might prove useful in the treatment of people with AIDS," says Gilla Kaplan, a scientist in the lab. The therapeutic studies, conducted at The Rockefeller University Hospital, used low doses of the genetically engineered IL-2, unlike the higher, toxic doses of IL-2 used by researchers elsewhere to treat cancer.

The results of the lab's first, short-term trials were encouraging: individuals who received IL-2 showed an increase (in some cases) or stabilization (in others) of CD4 T cell count—a clear benefit for those infected with HIV. They also showed an increase in the number and activity of both NKs and LAKs—increases

Dendritic cells scout out foreign invaders and present them to other immune-system cells, which can then respond. Above, T cells cluster around a dendritic cell.

THE CELLULAR IMMUNE SYSTEM



to CD4 T cells. As the CD4 T cells cluster around the dendritic cell and are activated by it in response to the antigen, they are also infected with the bystander HIV. Then, the CD4 T cells become a syncytium—one giant cell with many nuclei. The result: an "explosive" CD4 T cell infection and massive cell death.

Ironically, though, another aspect of dendritic cell functioning may actually help the body fight infection with HIV. In studies at The Rockefeller University Hospital, Steinman and his colleagues plan to remove dendritic cells from people infected with HIV, manipulate the

cells in the test tube so they present particular HIV antigens (not the whole virus), and then reintroduce the cells into the infected individual in an attempt to amplify CD8 T cell response. The researchers believe they will be able to manipulate the dendritic cells so that they present the HIV antigen only to CD8 T cells, and not to CD4 T cells.

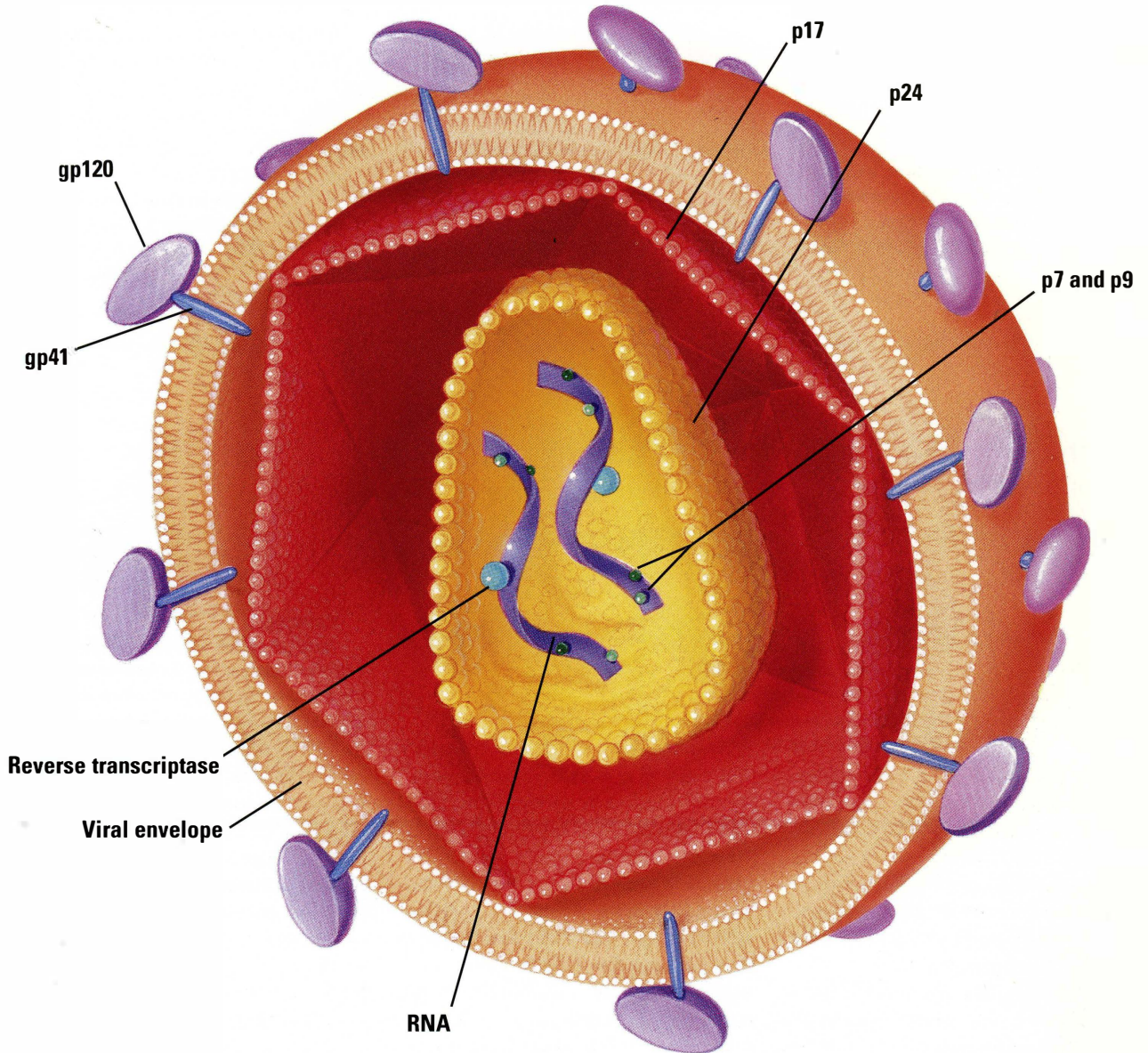
While one aim of the lab's research is to boost beneficial immune-system responses against HIV, a parallel aim is to diminish responses that may be harmful. One such harmful response is the overproduction of TNF-alpha, a potent

cytokine produced by macrophages and other immune system cells when viruses and bacteria invade the body. Some TNF-alpha is vital for the body's defensive maneuvers, but too much of the substance can produce debilitating fevers, weight loss, and fatigue that seriously impede a person's ability to combat disease. Patients with TB produce too much TNF-alpha. So do patients with AIDS, as a result of their opportunistic infections and, perhaps, as a result of infection with HIV itself.

The scientists' studies of TNF-alpha have their roots in the lab's long-standing interest in leprosy.

Illustrations by Terese Winslow.

THE STRUCTURE OF HIV



RNA

The material of which HIV's genes are made. RNA is a close molecular cousin of DNA, the more common genetic material.

Reverse transcriptase

The viral enzyme that makes a DNA copy of HIV's RNA genes.

Viral envelope

The virus's fatty membrane coat. It is derived from the membranes of the cells that HIV infects.

gp120 and gp41

Two proteins embedded in the virus's fatty coat (also called its envelope). The proteins are produced from a precursor protein called gp160. gp120 binds to CD4 receptor proteins on the surface of helper T cells and macrophages. In ways that remain unknown, gp41 is involved in the fusion of the virus with the cell it infects.

p17 and p24

Two proteins coded for by an HIV gene called *gag*. Molecules of p17 surround the inner portion of the HIV envelope. Molecules of p24 make up the capsid, or core — the structure that surrounds the virus's RNA genes.

p7 and p9

Two proteins coded for by an HIV gene called *gag*. They are both believed to bind to the virus's RNA genes.

While treatment for leprosy is effective, it can lead to serious side effects. For a quarter of a century, doctors successfully combated those side effects with the drug thalidomide, although they did not know why the therapy worked. Recently, however, research by Kaplan and her colleagues disclosed that thalidomide selectively inhibits TNF-alpha, while leaving other beneficial defensive responses intact. They have recently begun clinical trials of thalidomide to combat the effects of excess TNF-alpha in people with AIDS and/or TB. The initial results, which include dramatic weight gain and reductions in fever, have been

extremely promising.

The Rockefeller University researchers' most recent studies indicate that thalidomide may prove useful in combating not only the symptoms of AIDS, but the replication of its causative virus, as well. As it turns out, TNF-alpha is an extremely potent trigger for the activation of HIV genes that have been integrated into a host cell's genes and lie dormant there. (See illustration, page 18.) When TNF-alpha is produced in response to infection (such as with an opportunistic pathogen), the dormant HIV genes are turned on at the same time as immune-cell genes that are mobilized to fight the infection.

In this way, HIV "parasitizes" the regulatory mechanisms of immune cells for its own purposes.

Test-tube experiments by Kaplan and her colleagues have already indicated that thalidomide can stop HIV replication in certain laboratory cell lines, as well as in blood cells taken from patients and grown in culture. In ongoing clinical trials at The Rockefeller University Hospital, the researchers are now administering thalidomide to people with AIDS (with and without TB) to further explore the promising indications that thalidomide might provide an effective new therapy in the fight against AIDS.

A Budding Subject: How HIV Escapes from the Cell

In the Aderem lab, studies of interactions between proteins and membranes may yield strategies that keep HIV from assembling within an infected cell and moving out to infect others

Viral infections progress when viruses harbored within host cells multiply, leave, and go on to infect other cells. Before HIV can leave a cell, one of its proteins—called p55 gag (or p55, for short)—must get to the host cell's membrane. There, the virus assembles, with p55 providing the scaffold. Once assembled, the virus buds off from the membrane, and p55 is cleaved to form a number of proteins, including p24, p17, p9, and p7. (See illustration, page 14.)

In this process of assembly, as in many other processes, HIV hijacks the host cell's machinery for its own purposes. It induces a cellular enzyme to place a specialized substance on p55. This substance, called myristic acid, is a fatty molecule that decorates at least fifty cellular proteins and helps them bind to membranes. It

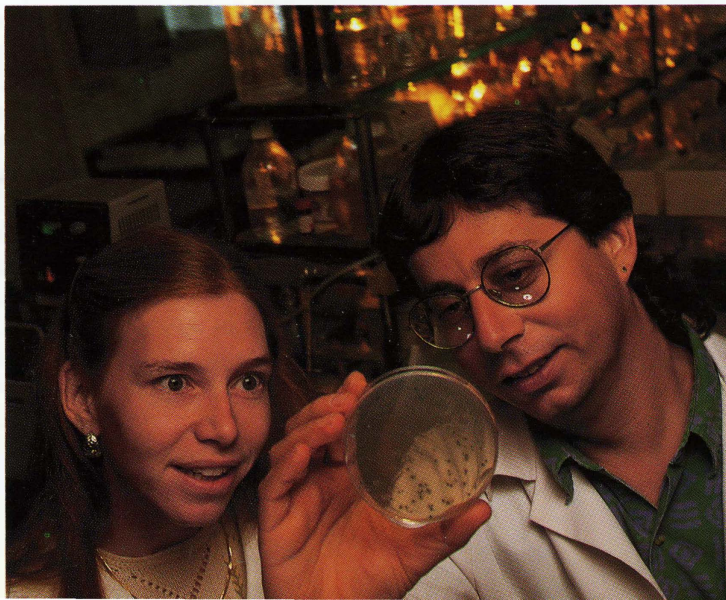
Because HIV hijacks the cell's own machinery, many anti-HIV strategies can impair the cell's normal functioning, too. The goal is to find a strategy that is specific to HIV.

serves the same purpose for p55.

Myristoylation—the addition of membrane-attaching myristic acid to proteins—has long been a subject of interest for Alan Aderem. As a researcher in the Cohn-Steinman lab, while investigating how immune cells called macrophages respond to external messages, he discovered two myristoylated proteins called MARCKS and MacMARCKS. These proteins are still under intensive study in his own lab, which he has headed since 1991.

MARCKS is now known to occur in virtually all cell types, while MacMARCKS' distribution is more limited. The specific function of these two "MARCKS brothers" varies among cell types, but in general they appear to facilitate interactions between cellular membranes and the cytoskeleton—the filamentous protein

Associate Professor Alan Aderem and Postdoctoral Fellow Jennifer Darnell are pursuing ways to keep HIV from assembling within infected cells.



backbone that gives a cell shape and allows it to move.

In macrophages, such membrane-cytoskeleton interactions are vital for phagocytosis and endocytosis—the processes by which the cells engulf and incorporate particles such as pathogens and dead or dying cells. The interactions may also be vital for viral budding—a process which, Aderem points out, appears to be the opposite of endocytosis. In one case, a particle—such as a pathogen—must be brought into the cell and somehow maneuvered through the cytoskeletal framework. In the other case, a particle—the virus—must make its way out through the cytoskeleton.

This intriguing analogy has led Aderem and his colleagues to add studies of HIV assembly and budding to the roster of projects under way in their lab. They are pursuing the subject in the two main cell types infected by HIV: CD4 T cells

and macrophages.

It is well established that newly assembled HIV buds off from the CD4 T cell's surface membrane, thereby killing the cell, but what happens in macrophages—where the virus does not bud outward—is less well understood. One model holds that in macrophages myristoylated p55 binds to internal membranes. The virus then assembles and “buds” into the compartments the membranes surround. Thus, macrophages can serve as HIV reservoirs. When these cellular storehouses eventually die, they release their burden of infectious virus. In the brain, where specialized macrophages called microglia are infected by HIV, such a process may cause the dementia that is often a symptom of AIDS.

What might direct myristoylated p55 to different membranes in different cell types? Aderem and his colleagues speculate that these proteins

might link up with specific receptors studding the membranes in question. They are currently hunting for these receptors, bolstered by the knowledge that they have already discovered candidate receptors for the myristoylated forms of MARCKS and MacMARCKS.

If receptors for myristoylated p55 can be found, it might eventually be possible to block them, thereby forestalling p55-membrane interactions. Such interventions would effectively stop HIV in its tracks, since the virus would no longer be able to assemble and bud. Experiments elsewhere have already shown that preventing the myristoylation of p55 also blocks HIV budding. But this strategy is not feasible for long-term therapeutic use, since it targets a cellular enzyme that controls many myristoylation events vital for normal cell functioning. “The goal,” says Aderem, “is to block a pathway that is absolutely specific for the virus.”

Fateful Paths: How CD4 T Cell Function Goes Awry in AIDS

Studies of T cell development and function in the Choi lab help illuminate the depletion of CD4 T cells, the immune-system orchestrators whose loss is so catastrophic in AIDS

As the disaster of AIDS attests, humans cannot survive without properly functioning T cells. But the critical importance of these cells is much better understood than the mechanisms by which they obtain, and maintain, their defensive capabilities. Yongwon Choi is investigating how T cells develop and function. Ultimately, his studies may also shed light on how their function goes awry in AIDS.

During development, immature T cells undergo a complicated process of selection that allows only some of them to survive. This complex enterprise involves the presentation to T cells of “self-antigens”—fragments of proteins that belong to the body's own tissues. In the course

of selection, Choi explains, “some developing T cells see self-antigen and live, while others see self-antigen and die. It's a very intriguing contradiction.”

Choi is pursuing the cellular signaling events at the heart of this contradiction, but his search is complicated by the difficulties of studying cells within developing mammals. One way he circumvents this problem is to study other immune system phenomena that may shed light on T cell development.

As part of his studies, Choi is investigating how CD4 T cells (the T cells depleted in AIDS) interact with a class of antigens known as superantigens. Rather than rousing one in 10,000 or 100,000 helper T cells, a superantigen goads 25 or 30 percent

of them into action. Oddly enough, once the superantigen-stimulated T cells have proliferated, they sometimes subsequently become inactive or self-destruct through a cell death program known as apoptosis—a process that recalls the death of T cells during development.

When Choi was a postdoctoral fellow, he discovered that a mouse retrovirus called MMTV encodes a protein that can act as a superantigen. When MMTV is incorporated into an animal's chromosomes and passed to the next generation, the superantigen it encodes can provoke the destruction, or deletion, of a whole subset of T cells in the developing animal. But if MMTV infects a mouse after it is born, the superantigen it encodes can cause the mas-

sive T cell proliferation characteristic of that induced by other superantigens. Like those superantigens, MMTV can also cause subsequent T cell inactivation or death.

Might HIV—which, like MMTV, is a retrovirus—also encode a superantigen? According to Choi, the evidence is suggestive, but mixed. Far more research is required to settle the

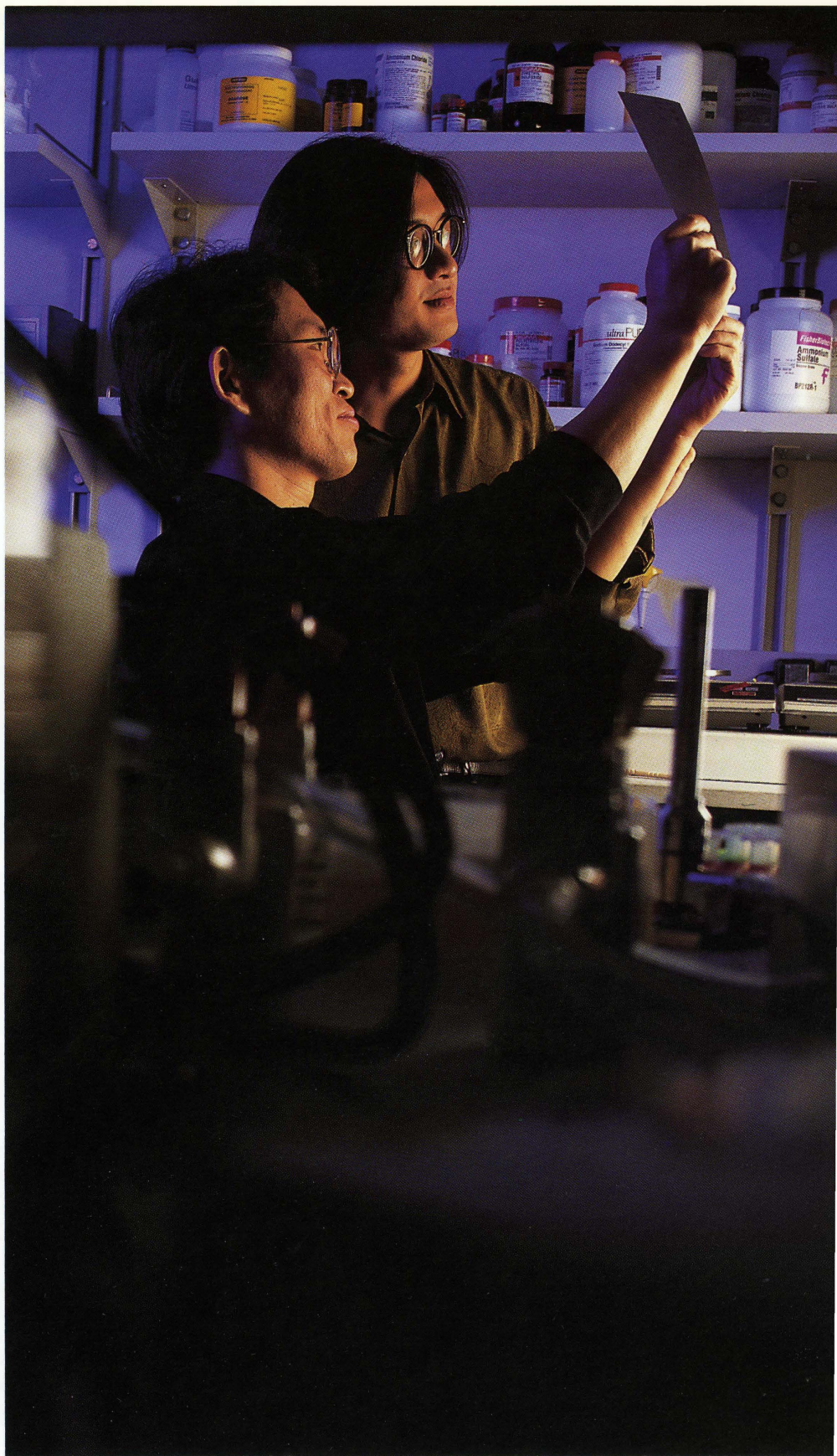
Many researchers believe that only a small proportion of CD4 T cells are infected with HIV. Why, then, do so many CD4 T cells eventually die?

question, and he hopes soon to begin such investigations. But should it turn out that HIV does not itself encode a superantigen, these immune-system superstimulators may still be involved in AIDS. “Many of the viruses already present in the body may encode superantigens that are only produced when HIV is also present,” says Choi. These activated superantigens could contribute to the CD4 T cell depletion seen in AIDS.

Whatever the ultimate role of superantigens in AIDS may turn out to be, Choi is hopeful that his fundamental interest in T cell development may yield clues about the disease. For instance, though the issue is currently being debated, many researchers (Choi among them) believe that only a small proportion of CD4 T cells are infected with HIV. Why, then, do so many CD4 T cells eventually die?

Choi points out, “If you present antigen to CD4 T cells from people who are HIV negative, the cells that are primed to recognize the antigen proliferate. But if you do the same thing with uninfected CD4 T cells of people who are HIV positive, the cells primed to recognize the antigen die, rather than proliferate.”

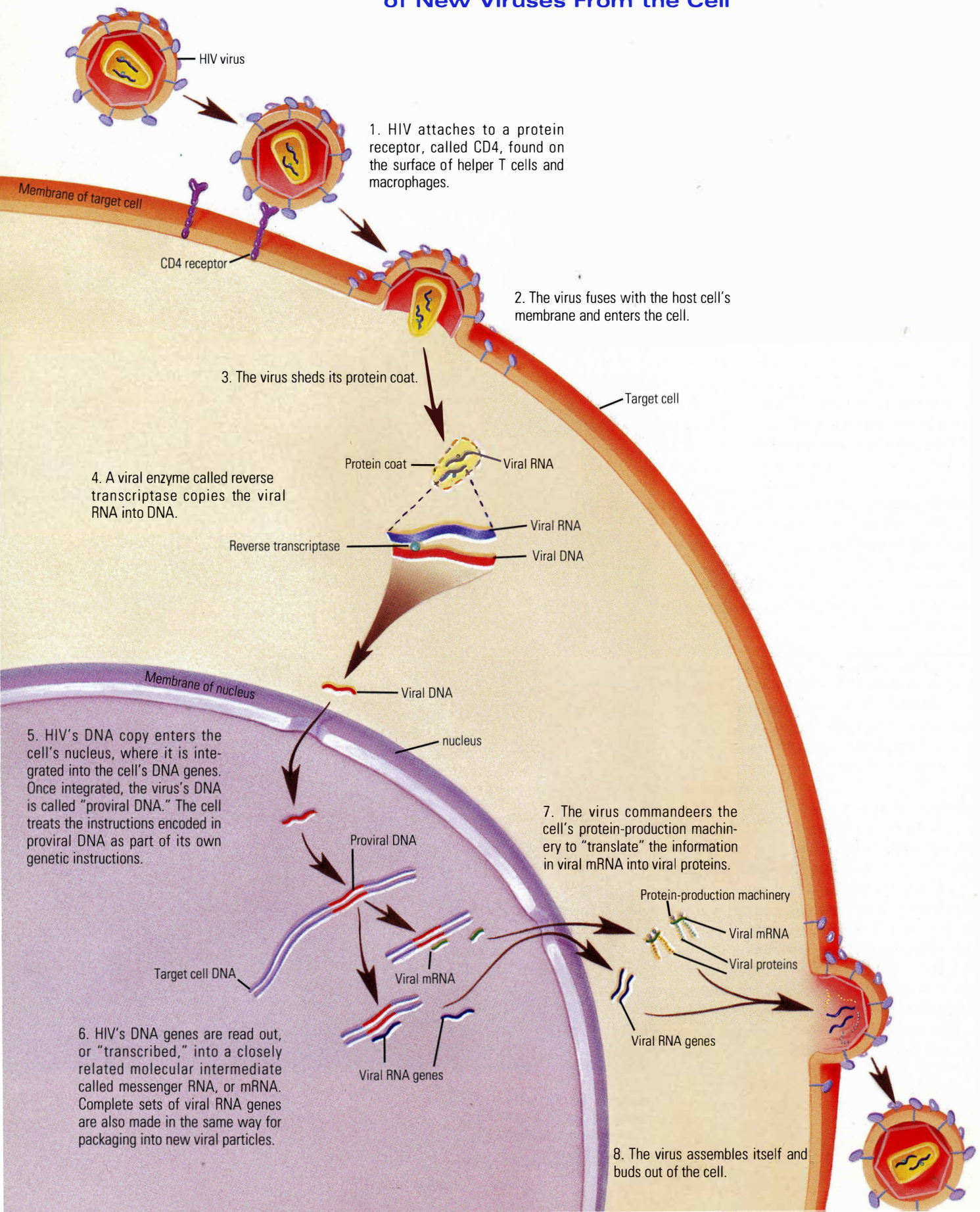
This paradox recalls the question that intrigues Choi about T cell maturation during development: Why do some T cells see self-antigen and live while others see self-antigen and die? The phenomena look to Choi like two sides of the same coin. The challenge is to figure out how the coin is minted.



Assistant Professor Yongwon Choi (*right*) and Postdoctoral Associate Jang Won Choi are researching the mechanisms of T cell development and death.

THE LIFE CYCLE OF HIV

From Infection of the Target Cell to the 'Budding' of New Viruses From the Cell



Revealing Differences: Studying the Human Retroviruses

Small in number but great in impact, the retroviruses that infect humans—including HIV—are the subject of study in the Hall lab

Numerous retroviruses infect animals, but only four are known to infect human beings: the human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) and the human T cell leukemia viruses I and II (HTLV-I and II). Virologist William Hall specializes in the study of these human retroviruses.

A physician as well as a basic researcher, Hall brings a special perspective to his studies. For instance, in studying HIV-positive intravenous drug abusers in the New York City area, he and his colleagues discovered that about 13 percent of them are also infected with either HTLV-I or HTLV-II, with the vast majority being infected with HTLV-II. A number of previous studies have shown that people infected with both HIV and HTLV-I progress to full-blown AIDS and deteriorate faster than the average patient. In contrast, Hall has noted, the course of disease tends to progress more slowly than average in those co-infected with HIV and HTLV-II.

What might contribute to these differences? Hall and his colleagues have found that HTLV-II infects CD8, or cytotoxic, T cells, unlike HIV and HTLV-I, which both infect CD4 T cells, otherwise known as helper T cells.

Profound differences in immune-system responsiveness may result from these different patterns of infection. As Hall explains, "Because HTLV-I and HIV both infect the same cell population, they may well activate each other's replication," thereby hastening the depletion of crucial CD4 T cells.

On the other hand, Hall points out, CD8 T cell counts are actually higher than normal in people who are infected with HTLV-II, and this may somehow bolster these persons'



Associate Professor William Hall (*left*) and Postdoctoral Fellows Shi Wei Zhu (*center*) and Takayuki Kubo study the human retroviruses.

abilities to fight back when they are co-infected with HIV.

Hall and his colleagues plan to pursue the role CD8 T cells may play in combating AIDS. He is intrigued by studies conducted in San Francisco showing higher-than-normal CD8 T cell counts in a group of homosexual men who are long-term survivors—that is, people who have remained well for over a decade, despite being infected with HIV. The men are not infected with HTLV-II, and the cause of their higher-than-normal CD8 T cell counts is not yet known. But the California researchers studying the men report that their CD8 T cells produce an as yet unidentified substance that inhibits HIV replication.

Though interested in these reported results, Hall remains circumspect. "We really don't know

what role CD8 T cells may be playing, but we want to find out," he says.

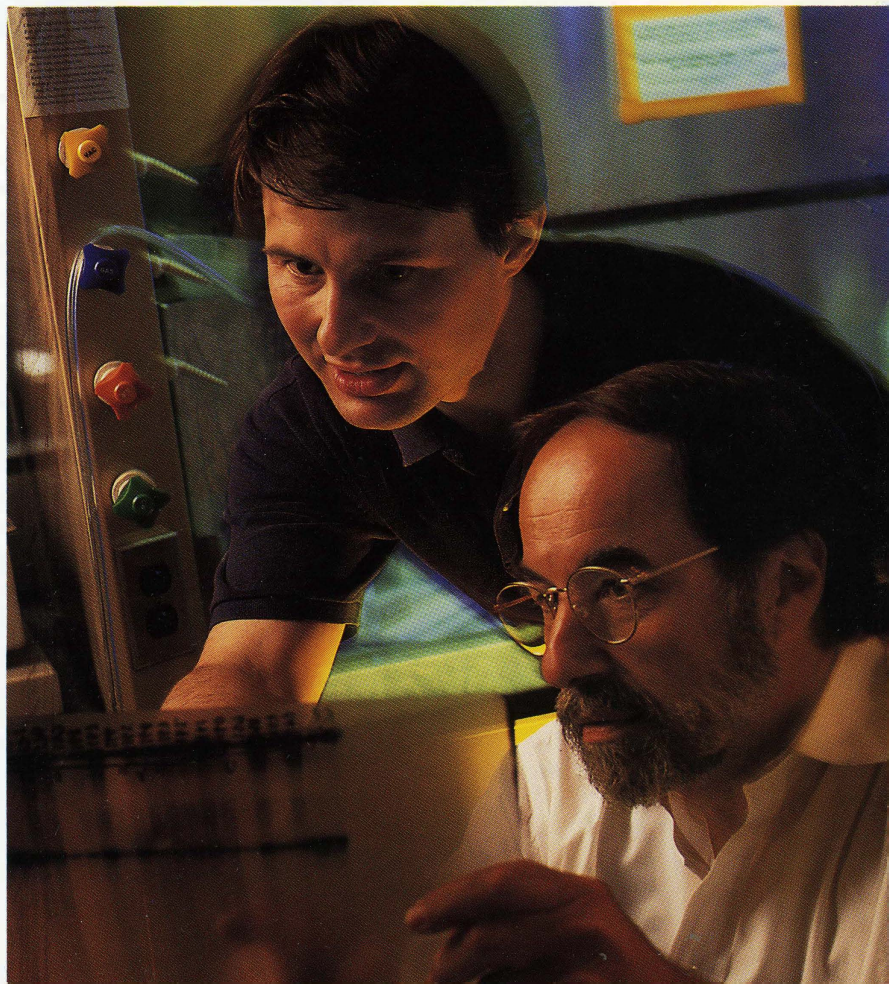
People infected with both HIV and another retrovirus called HTLV-I progress to AIDS and deteriorate faster than the average patient, while the course of disease progresses more slowly than average in those co-infected with HIV and HTLV-II. The question is why.

He and his colleagues are also studying HIV itself. While investigating how HIV's genes become integrated into the genes of the host cell, they found that an important part of this process involves the activity of an enzyme, called a topoisomerase, that is encoded by the HIV *gag* gene—the same gene that codes for a number of the virus's structural proteins. They are now studying a number of inhibitors of the topoisomerase to explore how they might be used as antiviral agents. In another

research direction, they are studying the process by which a viral precur-

sor protein called gp160 is processed into HIV's two envelope proteins, a step that is necessary to complete the virus's life cycle. They hope their research will disclose new ways to inhibit this process.

They are also exploring the possibilities for an oral vaccine against AIDS. Using HTLV-I as a model, they have identified regions of viral proteins that provoke neutralizing antibodies—antibodies that attach themselves to the virus and prevent it from infecting cells. They plan to link peptides (short proteins) mimicking these regions to the B subunit of cholera toxin, or CTB, which can be used as a vaccine delivery vehicle. CTB binds avidly to a molecule found in abundance on the surface of mucosal cells, the cells that line body cavities such as the mouth, gut, and genital tract. As a first step, Hall and his colleagues will test this model vaccine in rats, to see how well it evokes two different kinds of immune responses—one that works at mucosal surfaces, and one that operates systemically throughout the body.



A Slow and Stealthy Enemy: How HIV Remains Latent

Studies in the Baltimore lab aim to understand various aspects of latency—the ability of HIV to remain silently hidden within cells

Most interactions between virus and host occur rapidly. Within a few days or months of being infected, people in whom a particular virus proves pathogenic have usually either successfully combated the virus or—occasionally—succumbed to it.

But infection with HIV is different. During the course of AIDS, a person initially experiences a brief period of illness with certain symptoms (such as fever, chills, and malaise) and shows certain measurable signs (such as a dramatic increase of virus in the blood and a decrease of CD4 T cells). Then, although a slow decline of helper T cells may sometimes continue, the symptoms disappear, the blood's viral load abates, and the person usually stays well for a few years, a decade, or in rare cases even longer.

Eventually, though, the virus makes itself felt once again. The CD4 T cell count plummets, the viral load in the blood soars, and the rare cancers or opportunistic infections that will ultimately prove fatal set in.

This typical course of disease, which includes the period of well-being called “clinical latency,” is by now well established, but the molecular and cellular events underlying it are not. The basis of such molecular and cellular latency is a subject of study in the lab of David Baltimore, a virologist who earned his Ph.D. at Rockefeller and later served as its president.

Baltimore has long had an interest in retroviruses in general, and HIV in particular. For instance, he shared the Nobel Prize with Howard Temin in 1975 for their independent discoveries of reverse transcriptase, the enzyme that turns retroviral RNA genes into DNA. In the mid-1980s, his lab identified NF- κ B, the key protein that regulates the readout of HIV's genes into a molecular intermediate known as messenger RNA. And, in the late 1980s, his lab elucidated the complex, two-stage process by which some HIV proteins are produced later than others. Only when the later stage of protein production is finished can the virus assemble, leave the host cell, and go on to infect other cells.

Baltimore and his colleagues are now using versions of the assays they developed for these viral life-stage investigations to explore the molecular basis of latency as it is manifested in human disease. In one set of studies, they found dramatic differences between two subsets of HIV-positive individuals, all of whose CD4 T cell counts still looked normal. The researchers used an unusual treasure trove of blood samples provided by the New York

Blood Center. A detailed medical history of each donor was available, so that molecular and clinical data could be assessed in relation to one another.

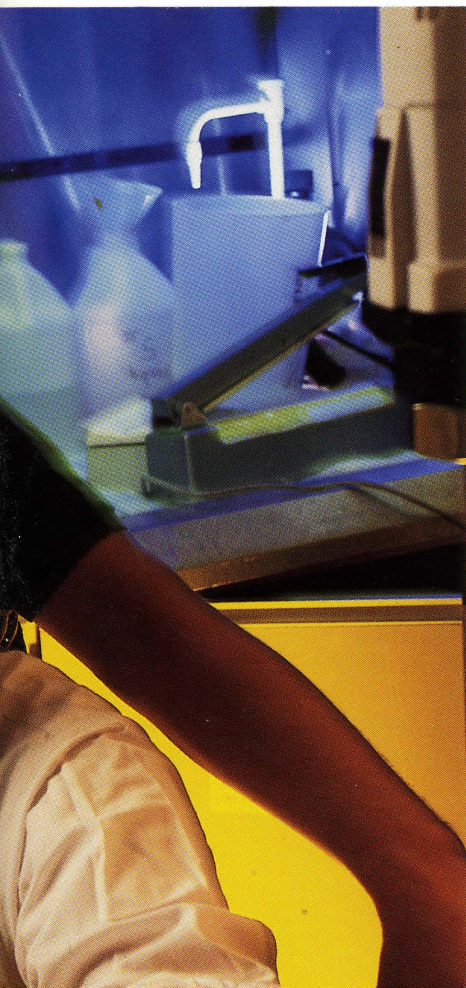
The scientists found that the blood of those people whose disease would progress within a few years contained the instruction molecules (messenger RNA) needed to produce both early- and late-stage viral proteins, while the blood of those who would be long-time survivors—staying well 10 years or more—showed either little or none of these messenger molecules. “Re-

searchers have been looking hard for a way to predict the fate of individual patients,” Baltimore comments. “We're hopeful these assays can be used to identify people who can benefit from earlier therapeutic intervention.”

Also yielding promising results are the lab's studies with chimpanzees, conducted in collaboration with a French group headed by Dr. Marc Girard, Baltimore's first postdoctoral fellow.

Chimps can be infected with HIV, but do not develop AIDS. So far, the blood of the animals under study by the Baltimore team looks like the blood of human long-term survivors: that is, it harbors little or no HIV messenger RNA. But messenger RNA can be found in the animals' lymph nodes—a significant finding in view of recent reports that the lymphatic tissue of humans sequesters HIV (and so may serve as a reservoir) during clinical latency. Thus, the

The typical course of infection with HIV—which includes the period of well-being called “clinical latency”—is by now well established, but the cellular events underlying it are not.



Basic research by Professor David Baltimore (*right*) and Postdoctoral Associate Kalle Saksela may yield ways to provide more individualized treatment for people infected with HIV.

chimp may turn out to provide what has long been missing in HIV studies—a good animal model for at least some stages of the human disease.

In addition to their studies of humans and chimpanzees, the researchers are conducting investigations using two test-tube models of cellular latency. This research indicates that more than one cellular

mechanism might maintain latency on the molecular level, including a hitherto unidentified cellular factor. Baltimore and his colleagues plan to explore how these test-tube models might relate to human infection with HIV. The researchers hope these studies will eventually result in ways to bolster natural mechanisms that maintain latency.

'Natural Allies': Exploring the Role of Natural Antibodies

Researchers in the Allfrey lab are studying 'host factors' that may help delay the progression to AIDS



In the laboratory of Professor Vincent Allfrey (*right*), adjunct faculty member Toby Rodman studies natural antibodies to a protein made by HIV.

The period of time between initial infection with HIV and the onset of AIDS varies greatly. Some people become ill within a year of infection, while others remain asymptomatic for a decade or longer.

Why does this disparity exist? Investigators in the lab of Vincent Allfrey aim to shed light on this question. There, Toby Rodman and colleagues are exploring the possibility that "natural antibodies" are host factors—that is, substances produced by HIV's human "host"—that may help retard the progression to AIDS.

Antibodies are proteins that travel through the blood, latching on to pathogens and facilitating their destruction. Exquisitely sensitive and specific to one particular pathogen, antibodies are normally produced after the virus or bacterium has invaded the body. But natural antibodies are different: they are produced by the body independently of any challenge from a virus, bacterium, or other agent. These preformed antibodies may act as an early defense system against infectious invaders. Although the existence of natural antibodies has been hypothesized for more than thirty years, until recently they received little attention from the scientific research community.

Rodman was not always involved in research on natural antibodies. Her initial studies focused on prota-

The period of time between initial infection with HIV and the onset of AIDS varies from person to person.

The existence of "natural antibodies" to a protein produced by HIV may help explain this disparity.

mine, a unique protein that packages DNA in the nucleus of sperm cells. Her interest in natural antibodies arose in the early 1980s from discussions with the late Henry Kunkel, a Rockefeller immunologist. At that time, AIDS had been primarily identified in the male homosexual population, and Kunkel and Rodman hypothesized that the constituents of semen, including sperm, might somehow play a role in infection with HIV. As an outgrowth of those discussions, Rodman and her colleagues discovered natural antibodies to protamine.

More recently, they discovered natural antibodies to a protein pro-

duced by HIV itself. This protein, called Tat, plays a vital role in the virus's ability to make more copies of itself. Two different anti-Tat natural antibodies have been found, each of which targets a region that is essential for the protein's function.

The anti-Tat natural antibodies are present in the blood of all HIV-negative people studied by the researchers. They are also present, in normal amounts, in people newly infected with HIV. But as HIV infection progresses, there is a corresponding decrease in anti-Tat natural antibodies, and people with full-blown AIDS have very low levels or none at all.

"The correlation between natural antibody level and stage of disease suggests that the antibodies may be a factor that helps delay the progression to AIDS," says Rodman. One question that remains to be answered is why the levels of these antibodies—which start out just about the same in all people—decline at variable rates in persons progressing toward AIDS.

Another question is how the antibodies might work. In studies of HIV-infected cells grown in a culture dish, Tat protein has been found in the fluid outside the cells. A number of research groups have speculated that in the body, Tat protein may also leave HIV-infected cells that are actively producing new virus. If this occurs, then the anti-Tat antibodies might prevent this extracellular Tat from entering HIV-infected cells that are not yet producing virus and activating the HIV genes that lie dormant within them. Another possibility is that the antibodies may have a direct, destructive effect on the Tat protein.

Much remains to be learned about the significance and function of the anti-Tat antibodies. But if they do prove to be a factor in delaying the progression to AIDS, their effects might eventually be boosted or mimicked to keep people who are infected with HIV asymptomatic for a longer time.

AIDS TERMS

CD4 ("helper") T cells: T cells that orchestrate a wide range of immune-system functions. CD4 T cells are one of HIV's targets, and are greatly diminished in AIDS.

Cytokines: Hormone-like proteins that induce immune-system cells to develop and become activated.

Cytotoxic CD8 ("killer") T cells: T cells that kill cells infected by viruses or bacteria.

Dendritic cells: Immune-system cells that serve as sentinels to scout out "foreigners" such as invading pathogens. They present fragments of these foreigners (called antigens) to CD4 and CD8 T cells, which can then respond.

DNA (deoxyribonucleic acid): The substance of which all genes, except those of some viruses (including retroviruses), are made.

HIV (human immunodeficiency virus): The virus that causes AIDS.

Macrophages: Immune-system cells that engulf and destroy pathogens and the cells they infect.

Opportunistic infections: Infections that can be combated by people with normally functioning immune systems, but that cause serious illness or death in people whose immune systems are compromised, as in people with AIDS.

Pathogens: Microorganisms that cause disease.

Proviral DNA: The DNA of retroviruses that has inserted itself into the genes of an infected cell.

RNA (ribonucleic acid): A closely related molecular "cousin" of DNA. RNA serves a number of functions, including those involved with the readout and implementation of the information in genes. In certain viruses (such as retroviruses) RNA also serves as the substance of genes.

Retroviruses: Viruses whose RNA genes are copied into DNA by an enzyme called reverse transcriptase. These DNA genes are then integrated into the DNA of the infected cell.

Reverse transcriptase: An enzyme that makes a DNA copy of RNA.

T cell: One member of a class of immune-system cells known as lymphocytes. The "T" in T cell reflects the fact that this type of lymphocyte develops and differentiates in an organ called the thymus.

Transcription: The process by which the information encoded in DNA is read out into a closely related molecular intermediate called messenger RNA (mRNA).

Virus: A pathogen consisting of nothing more than genes wrapped up in a protein coat. Unlike bacteria, viruses lack the machinery required to generate energy and produce proteins. Thus, a virus cannot survive or reproduce unless it is inside a cell whose biochemical apparatus it can "hijack" for its own purposes.

Defensive Maneuvers: The Search for a Vaccine

Researchers in the Fischetti lab are exploring a novel approach for a vaccine that homes in on sites where HIV first enters the body



Professor Vincent Fischetti and Postdoctoral Associate Donata Medagliani are setting their sights on a vaccine that elicits mucosal immunity.

Most vaccine research has been geared toward boosting defensive strategies that fight HIV once it has entered the body. But a different approach aims to prevent infection with the virus from occurring in the first place.

Twelve years into the AIDS epidemic, there is still no vaccine against the disease. So far, most vaccine research has been geared toward boosting defensive strategies that fight HIV once it has entered the body. But Vincent Fischetti and his colleagues are taking a different approach. “We’re targeting how to prevent the infection from occurring in the first place,” he says.

The researchers have their sights set on eliciting a type of response called mucosal immunity. The mucosal surfaces lining body cavities such as the mouth, gut, and genital tract provide one of the body’s first lines of defense. Among the protective factors found there are specialized antibodies, known as IgA antibodies, whose functions—though

still incompletely known—are believed to include preventing pathogens from attaching to cells. Such attachment is one of the first steps in infection.

For many years, researchers believed that a mucosal response initiated at one site—say, by swallowing an oral vaccine—would provide equally good protection at all mucosal surfaces, since the cells that produce IgA antibodies travel from one mucosal site to another in the body. But Fischetti and others have shown that antibody response is strongest at the site where it is initially evoked.

These findings are essential to work in Fischetti’s lab, where one of the goals is to devise live vaccine vectors, or delivery systems, that home in on the sites where a pathogen first gains entry. These sites vary, depending on the pathogen.

The roots of Fischetti’s research stretch back to studies conducted by Rockefeller investigator Rebecca Lancefield in the first half of the century. In a monumental achievement, she classified the multifarious strains, or serotypes, of streptococci, a type of gram-positive bacteria that cause a wide range of diseases in humans and animals. (Gram-positive bacteria are a subset of microorganisms that include the streptococci, staphylococci, and pneumococci.)

As part of her work on “Group A” streptococci, Lancefield discovered the M protein, a protein on the bacterium’s surface that contributes to its ability to cause disease. Over the years, more than eighty different forms of the M protein have been found among the various strains of Group A strep.

Fischetti and his co-workers analyzed the entire genetic and protein structure of a number of different M proteins. “By now, more is probably

known about this molecule than about any other surface protein on any other gram-positive bacterium," he says. For instance, the Rockefeller researchers have learned that although most regions of the M protein vary among serotypes, some regions are identical, or conserved. One such conserved region is not only identical among all M proteins, but also among nearly all the surface proteins of gram-positive bacteria. This conserved region is responsible for firmly attaching surface proteins to the bacterial wall.

The implications of this discovery are exciting. Using genetic engineering techniques, it should now be possible to fuse the conserved attachment region onto virtually any protein or protein fragment, and then prompt that fusion protein to become attached to the surface of any gram-positive bacterium.

By exploiting harmless bacteria that are normally found on specific mucous membrane sites in the

human body, the engineered proteins could then be delivered exactly where they are needed. Once at the site, the proteins would provoke the production of antibodies that would later help protect against infection by the pathogen that normally carries these proteins.

In collaboration with Italian researcher Gianni Pozzi (a former visiting professor at Rockefeller), Fischetti and his colleagues have already linked a number of genetically engineered proteins to a harmless bacterium found in the mouth, where it has evoked a strong antibody response when given orally to animals. Among those proteins is HIV's gp120 protein, which plays a central role in the virus's ability to link up with and infect host cells. (See *illustration, page 14.*)

The scientists are now starting experiments to attach gp120 to a harmless bacterium normally found in the vagina. They believe this strategy will prove particularly useful for

a vaccine against AIDS, since sexual contacts are the leading cause worldwide of the transmission of HIV. Because the bacteria carrying the vaccine are live and continue to propagate, one-time vaccination of difficult-to-reach populations (such as those in Asia and Africa) would be possible. Moreover, the engineered, live bacteria would be transmitted through sexual contact to those not originally vaccinated.

The researchers' previous studies have made them hopeful that the vaginal vaccine will evoke not only a strong mucosal immune response, but also a strong systemic immune response—one that induces the production of antibodies, known as IgG antibodies, that travel throughout the entire body via the blood. This "multiple warhead" approach may prove to be a real boon, given the unlikelihood that a mucosal immune response will be able to prevent every single viral particle from entering the body.

New Research Facility Provides State-of-the-Art Equipment and Safety

Rockefeller's new 'Biosafety Level 3' facility provides a research environment that is optimal for research on tuberculosis, HIV, and other human retroviruses

Rockefeller researchers studying TB and the human retroviruses will now have the benefit of a state-of-the-art "Biosafety Level 3" facility, slated to open in 1994. This facility, larger and more technologically sophisticated than any previously available on campus, provides a research environment that is optimal (in some cases) or required (in others) for the types of studies the scientists intend to conduct.

The 1500-square-foot facility is separated from all other rooms on the floor by an air lock leading into a central lab area. Five labs are currently connected to this area, and each lab has its own negative air pressure system to prevent the escape of microorganisms inadvertently released into the lab environment. This protection is necessary for the study of airborne pathogens, such as multidrug-resistant TB. The human retroviruses are not normally airborne, but the negative pressure serves as added protection.

State-of-the-art equipment in the labs also includes safety cabinets for work with infectious agents, special filtering and exhaust systems, a "pass-through" autoclave permitting all equipment to be sterilized before leaving the lab, special centrifuges and microscopes, and a facility to analyze cells by the state-of-the-art technique called fluorescence activated cell sorting, a method for separating different types of white cells from one another.

Researchers using the new facility will include Alan Aderem, Yongwon Choi, William Hall, Ralph Steinman, and Gilla Kaplan.

Spelling Disaster: The Readout of HIV's Genetic Code

Studies in the Roeder lab focus on the first step in the process that decodes information stored in the genes. That step is especially complex for HIV

Viruses differ from bacterial, plant, and animal cells in countless ways. But in one fundamental respect, these entities—considered by some to be less than alive—are identical to the most complex living cells in existence: the instructions encoded in their genes must be transformed into the proteins that allow them to function. For HIV, those proteins include the structural proteins that shelter the virus's genes, the envelope proteins that help it invade human cells, and the catalytic proteins essential for many events throughout its life cycle.

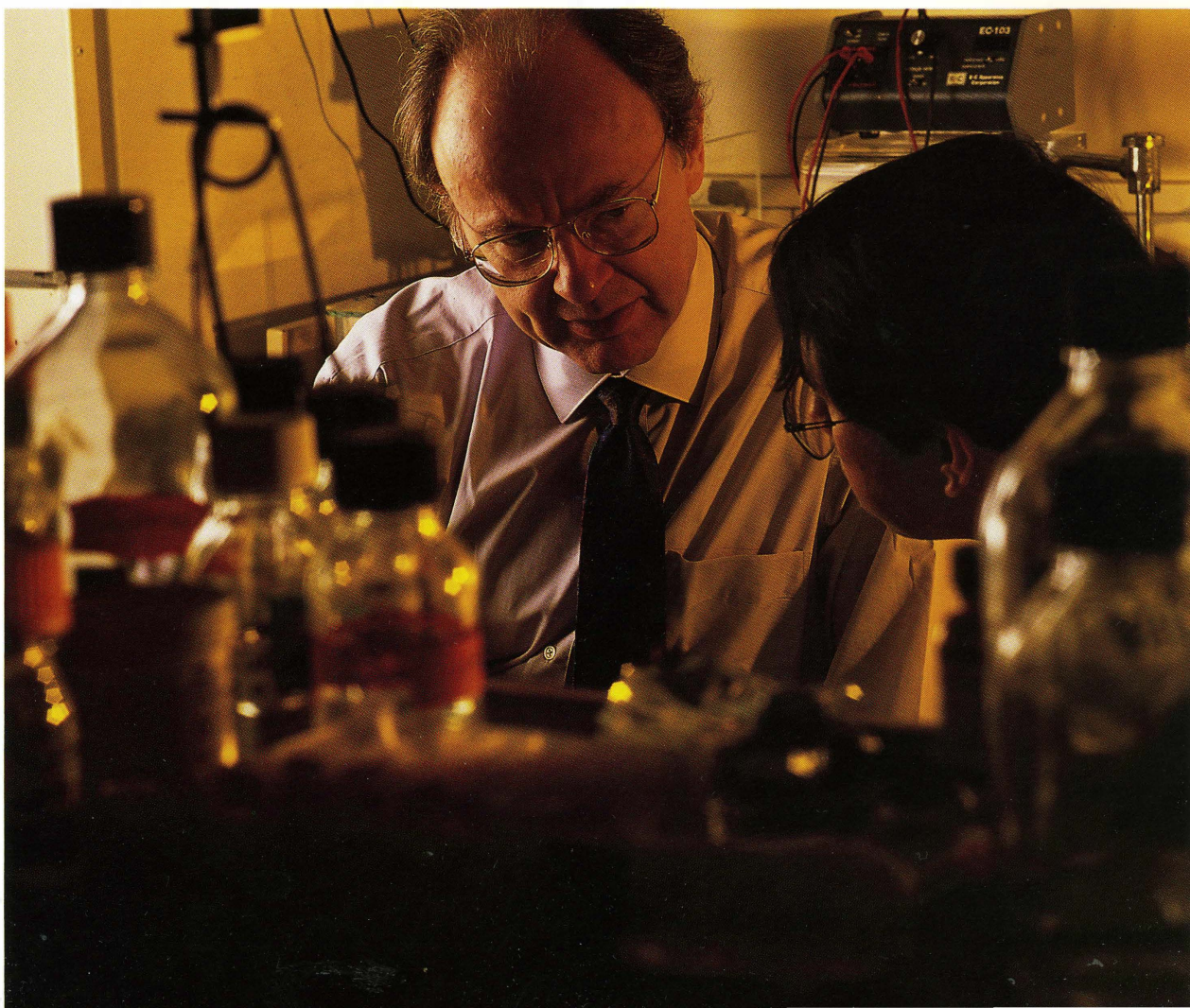
HIV's genetic instructions are carried in RNA rather than DNA. An enzyme called reverse transcriptase copies the RNA genes into DNA,

which is then integrated as so-called "proviral DNA" into the DNA of the host cell (*see illustration, page 18*). Once integrated, the proviral DNA can be transmuted into protein using the cell's own machinery. The first step in this process is transcription—the readout of DNA into a molecular intermediate called messenger RNA (mRNA), whose information is then "translated" into protein.

Transcription—an astoundingly complex process—involves the interaction of many proteins with one another and with DNA control elements located on the genes themselves. Rockefeller investigator Robert Roeder's pioneering research has already elucidated many of the molecular interactions that regulate

the complex process of transcription, and he continues to break new ground in understanding the transcription of many cellular and viral genes, including the genes of HIV. As part of his studies of HIV gene regulation, Roeder and his colleagues are investigating the function of a protein called Tat.

Tat is a "transcription factor," a type of protein that plays a major role in the complex interactions involved in transcription. Like all other proteins, the instructions for transcription factors are encoded by the genes themselves. As is the case for all viruses, HIV gene transcription involves some transcription factors encoded by cellular genes, and others encoded by genes of the virus.



Tat is a virally encoded transcription factor.

A number of cell-encoded transcription factors can prompt HIV's genes to be transcribed at a low level. But Tat is required for high levels of transcription. Once the first rounds of HIV gene transcription result in the production of enough Tat protein, HIV transcription is enhanced several hundred times.

Tat exerts its effects through a highly unusual mechanism—one so far seen only in the subset of retroviruses (called lentiviruses) to which HIV belongs. Tat interacts not with HIV's DNA genes, but rather with one of the very first mRNA regions to be transcribed from that DNA. This mRNA doubles back on itself to form a "hairpin" structure, called TAR, to which the Tat protein binds. Cellular factors also must bind to TAR in order for this "transactivation" to work.

Exactly how all these molecular interactions enhance the transcrip-

tion of HIV genes is still a matter of much debate. Roeder and his colleagues believe that Tat's interaction with TAR affects both the rate at which transcription is initiated, and the efficiency with which it continues along the entire length of HIV's genes. Researchers in the lab are continuing to tease out the details of how Tat-TAR interactions enhance HIV transcription. If such interactions could be blocked, the transcription of HIV genes would be dramatically limited—and so, therefore, would the production of the proteins HIV needs to survive.

The instructions in HIV's genes must be transformed into the proteins that allow the virus to function. Blocking the readout of those instructions could limit production of the substances the virus needs to survive.

A Deadly Connection: AIDS and Drug Abuse

Researchers at Rockefeller were among the first to view drug addiction as a physical disease. Today, researchers in the Kreek lab explore treatment for addiction, and aim to understand its biological basis

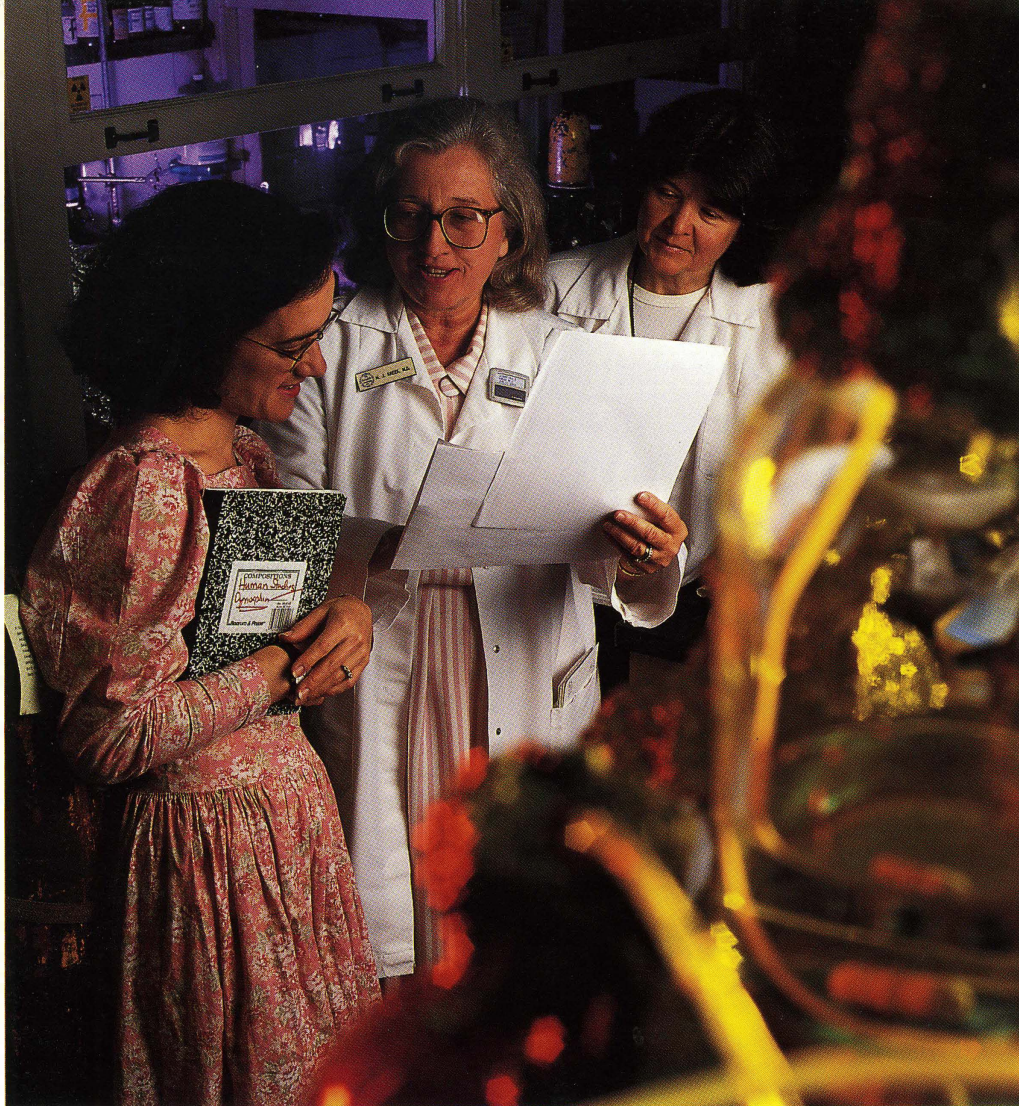
Drug addiction is a major factor fueling the AIDS epidemic. By sharing needles, HIV-infected drug users pass their infection to others; in addition, addicts often engage in unsafe sexual practices that can also lead to infection. The result of all these high-risk activities is grimly evident in the statistics. In New York City, for instance, intravenous drug use is the cause of almost half the AIDS cases reported, and more than 85 percent of children with AIDS were infected due to their parents' drug use.

How can this deadly connection be broken? One way is through effective treatment for drug abuse. As Rockefeller investigator Mary Jeanne Kreek explains, "With any epidemic, you must remove the

source of infection, and a major source of infection in AIDS is the drug addict. That is why treatment to end addiction is essential, both to treat the individual, and to prevent him or her from infecting others."

The treatment of drug addiction has a long history at Rockefeller, reaching back over thirty years to the pioneering work of Professor Emeritus Vincent Dole. Dole, the late Marie Nyswander, and Kreek were among the few at that time who viewed the drug addict not as an unsalvageable criminal but as a person afflicted by a physical disease. Their groundbreaking research showed that heroin addicts could be successfully treated with methadone, a long-acting opiate. As a result of these studies, hundreds of

Professor Robert Roeder (*left*) and Postdoctoral Fellow Camilo Parada study the complicated molecular interactions controlling the readout of HIV's genes.



Guest Investigator Lisa Borg (*left*), Associate Professor Mary Jeanne Kreek (*center*), and Senior Research Associate Ann Ho study the immune responses of drug addicts in treatment versus those not in treatment.

Drug addiction is a major factor fueling the AIDS epidemic. Treatment to end addiction is essential, both to treat the individual and to prevent him or her from infecting others.

methadone maintenance programs were established worldwide.

The Rockefeller tradition of addiction treatment and research continues today in the lab headed by Kreek, which is a National Institutes of Health-National Institute of Drug Abuse Center for Research in Addiction. Much of that work has a direct bearing on AIDS. For instance, using blood serum samples collected and stored as part of a

study conducted at Rockefeller, Kreek and her colleagues were the first to trace the advent of the HIV epidemic among drug abusers in New York City back to 1978. The scientists have also documented the effects of methadone maintenance on HIV infection in heroin addicts. Their 1984 studies, which have been confirmed by other labs, showed a dramatic impact: while approximately 50 percent of hard-core heroin addicts were HIV positive, only 9 percent of methadone-maintained addicts tested positive—and those were people who continued to use cocaine intravenously.

The concept of methadone maintenance programs has often met with resistance, but Kreek is optimistic

that the tide is now turning. “The AIDS epidemic has made the world awoken to the need for effective pharmacologic treatment of heroin addiction,” she says. “For the first time, people are really willing to see that methadone maintenance works.”

In addition to their research on methadone maintenance, Kreek and her colleagues are examining other links between AIDS and drug use, an approach that falls naturally into their studies of the biological bases of addiction. One of the lab’s main goals is to learn why some people who are exposed to a substance become addicted, while others do not. Says Kreek, “Provocative evidence suggests that there may be inherent vulnerabilities to many types of dependencies.” For instance, research in the lab has shown that addiction not only to heroin, but to cocaine and alcohol as well, may involve perturbations in the endogenous opioid system. The endogenous opioids are a class of peptides, or short amino acids, that are produced in the brain and that play roles in reproduction and the stress response.

Endogenous opioids also are involved in the complex biochemical pathways that affect immune function. Kreek and her colleagues have been examining the immune-system effects of drug use in HIV-negative heroin addicts. Their results show that untreated heroin addicts have abnormalities in many immune-system parameters, such as natural killer cell activity, T cell counts, and antibody levels. These abnormalities disappear, however, when the addicts are successfully treated with methadone.

Such findings may help explain why AIDS often progresses rapidly in untreated heroin addicts who become infected with HIV. They also underline the need to make methadone treatment available for all heroin addicts—including those infected with HIV—to bolster their immune systems as much as possible.

On the Trail of Disease: Tracking 'Emerging Viruses'

Research by Stephen Morse explores 'emerging viruses'—viruses, such as HIV, that seemingly strike out of nowhere. Better surveillance is needed to prevent the spread of such viruses

A decade ago, the world had barely heard of HIV. Now, at least fifteen million people are infected with the virus, and two and a half million have already died from the syndrome it causes. Where did HIV come from? How did it spread so fast? Are there other viruses like HIV out there just waiting to strike? These are some of the questions that interest Rockefeller investigator Stephen Morse.

Morse, a virologist, devotes much of his research energies to the study of the mouse thymic virus, a rodent virus that shares a number of similarities with HIV, including its ability to deplete CD4 T cells. But along with his laboratory studies of how this one particular virus acts on the molecular level, Morse is also pursuing viruses in a broader context. His quarry are "emerging viruses"—viruses, such as HIV, that seemingly strike out of nowhere.

His interest in emerging viruses grew out of a chance conversation held in the late 1980s with Joshua Lederberg, the Nobel Prize-winning scientist who was then Rockefeller's president. At a faculty gathering, Lederberg asked Morse about the potential dangers a particular mouse virus might pose for researchers working with lab rodents. This initial question grew into an interest so deep that, soon after, Morse led a multidisciplinary conference on emerging viruses at the National Institutes of Health. That conference seeded the growth of a new consensus about how emerging viruses make their mark on the world.

Most experts now agree that new viruses do not often emerge as the result of rapid mutations that quickly transform relatively benign viruses into dangerous ones. "Usually, emerging viruses are simply existing

infections that have been introduced from an animal population into a human one," Morse explains. When, for any number of reasons, the new human host is unable to combat the virus, the disease can rapidly become an epidemic, which is spread by the complex behaviors of humans.

Take the case of HIV-2, the viral strain that causes the majority of AIDS cases in West Africa. The genetic makeup of HIV-2 is virtually identical to a monkey virus called SIV. For many decades, humans in West Africa have probably occasionally been infected with SIV, due to their practices of eating monkey meat and keeping monkeys as pets. But demographic changes—moves to crowded urban centers, increases in the number of sexual partners, wider travel, and adoption of modern medical practices such as the use of needles—all contributed to a hastening spread of the disease in the last quarter of the twentieth century. The origin of HIV-1 (the most common cause of AIDS in Europe and the United States) is less clear, but many researchers believe that some other infection of primates (perhaps chimpanzees) is the root cause.

Much can be done to prevent the spread of viral disease, if the factors contributing to viral emergence can be monitored. Indeed, the need for dramatically improved global surveillance was stressed by a recent report on emerging infections sponsored by the National Academy of Science's Institute of Medicine. Morse, who helped write the report, is now spearheading efforts to implement its recommendations.

His concern for tracking and surveillance of emerging viruses also underlies the collaborative research he is conducting with researchers

elsewhere, such as Jeffrey Laurence of the Cornell University Medical College, and Irwin Gelman, a former Rockefeller postdoctoral fellow who is now a faculty member at the Mount Sinai Medical Center. These scientists were among the co-authors of the paper that caused an international sensation during the summer of 1992. The paper described the discovery of a small number of individuals with "AIDS-defining" illnesses—for instance, opportunistic infections in persons with low CD4 T cell counts—that could not be attributed to HIV identified by any test currently in use.

The researchers' report attracted tremendous media interest. So did a number of papers, published about six months later, that disputed the idea of an "HIV-negative" AIDS. While the press's attention to this issue has now died down, the commitment of Morse and his colleagues



HIV-2, the causative virus of most cases of AIDS in West Africa, is virtually identical to SIV, a virus found in monkeys such as this house pet.



Assistant Professor Stephen Morse (*right*) and Assistant for Research James Sullivan use highly sensitive assays to study the viral "family" to which HIV belongs.

to further investigate the subject has not. The scientists have been using assays developed at Rockefeller by Morse and Gelman that can detect genetic sequences from all viruses in

"Emerging viruses" such as HIV are usually existing infections that have been introduced from an animal population into a human one. The disease can then rapidly become epidemic when spread by the complex behaviors of humans.

the lentivirus subfamily, the class of retroviruses to which HIV belongs. The researchers believe they may have found evidence of such gene sequences in the blood of persons with "HIV-negative" AIDS, and that these patients' cases may represent infection with a variant of HIV-1 that less sensitive tests have failed to identify.

"These findings are not as strange or lurid as some of the initial press reports suggested," says Morse. "Given the rapid mutation rate of HIV, new variants are bound to arise, and it seems likely that some may be different enough to escape detection by tests currently in use." Noting that only a handful of cases have so far been reported, he says, "If there is a variant of HIV-1, its impact has clearly been far less dramatic than that of the well-recognized HIV-1." Nonetheless, Morse believes further research into this controversial question must continue, so that tests can be refined if necessary to help prevent the spread of AIDS.

Conclusion

Research Provides Hope for Future

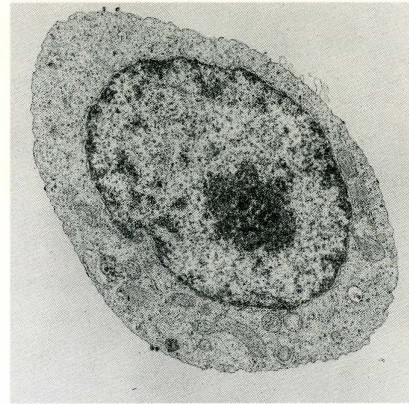
AIDS is a worldwide catastrophe whose toll will only worsen unless ways are found to better treat it, prevent it, and ultimately cure it. Until such discoveries are made, millions will continue to die from the disease—often, tragically, while they are still in the prime of life.

Many mysteries remain about AIDS, and creative new approaches are required to solve them. Perhaps no institution is better poised to foster such creative solutions than The Rockefeller University. Founded to conquer infectious diseases, Rockefeller has always encouraged interdisciplinary research that transcends rigidly defined scientific categories in the quest to improve human health. Today, Rockefeller researchers are drawing on their historic expertise in a wide range of fields such as molecular biology, virology, immunology, and infectious disease to meet the enormous scientific challenges posed by AIDS. At

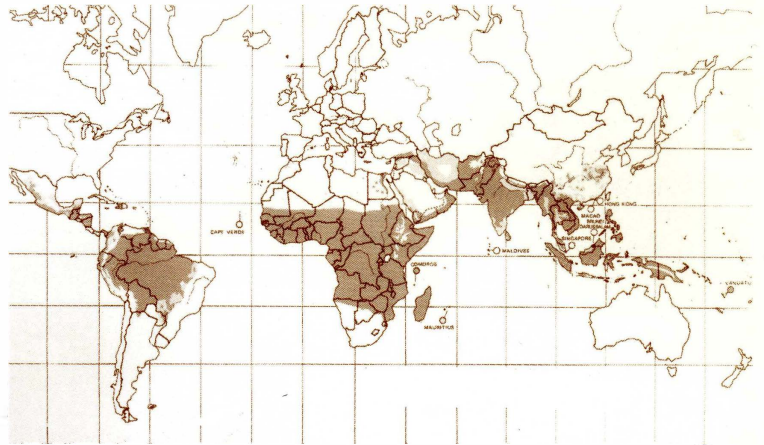
the heart of much of their work is The Rockefeller University Hospital, a unique facility where insights gained through basic biomedical research can be brought to bear on the treatment of people infected with HIV.

Just as research about AIDS draws on multiple disciplines, so the results of that research promise to shed new light on numerous scientific fields. Because HIV thrives by hijacking and subverting many normal cellular functions, research into the virus's modus operandi of necessity explores some of biology's most fundamental questions, such as how immune responses are generated and how the readout of genes is controlled. The results of this exploration promise new insight into many diseases, including cancer and autoimmune disease.

But the basic goal of AIDS research is, of course, to conquer AIDS. Drawing on their tradition of research "*Pro Bono Humani Generis*"—for the good of humankind—scientists at Rockefeller are aiming their sights at this goal.



Over the years, AIDS-related research at the university has been supported by a number of individuals, foundations, and corporations, including Alexander and Helene Abraham (through the Jacob Bleibtreu Foundation, Inc.), The Cremona Fund Inc., The Aaron Diamond Foundation, The Irvington Institute for Medical Research, the Abby R. Mauzé Charitable Trust, the Fannie E. Rippel Foundation, The Starr Foundation, and the Harry Winston Research Foundation, Inc. Significant support has also been provided by the federal government through the National Institutes of Health.



This world map illustrates the large portion of the globe in which malaria is commonly transmitted.

Scientists at Rockefeller Advance in Struggle Against

by Mika Ono Benedyk

Malaria now affects a staggering 300 million people. Over one million, mostly children, die from the disease every year. Others suffer from chronic, debilitating malarial attacks, characterized by fevers, chills, and violent shaking. While AIDS first appeared in the 1980s, malaria has plagued the population of tropical regions for centuries. Today, malaria is still counted among the world's deadliest diseases and leading public health problems. Recently it has become even more intractable due to the development of drug-resistant strains of the disease.

Scientists at The Rockefeller University have contributed a great deal to the understanding of malaria, which is caused by a parasite spread from person to person via mosquitoes. Over the last fifty years, Rockefeller scientists have tested new anti-malarial drugs, investigated the parasite's life cycle, discovered groundbreaking new research methods, and worked on the devel-

opment of an experimental vaccine. This work may ultimately help save countless lives.

World War II: Malaria Research for Their Country

Parasitologist William Trager arrived at Rockefeller in 1933 after graduating with a doctorate from Harvard University. In his initial studies of malaria, he provided the first direct evidence that lack of certain nutrients increases susceptibility to the illness. He

had just begun this work, however, when World War II broke out.

The U.S. Army was in desperate need of specialists in malaria, as the disease threatened to incapacitate American troops posted in the South Pacific. To make matters worse, the Japanese controlled supplies of quinine, the drug of choice at that time, which was harvested from cinchona tree plantations in parts of Asia.

Trager and two of his Rockefeller colleagues, virologist Frederick Bang and parasitologist Malcolm Ferguson, enlisted. They were posted in a special medical unit in Australia whose mission was to test a new antimalarial drug. The drug, called Atabrine, was developed by the Germans in 1932. Preliminary work by the U.S. Army suggested that Atabrine could be used safely as a suppressant drug.

Members of this unit administered Atabrine to malaria sufferers in the recommended dosages—one tablet a day, six days a week—and followed the patients' blood levels. The first subjects, men who had been fighting in New Guinea, were having an average of four attacks of malaria per year. Atabrine stopped the attacks completely. Further trials in Rockhampton with hundreds of men confirmed that the drug was a remarkably effective one: as long as the soldiers took Atabrine, they didn't have malarial attacks; if they stopped, symptoms would return within a few weeks.

"One of the young men we treated in the first trials had had some dozen attacks of malaria," said Trager. "He was in such bad shape when we first saw him that

Mika Ono Benedyk is a writer and editor in The Rockefeller University Public Affairs Office.

Malaria now affects a staggering 300 million people. Over one million, mostly children, die from the disease every year.

he needed a blood transfusion. With the help of Atabrine he recovered and became a technician with us. With his cooperation, we then did a little experiment. We asked him to stop taking his Atabrine. Like clockwork, the parasites came back. We could find them in the blood before he had any symptoms.”

On the basis of this and other work, the suppressant Atabrine became the standard army requirement in malarious regions, especially the Southwest Pacific. According to Trager, one of the medics later said that they had never had such a healthy army as in New Guinea at the time.

While Atabrine was critical for American troops in World War II, it did have one drawback that prevented

forty-eight hours—to develop fully. By that time, it has produced about a dozen daughter parasites (called merozoites in this stage) which then break out of the cell to invade others.

Working with samples of the malaria parasite that Trager personally brought back from Liberia, Trager and Rudzinska found that about half way through this cycle in the red blood cell, the cells develop knob-like structures on their membranes. Further biochemical work in the lab with Araxie Kilejian and Michael Wallach helped show that these structures are partially made up of a parasite-derived protein that is integrated into the host cell.

It turns out that the lab had identified the struc-

MALARIA

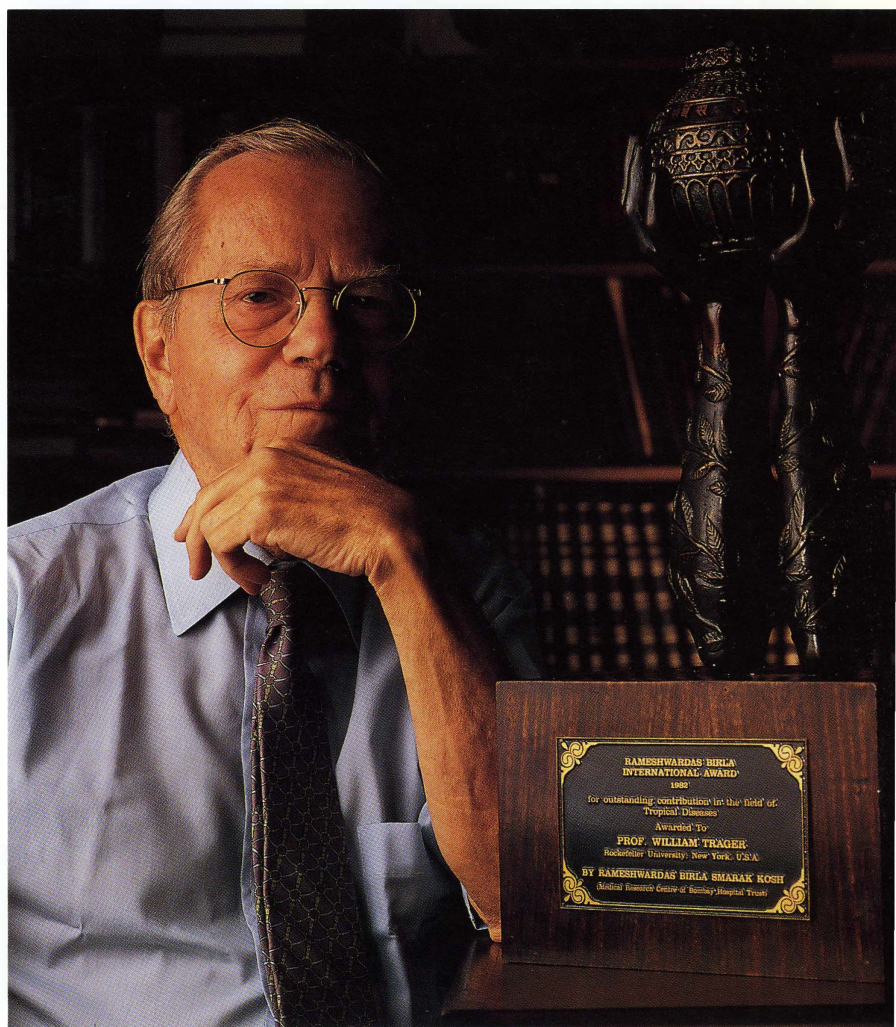
it from being widely used afterwards. “One reason it works so well is that it tends to accumulate in the tissue,” explained Trager. “It is a yellow-colored drug with a beautiful green fluorescence. After a couple of weeks of taking it and from then on, army personnel developed what was known as an ‘Atabrine tan.’ The skin had a peculiar yellow color, and in the sunshine, people’s ears showed a green fluorescence. Of course, they didn’t like that.”

Electron Microscopy Reveals New World

After the war, Trager returned to Rockefeller to continue his studies of bird malaria. A few years later, he was joined by Maria Rudzinska, a protozoologist who was the first to apply the newly developed electron microscope to the study of the malaria parasite.

Rudzinska’s work immediately revealed a whole new world. Long-standing mysteries such as how the parasite feeds on red blood cells were suddenly solved with ease. Although the fixation for the slides at that time often revealed nothing but big holes, she was able to see the parasite clearly the first time she tried. “In the very first sample I looked at, I made a real discovery,” Rudzinska said. “I could see the parasite engulfing big pieces of the red blood cell. I could see inside the food vacuole. It was a thrilling experience.”

Electron microscopy also allowed Trager and Rudzinska to understand how the malaria parasite develops within the red blood cell. After invading the cell, the parasite takes about two days—forty-four to



Rockefeller University parasitologist William Trager won worldwide acclaim for his work on malaria, most notably for his discovery of a method to culture the disease-causing parasite. He is shown here with a statuette he received as the first recipient of the Rameshwadas Birla Triennial International Award for outstanding contributions to the field of tropical diseases.

tures that are responsible for much of the pathology of severe malaria. The knoblike protrusions adhere to the walls of capillaries in various organs, causing organ damage. In the most serious cases, they may block capillaries in the brain, causing cerebral malaria, which is often fatal.

In very recent work, Dr. Irwin Sherman of the University of California at Riverside, who was a post-doctoral fellow with Trager in the 1960s and who has since become a leading authority on the biochemistry of malaria parasites, has synthesized specific peptides that interfere with this adhesion. These may provide therapy for cerebral malaria.

Cultivation: A Major Breakthrough

In the early 1970s, the most significant roadblock to developing a malaria vaccine was the lack of any means to culture the parasite. Scientists were limited to working with mice, birds or monkeys—an expensive as well as imperfect undertaking, as these strains of malaria differ from those that infect humans. Many scientists around the world struggled to grow the red blood cell stage of the parasite in culture.

Most researchers in the field are now optimistic that there will be a malaria vaccine in the future.

In 1976, Trager and his colleague James Jensen succeeded. “Many people were trying to grow the parasites in agitated cultures,” Trager said. “I thought that since the affected cells are attached to the walls of the capillaries half of the time, they are used to sitting quietly. So, instead of using an agitated culture, we created a device where a medium flowed slowly over a settled layer of red blood cells. Strangely enough, it worked!”

The finding generated a great deal of excitement. Trager and Jensen’s method, which is still used today, gave scientists a simple way to work with the malaria parasite in the lab. Trager soon received many awards for his work, including the Darling Medal and Prize of the World Health Organization, the First Rameshwardas Birla Triennial International Award of the Medical Research Centre of the Bombay Hospital Trust, the Leukart Medal of the German Society for Parasitology, and the Manson Medal of the Royal Society of Tropical Medicine and Hygiene.

The new culture method opened up a host of new research possibilities. It allowed the study of malarial drug resistance (most notably to chloroquine, a cheap and relatively safe drug widely used to combat the dis-

ease) and research into drugs that may negate this resistance. Some notable contributors in this area have included Trager and one-time members of his lab, including biochemist Bill Scheibel (now at the Uniformed Services University of the Health Sciences in Bethesda, Maryland) and scientist Virendra Bhasin (now at the University of Delhi, India).

The culture method also made possible a deeper understanding of the protective effect against malaria of the sickle hemoglobin mutation. Milton Friedman, one of Trager’s students, found that malaria parasites could not develop normally in red blood cells carrying the sickle cell trait when these cells were exposed to relatively low oxygen tension such as occurs in many organs of the body in people with the condition. This helps explain why people with the sickle cell trait rarely suffer from severe malaria. Malaria has supplied the evolutionary pressure that has maintained the high prevalence of this otherwise deleterious mutation in West Africa.

More recently, the culture method has facilitated basic work using newly developed molecular techniques on the genetics of the malaria parasite. Rockefeller alumnus Jeffrey Ravetch, now at Memorial Sloan-Kettering Cancer Institute, is one of those at the forefront of these studies.

As hoped, the ability to culture the parasite has also been invaluable in making progress toward developing a vaccine.

Toward a Vaccine

Rockefeller investigator Margaret Perkins, a former fellow in the Trager lab, is conducting research that may be directly applied to a future vaccine against malaria. Examining the biochemistry of the organism, Perkins has identified several proteins on the parasite’s surface that enable it to bind to, and enter, the red blood cell, as well as several other proteins that are secreted into the host cell membrane, facilitating invasion.

The hope is that some of these proteins—or important pieces of them—can be synthesized and used in a vaccine to stimulate the immune system against the parasite before it causes disease. One of the proteins Perkins identified, MSP1, may be tested as early as next year in human trials. If it proves successful, it will probably be used in combination with other immune-stimulating antigens from different stages of the parasite. These will most likely include other proteins from the merozoite stage, the form that invades the red blood cell. Proteins from the sporozoite stage, the form that travels from the site of the mosquito bite to the liver, and the sexual stages that develop while the parasite is

within the mosquito will probably also be included.

"It had been hoped that a vaccine against the sporozoite would be sufficient to stop development of subsequent stages in humans, as this would be intervention at the first point," said Perkins. "Unfortunately, we now realize that this is too simplistic. Escape of a few sporozoites from the liver will result in infection. The idea now is to develop a multiunit vaccine, made up of antigens or pieces of antigens from all stages of the parasite. This would block the parasite at each stage of development.

"Most researchers in the field are now optimistic that there will be a malaria vaccine in the future," she continued. "People who live in endemic areas do develop partial immunity when they reach adulthood. This suggests that there is hope that vaccine-induced immunity will be successful. It will be a matter of trial and error to determine which combination of antigens will work. Children are most at risk, so the most important vaccine to develop is one that is effective in early childhood."

Clinical Trials

While work continues on potential vaccine components, one experimental vaccine, developed by a Rockefeller adjunct faculty member, Manuel E. Patarroyo, has come far enough along to be tested in clinical trials.

Patarroyo, now head of the Institute of Immunology of the San Juan de Dios Hospital in his native Colombia, took a unique approach to making the vaccine. Virtually all vaccines to date, including those for polio, mumps, measles, and rubella, have been fashioned from mutated disease microbes. Instead, Patarroyo's vaccine is synthetic, consisting of four peptides (protein segments)—three from the merozoite stage of the parasite and one from the sporozoite stage—fused into a large molecule.

Patarroyo learned many of the techniques he applied to creating the vaccine at Rockefeller, from scientists such as immunologist Henry Kunkel, microbiologist John Zabriskie, and protein chemist Bruce Merrifield.

"If Patarroyo's vaccine turns out to be a successful one, it will be a good example of the kind of development that should happen more often," said Trager, who at age eighty-three is still active in his lab. "The basic work, namely the cultivating of the parasites and the peptide synthesis, was done at Rockefeller, and the putting together of a practical application was done in the country where it was needed."

Preliminary tests of Patarroyo's vaccine in Brazil,

Colombia, Ecuador, Venezuela, and Tanzania are promising. "We are getting 40 to 66 percent protection in field trials," said Patarroyo, whose work recently has received a great deal of publicity.

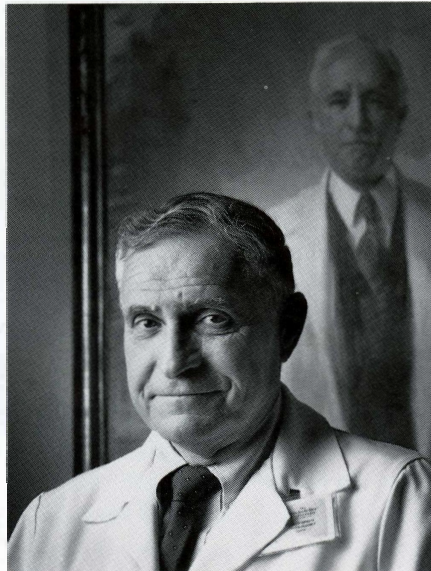
Another advantage of the vaccine is that it is cheap. A three-dose series costs thirty cents, "less than the price of a Coca-Cola," Patarroyo noted. Even less developed countries may be able to afford mass immunization if the vaccine proves feasible.

Although Patarroyo's vaccine will not eliminate malaria, it may prove to be one important victory in humankind's long war against infectious disease.



Margaret Perkins, a Rockefeller University investigator, is conducting research which may be directly applied to a future vaccine against malaria.

THE ROCKEFELLER UNIVERSITY HOSPITAL: **8** DECADES OF PATIENT- ORIENTED RESEARCH



The Rockefeller University Hospital, founded in 1910 as the first clinical research center in the country, pioneered a new kind of medical research in the United States. Rather than serving simply as a testing site for ideas generated in the lab, physicians were free to pursue experiments on the fundamental roots of disease. Over the last eight decades, the pioneering research that has come out of The Rockefeller University Hospital has included the discovery that genetic material is made of DNA, the isolation and testing of natural antibodies, the identification of specific genetic defects associated with atherosclerosis, and the development of a highly effective meningitis vaccine now used worldwide.

All photos are courtesy of The Rockefeller University Archives or Robert Reichert.

1993

Above, Jules Hirsch, professor and physician-in-chief, stands in front of a portrait of Rufus Cole, the first physician-in-chief of the hospital. Hirsch directs a laboratory that studies energy metabolism in man.

1923

Right, Rockefeller investigator Michael Heidelberger (*left*) and an unidentified technician work in the laboratory of Donald Van Slyke. Van Slyke brought a chemical approach to the study of human disease.





1992

Left, The late Professor and Senior Physician D. Martin Carter and Nurse Dorothea Caldwell-Brown speak with a young patient. Carter was a leading expert in clinical and experimental dermatology.

1922

Below, Rufus Cole, who was physician-in-chief from 1910 to 1937, stands at the bedside of one of the hospital's patients. Cole led important studies on the nature of acute lobar pneumonia, then one of the nation's leading killers.



1992

Left, Professor Jan Breslow (right) and Postdoctoral Associate Andrew Plump conducted research that resulted in the first transgenic mouse model of atherosclerosis, a major breakthrough in the study of this lethal disease.

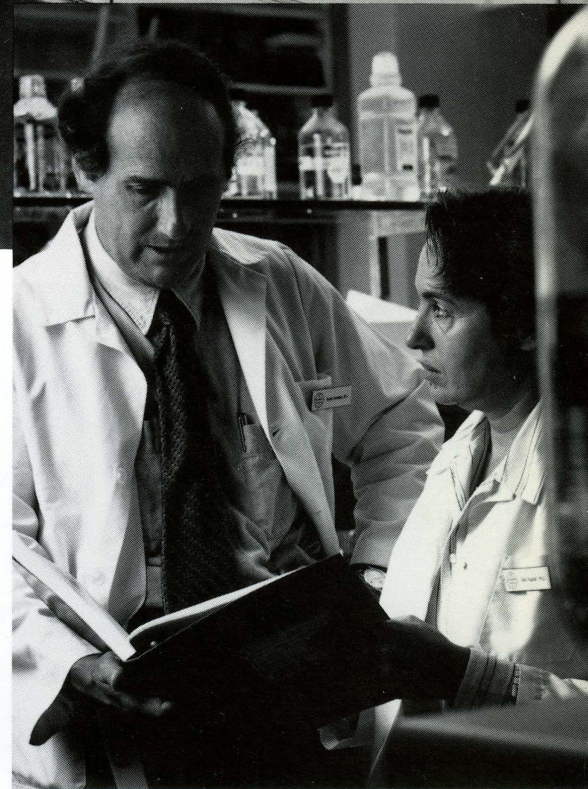


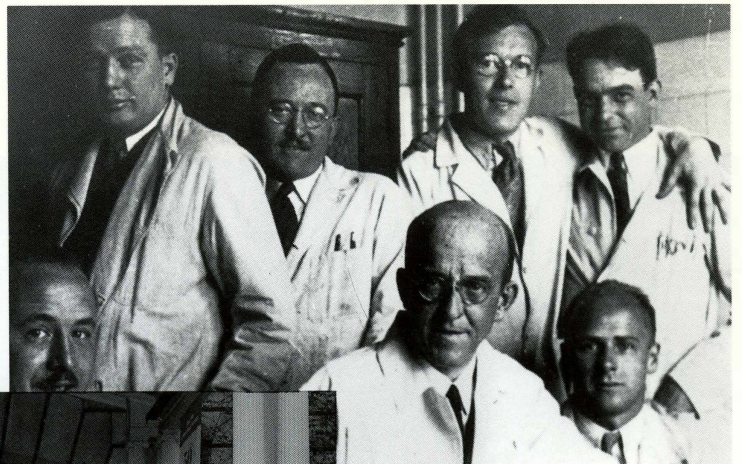
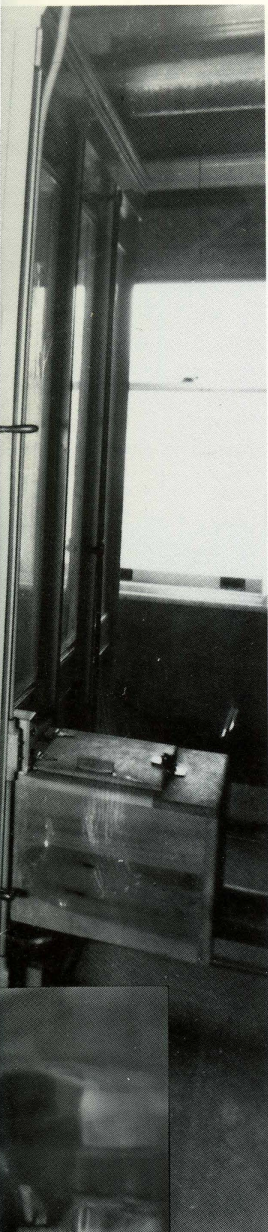
c. 1920

Right, William C. Stadie, a Rockefeller investigator, developed a new and far more effective way to administer oxygen to pneumonia patients. This special chamber enabled patients to breathe a regulated concentration of oxygen, greatly relieving their respiratory distress.

1918

Below, Rockefeller investigator and Nobel laureate Alexis Carrel demonstrates a new method for treating infected wounds. Carrel developed the procedure with Henry Dakin during World War I, when infection was a serious problem in treating wounded soldiers at the front.





1932

Above, Oswald Avery (center front) stands with some members from his lab.

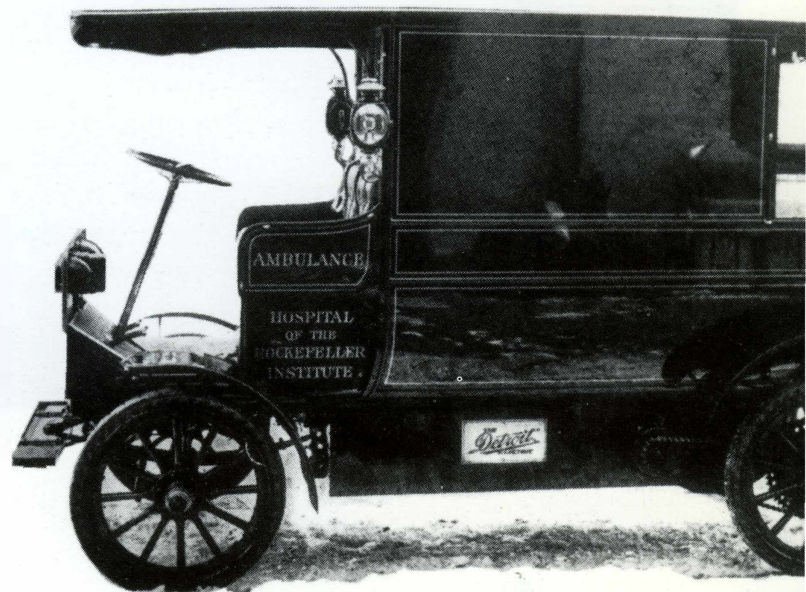


1993

Left, The current scientists and staff of The Rockefeller University Hospital bring the most modern techniques to bear on a variety of important clinical problems. Today's research topics include obesity, drug addiction, tuberculosis, aging, meningitis, strep, rheumatic fever, jaundice, psoriasis, wound healing, and AIDS.

1993

Left, Professor and Senior Physician Ralph Steinman and Associate Professor Cilla Kaplan work to elucidate the body's complex immune defense system which fights disease-causing microbes.



The Double- Edged Sword

The patients' cases were
by Susan Blum

highly unusual. All three had small-cell lung cancer, a deadly type of tumor that rapidly kills most of its victims and that almost never regresses, even with aggressive treatment. Yet—in some cases with therapy, in others without—these patients' tumors shrank or disappeared completely. Their good luck, though, was not unalloyed. Though their cancers resolved, the patients were plagued by an array of neurological symptoms, some so debilitating that they could no longer walk or feed themselves.

Assistant Professor Robert Darnell recently reported on these cases in the British medical journal, *The Lancet*. They are examples of the family of rare disorders, known as paraneoplastic neurological syndromes, under study in his lab. Darnell, an M.D.-Ph.D., says his research into the syndromes allows him to pursue his two compelling intellectual interests—gene regulation and neurobiology—and his

commitment to two scientific approaches—basic research and clinical investigation.

As a graduate student at the Washington University School of Medicine in St. Louis, Darnell was intrigued by the mechanisms that control how and when genes are transcribed (or read out) and how their instructions are used to make proteins.

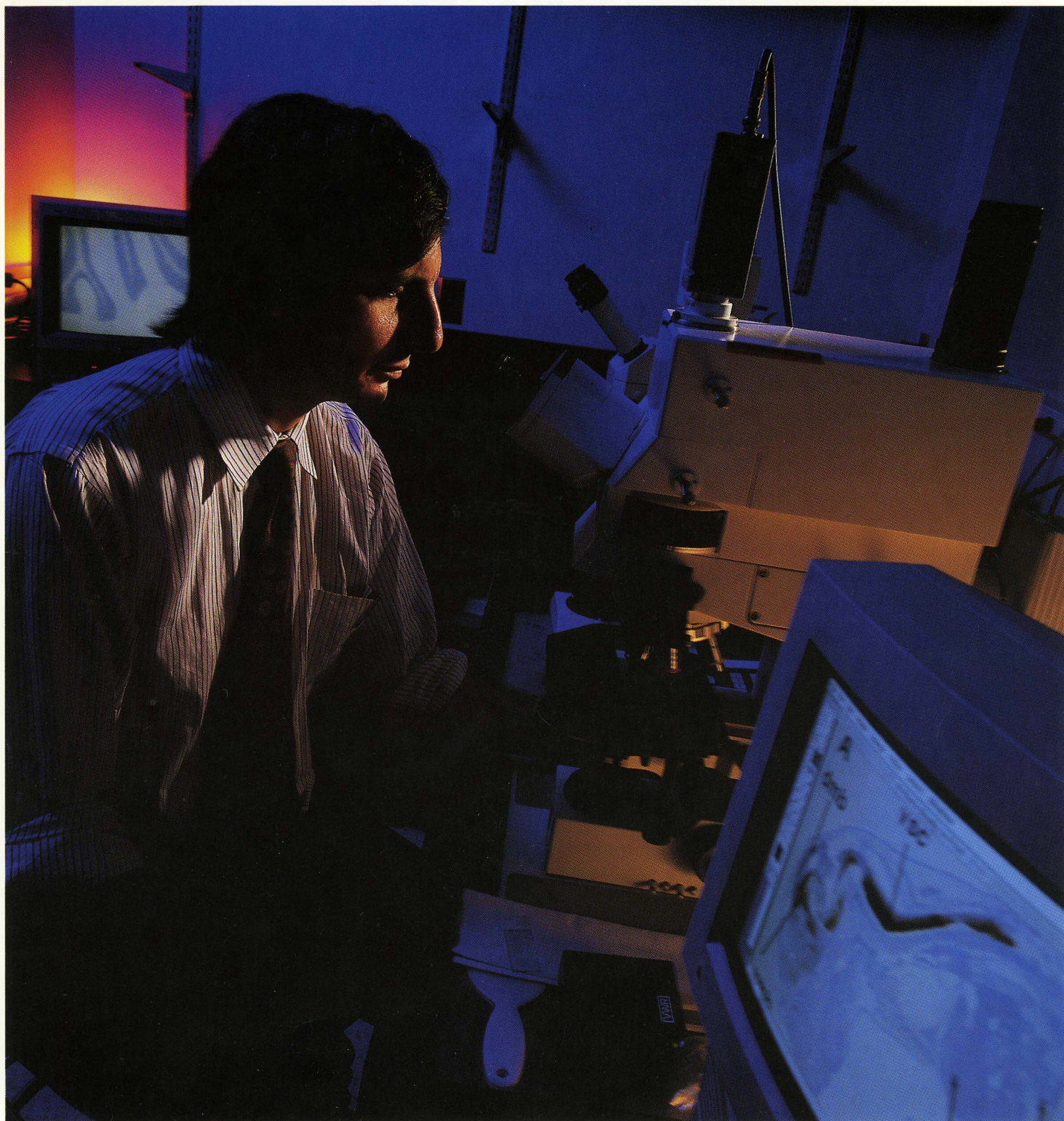
**Robert Darnell's
research explores
an unusual immune
response that
attacks cancer cells
but also wreaks
havoc in the brain.**

This interest may have had a genetic component. Darnell's father, Professor James E. Darnell, Jr., is a Rockefeller University faculty member whose research has elucidated some of the most fundamental aspects of transcriptional control.

Darnell explains that during the early 1980s, when he was earning his M.D. and Ph.D. degrees, "studies were just starting to focus on oncogenes, genes that play central roles in signaling a cell to differentiate or multiply in response to messages from its environment." When these genes are mutated, or when their regulation goes awry, cancer can result.

Compelling as they were, the oncogene studies were not the only ones that attracted Darnell's interest during his training. He was equally fascinated by research in neurobiology, which was starting to unravel the mechanisms by which neurons (nerve cells) change in response to signaling events. "It seemed to me that neurons and other cells in the body might both use the same kinds of signaling pathways for different purposes," he says. "For instance, in most cells, these signals normally culminate in regulated cell division, and their deregulation can lead to cancer. In neurons, which do not divide,

Assistant Professor Robert Darnell investigates a rare family of disorders called the paraneoplastic neurological syndromes. His studies may ultimately lead to strategies that boost the body's ability to fight cancer.



the functions of the signaling pathway are unknown, but are perhaps related to neurons' plasticity—their ability to change connections with other neurons in response to messages from their environment.”

To pursue the interconnections between neurobiology and cancer biology, and to conduct both basic research and clinical studies, Darnell became an attending neurooncologist at Memorial Sloan-Kettering Cancer Center. There he worked with Jerome Posner, a pioneer in the study of paraneoplastic neurologic syndromes—disorders that melded Darnell's two main interests.

In these diseases, small numbers of tumor cells of a particular type—say, tumors of the lung or of the breast—produce a protein normally made only by a particular type of nerve cell—say, a Purkinje cell in the region of the brain called the cerebellum. This protein production occurs due to the expression, or activation, in the tumor cell of a gene normally turned on only in the nerve cell.

The reason for this ectopic, or out-of-place, gene expression is not yet understood. Perhaps it is simply the random result of the cellular deregulation that is cancer's hallmark. But Darnell is investigating another possibility: that the ectopically expressed genes normally play important regulatory roles in nerve cells. Their aberrant expression would give cancer cells an advantage over normal ones, allowing them to circumvent the controls imposed by their own signaling networks. Darnell has termed such genes onconeural genes.

In the tumor, the proteins encoded by onconeural genes are perceived as foreign by the immune system, which mounts an effective attack

against them. Darnell believes that the vigor and success of this defense supports the long-held notion that many incipient cancers are nipped in the bud without our ever becoming aware that they have occurred.

But patients with paraneoplastic disease do become aware that they have (or had) cancer—usually not because of the cancer itself, but because of the immune response it elicits. For some reason—perhaps a break in the blood-brain barrier that normally screens the brain from many substances—antibodies

to the onconeural protein gain access to brain cells. The result: degenerative neurological diseases that, depending on the neurons involved, can impair balance, sight, motion, memory, and a host of other critical functions.

Why are the onconeural proteins perceived as foreign? “The immune system probably doesn't work in the brain exactly the same way it does in

the rest of the body,” Darnell speculates. Somehow, this difference prevents the immune system from “learning” that the onconeural proteins belong to the self. Thus, the proteins provoke an immune response both in the tumor cells, where they do not occur normally, and subsequently in the nerve cells, where they are usually produced.

Just as the immunologic rationale for the attack remains unknown, so does its mechanism. Do antibodies alone orchestrate the attack against onconeural proteins, or are immune cells known as cytotoxic T cells also involved? Darnell is conducting studies at The Rockefeller University Hospital to investigate—and design ways to modulate—the immune attack in patients with paraneoplastic syndromes.

Darnell is also exploring the syndromes

Studies of “out-of-place” proteins promise insights into cancer, the brain, and the immune system.

by cloning (that is, isolating) the onconeural genes and characterizing the proteins for which they code. So far, five main types of genes have been cloned, each one coding for a protein that was hitherto completely unknown. Darnell played a collaborative or leading role in the work on three of the genes and their products, and believes additional ones may yet be found.

Many of the onconeural genes now characterized give suggestive support to the hypothesis that they play critical roles in cellular regulation and signaling. For instance, one gene codes for a Purkinje neuronal protein with the "leucine zipper" motifs characteristic of many proteins that regulate gene transcription. Another gene codes for the first identified neuron-specific adaptin—a protein that forms a bridge between clathrin, an important cell-surface protein, and the "tails" of receptors that transmit signals coming from outside the cell. A third gene, characterized since Darnell came to Rockefeller, codes for a protein expressed in the parts of the developing central nervous system devoted to motor functions. Preliminary experimental results indicate that this protein plays a role after transcription is completed, by regulating the process by which newly transcribed genes get turned into proteins.

By investigating how onconeural proteins function in signaling and regulatory pathways, Darnell's

work may yield insights into some of neurobiology's most basic questions. And by investigating how the immune system's response to these proteins can trigger neuronal death, his research also holds promise for therapeutic advances on a number of fronts.

Insights gained through the clinical studies at The Rockefeller University Hospital may prove relevant not only to the paraneoplastic syndromes but to other autoimmune neurologic diseases such as multiple sclerosis. Moreover, the investigations into

how immune system responses can trigger neuronal death may ultimately shed light on degenerative diseases in which cell death is triggered by mechanisms other than direct immune attack.

Darnell's studies may also advance the fight against cancer. "The paraneoplastic syndromes identify bona fide tumor antigens that are targets of an immune response," he says.

Although other cancer cells express unusual antigens (cell surface proteins), none described to date elicits a strong enough counterattack to thwart the cancer's growth. Darnell is currently starting studies of breast tumors in mice—good animal models for cancer—to learn more about how onconeural proteins provoke such a vigorous immune response. A better understanding of this process may ultimately lead to strategies that enhance the body's ability to fend off a wider range of cancers.



At The Rockefeller University Hospital, Assistant Professor Robert Darnell (*center*) confers with M.D.-Ph.D. students Ronald Buckanovich and Lori Newman.

Zanvil A. Cohn, Henry G. Kunkel Professor and vice president for medical affairs at The Rockefeller University, died of an aortic dissection June 28.

A leading cell biologist and immunologist at Rockefeller for thirty-five years, Cohn was a pioneer in the modern study of the body's defense mechanisms against infection. His groundbreaking experiments have shaped the modern science of macrophages, the large white blood cells that are pivotal in inflammation and immunity. Cohn was best known for elucidating mechanisms whereby these cells identify, engulf, and destroy infectious microbes. His laboratory also discovered dendritic cells and demonstrated their potency in initiating an immune response.

Cohn was committed to supporting young scientists, and scores of men and women he trained have become leaders in universities throughout the world. He also helped establish the university's M.D.-Ph.D. program in the early 1970s, and his stewardship of the program continued until his death.

A graduate of Bates College (B.S., 1948) and Harvard Medical School (M.D., summa cum laude, 1953), Cohn came to Rockefeller in 1958 as a research associate and assistant physician. He was appointed professor and senior physician in 1962, the first Henry G. Kunkel Professor in 1986, and vice president for medical affairs in 1992. From 1977 until the time of his death, Cohn was also adjunct professor of medicine at Cornell University Medical College.

Z A N V I L A . C O H N 1 9 2 6 - 1 9 9 3

"Zan was a prince of a man who inspired all of us fortunate enough to have known him. He was an eminent scientist, a caring physician, and a great

human being guided by a clear philosophical stance. His studies of inflammation and immunity are classics and he used his knowledge and skill in the clinic to treat infectious diseases such as leprosy, TB, and AIDS. A man of great compassion,

Zan reached out to colleagues and patients in countries around the world. He was an invaluable colleague who helped build up and strengthen the

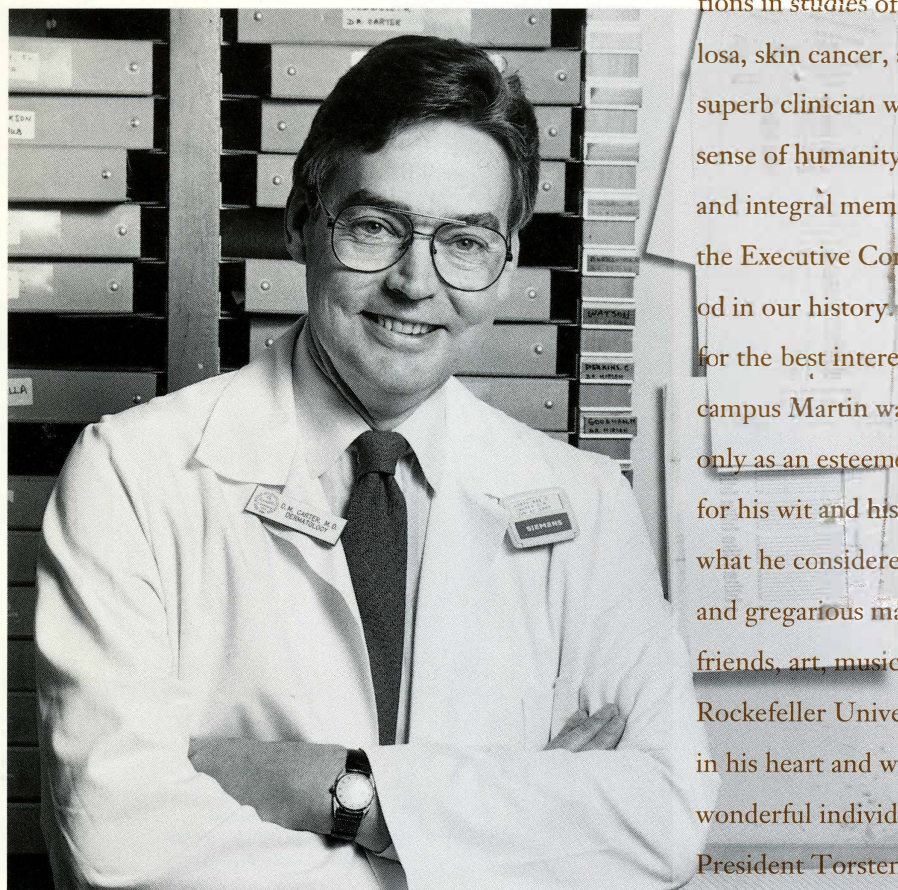
research and clinical activities associated with The Rockefeller University Hospital. As a teacher, Zan was greatly admired and respected by his students: you can hear them as one voice speaking of his scientific acumen, his wisdom, his sensitivity to their needs, his ability to encourage them to become independent scientists. Zan was

a member of the university faculty for thirty-five years and he came to embody what this university

stands for: *Pro Bono Humani Generis.*"

President Torsten Wiesel





“Martin was renowned throughout the world in the field of clinical and experimental dermatology, and he was recognized for his contributions in studies of psoriasis, epidermolysis bullosa, skin cancer, and wound healing. He was a superb clinician with profound insights and a sense of humanity. Martin was an important and integral member of our faculty, chairing the Executive Committee during a critical period in our history. He was always ready to work for the best interests of this institution. On the campus Martin was loved and respected not only as an esteemed clinical scientist but also for his wit and his readiness to stand up for what he considered right. He was a generous and gregarious man who enjoyed his family, friends, art, music, and civic activities. The Rockefeller University had a very special place in his heart and we have lost a very dear and wonderful individual.”

President Torsten Wiesel

D. MARTIN CARTER

1936 – 1993

D. Martin Carter, Carl J. Herzog Professor of Clinical Investigation and senior physician at The Rockefeller University, died Nov. 7 of a dissecting aortic aneurysm.

Renowned throughout the world in the field of clinical and experimental dermatology, Carter made many important contributions to the scientific understanding of genetic and environmental factors that influence the development of diseases of the skin, as well as to treatments for these conditions. Carter was a pioneer in the use of psoralens—photosensitizing drugs—in a variety of diseases. A pioneer also in cellular and molecular studies on epidermolysis bullosa, a group of rare hereditary disorders, he was the founder of the National Epidermolysis Bullosa Registry. Carter was also a highly respected physician and distinguished teacher.

A graduate of Dartmouth College (A.B., 1958), Harvard Medical School (M.D., 1961), and Yale University (Ph.D., 1971), Carter came to Rockefeller in 1981 to head the new Laboratory for Investigative Dermatology. He was previously affiliated with Yale University School of Medicine. At the time of his death, Carter also served as professor of dermatology and co-head of the Department of Dermatology at The New York Hospital-Cornell Medical Center at Cornell University Medical College.

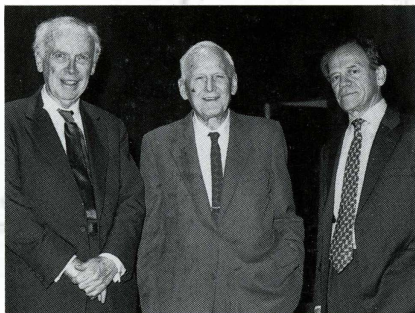
THE BEGINNING OF A YEAR-LONG CELEBRATION: 50 YEARS OF DNA



Jennifer Horne King

A banner on the portico of The Rockefeller University Hospital proclaims, "50 Years of DNA: Celebrating the Avery Laboratory and the Discovery of the Thread of Life."

Below, President Torsten Wiesel (right) with Professor Emeritus Maclyn McCarty (center), co-author of the paper with Oswald Avery and Colin MacLeod, and James D. Watson, director of Cold Spring Harbor Laboratory.



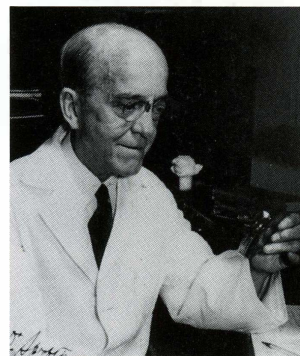
Leif Carlsson

To commemorate the fiftieth anniversary of the discovery at The Rockefeller University that genes are made of DNA—considered by many to be the single most important biological discovery of the twentieth century—the university has kicked off a year-long series of events that will run through May 1994.

"The 1944 discovery of Oswald Avery, Colin MacLeod, and Maclyn McCarty opened the gateway to the modern era of biology and medicine," said President Torsten Wiesel. "In

celebrating this historical event, we celebrate as well The Rockefeller University's enduring mission of diagnosing and curing sickness by uncovering the inner secrets of life."

The celebration was formally inaugurated in November 1993 with a lecture by Nobel laureate James D. Watson, best known for discovering the double-helical structure of DNA.



The Rockefeller University Archives

Above, Pioneering microbiologist Oswald Avery works in his lab, around 1944.

AVERY CELEBRATION EVENT SCHEDULE

WEDNESDAY, FEBRUARY 2, 1994 5:00 P.M.

Public Lecture

“The Human Genome Project in Its Scientific Context”
David Botstein, Stanford University School of Medicine

THURSDAY, FEBRUARY 3, 1994 4:00 P.M.

Historical Roundtable

Discussion with key scientists active between the publication of the 1944 Avery, MacLeod, and McCarty paper and the 1953 discovery of DNA's double-helical structure.

Moderator: Robert Olby, The Rockefeller University; author of *The Path to the Double Helix*

Speakers: Erwin Chargaff, College of Physicians and Surgeons of Columbia University (emeritus); Seymour S. Cohen, SUNY at Stony Brook (emeritus); Alfred Day Hershey, Carnegie Institute (former director); Rollin Hotchkiss, The Rockefeller University (emeritus); Joshua Lederberg, The Rockefeller University; Maclyn McCarty, The Rockefeller University (emeritus); Norton K. Zinder, The Rockefeller University

FRIDAY, FEBRUARY 4, 1994 3:45 P.M.

Scientific Symposium

Leading scientists in research areas pursued by the Avery laboratory—immunology, infectious disease, and molecular medicine.

Moderator: Emil Gotschlich, The Rockefeller University

Speakers: Robert Austrian, School of Medicine, University of Pennsylvania; John B. Robbins, National Institutes of Child Health and Human Development, National Institutes of Health

MONDAY, APRIL 18, 1994 6:00 P.M.

Public Lecture on Ethics and DNA Technology

Nancy Wexler, Columbia University

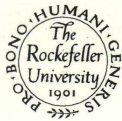
FRIDAY, MAY 6, 1994 3:45 P.M.

Scientific Symposium

Panel of young scientists working in key areas of DNA research at The Rockefeller University

Moderator: Jan L. Breslow, The Rockefeller University

Speakers: To be announced.



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