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### **AIDS AND ANIMAL RESEARCH**

#### **1. THE OUTLOOK**

It has been called the most devastating affliction to strike the human race in almost 700 years. Not since the Black Death swept through Europe in the middle of the 14<sup>th</sup> Century, wiping out a third of the population, has a single disease cut such a terrifying path.

By now, the statistics are grimly familiar. Since it was identified two decades ago, AIDS, or Acquired Immune Deficiency Syndrome, has killed nearly 28 million people—three million of them in 2001 alone. Worldwide, 60 million people, including an estimated 900,000 in the United States, are infected with Human Immunodeficiency Virus (HIV), the virus that causes AIDS. And each year, another five million—almost 14,000 every day – become infected.

The face of AIDS has changed dramatically since scientists first described it in 1981. In the developed countries, significant advances have been made in treating the disease and extending the lives of those with AIDS. Powerful new drugs developed in the mid-1990s have dramatically reduced mortality in the United States, from almost 45,000 in 1993 to 15,000 in 2001. But at the same time, AIDS has spread like wildfire in many poorer countries, especially sub-Saharan Africa. There, it has become not just a health crisis but a social catastrophe, infecting more than 20 percent of the people in seven countries, including South Africa. In Botswana, the most severely affected nation, fully 36 percent of the population is infected with HIV. At the same time, the populations most acutely affected have changed: from homosexual men and intravenous drug users in developed countries, to heterosexual men and women in their most productive, child-bearing years in much of the Third World. The disease has become so widespread in some of those countries that it threatens to destabilize entire societies. To date, some 14 million living children have been orphaned, prompting the United Nations to label the HIV/AIDS pandemic a “global emergency.”

At all stages in the 20-year fight against AIDS, research involving animals has been crucial. Much of our basic understanding of HIV comes from studying similar retroviruses in mice, cats and non-human primates. In the 1980s, medical researchers hoped that an effective

vaccine might be quickly found and conducted extensive trials involving animals that develop diseases similar to AIDS. Those early hopes were dashed, as AIDS proved to be a more difficult challenge than scientists had anticipated. Still, animal research and testing remains a vitally important element in the search for an AIDS vaccine. The drugs that revolutionized AIDS treatment in the late 1990s by extending and enhancing the length and quality of the lives of those living with the disease, were extensively tested on animals for efficacy and safety. Current research on microbicides, which hold out great promise for finding a low-cost way of slowing the spread of AIDS in poorer countries, involves tests with rhesus monkeys. And promising new work towards a vaccine—still considered the best hope for ultimately ending the worldwide pandemic—also includes extensive trials with non-human primates.

All these developments continue to change the focus of treatment and research. In the developed countries, available drug therapies have transformed AIDS from a death sentence into a long-term, manageable disease for many of those affected. But in less-developed countries, the need for inexpensive, accessible treatments is staggering. This devastating disease is spreading so fast, and the cost of cutting-edge treatment is so high, that medical breakthroughs are urgently needed to head off a human catastrophe of unprecedented proportions. At the heart of medical science is research and the foundation of that research is laboratory animals.

## **2. HOW HIV WORKS**

Scientists probably know more about HIV than any other virus they have ever studied. Yet the more they learn, the more they discover how insidious a foe it is. HIV replicates at blinding speed, making 10 billion copies of itself every day and generating constant mutations that quickly make it harder for the body to fight and less vulnerable to drugs designed to counter it. In fact, it changes so rapidly that the genetic diversity in the lifetime of a single infected person can be greater than the worldwide diversity of the influenza virus. Like other viruses, HIV uses the natural responses of the body's immune system to attack and eventually destroy it. HIV enters human cells by interacting with the CD4 antigen on the surface of T-lymphocytes (T-cells), whose principal function is to identify and destroy invading microbes. T-cells have high concentrations of CD4 proteins on their outer surfaces, so the HIV virus can easily attach itself to them. Once attached, the virus injects its own genetic material into the T-cell, where it binds with the cell's DNA. Paradoxically, the trigger that spreads HIV so rapidly is the body's own defense against such invaders. When T-cells mobilize to fight the infection, they begin to divide rapidly and make copies of themselves—and that spreads HIV throughout the body.

The disease works slowly at first. A person infected with HIV-1, the strain of the virus most common in North America and other developed countries, initially experiences symptoms that can feel like nothing more than the flu. They include fatigue, fever, skin rash and tender lymph nodes. During this stage, HIV increases to extremely high concentrations in the blood and mutates rapidly. After that, patients usually experience a long period, sometimes up to ten or more years, without symptoms. Eventually, however, the body's immune system is seriously weakened as HIV attacks a kind of T-cell known as a "helper" or "killer" cell (CD8). In a healthy person, these cells regulate the immune system by controlling the strength and quality of immune responses. As HIV kills off more and more helper cells, the patient's body loses the ability to fight off other infections. In the later stages of AIDS, a

general failure of the immune system occurs, leaving the infected person vulnerable to a range of opportunistic infections. The most common one seen among patients with advanced AIDS is a form of pneumonia caused by a respiratory pathogen known as *Pneumocystis carinii*. This organism normally exists in the airways of all people, but is often deadly to AIDS patients whose immune systems are so weakened that they are unable to fight it off. Many people with advanced AIDS also develop cancers, including Kaposi's sarcoma, a rare cancer of the blood vessels that produces purplish lesions on the skin and can spread to other organs.

Since its discovery, researchers have tried to find ways of fighting the AIDS menace at various stages of its progression through the body. They quickly understood that HIV was spread through the exchange of bodily fluids, mainly during sexual contact, blood transfusion, blood transfer through intravenous drug use and from mother to child during pregnancy. Soon after, a test was developed to detect antibodies to HIV in the bloodstream, so that people could find out whether they were infected. However, developing effective treatments has proven much more vexing. It was only in 1995-96, almost 15 years after the disease was first identified, that researchers successfully produced the revolutionary drugs known as protease inhibitors, which slow the production of an enzyme in HIV that is crucial to the virus's ability to replicate itself. Though these drugs are not a cure, their combination with other anti-AIDS drugs are prolonging the lives of many people with AIDS and dramatically reducing deaths from the disease in developed countries. But the fight is far from over: scientists have yet to develop other forms of treatment, such as the long-sought-after, elusive vaccine that would immunize people against HIV.

### **3. THE VITAL ROLE OF ANIMALS IN AIDS RESEARCH**

From the very beginning of the fight against AIDS, research involving animals has been at the center of scientists' efforts to understand the virus and discover strategies to fight it. The blood test that detects whether a person is carrying HIV was developed using animals. The drugs that now allow tens of thousands of people to live longer, more healthy lives while infected with the AIDS virus, were all tested for safety and efficacy safety with animals. And promising new research aimed at developing an effective vaccine against AIDS and topical microbicides that could be a major step towards slowing its spread in Africa and other parts of the developing world also involve animal research. This is required by both ethics and law: the Nuremberg Code of 1949 and the Helsinki Declaration of 1964 explicitly require that all new drugs be tested in laboratory animals before administering them to humans.

At an even more basic level, however, animal models have been crucial to scientists' understanding of how HIV works. Although HIV was not identified until the early 1980s, other viruses that produce AIDS-like diseases in animals had been recognized and have been studied since early in the 20th Century. Researchers began by trying to figure out what they could learn from these viruses. Like HIV, they are classified as retroviruses—meaning they cannot replicate without an enzyme known as “reverse transcriptase”—and are members of a subcategory called lentiviruses. Almost every mammal has its own lentivirus causing a disease similar to AIDS. There is no exact animal model for HIV disease in human beings. But many of the lentiviruses that attack mammals are remarkably similar to AIDS in the symptoms they cause, how they infect the body and how they spread, allowing researchers to build a valuable body of knowledge about these microscopic killers.

Many animals including sheep, horses, cats, mice, monkeys and apes have contributed to our knowledge of lentiviruses. Sheep and goats can be infected with a virus called Visna-maedi, that spreads through the lymphatic system and bloodstream, much like HIV, and causes similar symptoms, including severe pneumonia, wasting and secondary infections. Scientists studying Visna-maedi in sheep learned how the lentivirus controls its own rate of replication in order to survive more efficiently. Horses can suffer from a virus similar to HIV called the Equine Infectious Anemia Virus, which also causes comparable symptoms: weight loss, anemia, weakness and fatigue. However, the disease is not usually lethal because the horse's immune system recovers enough to control the infection, even though the virus remains in the blood. Researchers hope that their understanding of the horse's immune response will eventually help them to see where the human immune system fails, and suggest potential treatments.

Cats are also susceptible to an HIV-like virus called Feline Immunodeficiency Virus, or FIV. It causes many of the symptoms in cats that HIV causes in humans. It also takes a long time from infection to the time the illness becomes apparent, making cats a very useful model for studying the progression of the disease. Studying FIV has given scientists important insights into the early stages of AIDS-type viruses and how they affect the nervous system. At the same time, the feline virus provides a good model for observing how lentiviruses mutate and become resistant to the types of drugs developed to fight AIDS in people.

The mouse is the most commonly used animal in biomedical research, and this small rodent has provided invaluable knowledge to scientists. Through genetic engineering, scientists have developed a mouse born without an immune system—the so-called Severe Combined Immunodeficiency (SCID) mouse. The SCID mouse cannot reject foreign tissues, so cells from human organs can be transplanted into the mouse to establish an in vivo model of the human immune system. Researchers can then study the effect of drugs and viruses, including HIV, in an intact mammalian system.

Non-human primates have played and continue to play a vital role in AIDS research. Researchers caution that no animal perfectly mimics a human HIV infection; however, there are important differences between AIDS-like diseases in apes and monkeys and human AIDS. Still, they have a very good naturally occurring disease model in the form of Simian Immunodeficiency Virus, or SIV, which infects monkeys. The virus was discovered at about the same time HIV was first identified, and is genetically similar to the human virus. SIV produces symptoms that mimic the human disease: anemia, wasting, chronic diarrhea and swelling of the lymph nodes. And it attacks the body in strikingly similar ways. Like HIV, it zeroes in on the CD4 receptor in immune system cells, causing a rapid depletion of “helper” T-cells and severe decline in the function of the immune system. The advanced stage of the disease is also characterized by the outbreak of opportunistic infections and diseases, such as pneumonia. Unlike HIV, however, SIV typically acts within months instead of taking a decade or more to produce full-blown disease, allowing scientists to study the complete progression of the disease in a relatively short time.

Because SIV and HIV are related viruses, scientists have created a hybrid of SIV and HIV, known as SHIV. They have been able to infect monkeys with SHIV, and used them extensively in research aimed at developing a vaccine against HIV. Currently, scientists are

banking on the so-called “monkey model” as the most promising strategy for creating an AIDS vaccine that works in humans.

Chimpanzees are also important research models because they are the closest animals to humans in genetic makeup, and their immune system is very similar to ours. Research involving chimpanzees has provided important understanding of how HIV infects immune cells and which immune responses appear most successful. However, working with chimpanzees provokes a moral dilemma for some. HIV infection of chimpanzees has been traditionally associated with an absence of full-blown AIDS, and their immune systems are not as severely compromised. Another type of non-human primate, the sooty mangabey, is also resistant to AIDS. This suggests that chimpanzees and mangabeys have successfully adapted to HIV—while macaques and humans have not. If researchers can figure out exactly how some animals’ immune systems are able to defend against the infection, they would be closer to understanding why humans cannot control it.

#### **4. THE SEARCH FOR AN AIDS VACCINE**

As previously noted, extensive studies on the biology of HIV have heightened our understanding of the viral and host factors involved in establishing persistent infection. Scientists now know that HIV mutates rapidly releasing many different subtypes (clades) during the course of an infection and that these mutations may account for differences in immunological and biological properties, including multiple drug resistance. Such valuable basic information has provided researchers with unique targets and different approaches for designing new drugs and vaccines.

**Drugs:** Anyone who is ill or has a low CD4 lymphocyte count (the primary target of HIV infection) is advised to take treatments. Powerful drug combinations, often referred to as highly active anti-retroviral therapy (HAART), inhibit virus replication and cause blood levels to drop to barely detectable levels. They do not, however, eliminate HIV from the body. Combinations of anti-retroviral drugs (cocktails) are prescribed to attack HIV at various stages--asymptomatic to advanced. The expanded use of triple drug combination regimens, including protease inhibitors that work by blocking the activity of enzymes needed to form infectious virus particles, can slow the progression to full blown AIDS and extend the length and quality of the lives of those infected by 60% or more. For some patients, however, even the most rigorous treatment fails to reduce the virus load. And for others, the regimen may be too arduous (fatiguing), but cutting back on drugs or drug dosages allows the virus to bounce back. Given the current state of progress, there still remains an urgent and pressing need for research to prevent new infections and control the HIV/AIDS pandemic, especially in under-developed countries where 95% of all HIV infections occur.

**Vaccines:** Scientists are convinced that the development of an effective vaccine is possible and that global immunization is the most effective strategy to halt the spread of HIV/AIDS. Funding from one federal agency alone, the National Institutes of Health, for vaccine research has increased by 100% since 1997, reaching \$268 Million in 2001. The fact that some infected persons often carry the virus for many years and do not succumb to the disease suggests that human immune system can control the infection; while in others, there exists a very long latent period before significant damage to the immune system occurs. Vaccination may help people to live longer by priming the immune system to delay or prevent the onset of clinical disease. Moreover, through genetic engineering, the development of so-called

DNA vaccines presents a new approach for stimulating an immune response that may protect against infection.

In the long-term, an effective vaccine could provide the most affordable approach to halting the spread of HIV/AIDS, particularly in developing countries where health care delivery systems are rudimentary and health spending may be minimal. Many scientists believe that it would be cheaper and easier to administer vaccines than anti-retroviral drugs to poorer populations.

A wide variety of vaccination strategies are being pursued. These are aimed at fighting the HIV infection on two fronts: stimulating the immune system, (a) to produce neutralizing antibodies that block HIV from infecting cells, and (b) to speed proliferation of cytotoxic T lymphocytes (CTL) or so-called “killer T cells” that destroy cells infected with HIV.

1. Traditional vaccines composed of a weakened (attenuated) or inactivated (killed) preparation of the virus. Killed virus vaccines have not worked well because HIV mutates so rapidly that antibodies alone may not be effective in attacking the virus. The use of attenuated HIV isolates raises concerns about safety, since it is conceivable that the crippled virus could revert to virulence on its own.
2. Vaccines containing only a part(s) or subunit(s) of the virus. These can be produced by making synthetic copies from a subset of the virus’s genes. One example among several antigens being studied is gp120, a copy of a major outer envelope glycoprotein, which enables the virus to bond to the surface of certain target cells. Gp120 by itself lacks the elements required for infection but allows the host to recognize the virus.
3. Vaccines consisting of an HIV gene(s) that encode(s) for a viral protein(s) inserted into a plasmid or ring of naked DNA. The vaccine, administered by intradermal or intramuscular injection using a jet gun, enters the host cells, which subsequently manufacture the protein encoded by the viral gene (e.g., gp120, as described above). Scientists have recently turned to DNA vaccines after years of making whole cell or subunit vaccines that failed to confer immunity.
4. Vaccines consisting of an HIV gene(s) inserted into a weakened bacterium or harmless virus (several virus vectors are under study), which acts as a vector for the gene(s). Administered with a jet gun, the vector virus containing DNA for selected AIDS protein(s) stimulates an immune response against both the virus and the gene product(s) that may protect against subsequent infection by HIV.
5. Various combinations of vaccine strategies. By giving two vaccines together or in sequence, investigators aim to stimulate the immune system on two fronts.
6. Use of adjuvants, or substances given with the vaccine to enhance the immune response.

Any new drug or vaccine must be tested on animals to assess its efficacy and safety before clinical trials on human volunteers can proceed. At present, many diverse approaches are

being evaluated in the macaque model. While there is no ideal animal model, these animals can be infected with SHIV, the hybrid simian and human virus model that can destroy the animal's immune system in a short time. Scientists are encouraged by the early results of these trials which indicate that vaccination with various naked DNA and vector DNA vaccines appears to confer long-lasting immunity. Immunized monkeys given unusually high doses of SHIV did not develop the disease.

Most of the vaccines under development are "preventive," designed to protect against infection, rather than "therapeutic," designed to treat or control the disease, once contracted. Teams of scientists from several pharmaceutical organizations and leading research universities are currently testing DNA vaccines in small trials with healthy volunteers. If successful, clinical trials will then be conducted in high-risk groups which will be monitored to determine whether the vaccine prevents infection or slows the progression of AIDS.

## **5. CONCLUSION**

In the past 20 years, scientists have made tremendous progress in combating AIDS in the U.S.A. and other industrialized countries. Still, HIV infection cannot be eliminated with the currently available drug regimens. Fortunately, significant reductions in HIV-related morbidity and mortality are being achieved with potent antiviral drugs in combination and in appropriate sequence that reduce the viral burden and promote recovery of the immune function. Such treatments have changed the course of the disease, helping persons with HIV to significantly delay the progression to clinical AIDS and live longer. Despite these achievements, infection rates continue to climb in certain groups and present a grave public health problem, underscoring the need for novel vaccine strategies.

Traditional vaccines fail to provide protection from AIDS because they elicit a relatively weak immune response. With advances in genetic engineering, researchers are now turning to novel approaches including the use of viral and bacterial vectors to deliver HIV-DNA. In trials with nonhuman primates, several research groups report that immunization with naked DNA or DNA vaccines speeds the production of killer T cells (CD8 lymphocytes) that kill HIV infected cells. These vaccines hold out great promise for controlling AIDS in the future.

### **Additional Reading:**

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