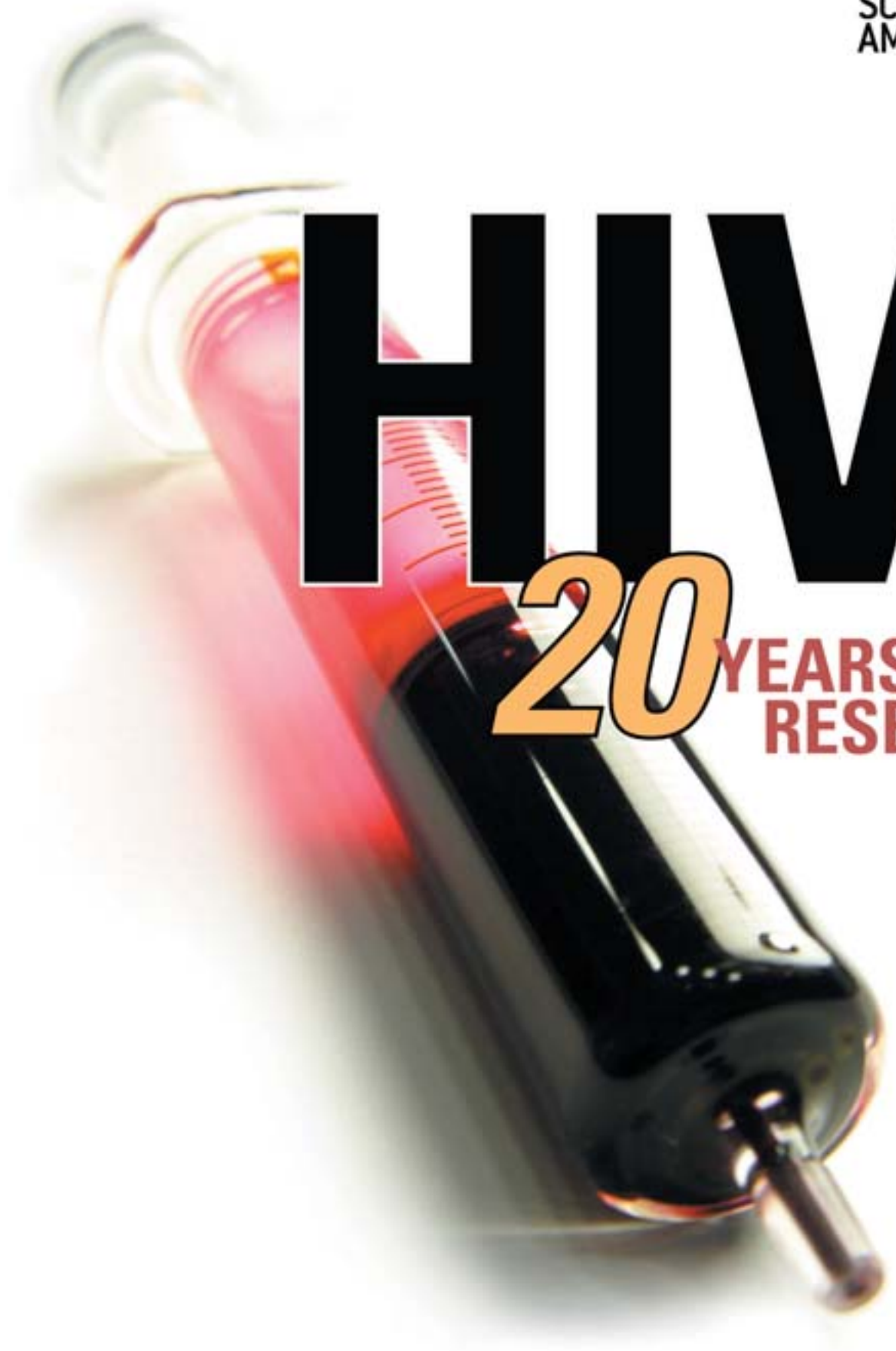
exclusive online issue | no.7

SCIENTIFIC AMERICAN.COM

HIV

20 YEARS of RESEARCH



COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

HIV: 20 YEARS OF RESEARCH

ScientiPcAmerican.com
exclusive online issue no. 7

Nearly 20 years ago researchers Þrst identiÞed HIV, the virus respon for AIDS. Since then, scientists have made great strides in understa how the virus operates and developing effective therapies. But vex challenges to better treatment and prevention remain. And with 42 mil people on the planet infected with HIV (more than three million died fro it last year alone), fresh insights can't come fast enough.

ScientiÞc American has regularly covered advances in HIV research. this anthology of articles published over the past decade, leading aut ties share their expertise on how HIV wreaks havoc on the body, how government might stem the HIV epidemic among drug users who inject, and what sorts of treatments the future might bring. Other articles cuss the hunt for HIV-resistance genes and ponder the development of a HIV vaccine. Ñ The Editors

TABLE OF CONTENTS

2 AIDS and the Use of Injected Drugs
BY DON C. DES JARLAIS AND SAMUEL R. FRIEDMAN; SCIENTIFIC AMERICAN, FEBRUARY 1994
The AIDS epidemic continues to grow among drug users who inject. It could be curbed if governments more re adopted effective prevention programs

7 How HIV Defeats the Immune System
BY MARTIN A. NOWAK AND ANDREW J. MCMICHAEL; SCIENTIFIC AMERICAN, AUGUST 1995
A plausible hypothesis suggests the immune devastation that underlies AIDS stems from continuous - and dar evolution of the human immunodeÞciency virus in the body

14 The African AIDS Epidemic
BY JOHN C. CALDWELL AND PAT CALDWELL; SCIENTIFIC AMERICAN, MARCH 1996
In parts of sub-Saharan Africa, nearly 25 percent of the population is HIV-positive as a result of heterosexual t sion of the virus. Could lack of circumcision make men in this region particularly susceptible?

20 In Search of AIDS-Resistance Genes
BY STEPHEN J. O'BRIEN AND MICHAEL DEAN; SCIENTIFIC AMERICAN, SEPTEMBER 1997
A genetic trait that protects against AIDS has now been uncovered, and others are emerging. The Þndings op new avenues for developing preventives and therapies

28 Improving HIV Therapy
BY JOHN G. BARTLETT AND RICHARD D. MOORE; SCIENTIFIC AMERICAN, JULY 1998
Today's optimal treatments can work magic, but they are costly and onerous and do not work for everyone. Wh the future bring?

38 Hope in a Vial
BY CARL ZIMMER; SCIENTIFIC AMERICAN, JUNE 2002
Will there be an AIDS vaccine anytime soon?

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Originally published in
February 1994

AIDS and the Use of Injected Drugs

The AIDS epidemic continues to grow among drug users who inject. It could be curbed if governments more readily adopted effective prevention programs

by Don C. Des Jarlais and Samuel R. Friedman

The tiny blood transfusions that take place when drug users share needles or syringes serve to transmit disease efficiently. Nothing has illustrated this fact more tragically than the rapid spread of HIV, the human immunodeÞciency virus and the causative agent of AIDS. More than 50 countries have documented HIV infection among persons who inject illicit drugs. An additional 30 countries have reported the practice of illegal drug injection; as a result, these places have populations at high risk of contracting or transmitting the AIDS virus. Once HIV is established among such a group, it moves into the community at large through sexual contact and pregnancy.

No hard Þgures exist for the number of these drug users throughout the world; the best estimate is about Þve million people. There are, however, hard Þgures for the impact of AIDS on this group in the U.S.: more than one third of all AIDS cases, or about 113,000, are associated with the injection of illicit substances. Even in the absence of deÞnitive numbers, the proliferation of HIV among these users has clearly become a medical catastrophe internationally as well. The most conservative predictions by public health institutions foresee increased use of illegal drugs via injection in many countries and increased transmission of HIV among people who take drugs in this way.

Fortunately, the efforts of some health care providers in North America, Europe and Australia show that the expansion of the AIDS epidemic among drug injectors can actually be curtailed. But a lack of political commitment often renders these valuable insights moot. Instead of designing policies that could effectively reduce the spread of HIV, many public health officials have desperately tried to avoid the controversy that surrounds drug use.

We hope that knowledge of the encouraging results of both pilot and full-scale programs may lead officials to strengthen policies to deal with this aspect of the AIDS pandemic. In this article, we review an array of strategies that should be more widely pursued and elaborated before the epidemic becomes uncontrollable among drug injectors, their sexual partners and their children. (The familiar phrase Òintravenous drug userÓ refers only to people who inject into their veins or arteries. Here we use the terms Òdrug users who injectÓ and Òinjecting drug usersÓ to include individuals who may administer the substance into the muscle or just below the skin surface as well.)

Many manifestations of AIDS in drug users who inject are quite different from those in homosexual and bisexual men who do not use drugs in this manner. Among injecting drug users the signs of the disease extend beyond the opportunistic infections and KaposiÕs sarcoma that traditionally served as the basis of a diagnosis of AIDS. The difference in diagnosis derives from the fact that the U.S. Centers for Disease Control primarily tracked the epidemic in homosexual and bisexual men in developing the original deÞnitions of AIDS.

Studies conducted by our research group and by Peter A. Selwyn and Gerald Friedland of YaleÐNew Haven Hospital and their colleagues at MonteÞore Medical Center in New York City have shown that in drug users who inject and in their sexual partners HIV infection is associated with substantially increased morbidity and mortality from bacterial infections. Patients suffer from diseases such as pneumonia and endocarditis (an inßammation of the inner membrane of the heart). In the U.S., much of the resurgence of tuberculosis is occurring among HIV-infected drug users who live in crowded conditions without access to good medical care. There are also indications that HIV infection increases the severity and frequency of gynecological illnesses, including cervical cancer and pelvic inßammatory disease. In the U.S., more than 80 percent of HIV-positive women inject drugs or are the partners of people who do.

The CDC deÞnition of AIDS has been periodically updated to incorporate these Þndings. The 1993 revision includes a diagnosis of AIDS based solely on severe immunosuppressionÑthat is, a CD4 cell count of less

DON C. DES JARLAIS and SAMUEL R. FRIEDMAN study drug addiction, drug abuse treatment and human immunodeficiency virus infection among drug users. Des Jarlais is director of research for the Chemical Dependency Institute of Beth Israel Medical Center and senior research fellow at the National Development and Research Institutes, Inc. He also serves as a member of the U.S. National Commission on AIDS. Friedman is a senior principal investigator at the National Development and Research Institutes, Inc., and has worked as a consultant for the National Institute on Drug Abuse and for the World Health Organization.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

than 200 cells per microliter of blood. (CD4 cells are a form of white blood cell and, normally, help the body protect itself against infection.) This criterion provides an alternative to diagnosing AIDS on the basis of a continually changing list of opportunistic infections and malignancies.

There appear to be two predominant routes by which HIV is introduced into populations of drug users. The virus canenter through overlapping, or bridge, groupsÑfor example, men who inject drugs and also have sex with men. This form of transmission appears to have occurred in New York City, Rio de Janeiro and Sydney. The second is travel. Contrary to the stereotypic view of injecting drug users, many travel frequently, for business and pleasure. For instance, a recent study found that 62 percent of drug users from Berlin had injected drugs outside their home city in the past two years; 14 percent of users from New York City had injected outside the area in the past two years.

Once HIV has been introduced into a community of drug users, it can diffuse extremely quickly. Rapid increases in HIV seroprevalence, or the percent of drug users who are infected, have been observed in both developed and developing nations. In cities such as Bangkok and Edinburgh and in the Indian state of Manipur, HIV seroprevalence among drug users has risen from 0 to 40 percent within two years or less.

This fast transmission is frequently associated with certain kinds of behavior: the use of shooting galleries and of dealersÕ works. Shooting galleries are places where drug injectors rent needles and syringes, administer drugs through the skin and then return the equipment to the operator of the gallery for rental to other customers. Payment may be in cash or in drugs. The term ÒdealersÕ worksÓ refers to the needles, syringes or paraphernalia that a seller will often lend to one customer, take back and then lend to the next.

Shooting galleries and dealersÕ equipment serve several functions within the drug subculture. They provide a source of needles and syringes, which are often scarce because of legal restrictions. In the U.S., for instance, most states that have large populations of such drug users require prescriptions for the sale of needles and syringes. In many other states and countries, over-the-counter sales are permitted, but pharmacists are often unwilling to sell to people they suspect of using illegal drugs.

Shooting galleries allow drug users to avoid toting around equipment. In almost all parts of the U.S., it is illegal for people to possess equipment for administering narcotics. In countries where such possession is legal, many drug users are reluctant to carry syringes because the police often conclude that the individual is engaged in illegal activities. Keeping needles at home circumvents some of these difficulties, but the practice can provoke confrontations between the drug user and the people he or she lives with.

At the same time that dealersÕ works and shooting galleries have served a utilitarian purpose within the drug-using community, they have also served as vectors for hepatitis and other blood-borne infections. The injecting devices are often shared by dozens of drug users who do not have any social relationship with one another and, therefore, do not know one anotherÕs HIV status or about any risky behavior that they may engage in. The materials are frequently used repeatedly until the needle or syringe becomes bent or too dull or clogged with dried blood and is discarded. With the advent of AIDS, such paraphernalia and places have taken on a deadly aspect.

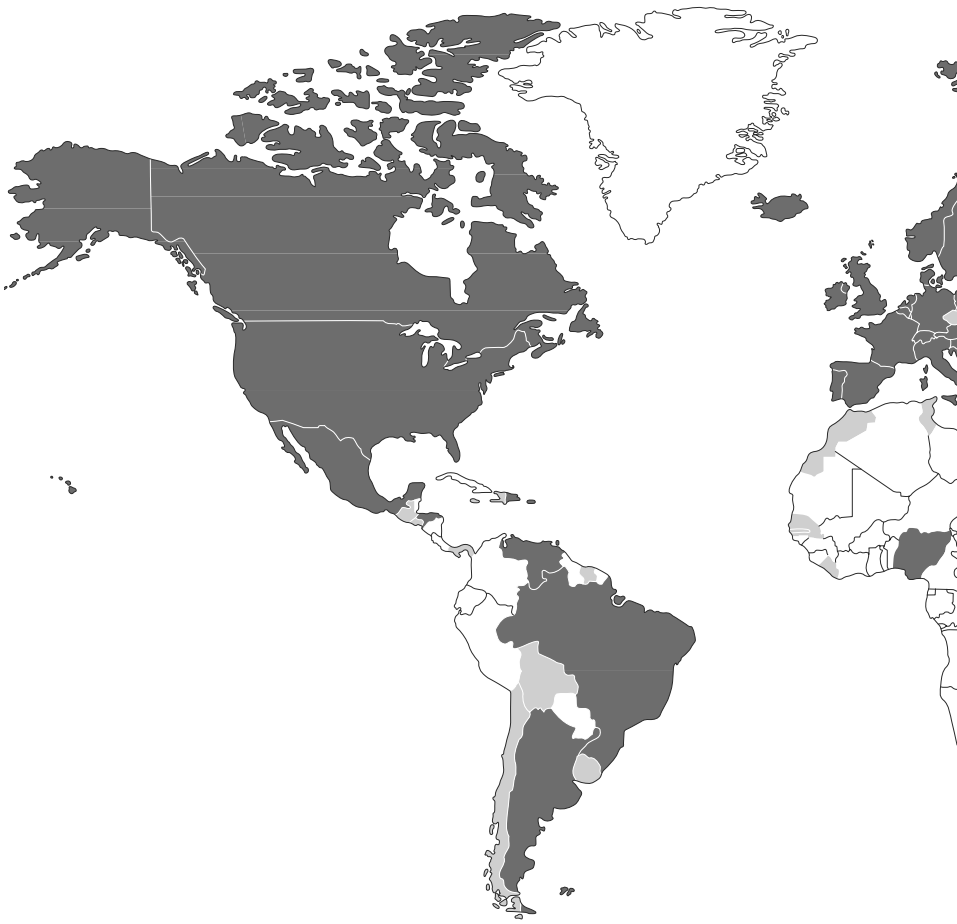
The reasons for the pervasiveness of injection as a form of illegal drug administration is a topic urgently in need of further research. Nevertheless, several features of its appeal are clear. During the first half of the 20th century, the consumption of illicit drugs through injection was relatively rare outside the U.S. But as with many parts of American cultureÑfrom T-shirts and blue jeans to fast foodÑmany different countries have adopted the practice.

The fact that cocaine, heroin and related drugs are illegal encourages the use of injection. Severe statutory restrictions greatly increase the cost of illicit substances to nonmedical users. Injecting provides a way to economize. Injectable forms of opiates and coca are much more concentrated than traditional forms, such as opium or coca tea. Injection provides an intense and economical effect by maximizing the amount of drug that reaches the brain. People who sniff or smoke drugs say that if they inject they need only one third of the amount of the drug to maintain a habit.

Because the injectable forms of illicit drugs are concentrated, they are relatively small in

Prevalence of Injecting Drug

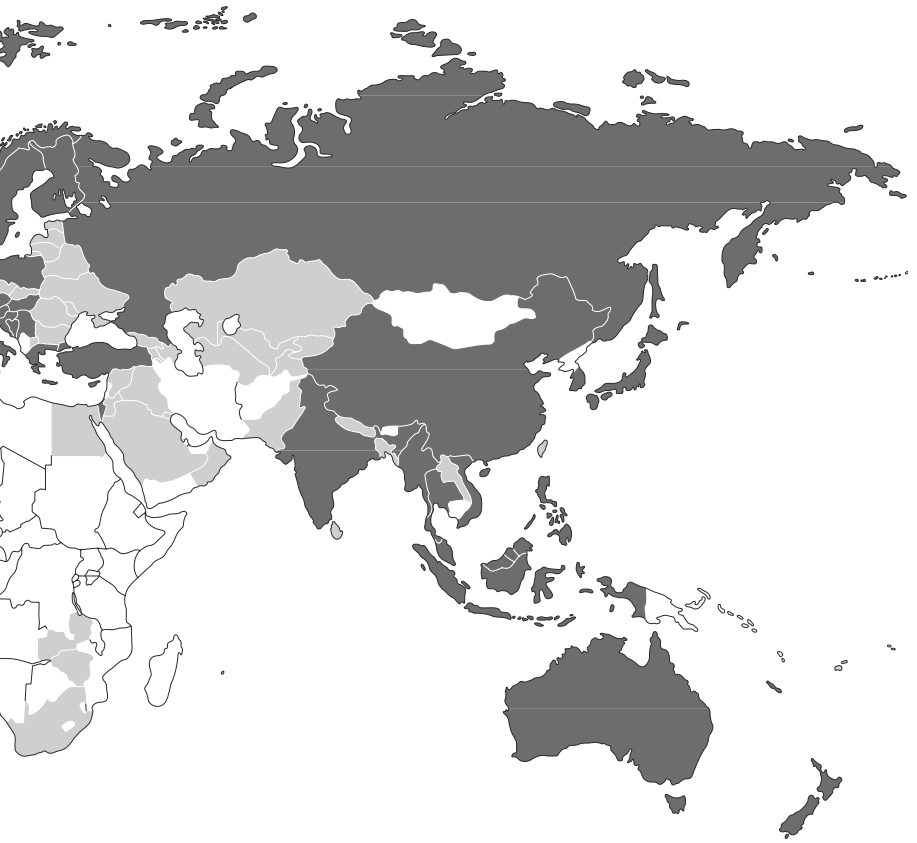
Use of injected drugs is associated with HIV infection in many countries (black), whereas other nations report illicit drug injection that is not currently linked to AIDS (yellow). According to the World Health Organization, some 15 million people are infected with the human immunodeficiency virus, but it is unclear how



COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Users and HIV Infection

many of them were infected through injection or through sex with a drug user. In the U.S., however, one third of all AIDS cases can be attributed to drug injection.



volume and easier to ship. Indeed, the movement of HIV can be traced along some drug distribution routes. Once established in the Golden Triangle of Southeast AsiaÑthe region where Thailand, Myanmar and Laos meetÑHIV radiated outward by traveling west into northeast India, south into Thailand, Malaysia and Vietnam and east across southern China toward Hong Kong. The virus has also moved along cocaine routes within Brazil.

When AIDS was Þrst detected among drug users who inject, there was tremendous skepticism about the ability of these individuals to change their behavior. This assumption was based on another stereotype: drug users are not at all concerned about their health. Fortunately, this attitude does not hold true for a great many of them.

In some cities, such as New York, drug users altered their behavior before any formal prevention program was implemented. Based on information obtained through the mass media and through the informal communication networks of the drug subculture, they started to use illicitly obtained sterilized needles: AIDS risk reduction was reported by 80 percent of drug users in New York City, 84 percent in Glasgow, 82 percent in Lund, Sweden, 84 percent in Sydney and 73 percent in Tacoma, Wash. Anecdotal reports found similar attempts among laborers in cities and villages throughout Asia. Although these Þndings make it clear that drug users who inject often seek to reduce risk, their success depends on whether they can obtain clean needles.

Prevention programs have also demonstrated that it is quite possible to encourage behavioral changes on the part of drug users. Yet these services have not always received the political attention, or funding, they deserve because it is difficult to quantify their effectiveness. The lack of control groups creates measurement problems. In addition, it is hard to evaluate behavioral changes brought about by prevention programs because different studies use different units to measure such shifts in behavior. Furthermore, actual HIV transmission has only seldom been used as an end point in these studies.

Despite these methodological problems, we have found it possible to make a set of generalizations with respect to developing effective AIDS prevention programs. First, drug users who inject are quite capable of learning the basic facts about HIV transmission and about how to reduce exposure to the virusÑindeed, one study found that 93 percent of the users of a particular methadone maintenance center knew about AIDS, and 59 percent had sought to protect themselves against infection. Second, programs are more effective if they involve drug users in design and implementation. Third, programs should provide the means for behavioral change, including treatment for users wishing to limit or stop their use of drugs as well as access to sterile injection equipment and condoms. Fourth, people should be given choices as to how they want to reduce the chances of HIV transmission: there is no one solution that works for everyone. Finally, it is apparent that to date no single program and no combination of programs have been able to eliminate high-risk behavior completely.

One point that emerges in an analysis of the programs is that their success appears to be a function of when they were implemented—that is, whether they were established when the HIV infection rate was minimal or after HIV was entrenched in the community. We have recently analyzed the relation between AIDS prevention and risk behavior in areas where HIV prevalence among drug users is low. Holly Hagan of the Tacoma-Pierce County Health Department, David Goldberg of the Communicable Diseases Unit of Ruchill Hospital in Glasgow, Kerstin Tunving of the University of Lund and Alex Wodak of St. VincentÕs Hospital in Sydney and their many colleagues provided information for our review. In each of these cities, HIV had been introduced into the population of drug users. Yet seroprevalence among heterosexual users remained low (less than 5 percent) and stable for at least four years. In each location, AIDS prevention activities were limited, but all included community outreach and access to sterile injection equipment through syringe exchanges and over-the-counter pharmacy sales.

Eliminating risky behavior was not necessary in order to keep HIV infection rates low in these four cities. Approximately half of the drug users whom we interviewed reported that they were at least occasionally injecting with needles and syringes that had been used by others. Most of the unsafe practices appeared to be contained within small groups of friends. Low and stable rates of HIV infection have also been reported in

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Athens, Toronto, Seattle, Perth and Melbourne. Thus, the experience of the four cities studied may well be generalized to many other places where prevention efforts were begun early.

In addition to cities where HIV infection rates were kept low, several teams of researchers have observed declining rates o new HIV infections and the stabilization of HIV seroprevalence in cities with high seroprevalence. These Þndings come from Johr K. Watters of the University of California at San Francisco, Kachit Choopanya of the Bangkok Metropolitan Administration, Roeland A. Coutinho of the Municipal Health Service of Amsterdam, Alfredo Nicolosi of the National Research Council in Milan and our group in New York. We found, for instance, that HIV seroprevalence in New York City has remained stable since 1983. Stabilization, however, does not imply a lack of new HIV infections. Rather stabilization occurs when there is a rough balance between new infections and the loss of HIV-infected drug users through death or the discontinuance of drug injection. It is not an ideal goal, but it does limit the extent of an epidemic.

Whereas the studies suggest that it is possible to prevent or stabilize epidemics among such drug users, reversing a well-establishec HIV epidemic is a much more formidable task. The situation in Amsterdam offers a good example of the difficulties of Þghting an entrenched HIV epidemic. HIV seroprevalence among drug users in Amsterdam had reached approximately 30 percent by the mid-1980s.

Public health officials quickly mounted a large AIDS prevention effort. This activity included enlisting and funding the local drug usersÕ group to do prevention work, syringe exchange, over-the-counter sales of needles and syringes and drug abuse treatment. (It is necessary to keep in mind one important criticism of the Amsterdam AIDS prevention program: many of the methadone maintenance clinics give methadone at dosages that are too low to alleviate the symptoms of drug withdrawal or craving.)

Coutinho and his colleagues found that the AIDS prevention programs in Amsterdam led to a reduction in the frequency of unsafe injections. The programs also brought about signiÞcant but smaller reductions in unsafe sexual activities. In addition, the rate of new HIV infections among drug users who inject declined from a peak of approximately eight per 100 person-years at risk during the early 1980s to a current rate of approximately four per 100 person-years at risk. This reduction in the rate of new infections is impressive, but incidence is still much too high.

A high prevalence of HIV infection among users who inject drugs increases the risk for everyone in a community of drug users. An individual who is usually careful is more likely to be infected if, for example, he or she forgets and injects with a needle that has been used by someone else. Sexual activity also becomes more dangerous, even though sex is less effective in transmitting HIV than is injection.

If addiction can be reduced by treatment programs, and if individuals can be discouraged from starting to inject drugs, the number of AIDS cases might be reduced even in areas where seroprevalence is high. The city of Edinburgh may have made the most progress in both these respects. The Community Drug Prevention Project there works with local general practitioners to administer drug abuse treatmentÑmostly in the form of methadone. Recent research by Sally J. Haw of City Hospital and her colleagues has shown that a large percentage of addicts who were previously injecting drugs are now in treatment. Many others are maintaining themselves on illicitly obtained orally administered narcotics.

Strategies for preventing individuals from starting to inject drugs have not been well studied. Indeed, the initiation into injecting may be the least investigated aspect of halting HIV infection among drug users. Estimating the rate at which new users start to inject illicit drugs is an extremely diffcult research task. One point is clear from the limited evidence available: knowledge of and concern about AIDS is not enough to deter persons from starting or returning to injection. In one study we conducted, people who were sniffing heroin and cocaine were given extensive AIDS education, including HIV counseling and testing. During a nine-month follow-up period, one quarter of the group injected drugs. Most of these individuals had a close relationship with a drug user who injected.

Despite the knowledge gathered from the studies described above as well as from other research efforts, treatment and prevention programs for drug users have not been widely implemented. Three obstacles stand in the way. In some developing countries, the lack of economic resources limits such prevention. Providing sterile injection equipment to drug users in societies that cannot make sterile equipment available to clinics and hospitals is not likely. Household bleach has been tried as a method for disinfecting, but the most recent studies fail to show any protective effect. Some of the difficulties may arise because needles and syringes need a relatively long contact time to be disinfectedÑthat is, two rinsings, each for 30 seconds or longer.

There are also regions in which greater knowledge of the dangers of drug injection and sexual behavior is needed for prevention of transmission. Present programs appear adequate for controlling HIV transmission in low seroprevalence areas, but a new gen-

Percent of Drug Users with HIV Infection*

	1978	'79	'80	'81	'82	'83	'84	'85	'86	'87	'88	'89	'90	'91
AMSTERDAM									33	31	31	34		
BANGKOK										1	43			
BERLIN								31	49	49	45			
BILBAO							42	42		44	45	27	30	
BOLOGNA		0	0	9	7.5	39	37							
DETROIT								13		16		16		
EDINBURGH					0	14	42	37						
GENEVA				7		27		52						
HAMBURG								0	23	16	13			
LONDON								5	6	4	5			
MANIPUR									0	0	0		54	
MILAN				11	28	61	67	69	73					
NEW YORK CITY	9	26	38		50		57		55				50	
PADUA						20	28	65	50					
RIO DE JANEIRO									4				20	37
ROME								34	42	33	31	32		
SAN FRANCISCO									7	12	12	11		
SARDINIA	0	0	0	1	10	18	32	43	57					
TOURS					0	0	15	17						
VIENNA									7	12	30	28	24	

*Figures come from myriad sources, including hospital records, stored blood samples and treatment programs. The information is incomplete because studies were not undertaken every year.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Some Successful Treatment Programs

NANTES, FRANCE
MILAN
STOCKHOLM
CONNECTICUT
NEW YORK CITY
SAN FRANCISCO
OTHER CITIES IN U.S.

CHICAGO
NEW JERSEY
BRISBANE, AUSTRALIA
GERMANY
PADUA
ROME
MEXICO
MADRID

NEW YORK CITY

INNSBRUCK
PARIS
GLASGOW

NEW YORK CITY
OTHER CITIES IN U.S.

ITALY
AMSTERDAM
STOCKHOLM
NEW YORK CITY
PHILADELPHIA
BALTIMORE
SAN FRANCISCO

CHICAGO
SACRAMENTO
SAN FRANCISCO
OTHER CITIES IN U.S.

NEW JERSEY
NEW YORK CITY
OTHER CITIES IN U.S.

AUSTRALIA
AMSTERDAM
LUND, SWEDEN
LONDON
SAN FRANCISCO
TACOMA
OTHER CITIES IN U.K.

eration of risk-reduction plans may be needed to reverse the situation in locations where seroprevalence is high. Among other components, these services would need to make sterile needles available, deter people from sharing injection equipment, offer drug abuse treatment and reduce the number of people who start to inject drugs. Encouraging the use of noninjectable forms of these drugs has also been suggested as a way to prevent the transmission of HIV. But workable strategies for implementing this idea have not yet been developed.

The biggest obstacle to reducing the spread of HIV among drug users is neither a lack of resources nor a lack of knowledge but a lack of political resolve to utilize already existing information. Many of the strategies for preventing HIV infection have reasonable evidence for their effectiveness but remain highly politically charged. In Europe, programs that offer maintenance treatment have remained contested, and that approach is not widely used. In some cities, such as Amsterdam, even when methadone maintenance is permitted, it is provided at dosages that are too low to be effective and for periods that are too short. In other places, a user must already be infected with HIV before he or she is permitted to receive methadone maintenance treatment.

In the U.S., and to a lesser extent in Sweden, syringe exchanges and over-the-counter sales of sterile injection equipment have remained very controversial. Opponents of legal access to such equipment have claimed that these programs would promote illicit drug injection and would be taken as an official condoning of the behavior. Yet no study has found any evidence to support this contention. In the U.S., the debate over syringe exchange has been complicated by our historical tendency to stigmatize certain ethnic groups for their illicit drug use, adding racial hostility to the debate over AIDS prevention [see ÒOpium, Cocaine and Marijuana in American History,Ó by David F. Musto; SCIENTIFIC AMERICAN, July 1991].

Some of the opposition to syringe exchange, over-the-counter sales of injection equipment and other less dangerous injection programs does not consider research or scientiÞc evidence. In Sweden the Parliament rejected the Þndings of an evaluation study that called for the expansion of the existing syringe exchange plans into a national system. In the U.S., federal legislation prohibited funding of similar exchange services unless the surgeon general found such practices to be safe and effective. But until 1992, the federal government refused to fund research to examine the safety and effectiveness of the locally funded efforts.

These counterproductive attitudes are slowly changing. The debate about methods to prevent AIDS among drug users has led to a general rethinking of how best to regulate the use of licit and illicit drugs. An approach called harm reduction has developed from work done in the Netherlands, the U.K. and Australia. The harm-reduction perspective pragmatically acknowledges the difficulties of ending all misuse of psychoactive drugs. Nevertheless, it emphasizes the possibilities for reducing the individual and social harm associated with drug use.

Harm-reduction programs consider it possible to lower the chance of HIV infection among persons who continue to take drugsÑjust as it is possible to reduce the chances of automobile accidents among persons who continue to drink alcohol. Harm-reduction programs are particularly compatible with scientiÞc research on behavior stemming from drug use because such data are critical to determining which social and public health policies work.

HIV infection among drug users who inject, their sexual partners and their children is now a global public health problem [see ÒUnderstanding the AIDS Pandemic,Ó by Roy M. Anderson and Robert M. May; SCIENTIFIC AMERICAN, May 1992]. It is very likely that the situation will continue to worsen as both drug use and HIV infection among users extends to more developing countries. Present risk-reduction strategies offer a good possibility for preventing epidemics in places where HIV prevalence is low and where adequate resources for community outreach and sterile injection equipment are available. Actually reducing further HIV transmission among drug users, however, requires a political philosophy that enables officials to implement the Þndings of current research. So far, and at great social cost, such enlightenment has eluded many governments. SA

FURTHER READING

HIV-1 INFECTION AMONG INTRAVENOUS DRUG USERS IN MANHATTAN, NEW YORK CITY, FROM 1977 THROUGH 1987. D. C. Des Jarlais et al. in *Journal of the American Medical Association*, Vol. 261, No. 7, pages 1008Ð1012; February 17, 1989.

HIV AMONG DRUG INJECTORS: THE EPIDEMIC AND THE RESPONSE S. R. Friedman and D. C. Des Jarlais in *AIDS Care*, Vol. 3, No. 3, pages 239Ð250; September 1991.

INTERNATIONAL EPIDEMIOLOGY OF HIV AND AIDS AMONG INJECTING DRUG USERS D. C. Des Jarlais, S. R. Friedman, K. Choopanya, S. Vanichseni and T. P. Ward in *AIDS*, Vol. 6, No. 10, pages 1053Ð1068; 1992.

HARM REDUCTION: A PUBLIC HEALTH RESPONSE TO THE AIDS EPIDEMIC AMONG INJECTING DRUG USERS D. C. Des Jarlais, S. R. Friedman and T. P. Ward in *Annual Review of Public Health*, Vol. 14; 1993.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Originally published in
August 1995

How HIV Defeats the Immune System

A plausible hypothesis suggests the immune devastation that underlies AIDS stems from continuous—and dangerous—evolution of the human immunodeficiency virus in the body

by Martin A. Nowak and Andrew J. McMichael

The interplay between the human immunodeficiency virus (HIV) and the immune system turns out to be significantly more dynamic than most scientists would have suspected. Recent research indicates that HIV replicates prodigiously and destroys many cells of the immune system each day. But this growth is met, usually for many years, by a vigorous defensive response that blocks the virus from multiplying out of control. Commonly, however, the balance of power eventually shifts so that HIV gains the upper hand and causes the severe immune impairment that defines full-blown AIDS.

We have put forward an evolutionary hypothesis that can explain the ultimate escape of the virus from immune control, the typically long delay between infection and the onset of AIDS, and the fact that the extent of this delay can vary considerably from patient to patient. Most infected individuals advance to AIDS over the course of 10 years or so, but some patients are diagnosed within two years of infection, and others avoid AIDS for 15 years or more.

We argue that the powerful immune response enabling many patients to remain healthy for years is finally undermined by continuous mutation of the virus. As will be seen, within any given individual, new viral variants may emerge that are able to evade the protective forces somewhat. In our view, the accumulation of many such variants can muddle the immune system to the point that it can no longer fight the virus effectively.

To understand how we came to this hypothesis, which is gaining clinical support, it helps to know a bit about how the immune system eradicates viruses in general and how it responds to HIV in particular. When any virus enters the body and colonizes cells, defensive forces launch a multipronged but highly targeted attack. Macrophages and related cells engulf some of the free particles and break them up. Then the cells fit certain protein fragments, or peptides, into grooves on proteins known as human leukocyte antigens (HLAs). The cells subsequently display the resulting complexes on their surface for perusal by the white blood cells called helper T lymphocytes.

The Body Fights Back

Each helper cell bears receptors able to recognize a single displayed peptide, or epitope. If it encounters the right epitope on a macrophage or similar cell, it binds to the peptide, divides and secretes small proteins. The proteins help to activate and promote replication of still other components of the immune system—notably cytotoxic, or killer, T lymphocytes and B lymphocytes.

Under the right circumstances, the killer T cells directly attack infected cells. Like macrophages, infected cells break up some viral particles, combine certain of the fragments with HLA molecules and exhibit the complexes on the cell surface. If a cytotoxic T lymphocyte, through its receptors, recognizes one of the epitopes on a diseased cell, it will bind to the epitope and destroy the cell before more viral particles can be generated. Activated B lymphocytes secrete antibodies that recognize specific peptides on the viral surface. The antibodies mark free viral particles, those not yet sequestered in cells, for destruction.

All these responses are believed to participate in the defense against HIV. In the initial stage of HIV infection, the virus colonizes helper T cells and macrophages. It also replicates unchecked for a while. As the amount of virus soars, the number of helper cells falls; macrophages die as well, but the effects on them have been less studied. The infected

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

T cells perish as thousands of new viral particles erupt from the cell membrane. Soon, though, cytotoxic T and B lymphocytes mount a strong defense and kill many virus-infected cells and viral particles. These effects limit viral growth and give the body an opportunity to restore temporarily its supply of helper cells to almost normal concentrations. Nevertheless, the virus persists. In the early phase, which may last for a few weeks, about 30 percent of infected patients display some symptoms, often a fever that may be accompanied by a rash and swollen lymph glands. Even those individuals, though, usually go on to enter a prolonged symptom-free stage.

Throughout this second phase the immune system continues to function well, and the net concentration of measurable virus remains relatively low. Nevertheless, the viral level rises gradually, in parallel with a decline in the helper population. Accumulating evidence indicates that helper cells are lost because the virus and cytotoxic T cells destroy them, not because the body's ability to produce new helper cells becomes impaired. It is a sad irony that the killer cells required to control HIV infection also damage the helper T cells they need to function efficiently.

Patients are generally said to cross the line to AIDS when the helper cell count, which in healthy individuals measures 1,000 cells per microliter of blood, falls below 200. During this stage, the viral level climbs sharply, and measures of immune activity drop toward zero. It is the loss of immune competence that enables normally benign microorganisms (particularly protozoa and fungi) to cause life-threatening diseases in AIDS patients. Once AIDS develops, people rarely survive for more than two years.

Persistence of a good immune response in the face of constant attack by HIV raises the issue of why the immune system is unable to eradicate HIV completely in most, if not all, cases. Several years ago various features of HIV led one of us (Nowak) and his colleagues in the zoology department of the University of Oxford to suspect the answers lay with an ability of the virus to evolve in the human body.

Evolutionary Theory Predicts Trouble

Evolutionary theory holds that chance mutation in the genetic material of an individual organism sometimes yields a trait that gives the organism a survival advantage. That is, the affected individual is better able than its peers to overcome obstacles to survival and is also better able to reproduce prolifically. As time goes by, offspring that share the same trait become most abundant in the population, outcompeting other members—at least until another individual acquires a more adaptive trait or until environmental conditions change in a way that favors different characteristics. The pressures exerted by the environment, then, determine which traits are selected for spread in a population.

When Nowak and his co-workers considered HIV's life cycle, it seemed evident that the microbe was particularly well suited to evolve away from any pressures it confronted (namely, those exerted by the host's immune system). For example, its genetic makeup changes constantly; a high mutation rate increases the probability that some genetic change will give rise to an advantageous trait. This great genetic variability stems from a property of the viral enzyme reverse transcriptase. In a cell, HIV uses reverse transcriptase to copy its RNA genome into double-strand DNA. This DNA is inserted into a chromosome of the host, where it directs the production of more viral RNA and viral proteins. These elements, in turn, assemble themselves into viral particles that can escape from the cell. The virus mutates readily during this process because reverse transcriptase is rather error prone. It has been estimated that each time the enzyme copies RNA into DNA, the new DNA on average differs from that of the previous generation in one site. This pattern makes HIV the most variable virus known.

HIV's high replication rate further increases the odds that a mutation useful to the virus will arise. To appreciate the extent of HIV multiplication, consider findings released early this year from teams headed by George M. Shaw of the University of Alabama at Birmingham and by David D. Ho of the Aaron Diamond AIDS Research Center in New York City. The groups reported that at least a billion new viral particles are produced in an infected patient each day. They found that in the absence of immune activity, the viral population would on average double every two days. Such numbers imply that viral particles present in the body 10 years after infection are several thousand generations removed from the original virus. In 10 years, then, the virus can undergo as much genetic change as humans might experience in the course of millions of years.

A Scenario of Disease Progression

With knowledge of HIV's great evolutionary potential in mind, Nowak and his colleagues conceived a scenario they thought could explain how the virus resists complete eradication and thus causes AIDS, usually after a long time span. Their proposal assumed that constant mutation in viral genes would lead to continuous production of viral variants able to evade to some extent the immune defenses operating at any given time. Those variants would emerge when genetic mutations led to changes in the structure of viral peptides—that is, epitopes—recognized by the immune system. Frequently such changes exert no effect on immune activities, but sometimes they can cause a peptide to become invisible to the body's defenses. The affected viral particles, bearing fewer recognizable epitopes, would then become more difficult for the immune system to detect.

The hypothesis proposed that a mutation able to reduce recognition of an epitope would give a viral variant a survival advantage, at least until the immune system discovered and reacted to the altered peptide. This response would reduce the viral load for a time, but meanwhile other "escape mutants" would begin to break out, and the cycle would continue, preventing full elimination of the infection.

Such a scheme is extremely hard to verify with clinical tests alone, largely because the nonlinear interactions between the virus and the immune system are impossible to monitor in detail. Consequently, Nowak turned to a computer simulation in which an initially homogeneous viral population evolved in response to immunologic pressure. He reasoned that if the mathematical model produced the known patterns of HIV progression, he could conclude the evolutionary scenario had some merit.

The equations that formed the heart of the model reflected features that Nowak and his colleagues thought were important in the progression of HIV infection: the virus impairs immune function mainly by causing the death of helper T cells, and higher levels of virus result in more T cell death. Also, the virus continuously produces escape mutants that avoid to some degree the current immunologic attack, and these mutants spread in the viral population. After a while, the immune system finds the mutants efficiently, causing their populations to shrink. The model additionally distinguished between two kinds of immune responses: those recognizing epitopes that undergo mutation readily and those recognizing conserved epitopes (ones that appear in an unchanging form on every viral particle in the body, because the virus cannot tolerate their loss or alteration).

The simulation managed to reproduce the typically long delay between infection by HIV and the eventual sharp rise in viral levels in the body. It also provided an explanation for why the cycle of escape and repression does not go on indefinitely but culminates in uncontrolled viral replication, the almost complete loss of the helper T cell population and the onset of AIDS.

In particular, the model indicated that the immune system can often mount a strong defense against several viral variants simultaneously. Yet there comes a point, usually after many years, when there are too many HIV variants. When that threshold is crossed, the immune system becomes incapable of controlling the virus. This "diversity threshold,"

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

as we call the breaking point, can differ from person to person. For instance, if the immune system is relatively weak from the start, a few variants may be sufficient to overcome the body's defenses.

There is an intuitive explanation for why the presence of multiple HIV variants in an individual can impair the efficiency of the immune system. This explanation considers the battle between HIV and the body's defensive forces to be a clash between two armies. Each member of the HIV army is a generalist, able to attack any enemy cell it encounters. But each member of the immune army is a specialist, able to recognize an HIV soldier only if the soldier is waving a flag of a precise color.

Suppose the armies would be equally powerful if every specialist in the immune army recognized the same flag and every HIV soldier carried that flag. Now suppose that the HIV army consisted of three groups, each carrying a different flag and that, in response, the immune specialists also divided into three groups, each recognizing a separate flag. Under these conditions, the immune army would be at a significant disadvantage. Any given immune specialist would recognize and attack only one out of every three enemy soldiers it encountered—the one carrying the right flag. The HIV soldiers, meanwhile, would continue to pick off every specialist they met and would ultimately win the war.

Predicting the Course of Disease

Beyond giving us the concept of a diversity threshold, the model offered a possible explanation for why some patients progress to AIDS more quickly than do others. If the initial immune response to conserved epitopes is strong, the efficiency of the defensive attack on HIV will not be undermined very much by mutation in other epitopes. (Many active members of the immune system will continue to recognize every infected cell or viral particle they encounter.) Hence, the body should control the virus indefinitely, in spite of quite high levels of viral diversity. In such individuals, progression to AIDS is likely to be slow (or may not happen at all).

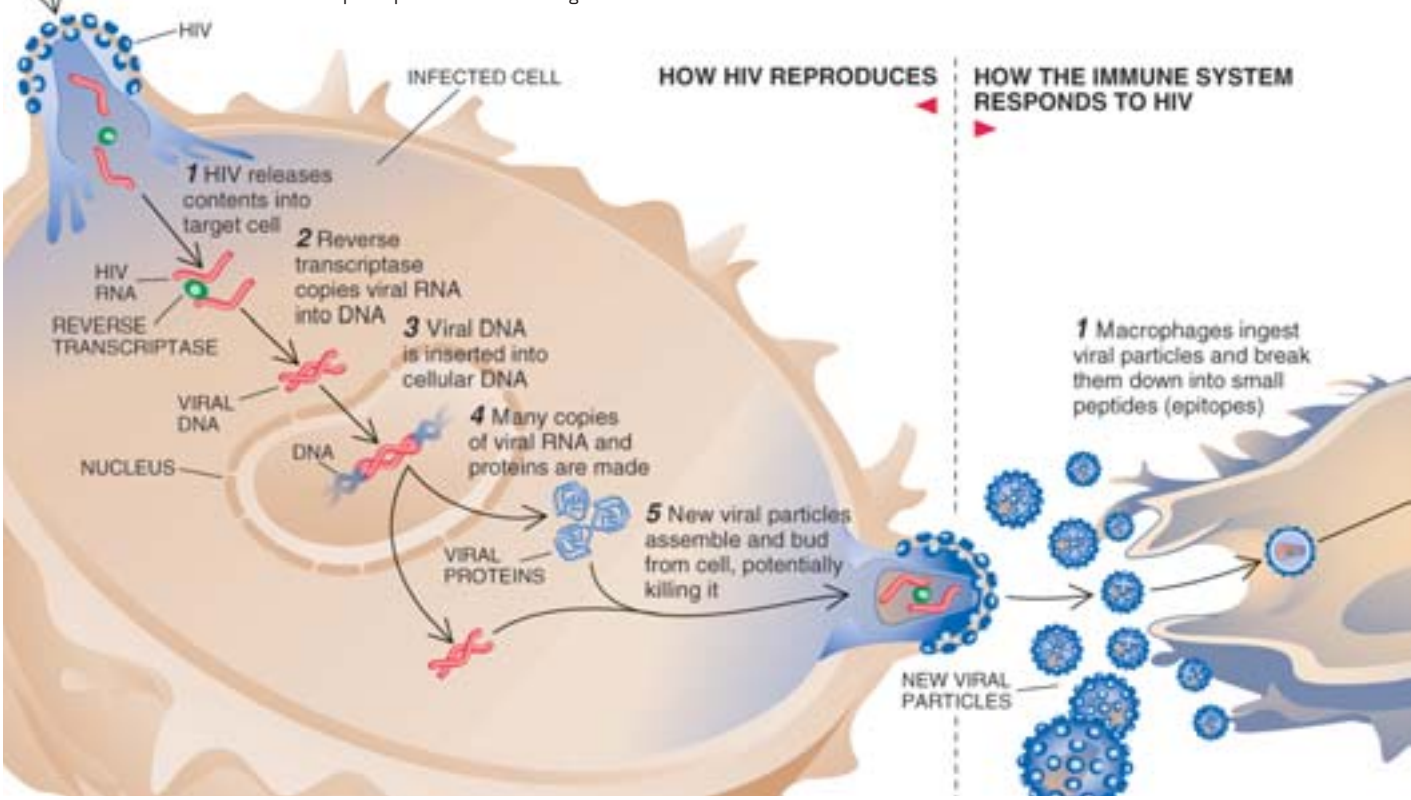
If the immune response to conserved epitopes is not strong enough to control the viral population on its own, but the combined effort of the responses against conserved and variable epitopes can initially manage the virus, the defensive forces could do well for quite a while. But the reaction against variable epitopes should eventually be undermined by the emergence of escape mutants and increasing viral diversity. In this case, HIV levels should rise as the response to variable epitopes becomes less efficient. This is the pattern that apparently occurs in most patients.

If the combined immune responses to conserved and variant epitopes are too weak to control HIV replication from the start, AIDS should develop rapidly. In that situation, the original viral particles would proliferate without encountering much resistance, and so the virus would be under little pressure to generate mutants able to escape immune reconnaissance. Such patients might progress to AIDS even in the absence of significant viral diversity.

The simulation also provided insight into probable properties of the viral population during each stage of HIV disease. In the earliest days, before the immune system is greatly activated, the viral variants that replicate fastest will become most abundant. Hence,

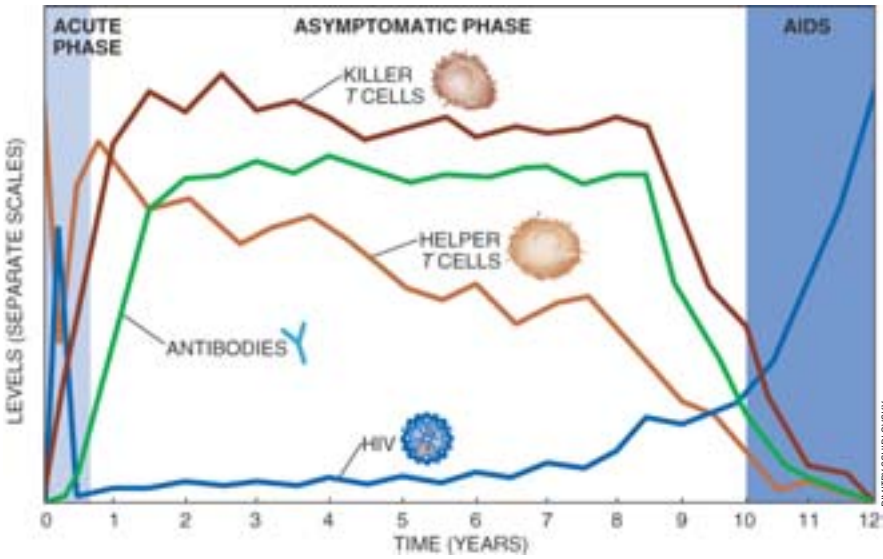
HIV versus the Immune System

The battle between HIV and the immune system begins in earnest after the virus replicates in infected cells and new particles escape (1–5, left panel). Rising levels of HIV in the body induce a response from many components of the immune system (right panel). Such responses can destroy free viral particles (1–5) as well as virus-infected cells (4a and 5a). But they generally are unable to eliminate HIV completely. One reason for the failure is that the virus infects, and depletes the levels of, helper T cells and macrophages, two central participants in the defense against HIV.



COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

COURSE OF HIV INFECTION typically runs many years, during most of which the patient has no symptoms. Strikingly, the body's defenses—as indicated by levels of antibodies, killer T cells and helper T cells in the blood—remain strong throughout much of the asymptomatic period, eradicating almost as much virus as is produced. At some point, however, the immune defenses lose control of the virus, which replicates wildly and leads to collapse of the immune system.

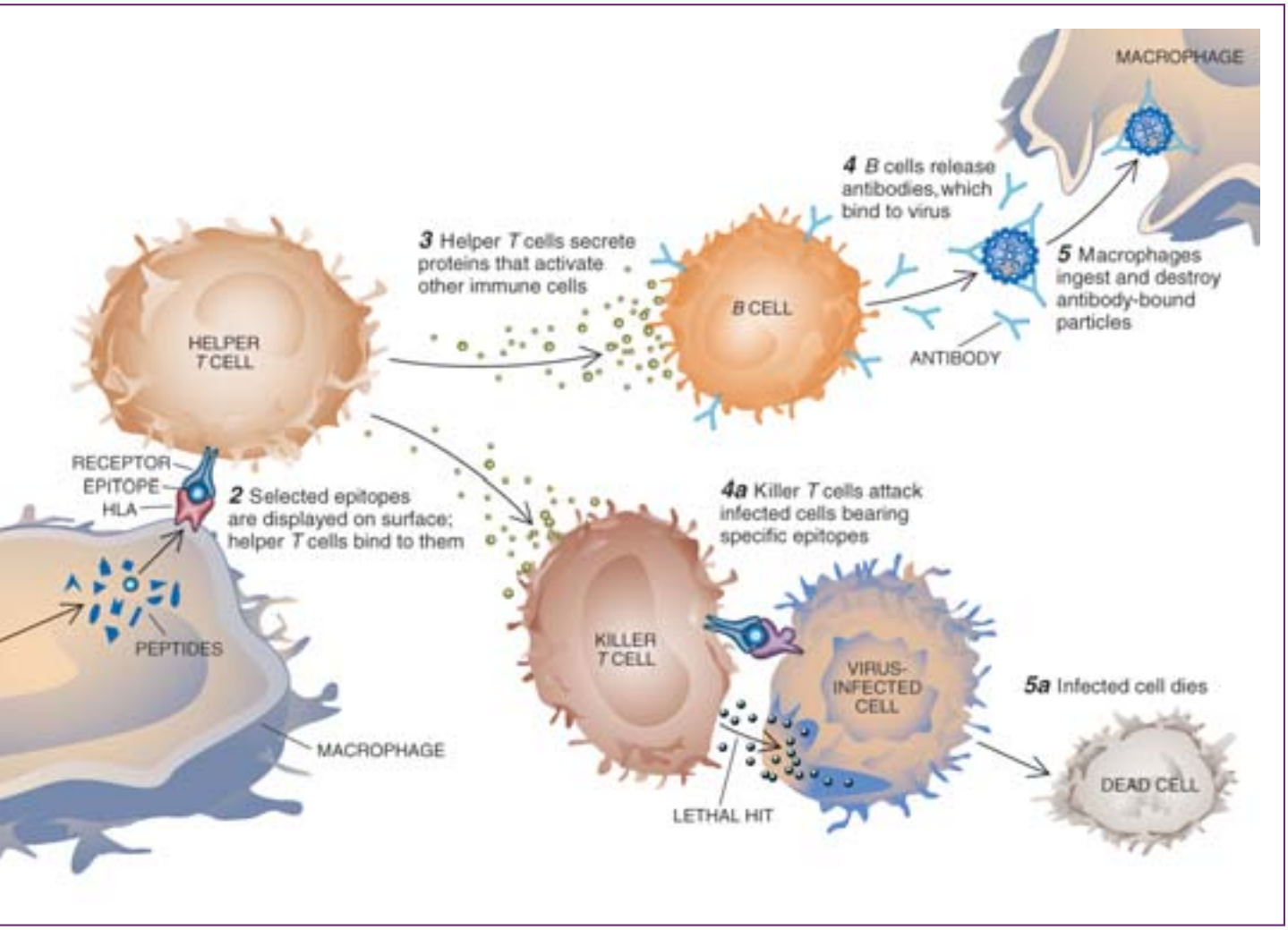


DIMITRY SCHIDLOVSKY

even if a patient were infected by several variants at once, after a short time most of the virus in the body would probably derive from the fastest-growing version. And so we expect little genetic diversity during the acute phase of disease.

After the immune system becomes more active, survival becomes more complicated for HIV. It is no longer enough to replicate freely; the virus also has to be able to ward off immune attacks. Now is when we predict that selection pressure will produce increasing diversity in epitopes recognized by immune forces. Once the defensive system has collapsed and is no longer an obstacle to viral survival, the pressure to diversify evaporates. In patients with AIDS, then, we would again anticipate selection for the fastest-growing variants and a decrease in viral diversity.

Long-term studies involving a small number of patients have confirmed some of the modeling predictions. These investigations, done by several researchers—including Andrew J. Leigh Brown of the University of Edinburgh, Jaap Goudsmit of the University of



DIMITRY SCHIDLOVSKY

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

power of the peptide. (Some epitopes evoke more T cell replication than do others.)

The results of the multiple-epitope models were complex, to say the least. In essence, though, the overall efficacy of the immune system declined over time, and the drop resulted from much the same kind of fluctuation in immune reactivity seen in the two patients who produced HLA molecules of the B8 type. The fluctuation seemed to derive from a kind of competition among killer T cell populations.

Our calculations suggest that in the body, one clone of killer T cells (a population recognizing one epitope) essentially vies with all others for dominance. As the initial killer cell response, which involves many clones, takes effect, the viral population gets smaller, thereby reducing the number of stimulatory signals received by the T cells. Ultimately, only the T cell clones recognizing the most stimulatory epitopes remain active, and the T cell response may even be dominated by a single clone.

Such a process could be beneficial and could potentially eliminate a virus if the microbe did not change. On the other hand, if the epitope fueling the dominant response mutates, the corresponding T cell clone may not recognize the mutant. Viral particles bearing this peptide may then multiply virtually unnoticed. Sometimes the immune system will catch up with the renegade group and mount a defense targeted against the new version of the epitope, but other times the defensive system may switch its attention to a different, and originally less stimulating, epitope. This switching can be repeated many times, producing a very intricate pattern in which the relative abundances of T cell clones fluctuate continuously. Emergence of an unrecognized form of an epitope can thus cause trouble in at least two ways. In addition to reducing directly the strength of the attack on the altered viral variant, it can induce the immune system to shift its efforts toward less stimulating epitopes.

The global picture taking shape from our recent simulations is one in which diversity of epitopes gives rise to fluctuations of immune responses and diversion to weaker and weaker epitopes. Such diversion results in high levels of HIV, leading to faster killing of helper cells and macrophages and to reduced control of the overall viral population. Put another way, viral diversity seems to drive disease progression. These multiple-epitope simulations can be applied to antibody responses as well.

Thoughts on Therapy

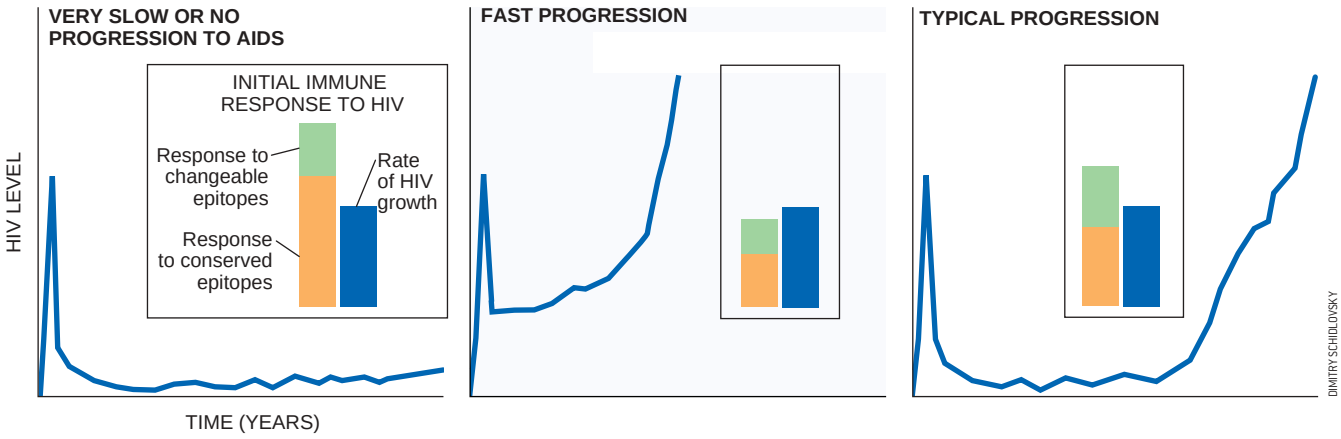
Someone unfamiliar with such findings might reasonably suspect that patients who respond to many different epitopes will enjoy better control of a viral population, because a microbial particle not noticed by one clone of immune cells would probably be noticed by another clone. Yet our models predict that in the case of HIV, a response to many different epitopes can be a bad sign—an indication that important epitopes may have undergone unrecognized mutations. The simulations imply that patients whose immune defenses stably recognize one or a few epitopes probably control the virus better than those who respond to a large number of epitopes. This view is supported by an interesting finding from the HLA study described earlier. The two patients who displayed fluctuating T cell responses progressed toward AIDS more quickly than did patients who had consistent responses to a single epitope. This study involved too few patients to allow for definitive conclusions, however.

If the models reflect the course of HIV disease accurately, the findings have implications for the development of vaccines (for prevention or treatment) and chemical-based therapies. In the case of vaccines, it would probably be counterproductive to stimulate immune activity against a variety of HIV epitopes in an individual. After all, such stimulation would probably elicit an undesirable competition among immune forces. Rather it may be better to boost the response against a single conserved epitope, even if that epitope is not normally recognized most readily. This response could ideally evoke a persistent, controlling response to HIV. The trick, of course, would be to identify conserved epitopes and find the best way to deliver them.

Another striking implication relates to the fact that the virus replicates quickly and continuously in all stages of infection. This realization has made many physicians conclude that chemical agents able to halt viral replication are probably most effective when delivered early, before the virus has a chance to expand too much. Combination therapies may also be more effective than single drugs, because even if the virus generated a mutant population resistant to one of the substances, the other drugs could still continue to be effective. By retarding the rate of replication, such strategies should slow the speed at which mutants are produced and so limit viral diversity. Our models further suggest that reducing viral levels and curtailing diversity in this way would help the natural immune system to contain the virus.

A Broad View of HIV Dynamics

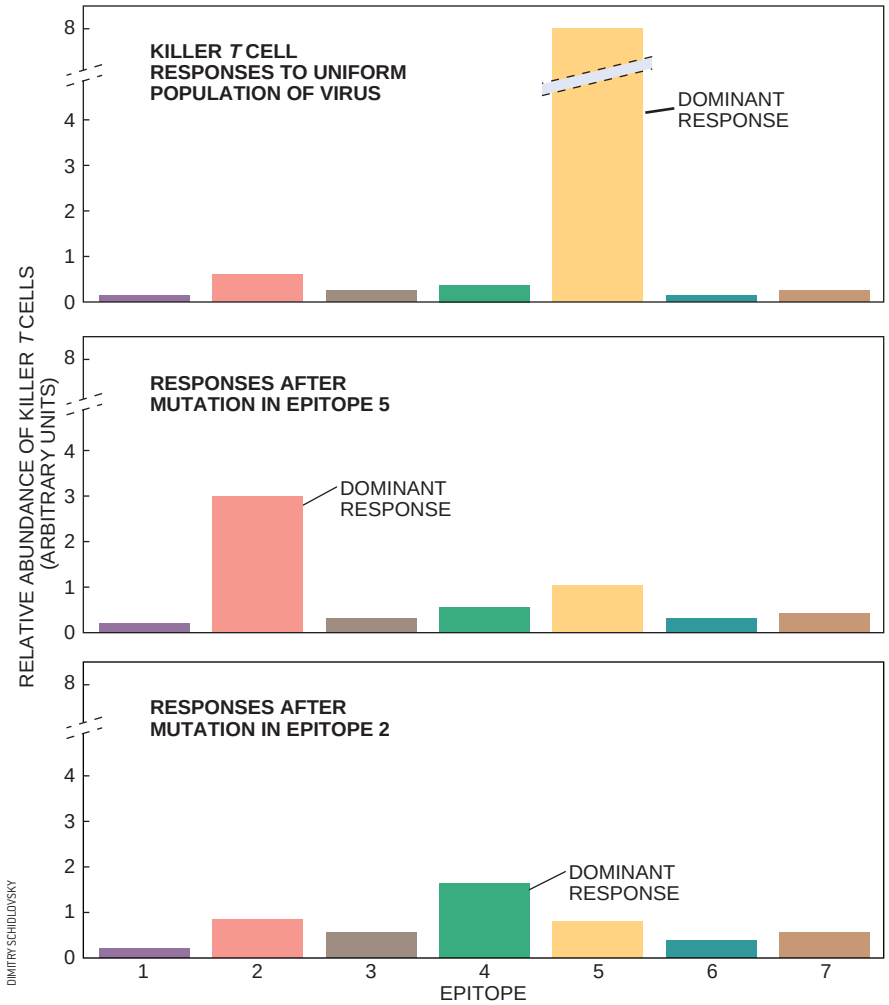
The collected clinical and mathematical findings show that in addition to replicating massively in infected patients, HIV



DIMITRY SCHILOVSKY

SPEED AT WHICH HIV LEVELS RISE (linear plots) over the years may depend greatly on the composition of the initial immune response (insets). Modeling suggests that if the immune attack directed against conserved epitopes (ones found on every viral particle) can limit viral growth on its own (left), the body might keep viral levels low indefinitely—even after the response to readily changeable epitopes inevitably decays. This pattern is uncommon. If the combined responses are weak (center), viral levels will rise quickly. If the combined responses are strong but the "conserved" response cannot by itself control the virus (right), the typical, fairly slow course of viral multiplication should result. In that situation, levels will begin to soar when the ability to respond e ciently to changeable epitopes is lost.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.



COMPUTER SIMULATION tracked levels of killer T cells in a hypothetical patient. Initially (top) the T cells responded to a homogeneous population of HIV particles, each of which carried seven recognizable epitopes; epitope 5 elicited the strongest response (yellow). After a viral mutant carrying an altered, unrecognized version of this epitope emerged (middle panel), the dominant response became focused on a less stimulatory epitope—number 2 (red). And after epitope 2 mutated (bottom), dominance shifted again, to number 4 (green), an even weaker epitope. Such shifts could contribute to reduced immunologic control in HIV-infected patients.

mutates repeatedly and thus spawns an enormous diversity of viral populations. These features enable the virus to evolve in response to the threats it encounters during the course of an individual infection. Mutants able to evade immune attack to some degree appear and predominate until the immune system gathers the strength to quell them—but meanwhile new escape mutants begin to multiply. Power thus moves repeatedly from the virus to the immune system and back for a time.

The reversals do not go on endlessly, though, apparently because the evolution of viral diversity gradually tilts the balance toward the virus. Diversity favors the microbe in part because the variability befuddles the patient's immune system, which becomes less efficient and therefore enables the viral population to grow and to kill increasing numbers of helper cells.

Of course, killing of helper cells impairs the functioning of killer T cells and B cells, which react strongly only when they are stimulated by proteins released from helper cells. As these two cell types become even less effective, a potentially lethal spiral ensues in which viral levels rise further, more helper T cells are killed and the overall responsiveness of the immune system declines.

Generation of mutants thus stimulates a continuous reduction in the efficiency of the immune system. At some point, the diversity becomes too extensive for the immune system to handle, and HIV escapes control completely. As the viral load increases, the killing of helper cells accelerates, and the threshold to AIDS is crossed. Finally, the immune system collapses. In short, it seems that an evolutionary scenario can go a long way toward explaining why HIV infection usually progresses slowly but always, or almost always, destroys the immune system in the end. SA

The Authors

MARTIN A. NOWAK and ANDREW J. MCMICHAEL are collaborators at the University of Oxford. Nowak is a Wellcome Trust Senior Research Fellow in the department of zoology and at Keble College. He earned his Ph.D. from the University of Vienna, where he studied biochemistry and mathematics. Although Nowak concentrates on the interactions between HIV and the immune system, he has developed a wide variety of mathematical models relating to evolutionary biology. McMichael, who became excited by science after reading a series of Scientific American articles on DNA in the 1960s, is a Medical Research Council Clinical Research Professor of Immunology at Oxford and head of the Molecular Immunology Group at Oxford's Institute of Molecular Medicine. He is also a consultant to Celltech and a Fellow of the Royal Society. McMichael has climbed the highest mountain in Austria, Nowak the highest mountain in England.

Further Reading

Antigenic Diversity Thresholds and the Development of AIDS. M. A. Nowak, R. M. Anderson, A. R. McLean, T.F.W. Wolfs, J. Goudsmit and R. M. May in Science, Vol. 254, pages 963–969; November 15, 1991.

Human Immunodeficiency Virus : Genetic Variation That Can Escape Cytotoxic T Cell Recognition . R. E. Phillips et al. in Nature, Vol. 354, No. 6353, pages 453–459; December 12, 1991.

How Does HIV Cause AIDS? Robin A. Weiss in Science, Vol. 260, pages 1273–1279; May 28, 1993.

Viral Quasispecies. Manfred Eigen in Scientific American, Vol. 269, No. 1, pages 42–49; July 1993.

Multifactorial Nature of Human Immunodeficiency Virus Disease: Implications for Therapy . Anthony S. Fauci in Science, Vol. 262, pages 1011–1018; November 12, 1993.

Antigenic Oscillations and Shifting Immunodominance in HIV-1 Infections . M. A. Nowak, R. M. May, R. E. Phillips, S. Rowland-Jones, D. Lalloo, S. McAdam, P. Klenerman, B. Köppe, K. Sigmund, C.R.M. Bangham and A. J. McMichael in Nature, Vol. 375, pages 606–611; June 15, 1995.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Originally published in August 1995

The African AIDS Epidemic

In parts of sub-Saharan Africa, nearly 25 percent of the population is HIV-positive as a result of heterosexual transmission of the virus. Could lack of circumcision make men in this region particularly susceptible?

by John C. Caldwell and Pat Caldwell

AIDS has swept across sub-Saharan Africa on an extraordinary scale. Two thirds of the roughly 16 million people in the world infected with the human immunodeficiency virus (HIV), which causes AIDS, live there. Half of the world's cases are found in what we call the AIDS belt—a chain of countries in eastern and southern Africa that is home to only 2 percent of the global population.

Heterosexual intercourse serves as the main vehicle for spreading HIV throughout sub-Saharan Africa. This is in stark contrast to the developed world, where the virus is most frequently transmitted during homosexual intercourse or when intravenous drug users share contaminated syringes. Attempts to halt the flood of AIDS cases in Africa will not succeed until researchers can determine what factors contribute to the remarkable prevalence of the disease among heterosexuals. Such a diagnosis will also help us figure out how likely it is that heterosexual epidemics might extend into Asia or the West.

One frequently mentioned explanation for the severe epidemic in the AIDS belt is that the virus originated here and continues to move outward from an epicenter of disease. But AIDS cases appeared in hospitals in Uganda and Rwanda at the same time they did in the West, and no stored human-tissue samples taken from Africans during the 1970s are HIV-positive. Furthermore, the AIDS belt is not circular but elongated, clearly not the pattern of expansion from an epicenter. (A related virus, HIV-2, most likely did originate in Africa, but it infects fewer people and kills much more slowly; for these reasons we do not deal with it in this article.)

To determine what factors might be spurring the rapid progress of HIV in sub-Saharan Africa, we decided to reexamine everything we knew, and thought we knew, about the epidemic. Were we sure that HIV was transmitted primarily by heterosexual intercourse? Were there differences between behavior in the AIDS belt and in the rest of the region that might account for the severity of the epidemic in certain countries? Could susceptibility to AIDS be linked to other health problems that were common in the heavily infected areas?

We were in a good position to study the African AIDS epidemic, because for more than three decades we have examined family dynamics, fertility and fertility control in sub-Saharan Africa. In the late 1970s we also worked on sexuality and sexually transmitted diseases there. And since 1989 we have studied the epidemiological, social and behavioral aspects of the African AIDS epidemic. Our collaborators in Africa have been, since 1989, I. O. Orubuloye of Ondo State University in Nigeria and the West African Research Group on Sexual Networking and, since 1991, James P. Ntozi and Jackson Mukiza-Gapere, both at Makerere University in Uganda, as well as John K. Anarfi of the University of Ghana and Kofi Awusabo-Asare of the University of Cape Coast in Ghana.

Heterosexual Transmission

The first assumption we had to scrutinize was the notion that AIDS in sub-Saharan Africa spreads primarily through heterosexual intercourse. We were skeptical because elsewhere the risk of acquiring the virus during heterosexual sex is extremely low. If a man and a woman are otherwise healthy except for the fact that one is HIV-positive, then in a single act of unprotected vaginal intercourse, the chance of transmission from the man to the woman is one in 300 and from the woman to the man, possibly as low as one in 1,000. This level of risk contrasts sharply with the much greater likelihood of infection during unprotected anal intercourse; when sharing needles during drug use; or from a transfusion of infected blood. These means of transfer are sufficiently risky to sustain an epidemic among small segments of a population—among homosexual men and intravenous drug users, for example. But these methods cannot sustain a society-wide epidemic in the manner that heterosexual transmission would allow.

Despite our initial skepticism, evidence for

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

a heterosexual epidemic in Africa is convincing. The most careful studies have shown that the infection rate among females is probably 1.2 times higher than the infection rate among males and that most HIV-positive women caught the virus from their spouse. In the West, however, the number of infected men (who are more likely than women to contract HIV from intravenous drug use or homosexual intercourse) is Þve to 10 times greater than the number of women.

Additional studies have ruled out other typical methods of transmission in most of Africa. For instance, we have found that because anal intercourse is considered abhorrent for a variety of reasons, including its connection with witchcraft, the practice is almost completely suppressed in much of sub-Saharan Africa. Furthermore, intravenous drugs are seldom used there: marijuana is widely consumed, but injected opiates are too expensive for these impoverished societies.

Many researchers in the West have assumed that the heterosexual AIDS epidemic reßects unusual sexual practices that facilitate transmission. But by global standards, sexual activity in sub-Saharan Africa is fairly simple: even in commercial sex, there is little foreplay or violence that can cause bleeding. We did worry, however, that perhaps other traditions might make even straightforward intercourse unusually dangerous. For example, in some parts of Africa, women apply astringents such as alum (ßong used in the West to dry blood from shaving cuts), cloth or leaves to dry their vaginas, according to local traditional male demands. Vaginal drying is also used to remove discharge caused by infections, which occur frequently in tropical situations where hygiene is difficult and medication rare. Such methods of drying can scratch or alter the vaginal wall, making it more susceptible to bleeding during intercourse and possibly rendering HIV infection more likely. Yet we have not found any evidence linking these practices with an increased risk for contracting HIV.

Role of Circumcision Suggested

Although we were initially stymied in our search for what might be fueling the heterosexual epidemic, we found a new lead in 1989. A joint Canadian-Kenyan medical research team working at Kenyatta Medical School in Nairobi, where the epidemic is intense, had reported a year earlier that AIDS rates were higher among Luo migrants from western Kenya than among the Kikuyu from central Kenya. At Þrst, the researchers assumed that the Luo ethnic group was at greater risk of the disease because they migrated from an area that is close to Uganda, which the researchers believed might be the epicenter of the HIV epidemic.

But as the epicenter idea became less tenable, the researchers proposed an alternative explanation: they surmised that the Luo were at greater risk because, unlike the Kikuyu, they were not circumcised. Apparently, uncircumcised Luo men were more likely to have chancroid Ñ a sexually transmitted disease characterized by soft sores on the genitals Ñ or syphilis. These men also had an unexpectedly elevated risk of contracting HIV [see box on page 18]: in the capital city of Nairobi, a Luo man with chancroid who had sex once with an HIV-positive prostitute had a 50 percent chance of contracting the virus.

Drawing on these Þndings, in 1989 an American team led by John Bongaarts of the Population Council published a paper showing that the regions across sub-Saharan Africa with high levels of HIV infection among local peoples corresponded remarkably well with the areas where men were typically uncircumcised. The next year Stephen Moses and his colleagues from the Canadian-Kenyan group completed their own analysis of the AIDS epidemic and arrived at the same conclusion.

Surprisingly, the publication of these two papers did not excite much interest. The World Health OrganizationÕs Global Program on AIDS emphasized that Moses and his team could not point to any physiological mechanism to explain how lack of circumcision was implicated in an increased risk of HIV infection; the research simply demonstrated a statistical correlation between circumcision, chancroid or syphilis, and HIV infection. Consequently, other researchers gave the Þnding little weight.

Instead many AIDS experts seemed to assume that African conditions and behavior were so Òbad,Ó in some unspeciÞed way, that the disease would spread inexorably throughout the continent. But we felt that this explanation was wrong. We believed that we could establish what made HIV transmission so common in Africa, particularly in the AIDS belt. Was it lack of circumcision, or something else? Our team redoubled its efforts to examine the culture of sub-Saharan Africa, looking for any traditions that might account for the rampant spread of heterosexually transmitted AIDS cases.

Background Research in Nigeria

We decided to look Þrst at the country of Nigeria, outside the AIDS belt, where we have been working over a 30-year period, studying the very traditions and sexual behavior that might affect AIDS transmission. According to established thinking about sexually transmitted diseases, much of the sexual behavior in Nigeria Ñ where most men and many women have multiple sex partners, for example Ñ seemed to make the country a prime candidate for a heterosexual epidemic of AIDS. Yet the percentage of HIV-positive people (0.5 percent of the population) is the same as in the U.S. and only slightly above that in other Western countries. Perhaps our work in Nigeria could help us isolate unique conditions found in the AIDS belt that contribute to the extraordinary epidemic there.

For reasons embodied in traditional culture and religion, and probably associated with certain types of farming, land tenure and inheritance, sub-Saharan African culture has always placed greater emphasis on fertility than on the repression of womenÕs premarital and extramarital sexuality [see ÒHigh Fertility in Sub-Saharan Africa,Ó by John C. Caldwell and Pat Caldwell; SCIENTIFIC AMERICAN , May 1990]. InÞdelity might occasionally spark Þghts, punishment and, more rarely, marital dissolution, but it was never equated with sin and excoriated in the way that it was in traditional Western and Asian societies. Much good ßowed from this permissive attitude: women were not suppressed and hidden, and girls had survival chances as great as their brothersÕ. But eventually these cultural traditions and attitudes did make the societies susceptible to attack by sexually related diseases.

Our work in Nigeria showed that in the southern part of the country, most married men with one wife and more than 25 percent of married men with multiple wives had experienced extramarital sexual relations within the previous year; 75 percent of single men had had relations with at least two women in that time. In addition, around 30 percent of married women had had intercourse with at least two men in the previous year, as had 50 percent of all single women. Translated into terms of the number of lifetime partners, these Þndings are similar to the results of recent social surveys of the U.S., Britain and France, although in these countries, a greater proportion of partnerships occurred before marriage. Apparently, even though sexuality is becoming a bit more liberated in Western societies, the culture is still more successful in discouraging extramarital sex. In Western countries, heterosexual HIV transmission remains low, however, because of better prevention measures.

Sexually transmitted diseases and AIDS spread rapidly among people with many sexual partners, poor facilities to combat infection and lack of access to preventive means, such as condoms. Not surprisingly, we found such illnesses as gonorrhea and, to some extent, syphilis to be common in Nigeria; chancroid, however, is almost unknown there. Somehow the country was not suffering the kind of AIDS epidemic raging elsewhere. Throughout the parts of sub-Saharan Africa that lay outside the AIDS belt, men and women had multiple sex partners, untreated sexually transmitted diseases were widespread, and a signiÞcant proportion of males visited prostitutes, among whom levels of HIV infection were sufficient to start

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

AIDS in Sub-Saharan Africa

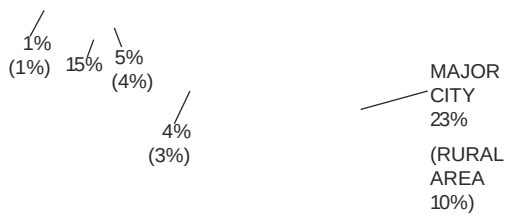
The impact of AIDS on the continent of Africa has been anything but uniform. In the stretch of countries known as the AIDS belt (large map on this page), nearly 25 percent of the urban population is infected with HIV, the AIDS-causing virus. Yet in other regions, the rate of infection is as low as 1 percent (map A), comparable to numbers seen in some Western countries. To account for the extraordinary epidemic in the AIDS belt, we examined a variety of factors to determine what customs were both common throughout this region and unique to it and thus might explain the severity of

THE AIDS BELT

PERCENTAGE OF POPULATION WITH HIV

HIGHEST PERCENTAGE OF HIV CASES (SHOWN IN ALL MAPS)

A



POTENTIAL EXPLANATIONS FOR AIDS EPIDEMIC

B

AIDS BELT: CENTRAL AFRICAN REPUBLIC, SOUTHERN SUDAN, UGANDA, KENYA, RWANDA, BURUNDI, TANZANIA, ZAMBIA, MALAWI, ZIMBABWE, BOTSWANA

ABIDJAN IN IVORY COAST

MALI, BURKINA FASO, PARTS OF IVORY COAST, GHANA

CHAD, CAMEROON, EQUATORIAL GUINEA, GABON, CONGO, ZAIRE, ANGOLA, NAMIBIA, SOUTH AFRICA, SWAZILAND, LESOTHO, MOZAMBIQUE

SENEGAL, GAMBIA, GUINEA-BISSAU, GUINEA, SIERRA LEONE, LIBERIA, NIGER, TOGO, BENIN, NIGERIA

HIGH LEVELS OF STERILITY CAUSED BY WIDESPREAD SEXUALLY TRANSMITTED DISEASES

C

LATE AGE AT MARRIAGE

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

REGIONS WHERE MOST MEN ARE UNCIRCUMCISED

MEN TYPICALLY UNCIRCUMCISED

CITIES WHERE MEN
ARE TRADITIONALLY
CIRCUMCISED BUT WHERE LARGE
POPULATIONS
OF UNCIRCUMCISED MEN HAVE
RECENTLY MIGRATED; HIGH HIV LEVELS

MEN WERE NOT CIRCUMCISED
UNTIL RECENTLY

the disease there.

For example, high levels of sexually transmitted diseases (map B) often signify regions where sexual behavior is particularly risky (people have multiple partners or engage in sex with prostitutes). Also, premarital and extramarital sex—generally more conducive to spreading such diseases than sex within marriage—tend to be more common where people marry late (map C) or where men marry more than one wife (map D), thus forcing some men to stay single for many years. Sex outside marriage can be less common where women's sexual behavior is not restricted after giving birth (map E). Matrilineal societies or communities that allow women to participate freely in commerce foster independence in women, which may in turn promote premarital and extramarital sex (map F).

Most of the ideas we investigated failed to explain the extraordinarily high rate of infection in the AIDS belt. One factor did stand out, however: lack of male circumcision (large map on this page). In the area where men are typically uncircumcised, HIV rates are among the highest in the AIDS belt. Furthermore, the migration of uncircumcised men from this area to cities outside the region of rampant infection has led to exceedingly high numbers of AIDS cases in many urban locales as well. —J.C.C. and C.P.

D

HIGHEST LEVELS
OF POLYGYNY

E

SHORTEST
OF POSTPARTUM ABSTINENCE

F

WOMEN ALLOWED TO PARTICIPATE
IN COMMERCE; MAINTAIN SEPARATE BUDGETS FROM
HUSBANDS

MATRILINEAL SOCIETIES

LAURIE GRACE AND PETE SAMEK

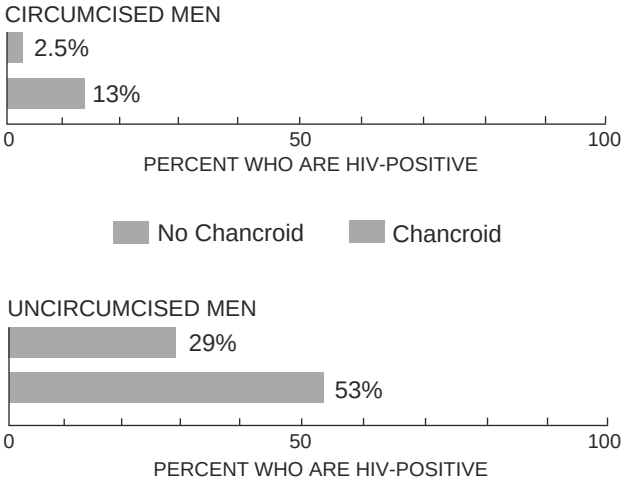
COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Circumcision, Chancroid and AIDS

So far no one has been able to determine precisely how lack of circumcision may make some men more susceptible to HIV infection. But current thinking emphasizes both the distal part of the foreskin and the likely importance of penile hygiene. Certain sexually transmitted diseases, particularly chancroid, which causes large, soft sores on the genitals, tend to occur more frequently among uncircumcised men in poor areas where maintaining personal cleanliness is difficult. Chancroid disappeared in the West around the beginning of this century, apparently as the society became more affluent, making hygiene easier to maintain.

Studies of American and Australian soldiers during the Vietnam and Korean wars support the connection between lack of circumcision, poor hygiene and high levels of chancroid. In a 1952 study of U.S. soldiers in Korea, for instance, Joseph Asin of the U.S. Army wrote that the men were more susceptible to chancroid infection as a result of inadequate cleaning, Òespecially washing the genitals with soap and water following exposure.Ó For uncircumcised men, thorough cleaning of the genitals can be particularly challenging. Only about 33 percent of U.S. soldiers in Korea were uncircumcised, but 96 percent of those infected with chancroid were uncircumcised.

Recent research in Kenya has demonstrated that uncircumcised men with chancroid are at greater risk for HIV [see graphs at left]. Still, these surveys cannot determine whether lack of circumcision itself makes men more susceptible to AIDS or whether lack of circumcision promotes chancroid infection, which then catalyzes transmission of HIV. We suspect that both scenarios account for high levels of HIV infection in the AIDS belt. Once men are infected with chancroid, they are at greater risk of contracting HIV because the presence of genital sores makes transmission during intercourse more likely. In contrast to healthy foreskin, infected foreskin can be penetrated more easily by HIV. J.O. and J.C.



SOURCE: F. A. Plummer et al. in AIDS and Women's Reproductive Health, *edited by L. C. Chen et al. Plenum Press, 1991.*

JESSIE NATHANS

an epidemic. But the disease had reached a crisis level only in the AIDS belt. What was the additional factor that characterized this area?

Rejecting Other Options

Along with other groups, we explored a variety of hypotheses. Perhaps AIDS might spread along routes of unusually high levels of sexually transmitted diseases, which tend to be most pervasive where risky sex practices, such as sex with prostitutes or multiple partners, are rampant. A test was at hand. Immediately to the west of Uganda and Rwanda, but outside the AIDS belt, lies an area known as the worldÕs major infertility belt. In this region, an epidemic of sexually transmitted diseases, persisting for more than a century, has devastated the population, leaving many people sterile. SigniÞcantly, though, despite the extreme levels of certain sexually transmitted diseases, few people in this region suffer from chancroid, and the prevalence of AIDS here is, at most, moderate.

We looked next at the custom of scariÞcation. In this practice, found widely throughout Africa, incisions made on the limbs or face are believed to prevent or cure illness. In some areas, all members of a household where sickness has occurred are scariÞed one after another with the same instruments, so the possibility of contaminating one person with another Õs blood is high. But this practice is not popular throughout the AIDS belt, nor is it unique to it. Thus, scariÞcation could not explain the epidemic.

We then tried to determine what might prompt men to resort to sexÑand particularly commercial sexÑoutside marriage. For example, in societies where men marry more than one wife, there are typically large numbers of single men seeking sex; for many years, these men are simply unable to Þnd any available women to wed. The practice of polygyny, however, is more common outside the AIDS belt. We also looked at average male age at the time of marriageÑtheorizing that the longer a man remained unmarried, the longer, and the more frequently, he might resort to visiting prostitutesÑbut this idea, too, failed to provide an explanation.

We examined the duration of abstinence among women after they give birth: in some regions, men have no sexual access to their wives for up to 60 percent of their married life because of traditions regarding childbirth and sexual relations. But in certain parts of the AIDS belt, postpartum abstinence is rather brief compared with the practice elsewhere in Africa, reducing the time when husbands can turn only to extramaritalÑand hence riskyÑsex.

We followed up a host of other ideas: The tradition of young men participating in warrior societies, which tend to be critical of premarital pregnancies, might encourage men to visit prostitutes, among whom accidental pregnancies would have little impact on the men involved. The custom of demanding large bride-prices makes parents more worried about a marriage arrangement falling apart if the bride becomes pregnant before marriage; such concern might also prompt men to visit prostitutes before marriage. Female autonomy, found in matrilineal societies or reßected by womenÕs participation in commerce and trade, might foster womenÕs sexual independence from their husbands, possibly resulting in risky extramarital sex on the part of both husbands and wives. Populations with more men than women (most frequently found in urban areas as a result of immigration) might also promote prostitution. But none of these situations were both common throughout the AIDS belt and unique to itÑwe still had not isolated the factor that was promoting the rapid heterosexual transmission of HIV.

Back to Circumcision

In frustration, we turned once again to the earlier research that suggested a connection between lack of male circumcision and the

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

spread of AIDS. We updated our maps with more recent data on levels of HIV infection and noted that the areas of Africa with large numbers of uncircumcised men were almost exactly the same as the regions suffering from the severe AIDS epidemic (see box on pages 16 and 17). But other researchers had discounted these reports, claiming that the original source of the circumcision data was out-of-date (the information on menÕs circumcision status was Þrst presented in Ethnographic Atlas, by George P. Murdock of the University of Pittsburgh, published in 1967) or that the analysis of the proposed relationship was faulty. And some critics felt that Þndings were merely coincidental. Further complicating the debate was the controversy in the Western medical profession about whether circumcision was meaningless mutilation; many did not wish to revive that discussion. In addition, some health administrators feared that linking circumcision status, susceptibility to AIDS and ethnicity might lead to greater ethnic hostility rather than improved AIDS-prevention strategies.

Over the past three years, however, we have reexamined the methodology of the papers as well as the anthropological sources and determined that the Þndings are sound. Also, in continuing investigations we have found very little support for the charge that the circumcision data are no longer relevant. The link between lack of circumcision and elevated levels of HIV infection appears robust. In some parts of the AIDS belt, nearly all men are uncircumcised Ñ a situation unlike almost anywhere else in Africa. Outside the AIDS belt, in the city of Abidjan, the capital of Ivory Coast, levels of HIV infection are as high as they are in some cities of the AIDS belt; we believe the epidemic in Abidjan is very likely sustained by immigrants who come from a surrounding area where a majority of men are uncircumcised.

Thus, we concluded that in the AIDS belt, lack of male circumcision in combination with risky sexual behavior, such as having multiple sex partners, engaging in sex with prostitutes and leaving chancroid untreated, has led to rampant HIV transmission. Unsafe sexual practices have certainly contributed to the spread of AIDS across Africa and indeed around the world. But risky behavior alone cannot sustain the epidemic seen in the AIDS belt.

Caution is warranted regarding the connection between male circumcision and AIDS. Lack of circumcision seems primarily to amplify the dangers of unsafe sexual practices: men in Asia, for example, are frequently uncircumcised, but the region has not seen the number of AIDS cases that Africa has, probably because of different sexual customs. And circumcision is not an absolute protection against AIDS; circumcised men are certainly still at risk. Everywhere in sub-Saharan Africa, even where all men are circumcised, levels of HIV infection are high among prostitutes and their customers. The epidemic in this group could potentially serve as a starting point for the virus to move into other communities.

Combating AIDS Globally

Because the virus progresses through different segments of the population at markedly distinct rates, the best chances for combating AIDS everywhere lie in targeting education and prevention programs at high-risk groups Ñ homosexuals, prostitutes and their clients, intravenous drug users, and promiscuous men and women. In sub-Saharan Africa, circumcision could be offered as a reinforcement of other protective measures.

Universal male circumcision, which is likely to prove unacceptable, would nevertheless probably reduce the level of infection in the AIDS belt to the much lower numbers seen in the rest of sub-Saharan Africa. But eradication of the disease will require a multipronged assault: people must be encouraged to have fewer partners and practice safer sex. Improved treatment for sexually transmitted diseases, particularly chancroid, is also crucial.

There are signs of change in East Africa. Average numbers of sexual partners appear to be falling. Women in Uganda, for example, are offering alternatives to vaginal sex or refusing to have unsafe sex. And condom use has become much more accepted. No doubt as a result, the disease appears to be slowing in Uganda.

In rural southwest Tanzania, and surely elsewhere in the AIDS belt, uncircumcised men have not waited for agreement among researchers about the connection between circumcision and AIDS. Based on observations of their community and neighboring ones, they have concluded that they are at greater risk for AIDS than circumcised men. These men are appearing at hospitals in sharply increasing numbers, requesting circumcision for themselves and often for their sons. Clinics that offer adult male circumcision as a protection against AIDS now advertise in Tanzanian newspapers.

Thankfully, ethnic rivalries do not appear to have worsened as a result of this issue. If health authorities state that lack of circumcision appears to make men more susceptible to HIV infection, it now seems probable that some men will seek circumcision. At the same time, however, medical experts will have to stress to everyone that circumcision is not in itself protection against AIDS. Although the epidemic in sub-Saharan Africa may subside somewhat, because of greater use of condoms and probably increased incidence of circumcision, Africans in the AIDS belt remain at extremely high risk of HIV infection.

For AIDS programs worldwide, the sub-Saharan epidemic demonstrates an important lesson. Because a number of unusual conditions must combine to sustain a society-wide heterosexual epidemic, it seems unlikely that in the West, AIDS will spread signiÞcantly beyond high-risk groups such as homosexual men and intravenous drug users. Consequently, prevention programs Ñ particularly those in Asia, where the epidemic may worsen Ñ should be addressed speciÞcally to people who are most likely to contract HIV. Such efforts should slow the ongoing epidemic in Africa and prevent similar conditions from developing elsewhere. SA

The Authors

JOHN C. CALDWELL and PAT CALDWELL work at the Health Transition Center of the National Center for Epidemiology and Population Health at the Australian National University in Canberra. Both John Caldwell, a demographer, and Pat Caldwell, an anthropologist, are principal investigators in the Swedish Agency for Research Cooperation project on sexual networking, sexually transmitted disease and HIV in sub-Saharan Africa. Between 1970 and 1988 John Caldwell served as head of the Australian National UniversityÕs department of demography. He is also adjunct professor at Harvard UniversityÕs department of population sciences and international health.

Further Reading

THE RELATIONSHIP BETWEEN MALE CIRCUMCISION AND HIV INFECTION IN AFRICAN POPULATIONS. John Bongaarts, Priscilla Reining, Peter Way and Francis Conant in AIDS, Vol. 3, No. 6, pages 373Ð377; 1989.

Geographical Patterns of Male Circumcision Practices in Africa: Association with HIV Seroprevalence. Stephen Moses, Janet E. Bradley, Nico J. D. Nagelkerke, Allan R. Ronald, J. O. Ndinya-Achola and Francis A. Plummer in International Journal of Epidemiology, Vol. 19, No. 3, pages 693Ð697; September 1990.

SEXUAL NETWORKING AND AIDS IN SUB-SAHARAN AFRICA: BEHAVIOURAL RESEARCH AND THE SOCIAL CONTEXT. Edited by I. O. Orubuloye, John C. Caldwell, Pat Caldwell and Gigi Santow. Australian National University, 1994.

Forum: The East African AIDS Epidemic and the Absence of Male Circumcision: What is the Link? In Health Transition Review, Vol. 5, No. 1, pages 97Ð117; April 1995.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Originally published in
September 1997

In Search of AIDS-Resistance Genes

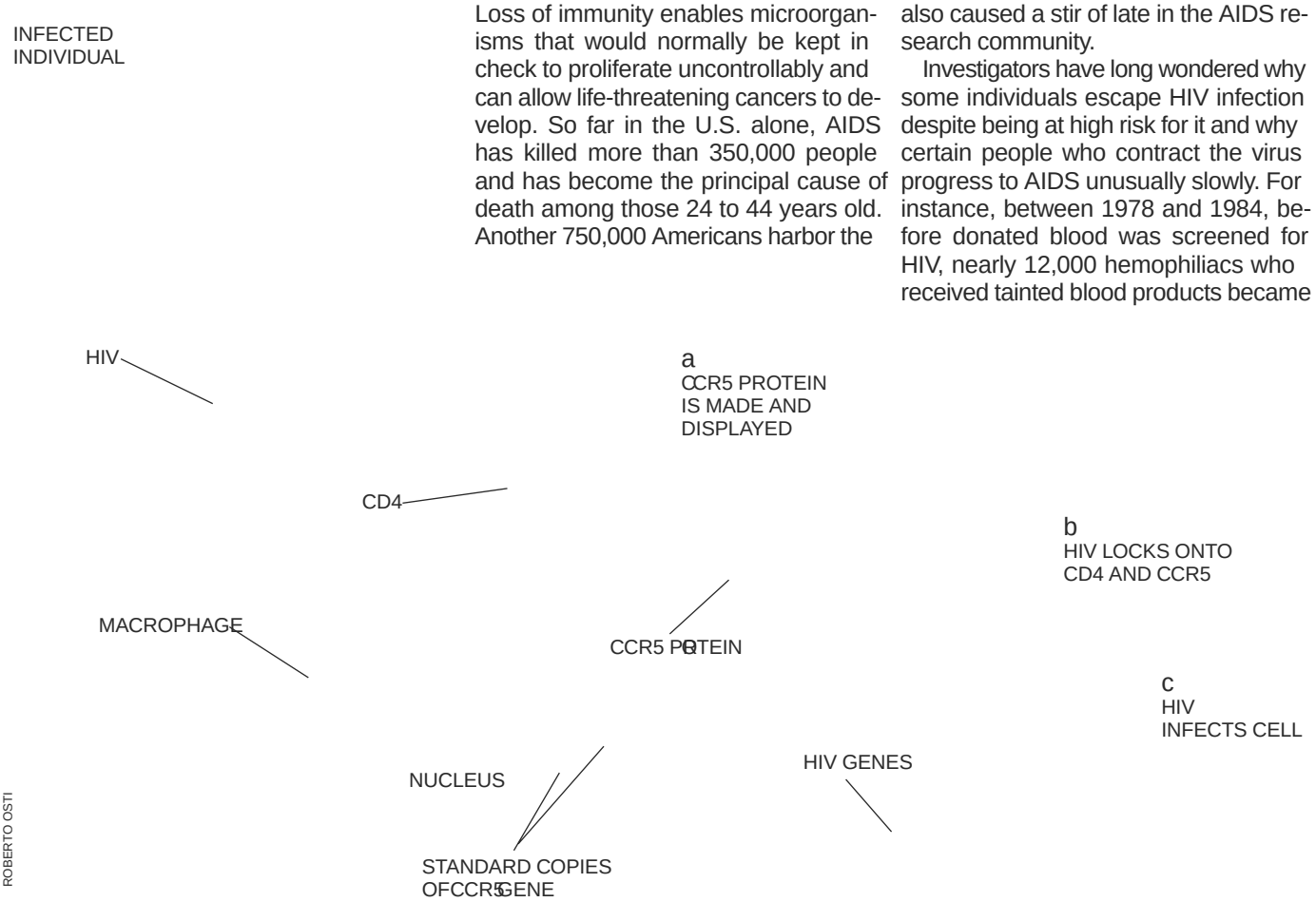
A genetic trait that protects against AIDS has now been uncovered, and others are emerging. The Þndings open entirely new avenues for developing preventives and therapies

by Stephen J. OÕBrien and Michael Dean

Most people today are all too familiar with the devastating effects of HIV, the human immunodeÞciency virus. The virus, which is transmitted by blood-to-blood contact, may produce no symptoms for years. But typically within 10 to 15 years it destroys key cells of the immune system and causes AIDS (acquired immunodeÞciency syndrome). Loss of immunity enables microorganisms that would normally be kept in check to proliferate uncontrollably and can allow life-threatening cancers to develop. So far in the U.S. alone, AIDS has killed more than 350,000 people and has become the principal cause of death among those 24 to 44 years old. Another 750,000 Americans harbor the virus, part of some 30 million who are affected worldwide.

In the past few years, advances in drug therapy have enabled a number of patients to cheat death. Sophisticated combinations of medicines have diminished the levels of virus in the body and restored immune function. Those feats have been justiÞably well publicized, but Þndings less known to the public have also caused a stir of late in the AIDS research community.

Investigators have long wondered why some individuals escape HIV infection despite being at high risk for it and why certain people who contract the virus progress to AIDS unusually slowly. For instance, between 1978 and 1984, before donated blood was screened for HIV, nearly 12,000 hemophiliacs who received tainted blood products became



ROBERTO OSTI

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

infected, but 10 to 25 percent of the recipients evaded the virus. And about 1 percent of individuals who carry HIV remain relatively healthy, with few or no symptoms and with adequate immune functioning, for atypically long spans of 15 years or more.

The recent Þndings reveal that some people who are partly or fully resistant to HIV infection owe their good fortune to their genesÑ or, more precisely, to possession of a particular variant of a gene involved in immunologic function. This discovery has already sparked intensive efforts to translate the new genetic understanding into innovative strategies for preventing and controlling HIV infection. (We should note that we are using the term ÒHIVÓ to mean HIV-1, the virus responsible for most AIDS worldwide. Another form, HIV-2, causes AIDS more slowly and is restricted to certain parts of Africa; genetic resistance to HIV-2 has not yet been studied.)

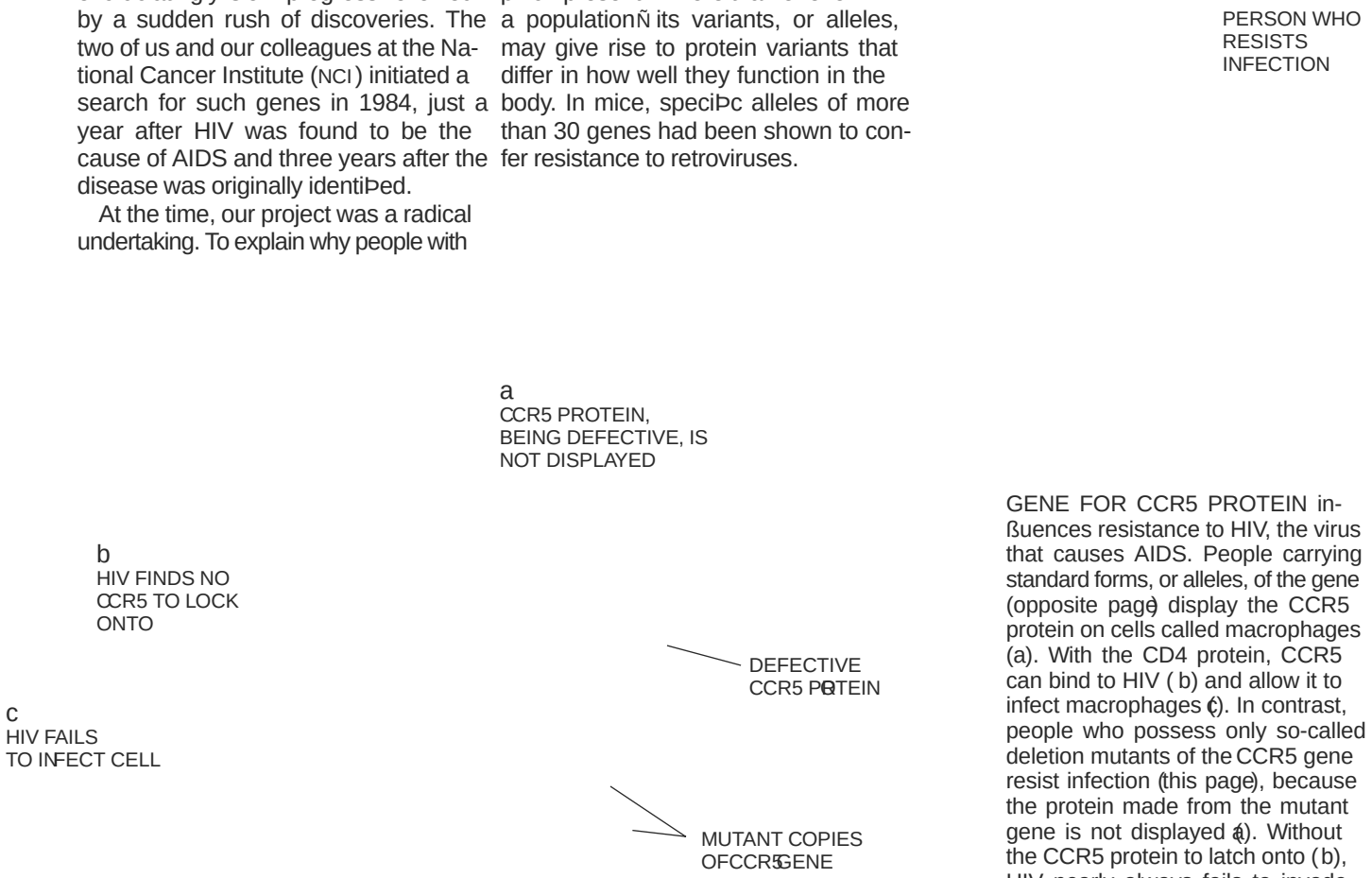
Precedents in Animals

The story of how the Þrst HIV-resistance gene was unmasked is one of excruciatingly slow progress followed by a sudden rush of discoveries. The two of us and our colleagues at the National Cancer Institute (NCI) initiated a search for such genes in 1984, just a year after HIV was found to be the cause of AIDS and three years after the disease was originally identiÞed.

At the time, our project was a radical undertaking. To explain why people with equal exposure to HIV could have different fates, most workers in the 1980s focused on genetic characteristics of the virus (such as variations in the virulence of different strains) or on nongenetic Òco-factorsÓ that might inßuence the disease-causing power of the virus (such as infection of the host by another microbe). And we had little solid evidence that humans could possess genetic protection from AIDS. Indeed, certain of our colleagues doubted we would Þnd anything on our genetic ÒÞshingÓ expedition, a hunt on which we were wagering considerable time and resources.

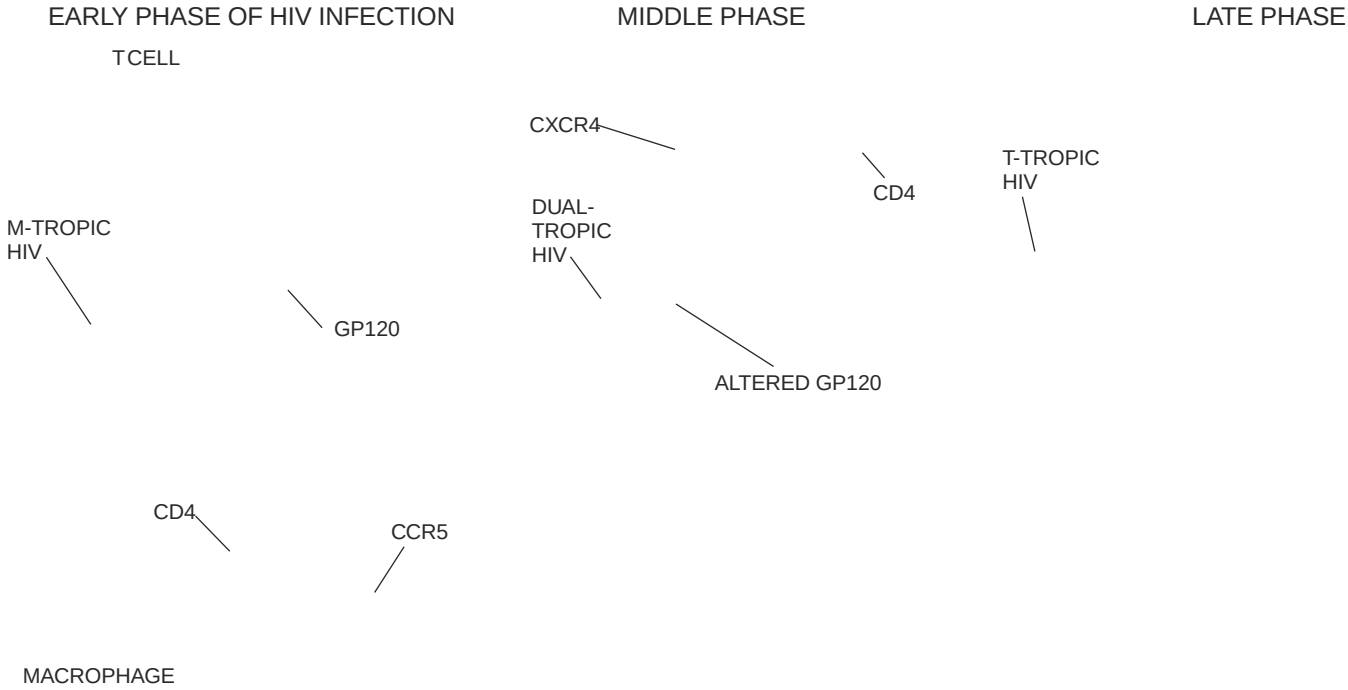
Yet we were not operating blindly. Research in animals had clearly established that genes often affect the acquisition and development of infections, especially those caused by retroviruses, the family that includes HIV. Most genes serve as blueprints for proteins, the molecules that perform the majority of activities in cells. When a protein-coding gene is switched on, its sequence of building blocks, or DNA nucleotides, is used as a guide for stringing together the unique sequence of amino acids in the speciÞed protein. If the gene is polymorphicÑ present in more than one form in a populationÑ its variants, or alleles, may give rise to protein variants that differ in how well they function in the body. In mice, speciÞc alleles of more than 30 genes had been shown to confer resistance to retroviruses.

Other animal work had also demonstrated a genetic component to infectious disease. Inbred mice, rats and livestock are notoriously sensitive to communicable disorders, mainly because inbreeding leaves them with a limited repertoire of disease-resistance alleles. In outbred groups, some fraction of a population is likely to have an allele that protects against a given pathogen; that allele will enable its owners to survive an epidemic and perpetuate the group.



GENE FOR CCR5 PROTEIN inßuences resistance to HIV, the virus that causes AIDS. People carrying standard forms, or alleles, of the gene (opposite page) display the CCR5 protein on cells called macrophages (a). With the CD4 protein, CCR5 can bind to HIV (b) and allow it to infect macrophages (c). In contrast, people who possess only so-called deletion mutants of the CCR5 gene resist infection (this page), because the protein made from the mutant gene is not displayed (a). Without the CCR5 protein to latch onto (b), HIV nearly always fails to invade macrophages (c).

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.



ROBERTO OSTI

HIVÕS AFFINITY FOR IMMUNE CELLS changes over time inside infected patients. Initially the virus is ÒM-tropicÓ (left): it favors macrophages, which it invades by binding (through its gp120 protein) to the molecules CD4 and CCR5 on the macrophage surface. Eventually, however, HIV can become Òdual-tropicÓ (center). Such strains produce gp120 molecules able to recognize the CXCR4 protein on CD4-bearing T cells and may infect both macrophages and T cells. Still later, the bulk of the viral population can switch its preference to the CXCR4 receptor and become ÒT-tropicÓ (right). T-tropic viruses readily destroy infected T cells and thereby contribute to the collapse of the immune system and the onset of AIDS.

Because human populations are genetically diverse, we suspected that they, like other outbred species, possessed many powerful disease-resistance alleles. Those alleles, perhaps including defenses against HIV, simply remained to be discovered.

Further, although few pathogen-resisting alleles had been deÞned convincingly in humans, several epidemiological studies had noted a strong genetic inßuence on disease susceptibility. For instance, one analysis showed that if a biological parent of an adoptee died of an infectious disease before age 50, the adoptee had a markedly increased risk of also dying from an infection.

Unfortunately, science had provided no simple blueprint for Þnding HIV-resistance alleles in humans. We therefore combined knowledge and techniques from three disparate disciplines: AIDS epidemiology, human molecular genetics and population genetics theory.

High-Tech Gene Prospecting

First, we needed a source of genes from the populations of interest to us, such as individuals at high risk for HIV infection who did or did not become infected after exposure to the virus. If the two groups differed in their genetic makeupÑ in their alleles for speciÞc genesÑ we would suspect that the genes displaying the variation inßuenced susceptibility to HIV infection.

To obtain human DNA for study, we joined forces with public health epidemiologists who were trying to track the pattern of the still new epidemic. As part of that effort, the epidemiologists were enlisting cohorts, or groups of several hundred individuals, at high risk for HIV infection Ñ notably, homosexual men, users of intravenous drugs and hemophiliacs who had received contaminated blood products. These cohorts were to be monitored for years by physicians, who (with the patientsÕ permission) would supply blood, tissue samples and case reports to researchers. As blood was collected, our cell biology team, led by Cheryl Winkler, carefully produced immortal lines of cultured cells that would provide an unlimited supply of DNA for genetic testing.

To determine which genes to compare, we took advantage of recent advances in gene mapping, a set of procedures that pinpoints the location of genes on chromosomes and determines their nucleotide sequences. More than 6,000 of the approximately 50,000 to 100,000 genes in human chromosomes have now been mapped. Back in 1984 fewer than 1,000 had been found. Nevertheless, to test even 1,000 genes in our AIDS cohorts was an impossible task.

We narrowed the choice by drawing on established knowledge of how retroviruses behave in their hosts. The host is always an unsuspecting collaborator in establishing infection and enabling pathogens to spread through tissues. To enter cells, all viruses must recognize (bind to) certain proteins encoded by host genes and displayed on the cell. These proteins normally act as receptors for other host molecules, but viruses can co-opt the receptors, using them as springboards for entry into a cell.

Once in a cell, retroviruses insidiously insert their genes into a hostÕs chromosomes. They thereby ensure that viral genesÑ which can direct the synthesis of an endless supply of viral particlesÑ are

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

passed to each new generation of cells whenever the initial host cell replicates. Here, again, the viruses require help from the host. They must recruit several cellular enzymes to splice viral genes into chromosomes, to produce fresh viral particles and even to evade the hostÕs immune defenses.

With such understanding to guide us, we originally decided to concentrate on about 50 genes whose proteins could potentially inßuence HIVÕs life cycle. We also examined 250 polymorphic (variable) DNA segments that had been identiÞed in chromosomal sites between genes. If our subjects differed in these segments, those differences would indicate that alleles of nearby genes might also vary systematically between the groups. We could then perform a fairly narrow search for those genes and try to determine their function in cells and their role in HIV infection.

Finally, to pinpoint genetic traits that confer resistance to HIV, we borrowed strategies from human population genetics. We divided each cohort into two groups, according to selected aspects of their healthÑ for example, those infected with HIV versus those who remained free of it after extensive exposure; infected patients who progressed to AIDS rapidly versus those who progressed slowly if at all; or infected patients who acquired a speciÞc AIDS-related disease (such as *Pneumocystis cariniipneumonia* or KaposiÕs sarcoma) versus those who did not.

Having made these divisions, we compared how often each known allele or polymorphic segment appeared in the groups. We also compared what are called genotypes. An individual inherits two copies of all genes outside the sex chromosomes (one copy from the mother and one from the father). The pair of alleles at a particular chromosomal locus, or gene address, constitutes the genotype. Someone who inherits two identical alleles of a given gene is said to be a homozygote; someone who inherits two distinct alleles is said to be a heterozygote. In our screening tests, we noted the percentage of patients in each group who were homozygous for a known allele and the percentage of patients that were heterozygous. Appreciable differences in allele or genotype frequencies, or both, in two subject groups would indicate that the gene under study probably accounted for the divergent fates of the subject groups.

For years we continued to add more patients, more genes, more polymorphic segments and more sophisticated computer programs to analyze the data. Periodically, we thought we noted genetic differences, but they nearly always evaporated under close inspection. Meanwhile we monitored the many advances in understanding of human immunology and in the behavior of HIV in the body, always seeking ideas for other genes to study. Late in 1995 and early in 1996Ñ more than a decade after we began this massive and tedious effortÑ cracks Þnally appeared in the dike.

Good Clues, at Last

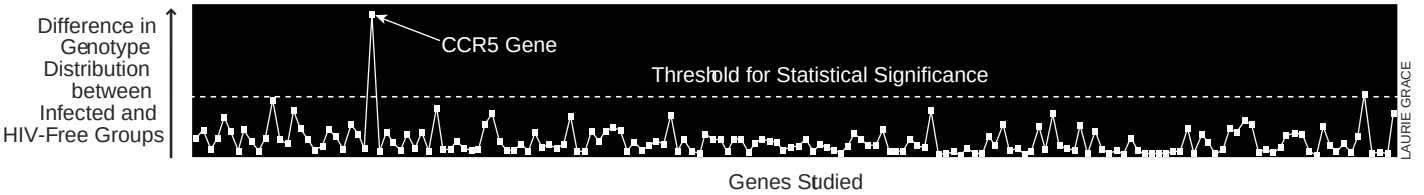
Those cracks were created by other research teams who resolved two long-standing mysteries relating to HIVÕs molecular interaction with host cells. With those solutions came clues to genes involved in resistance to HIV.

By the mid-1990s scientists and nonscientists alike were well aware that HIV caused immune deÞciency mainly by depleting white blood cells known as T lymphocytes that displayed a protein called CD4 on their surface. These T cells normally orchestrate many aspects of the immune response to viruses. It was also known, albeit less widely, that HIV can infect and persist for years in another class of CD4-carrying immune cells called macrophages. HIV does not destroy macrophages and Þnds a safe haven in them.

The CD4 molecules on T lymphocytes and on macrophages usually participate in signaling between immune cells. But when HIV enters the picture, CD4 molecules bind to a sugary protein (gp120) protruding from HIVÕs outer envelope and, in so doing, help the virus to gain entry into the bound cells. Yet experiments had shown that CD4, though necessary for HIV inÞltration of cells, was not sufÞcient; the cells also had to display at least one more protein to which the virus could bind. More than 10 years after the discovery of HIV, however, scientists still had no clue to the nature of that second receptor.

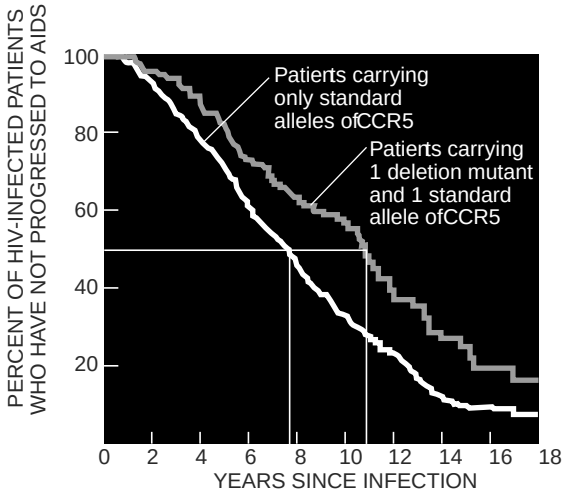
The other puzzle related to a discovery reported in 1986 by Jay A. Levy of the University of California at San Francisco. He found that a class of T lymphocytes displaying a different protein Ñ CD8Ñ secreted molecules, termed suppressive factors, that blocked HIV from invading normally susceptible cells in culture. Suppressive factors that limited virus infection had also been shown to exist in African monkeys that harbored SIV (the simian form of HIV) yet did not advance to AIDS, as well as in people who survive HIV infection for an unusually long time. The identity of these sundry suppressive factors remained to be determined, however.

	2 Copies of Standard CCR5 Allele	2 Copies of Mutant CCR5 Allele	1 Standard and 1 Mutant CCR5 Allele
HIV-Infected Individuals	85 percent	0 percent	15 percent
Uninfected Individuals	83 percent	3 percent	14 percent



DIFFERENCES IN THE GENETIC PROFILE of two populations (graph) implicated the CCR5 gene in resistance to HIV infection. The authors Þrst identiÞed the genotypes, or combinations of alleles, for each of 170 genes in a group of HIV-infected patients and, separately, in a group of uninfected individuals. For instance, they measured the percentages of people having two copies of the normal CCR5 allele, two copies of the truncated, deletion mutant, or one copy of each (table). Then, for every gene, they plotted the statistical difference (dots) in the genotype distribution between the two populations under study. Of all the genes that were examined, only the difference for the CCR5 gene turned out to be highly signiÞcant.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.



LAURIE GRACE

COMPARISON of how long HIV-infected individuals lived without progressing to AIDS revealed that patients harboring one deletion mutant of the CCR5 gene (green line) avoided AIDS longer than patients carrying only standard CCR5 alleles (yellow line). For instance, it took about 11 years for 50 percent of the first group to advance to AIDS but about eight years for half of the second group to reach that point.

In December 1995 Robert C. Gallo, then at the NCI, and other collaborators announced that they had identified three related suppressive factors that could block infection by HIV variants that prefer to colonize macrophages (so-called M-tropic strains). All three factors turned out to be known chemokines: short strings of amino acids responsible for luring immune cells to injured or diseased tissues.

Many investigators still grappling with the first puzzle—the search for HIV's second receptor—understood that chemokines work their effects on defensive cells by binding to surface proteins. It seemed possible that the chemokines isolated by Gallo's group—named RANTES, MIP-1 and MIP-1 —might interfere with HIV entry into immune cells by binding to and blocking some cell surface protein that HIV required for access to the interior. In other words, the cell-surface receptor (or receptors) for Gallo's chemokines could well lead a double life as the second receptor for HIV on macrophages and perhaps on other CD4-bearing cells.

The notion defied immediate testing because the cellular receptor for RANTES and its cousins had not yet been isolated. But discoveries reported early in 1996 made such tests possible and provided us, and others, with new genes to screen as resistance factors.

First, Edward A. Berger and his colleagues at the National Institute of Allergy and Infectious Diseases isolated the second receptor for HIV variants that prefer to colonize T lymphocytes (T-tropic strains). It was a chemokine receptor, albeit one (now called CXCR4) that bound a chemokine distinct from RANTES, MIP-1 and MIP-1 . If Gallo's findings had not convinced AIDS researchers that chemokine receptors played a part in HIV infectivity, Berger's results drove the point home.

Almost simultaneously, Michael Samson and Marc Parmentier of the Free University of Brussels and their collaborators isolated the gene for a receptor onto which RANTES, MIP-1 and MIP-1 all hook when they draw defensive cells to damaged tissue. Within two months, five separate groups proved that the encoded protein, now known as CCR5, was also the elusive second receptor for M-tropic strains of HIV.

Combined with observations from other studies, the new chemokine receptor findings critically refined understanding of how HIV infections become established and progress. HIV initiates infection by setting up residence primarily in macrophages. It enters these cells by linking its gp120 protein with two receptors on macrophages: CD4 and CCR5. Once inside the macrophages, HIV synthesizes large quantities of virus and challenges the immune system to its limits.

Years later the constantly mutating virus can alter the gene for gp120 in a way that causes the gp120 protein to change its second-receptor allegiance. The genetic change causes the region that recognizes CCR5 to bind more effectively to CXCR4 on T lymphocytes. Now the HIV population becomes dominated by T-tropic variants—those preferring to infect T cells.

This shift in attraction soon becomes deadly, because T-tropic viruses kill the cells they infect. Not surprisingly, the shift is often followed swiftly by an overall drop in CD4 T cell concentrations in patients and, simultaneously, by the onset of the opportunistic infections and cancers that for many years defined progression to AIDS. Today the Centers for Disease Control and Prevention formally defines AIDS by the presence of AIDS-defining illnesses or by a drop in CD4 T cells to fewer than 200 per cubic millimeter of blood; normal levels are about 1,000 per cubic millimeter.

The Expedition Succeeds

As soon as we knew that CCR5 and CXCR4 were co-receptors for HIV, we immediately decided to see whether the genes for those proteins affected resistance to HIV in our cohorts. To pursue this idea, we had to determine whether the CCR5 and CXCR4 genes were polymorphic. If everyone had identical versions of those genes, the genes could not account for differences in susceptibility to HIV.

All copies of the CXCR4 gene we examined were the same. But in July 1996 Mary Carrington of our group discovered that a major variant of the normal CCR5 gene occurred in about one in five individuals. Comparisons of the nucleotide sequences of the two CCR5 alleles revealed that the less common one was missing 32 nucleotides. Because of the way the genetic code works, we knew that the loss would result in the premature creation of a "stop" code in the gene and would, in turn, cause the cells to manufacture a severely foreshortened version of the CCR5 protein.

When we divided nearly 2,000 high-risk patients into infected and noninfected groups and compared their CCR5 genotypes, we found dramatic differences. Some 3 percent of the noninfected individuals carried only the deletion mutant of CCR5 in their cells (that is, were homozygous for the mutant). In contrast, not one patient out of 1,343 in the infected group was homozygous for the deletion mutant. The difference—which indicated homozygosity for the deletion mutant was protective against HIV—was highly significant statistically and was certainly no coincidence.

Moreover, the apparent protection provided by having solely mutant CCR5 alleles did not depend on the route of infection: no hemophiliacs, homosexuals or drug users who were homozygous contracted HIV. We suspected that homozygosity for the deletion mutant shielded patients because they manufactured only truncated CCR5 proteins that either failed to reach the cell surface or were so deformed that they could not dock with HIV.

Within a few weeks after submitting a paper on these remarkable findings to

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

The HIV-resistance allele, or deletion mutant, of the CCR5 gene is not distributed equally among the worldÕs peoples. It is virtually absent in African and eastern Asian populations and in Native Americans and is rare in African-Americans [see second column in table below]. It is, however, fairly prevalent among Caucasians (descendants of the early settlers of Europe and western Asia).

Yet even among Caucasians the distribution varies. A plot of the alleleÕs frequency among Caucasians in Eurasia [see map below] reveals a gradient, or cline, that is highest in the north and drops to an undetectable level in Saudi Arabia. The frequency is calculated by counting the number of mutant copies in a population and dividing by the total of all CCR5 copiesÑthe sum of mutant and standard copies combined.

These patterns answer some questions and raise others about the origin and prevalence of the mutant, which codes for a defective CCR5 protein. The apparent absence of the mutant in Africa indicates that it arose some time after humans left AfricaÑa split widely believed to have taken place 130,000 to 200,000 years ago. But what caused the deletion mutant to reach such a high frequency in Caucasians, and when did that event occur?

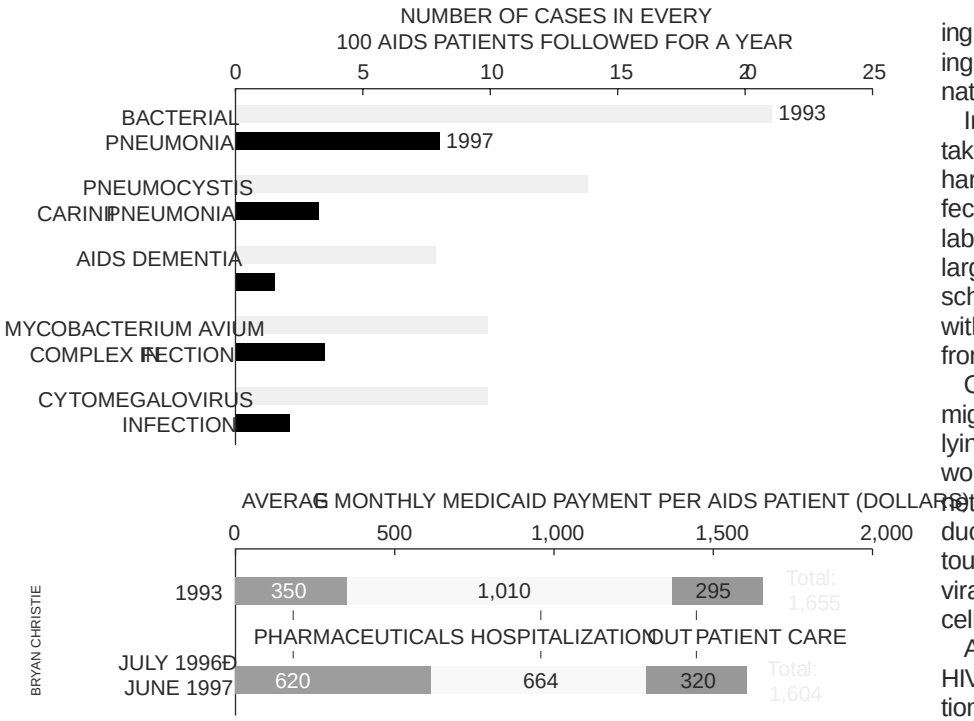
The surprisingly high frequency of the allele in parts of Europe and Asia suggests that some devastating event in these locales gave originally rare individuals who harbored the mutant a dramatic survival advantage. Those individuals then lived to reproduce, causing the fraction of the population bearing the allele to become larger than before. As survivors of this historic cataclysm procreated, the CCR5 mutation persisted and accumulated to higher levels.

We suspect that the catastrophic event was a major epidemic caused by an agent that, like HIV, makes use of the normal CCR5 protein, but not the defective form, to infect cells. This hypothesis makes sense to us because rare alleles often become more common in animals after they provide resistance against a newly encountered pathogen. And the longer the epidemic lasts, the higher the allele frequency rises.

We also think the Eurasian epidemic occurred very long ago. Indeed, using genetic dating methods, we have estimated that the catastrophic challenge struck about 4,300 years ago and certainly no more recently than 1,200 years ago. The mutant is less common in southern Eurasia than in the north, perhaps because the eye of the epidemic was concentrated in the

-0.eTc -mmd.3.5oonc1j atf1oojece5isa4rs n.(m,)06e -01-0.2(ar)4M4
1.1579tant isivru g6.5(ru g)5.5(ot)5aajor ep1792ivCr Tca0095 TTw sio

the journal Science, we learned we were not alone in searching for polymorphisms in chemokine receptors. Nathaniel R. Landau and Richard A. Koup of the Aaron Diamond AIDS Research Center in New York City and their co-workers had independently discovered the same 32-base-pair deletion allele. They had been studying a group of homosexual men who had many high-risk sexual exposures to HIV but had never become infected. Examination of white blood cells from two of these men indicated that the CCR5 protein was absent from the cell surface. A look at the nucleotide sequence of the CCR5 genes revealed that both men were homozygous for the deletion mutant. Further, in other work, Samson and ParmentierÕs team had searched for and failed to turn up any homozygotes for the dele-



VALUE OF AGGRESSIVE HIV THERAPY, which came into wide use in 1996, is reflected in declining frequencies of several opportunistic infections in AIDS patients (as defined by CD4 T cell levels) at the HIV clinic of Johns Hopkins University (top). Although costs per patient did not decline much (bottom), less money had to be spent on hospital care—another sign that patients were healthier.

Because current treatment plans seem unlikely to cure HIV infection within several years and because of HAART's various drawbacks—including side effects, complicated regimens and resistance—the search is on for additional drugs. The first entries to reach the market will add new choices within existing drug classes, and some will combine existing drugs into a single pill or will otherwise reduce the complexity or toxicity of current regimens. And at least two custom-designed protease inhibitors in clinical trials appear to work against strains of HIV that are resistant to existing protease inhibitors.

Other Therapeutic Ideas

Other agents under consideration would stall HIV replication in new ways. Some would block the integrase enzyme from inserting HIV DNA into a cell's chromosomes. A different strategy attempts to knock zinc out of a protein that needs the metal in order to draw HIV RNA into new particles.

Teams are also exploring ways to stop infected cells from making critical viral proteins. One such approach deploys so-called antisense DNA to inactivate two genes (tat and rev) that normally give rise to proteins needed for the efficient manufacture of other viral proteins. In rhesus macaques exposed to the simian version of HIV, this therapy limited viral replication and CD4 T cell depletion.

Many investigators are attempting to interdict HIV's entry into cells. Recall that to fuse with, and gain entry into, a target cell, the virus must attach both to CD4 and to a co-receptor on the cell surface. Initial attempts to interfere with binding to CD4 were disappointing, but new possibilities have recently come to light, and many groups are examining compounds that might sheath the HIV-docking site on co-receptors to keep HIV at bay [see "In Search of AIDS-Resistance Genes," by Stephen J. O'Brien and Michael Dean; Scientific American, September 1997].

A number of scientists are focusing on the other part of the equation: the immune system. They are trying to augment the body's remaining forces or to restore lost powers. For instance, some patients are receiving low doses of a biological molecule called interleukin-2, which enhances the proliferation of T lymphocytes. It is also hoped that interleukin-2 can help force immature "stem cells" to spawn a full repertoire of fresh new cells of the immune system, including T lymphocytes and antibody-producing cells that can recognize and eliminate HIV.

Immune-reconstitution efforts are taking additional forms as well—such as harvesting stem cells from an HIV-infected patient, multiplying them in the laboratory and then returning the enlarged cell population. A twist on this scheme would supply the infused cells with a gene that would protect them from later succumbing to HIV.

Other approaches being pondered might kill HIV-infected cells without relying on the immune system. One would deliver a virus that had been genetically altered to enter only HIV-producing cells, leaving HIV-free cells untouched. It would enter by attaching to viral proteins displayed on a colonized cell and would then destroy the cell.

As time goes by, patients infected with HIV are sure to have more and more options for therapy. Specific recommendations may change from time to time, but the principles of therapy, now grounded in solid research, will remain steadfast: until there is a cure or a vaccine, controlling HIV replication offers the best chance for a long, productive life.

The fact that such insight and the tools to suppress HIV reproduction are available is both astonishing and wonderful. The therapeutic advancement achieved since late 1995 has few parallels in the history of medicine, save perhaps for the revolution sparked by the introduction of penicillin. Just three years ago those of us who cared for HIV patients mainly devoted our time and energy to easing symptoms and preparing patients to die. Now we help patients to live. The war against HIV is far from over, but the success of aggressive therapy is certainly a victory to be savored. SA

The Authors

JOHN G. BARTLETT and RICHARD D. MOORE are colleagues at the Johns Hopkins University School of Medicine. Bartlett is professor of medicine, chief of the division of infectious diseases and director of the AIDS service at the university. He is also co-chair of the panel that wrote and updates the U.S. guidelines for using anti-HIV drugs in adults and adolescents. Moore is associate professor of internal medicine, infectious diseases and clinical pharmacology at Johns Hopkins and is director of the program in pharmacoepidemiology and pharmacoeconomics there.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Originally published in
June 2002

HOPE IN A VIAL

Will there be an AIDS vaccine anytime soon?

By Carol Ezzell

It wasn't supposed to be this hard.

When HIV, the virus responsible for AIDS, was first identified in 1984, Margaret M. Heckler, then secretary of the U.S. Department of Health and Human Services, predicted that a vaccine to protect against the scourge would be available within two years. Would that it had been so straightforward.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

Roughly 20 years into the pandemic, 40 million people on the planet are infected with HIV, and three million died from it last year (20,000 in North America). Although several potential AIDS vaccines are in clinical tests, so far none has lived up to its early promise. Time and again researchers have obtained tantalizing preliminary results only to run up against a brick wall later. As recently as two years ago, AIDS researchers were saying privately that they doubted whether even a partially protective vaccine would be available in their lifetime.

No stunning breakthroughs have occurred since that time, but a trickle of encouraging data is prompting hope to spring anew in the breasts of even jaded AIDS vaccine hunters. After traveling down blind alleys for more than a decade, they are emerging battered but not beaten, ready to strike out in new directions. "It's an interesting time for AIDS vaccine research," observes Gregg Gonsalves, director of treatment and prevention advocacy for Gay Men's Health Crisis in New York City. "I feel like it's Act Two now."

In the theater, Act One serves to introduce the characters and set the scene; in Act Two, conflict deepens and the real action begins. Act One of AIDS vaccine research debuted HIV, one of the first so-called retroviruses to cause a serious human disease. Unlike most other viruses, retroviruses insinuate their genetic material into that of the body cells they invade, causing the viral genes to become a permanent fixture in the infected cells and in the offspring of those cells. Retroviruses also reproduce rapidly and sloppily, providing ample opportunity for the emergence of mutations that allow HIV to shift its identity and thereby give the immune system or antiretroviral drugs the slip.

Act One also spotlighted HIV's opposition—the body's immune response—which consists of antibodies (Y-shaped molecules that stick to and tag invaders such as viruses for destruction) and cytotoxic, or killer, T cells (white blood cells charged with destroying virus-infected cells). For years after infection, the immune system battles mightily against HIV, pitting millions of new cytotoxic T cells against the billions of virus particles hatched from infected cells every day. In addition, the immune system deploys armies of antibodies targeted at HIV, at least early in the course of HIV infection, although the antibodies prove relatively ineffectual against this particular foe.

As the curtain rises for Act Two, HIV still has the stage. Results from the first large-scale trial of an AIDS vaccine should become available at the end of this year, but few scientists are optimistic about it: a preliminary analysis suggests that it works poorly. Meanwhile controversy surrounds a giant, U.S.-government-sponsored trial of another potential vaccine slated to begin this September in Thailand. But waiting in the wings are several approaches that are causing the AIDS research community to sit up and take notice. The strategies are reviving the debate about whether, to be useful, a vaccine must elicit immune responses that totally prevent HIV from colonizing a person's cells or whether a vaccine that falls somewhat short of that mark could be acceptable. Some scientists see potential value in vaccines that would elicit the kinds of immune responses that kick in soon after a virus establishes a foothold in cells. By constraining viral replication more effectively than the body's natural responses would, such vaccines, they argue, might at least help prolong the lives of HIV-infected people and delay the onset of the symptomatic, AIDS phase of the disease.

In the early 1990s scientists thought they could figure out the best vaccine strategy for preventing AIDS by studying long-term nonprogressors, people who appeared to have harbored HIV for a decade or more but who hadn't yet fallen ill with AIDS. Sadly, many of the nonprogressors have become ill after all. The key to their relative longevity seems to have been "a weakened virus and/or a strengthened immune system," says John P. Moore of Weill Medical College of Cornell University. In other words, they were lucky enough to have encountered a slow-growing form of HIV at a time when their bodies had the ammunition to keep it at bay.

Not Found in Nature?

AIDS VACCINE developers have struggled for decades to find the "correlates of immunity" for HIV—the magic combination of immune responses that, once induced by a vaccine, would protect someone against infection. But they keep coming up empty-handed, which leaves them with no road map to guide them in the search for an AIDS vaccine. "We're trying to elicit an immune response not found in nature," admits Max Essex of the Harvard School of Public Health. As a result, the quest for an AIDS vaccine has been a bit scattershot.

To be proved useful, a candidate AIDS vaccine must successfully pass through three stages of human testing. In phase I, researchers administer the vaccine to dozens of people to assess its safety and to establish an appropriate dose. Phase II involves hundreds of people and looks more closely at the vaccine's immunogenicity, its ability to prompt an immune response. In phase III, the potential vaccine is given to thousands of volunteers who are followed for a long time to see whether it protects them from infection. Phase III trials for any drug tend to be costly and difficult to administer. And the AIDS trials are especially challenging because of an ironic require-

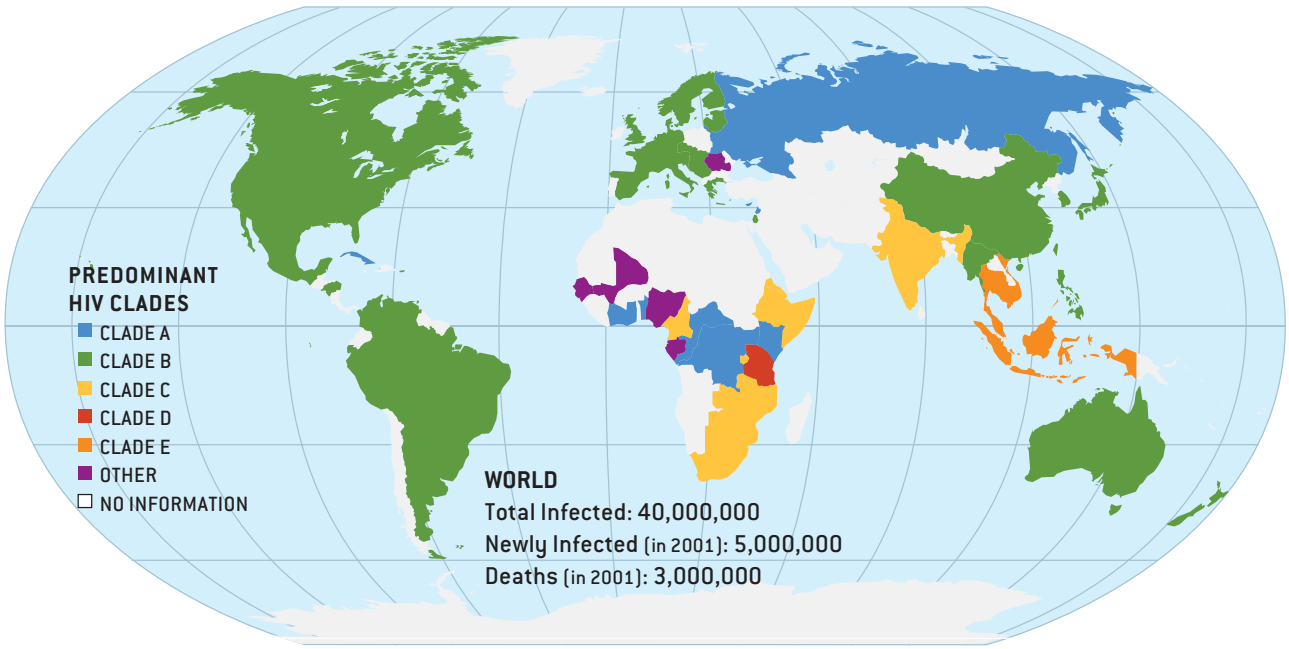
Overview/*AIDS Vaccines*

- Final results from the first large-scale test of a possible AIDS vaccine will be available at the end of this year, but few researchers are optimistic it will work.
- Scientists are now aiming to generate potential AIDS vaccines that stimulate both arms of the immune system: killer cells and antibodies.
- There are five main subtypes, or clades, of HIV. Researchers are debating whether it will be important to devise vaccines for a given area based on the predominant clade infecting that area.

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

WORLD AIDS SNAPSHOT

MOST OF THE GLOBE'S 40 million people infected with HIV live in sub-Saharan Africa and South and Southeast Asia, as reflected in the ranking below, which is based on 2001 data from the Joint United Nations Program on HIV/AIDS. There are five major strains of HIV, which are also called clades. Although more than one clade can usually be found in any given area, the map highlights the predominant clade affecting each region. The boundaries between prevailing clades are not exact; they change frequently.



1 SUB-SAHARAN AFRICA
Total Infected: 28,100,000
Newly Infected: 3,400,000
Deaths: 2,300,000

2 SOUTH/SOUTHEAST ASIA
Total Infected: 6,100,000
Newly Infected: 800,000
Deaths: 400,000

3 LATIN AMERICA
Total Infected: 1,400,000
Newly Infected: 130,000
Deaths: 80,000

4 EAST ASIA/PACIFIC IS.
Total Infected: 1,000,000
Newly Infected: 270,000
Deaths: 35,000

5 E. EUROPE/C. ASIA
Total Infected: 1,000,000
Newly Infected: 250,000
Deaths: 23,000

6 NORTH AMERICA
Total Infected: 940,000
Newly Infected: 45,000
Deaths: 20,000

7 WESTERN EUROPE
Total Infected: 560,000
Newly Infected: 30,000
Deaths: 6,800

8 N. AFRICA/MIDDLE EAST
Total Infected: 440,000
Newly Infected: 80,000
Deaths: 30,000

9 CARIBBEAN
Total Infected: 420,000
Newly Infected: 60,000
Deaths: 30,000

10 AUSTRALIA/NEW ZEALAND
Total Infected: 15,000
Newly Infected: 500
Deaths: 120

LAURIE GRACE; SOURCES: UNAIDS (*statistics*) AND VADIM ZALUNIN *Los Alamos National Laboratory* (*clade boundaries*)

ment: subjects who receive the vaccine must be counseled extensively on how to reduce their chances of infection. They are told, for instance, to use condoms or, in the case of intravenous drug users, clean needles because HIV is spread through sex or blood-to-blood contact. Yet the study will yield results only if some people don't heed the counseling and become exposed anyway.

The first potential vaccine to have reached phase III consists of gp120, a protein that studs the outer envelope of HIV and that the virus uses to latch onto and infect cells. In theory, at least, the presence of gp120 in the bloodstream should activate the recipient's immune system, causing it to quickly mount an attack targeted to gp120 if HIV later finds its way into the body.

This vaccine, which is produced by VaxGen in Brisbane, Calif.—a spin-off of biotech juggernaut Genentech in South San Francisco—is being tested in more than 5,400 people (mostly homosexual men) in North America and Europe and in roughly 2,500 intravenous drug users in Southeast Asia. The results from the North American/European trial, which began in 1998, are expected to be announced near the end of this year.

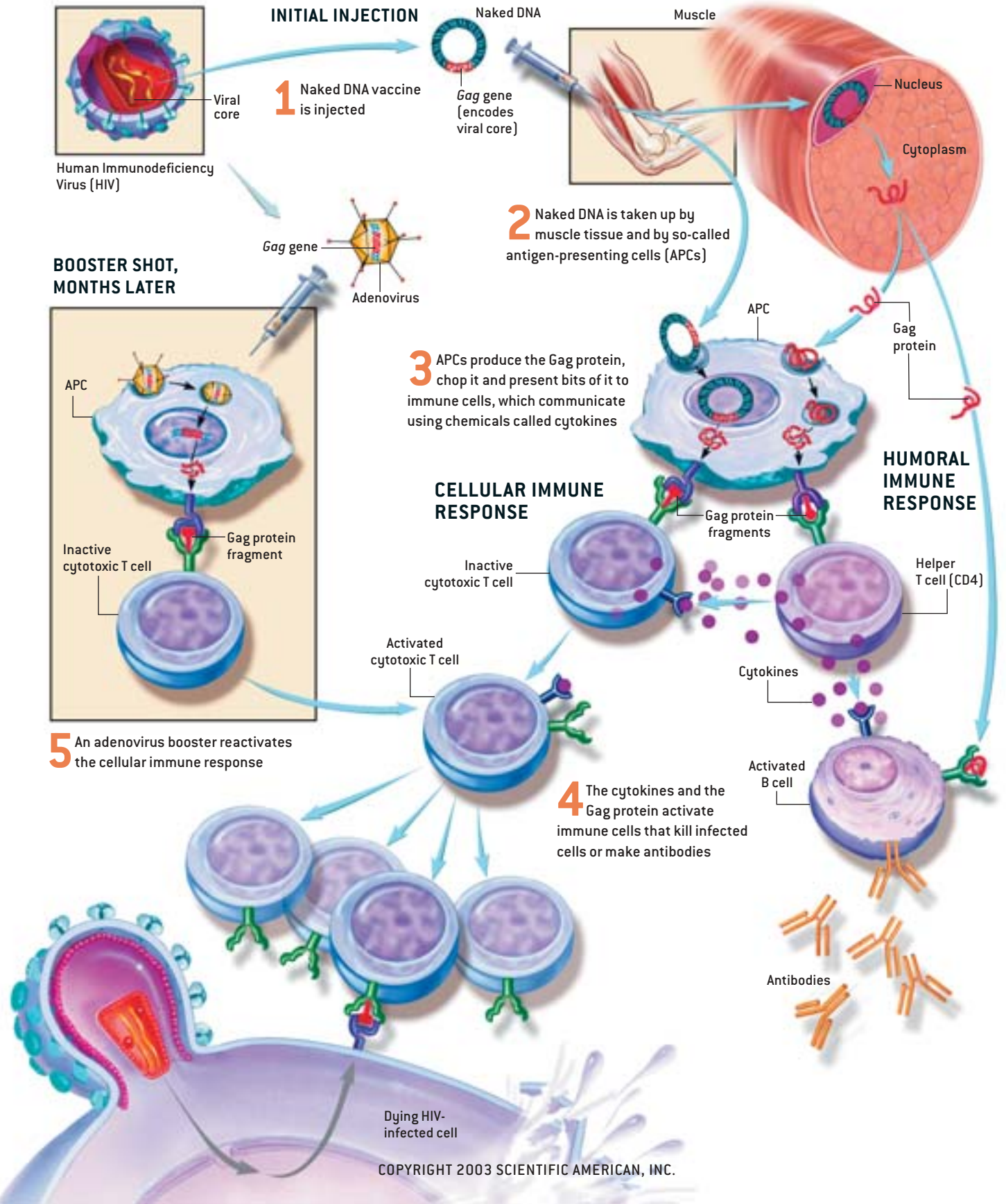
Many AIDS researchers are skeptical of VaxGen's approach because gp120 normally occurs in clumps of three on the surface of the virus, and the company's vaccine employs the molecule in its monomeric, or single-molecule, form. Moreover, vaccines made of just protein generally elicit only an antibody, or humoral, response, without greatly stimulating the cellular arm of the immune system, the part that includes activity by cytotoxic T cells. A growing contingent of investigators suspect that an antibody response alone is not sufficient; a strong cellular response must also be elicited to prevent AIDS.

Indeed, the early findings do not seem encouraging. Last October an independent data-monitoring panel did a preliminary analysis of the results of the North

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

One AIDS Vaccine Strategy

A VACCINE APPROACH being pioneered by Merck involves an initial injection of a naked DNA vaccine followed months later by a booster shot of crippled, genetically altered adenovirus particles. Both are designed to elicit an immune response targeted to the HIV core protein, Gag, and to primarily arouse the cellular arm of the immune system—the one that uses cytotoxic T cells to destroy virus-infected cells. The naked DNA vaccine also results in the production of antibody molecules against Gag, but such antibodies are not very useful in fighting HIV.



TERESE WINSLOW

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

American/European data. Although the panel conducted the analysis primarily to ascertain that the vaccine was causing no dangerous side effects in the volunteers, the reviewers were empowered to recommend halting the trial early if the vaccine appeared to be working. They did not.

For its part, VaxGen asserts that it will seek U.S. Food and Drug Administration approval to sell the vaccine even if the phase III trials show that it reduces a person's likelihood of infection by as little as 30 percent. Company president and co-founder Donald P. Francis points out that the first polio vaccine, developed by Jonas Salk in 1954, was only 60 percent effective, yet it slashed the incidence of polio in the U.S. quickly and dramatically.

This approach could backfire, though, if people who receive a partially effective AIDS vaccine believe they are then protected from infection and can engage in risky behaviors. Karen M. Kuntz and Elizabeth Bogard of the Harvard School of Public Health have constructed a computer model simulating the effects of such a vaccine in a group of injection drug users in Thailand. According to their model, a 30 percent effective vaccine would not slow the spread of AIDS in a community if 90 percent of the people who received it went back to sharing needles or using dirty needles. They found that such reversion to risky behavior would not wash out the public health benefit if a vaccine were at least 75 percent effective.

The controversial study set to begin in Thailand is also a large-scale phase III trial, involving nearly 16,000 people. It combines the VaxGen vaccine with a canarypox virus into which scientists have stitched genes that encode gp120 as well as two other proteins—one that makes up the HIV core and one that allows it to reproduce. Because this genetically engineered canarypox virus (made by Aventis Pasteur, headquartered in Lyons, France) enters cells and causes them to display fragments of HIV on their surface, it stimulates the cellular arm of the immune system.

Political wrangling and questions over its scientific value have slowed widespread testing of the gp120/canarypox vaccine. Initially the National Institute of Allergy and Infectious Diseases (NIAID) and the U.S. Department of Defense were scheduled to conduct essentially duplicate trials of the vaccine. But NIAID pulled the plug on its trial after an examination of the data from a phase II study showed that fewer than 30 percent of the volunteers generated cytotoxic T cells against HIV. And in a bureaucratic twist, this past January the White House transferred the budget for the Defense Department trial over to NIAID as part of an effort to streamline AIDS research.

Peggy Johnston, assistant director of AIDS vaccines for NIAID, says she expects there will be a trial of the vaccine but emphasizes that "it will be a Thai trial; we won't have any [NIAID] people there on the ground running things."

Critics cite these machinations as a case study of politics getting in the way of progress against AIDS. "There's little science involved" in the trial, claims one skeptic, who wonders why the Thais aren't asking, "'If it's not good enough for America, how come it's good enough for us?'" Others point out that the trial, which was conceived by the Defense Department, will answer only the question of whether the vaccine works; it won't collect any data that scientists could use to explain its potential failure.

Partial Protection

INTO THIS SCENE comes Merck, which is completing separate phase I trials of two different vaccine candidates that it has begun to test together. In February, Emilio A. Emini, Merck's senior vice president for vaccine research, wowed scientists attending the Ninth Conference on Retroviruses and Opportunistic Infections in Seattle with the company's initial data from the two trials.

The first trial is investigating a potential vaccine composed of only the HIV *gag* gene, which encodes the virus's core protein. It is administered as a so-called naked DNA vaccine, consisting solely of DNA. Cells take up the gene and use it as a blueprint for making the viral protein, which in turn stimulates a mild (and probably unhelpful) humoral response and a more robust cellular response [*see illustration on page 41*]. Emini and his colleagues reported that 42 percent of volunteers who received the highest dose of the naked DNA vaccine raised cytotoxic T cells capable of attacking HIV-infected cells.

The second trial employs the HIV *gag* gene spliced into a crippled adenovirus, the class responsible for many common colds. This altered adenovirus ferries the *gag* gene into cells, which then make the HIV core protein and elicit an immune response targeted to that protein. Emini told the conference that between 44 and 67 percent of people who received injections of the adenovirus-based vaccine generated a cellular immune response that varied in intensity according to the size of the dose the subjects received and how long ago they got their shots.

Merck is now beginning to test a combination of the DNA and adenovirus approaches because Emini predicts that the vaccines will work best when administered as part of the same regimen. "The concept," he says, "is not that the DNA vaccine will be a good vaccine on its own, but that it may work as a primer of the immune system," to be followed months later by a booster shot of the adenovirus vaccine. A possible stumbling block is that most people have had colds caused by adenoviruses. Accordingly, the immune systems of such individuals would already have an arsenal in place that could wipe out the adenovirus vaccine before it had a chance to deliver its payload of HIV genes and stimulate AIDS immunity. Increasing the dose of the adenovirus vaccine could get around this obstacle.

Emini says he and his co-workers are emphasizing cellular immunity in part because of the disappointing results so far with vaccines designed to engender humoral responses. "Antibodies continue to be a problem," he admits. "There are a handful of reasonably potent antibodies isolated from HIV-infected people, but we haven't figured out how to raise those antibodies using a vaccine."

Lawrence Corey of the Fred Hutchinson Cancer Research Center in Seattle agrees: "You'd like to have both [a cellular and an antibody response], but the greatest progress has been in eliciting a cellular response," says Corey, who is

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.

also principal investigator of the federally funded HIV Vaccine Trials Network.

Antibodies are important, too, because they are the immune system's first line of defense and are thought to be the key to preventing viruses from ever contacting the cells they infect. Corey says that vaccines that are designed primarily to evoke cellular immunity (as are Merck's) are not likely to prevent infection but should give someone a head start in combating the virus if he or she does become infected. "Instead of progressing to AIDS in eight years, you progress in 25 years," he predicts. But, Corey adds, it is unclear whether a vaccine that only slowed disease progression would stem the AIDS pandemic, because people would still be able to spread the infection to others despite having less virus in their bloodstream.

Finding a way to induce the production of antibodies able to neutralize HIV has been hard slogging for several reasons. For one, the virus's shape-shifting ways allow it to stay one step ahead of the immune response. "The thing that distinguishes HIV from all other human viruses is its ability to mutate so fast," Essex says. "By the time you make a neutralizing antibody [against HIV], it is only against the virus that was in you a month ago."

According to many scientists, vaccines using a logical molecule, gp120—the protein the virus uses to invade immune cells, as discussed above—haven't worked, probably because the antibodies that such vaccines elicit bind to the wrong part of the molecule. Gp120 shields the precise binding site it uses to latch onto CD4, its docking site on immune cells, until the last nanosecond, when it snaps open like a jackknife. One way to get around this problem, suggested in a paper published in *Science* three years ago by Jack H. Nunberg of the University of Montana and his colleagues, would be to make vaccines of gp120 molecules that have previously been exposed to CD4 and therefore have already sprung open. But those results have been "difficult to replicate," according to Corey, making researchers pessimistic about the approach.

Another possible hurdle to getting an AIDS vaccine that elicits effective anti-HIV antibodies is the variety of HIV subtypes, or clades, that affect different areas of the world. There are five major clades, designated A through E [*see illustration on page 40*]. Although clade B is the predominant strain in North America and Europe, most of sub-Saharan Africa—the hardest-hit region of the globe—has clade C. The ones primarily responsible for AIDS in South and Southeast Asia—the second biggest AIDS hot spot—are clades B, C and E.

Several studies indicate that antibodies that recognize AIDS viruses from one clade might not bind to viruses from other clades, suggesting that a vaccine made from the strain found in the U.S. might not protect people in South Africa, for example. But scientists disagree about the significance of clade differences and whether only strains that match the most prevalent clade in a given area can be tested in countries there. Essex, who is gearing up to lead phase I tests of a clade C–based vaccine in Botswana later this year, argues that unless researchers are sure that a vaccine designed against one clade can cross-react with viruses from another, they must stick to testing vaccines that use the clade prevalent in the populations being studied. Cross-reactivity could occur under ideal circumstances, but, he says, "unless we know that, it's important for us to use subtype-specific vaccines."

Using the corresponding clade also avoids the appearance that people in developing countries are being used as guinea pigs for testing a vaccine that is designed to work only in the U.S. or Europe. VaxGen's tests in Thailand are based on a combination of clades B and E, and in April the International AIDS Vaccine Initiative expanded tests of a clade A–derived vaccine in Kenya, where clade A is found.

But in January, Malegapuru William Makgoba and Nandipha Solomon of the Medical Research Council of South Africa, together with Timothy Johan Paul Tucker of the South African AIDS Vaccine Initiative, wrote in the *British Medical Journal* that the relevance of HIV subtypes "remains unresolved." They assert that clades "have assumed a political and national importance, which could interfere with important international trials of efficacy."

Early data from the Merck vaccine trials suggest that clade differences blur when it comes to cellular immunity. At the retrovirus conference in February, Emini reported that killer cells from 10 of 13 people who received a vaccine based on clade B also reacted in laboratory tests to viral proteins from clade A or C viruses. "There is a potential for a substantial cross-clade response" in cellular immunity, he says, "but that's not going to hold true for antibodies." Corey concurs that clade variation "is likely to play much, much less of a role" for killer cells than for antibodies because most cytotoxic T cells recognize parts of HIV that are the same from clade to clade.

Johnston of NIAID theorizes that one answer would be to use all five major clades in every vaccine. Chiron in Emeryville, Calif., is developing a multiclade vaccine, which is in early clinical trials. Such an approach could be overkill, however, Johnston says. It could be that proteins from only one clade would be recognized "and the other proteins would be wasted," she warns.

Whatever the outcome on the clade question, Moore of Weill Medical College says he and fellow researchers are more hopeful than they were a few years ago about their eventual ability to devise an AIDS vaccine that would elicit both killer cells and antibodies. "The problem is not impossible," he says, "just extremely difficult." SA

Carol Ezzell is a staff editor and writer.

MORE TO EXPLORE

HIV Vaccine Efforts Inch Forward. Brian Vastag in *Journal of the American Medical Association,* Vol. 286, No. 15, pages 1826–1828; October 17, 2001.

For an overview of AIDS vaccine research, including the status of U.S.-funded AIDS clinical trials, visit **www.niaid.nih.gov/daids/vaccine/default.htm**

A global perspective on the AIDS pandemic and the need for a vaccine can be found at the International AIDS Vaccine Initiative Web site: **www.iavi.org**

Joint United Nations Program on HIV/AIDS: **www.unaids.org**

COPYRIGHT 2003 SCIENTIFIC AMERICAN, INC.