# Stalking the AIDS Virus

A Killer with Many Faces

by Vin LoPresti

Developing an AIDS vaccine is "the moral obligation of our times."

An improved understanding of the interaction between HIV and the immune system has brought Lab researchers closer to identifying key parameters in AIDS vaccine development.

D iscussing her research with the compassionate zeal of one attuned to human suffering, biologist Bette Korber talks compellingly of her campaign against modern medicine's most formidable infectious adversary—the AIDS virus, HIV (human immunodeficiency virus): "Even if we fail, we have to try as hard as we can. We owe it to future generations. I do think it's the moral obligation of our times."

Illustrations by Vicente Garcia
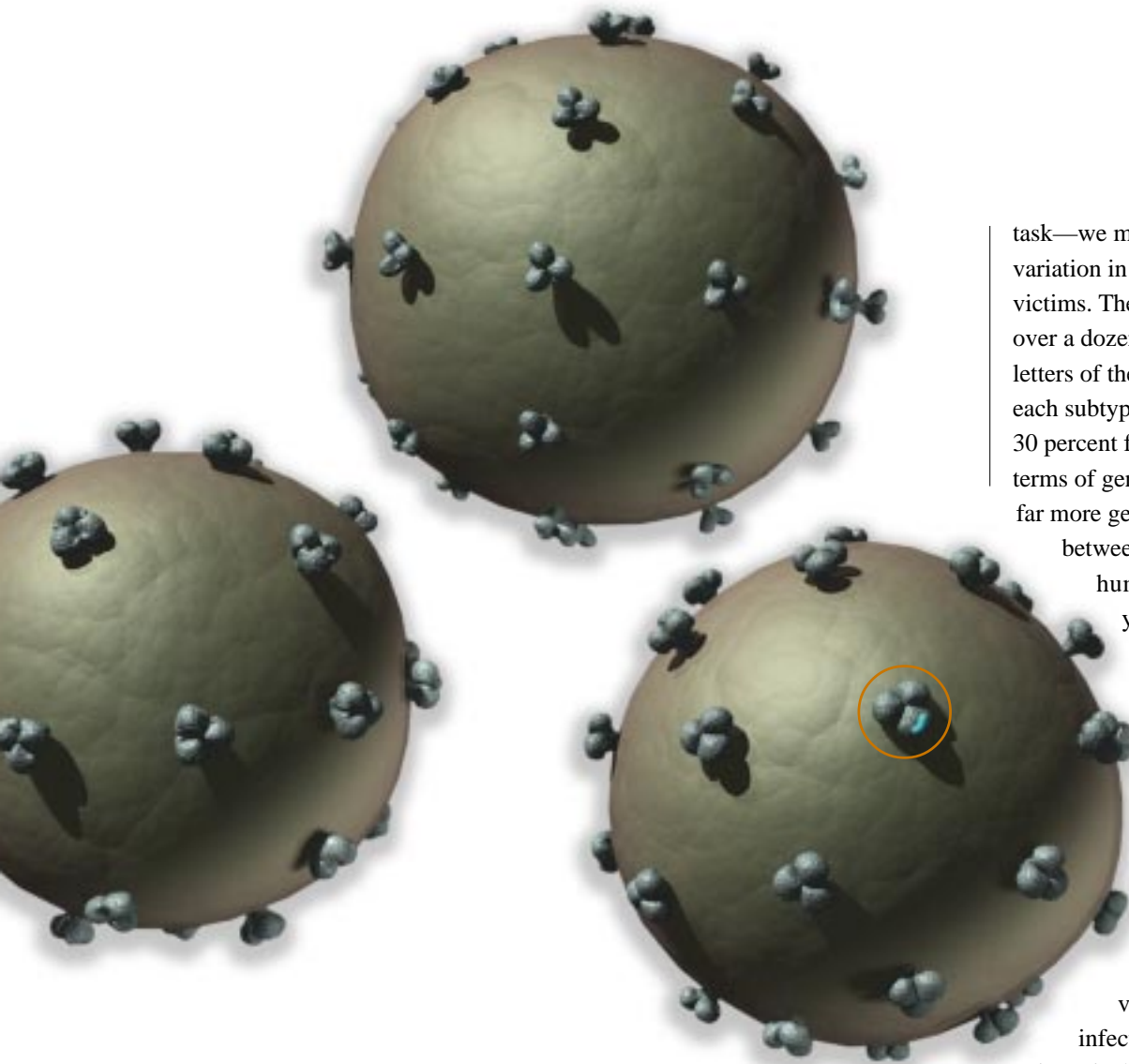
Artist's conception of three mutant forms of the AIDS virus, HIV, illustrating subtle differences in the shape of the virus' surface glycoprotein (gp120). Although similar enough in overall shape to allow all viruses to remain infectious, the surface proteins vary in their conformation (three-dimensional geometry). Similar variation is also found in the virus' internal proteins, which are not shown in this view. Each virus' outer membrane is derived from a cell that it had previously infected. As shown in the circled protein on the surface of the right-most virus, only a very small fragment of the viral protein (in blue) is ultimately recognized by cytotoxic T cells, part of the immune system's viral defense (see the illustration on page 16).

Korber is part of a growing cadre of immunology researchers who view an effective AIDS vaccine as the holy grail, and she collaborates with a diverse group of colleagues. Their recent research offers hope that important brushstrokes in the picture of HIV's interaction with the immune system are beginning to delineate a more detailed backdrop against which to intervene in the pathological drama of AIDS.

Variation in the Virus and Its Victims

To understand how HIV can so completely compromise its victims' immune systems—and why designing an effective vaccine is such a difficult task—we must appreciate the genetic variation in both the virus and its victims. The virus exists globally as over a dozen subtypes (named with letters of the alphabet), and genes in each subtypes typically differ by 25 to 30 percent from all other subtypes in terms of genetic information. This is far more genetic variation than is found between most of the genes of humans and chimpanzees; yet humans and chimps are considered separate species, while the HIV subtypes are considered parts of a single viral species. Moreover, even within the same subtype—the predominant B subtype in the United States, for example—the genetic variation between viruses infecting different individuals is typically 10 to 15 percent and steadily increasing. Such genetic variability poses an enormous challenge to both vaccine developers and to the immune system defenses of infected individuals. As a service to the AIDS research community, Korber and her Los Alamos colleagues maintain an extensive online database of the genetic sequences found in strains of these HIV subtypes (www.hiv.lanl.gov). This database is accompanied by a variety of analytical software tools and is extensively used by scientists worldwide.

For HIV victims, this viral variability is further complicated by human genetic variation. Since only identical twins have exactly the same copies (or alleles) of every human gene, the rest of us are, by definition, variant to a greater or lesser degree. With respect to HIV
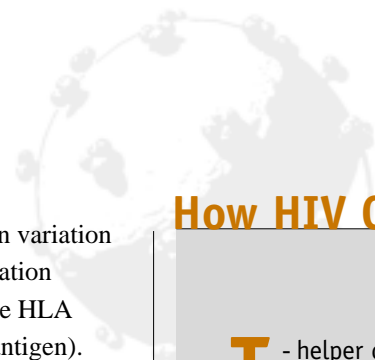
infection, the relevant human variation rests primarily in the information contained in the genes for the HLA proteins (human leukocyte antigen). These proteins form a collection of regulatory macromolecules on the surface of virtually all human cells. For example, HLA proteins have long been known as the culprits in instigating certain types of graft rejection. In the context of normal immune system function, they are key molecular intermediaries in the process by which the two major types of immune system T cells—T-helper cells and cytotoxic T cells—recognize invading pathogens such as bacteria and viruses (see the sidebar on this page).

The HIV-HLA Connection

We receive genes for two sets of HLA proteins, one set from each parent. Each set consists of proteins denoted as A, B, C, and D. Since each protein comes in many slightly different forms (or is polymorphic), each person's cell surfaces most commonly carry two distinct sets of HLA proteins. These protein sets partly define an individual's immunological uniqueness. For example, if we ignore D, whose biology is more complex, one person's cells might carry on their surfaces A1 and A24, B27 and B57, and C3 and C5. The numbers simply indicate structural variants of the A, B, and C proteins inherited from each parent. The cells of a second, biologically unrelated individual would likely carry a different set of HLA proteins (for example, A2 and A15, etc.).

Korber and her colleagues have been studying the HLA-A, -B, and -C proteins (denoted HLA Class I) because they regulate the immune system's response to viruses, including HIV.

How HIV Cripples

T - helper cells function as the central processing unit of the immune system. They respond to invading pathogens by releasing signals (cytokines) that regulate a broad spectrum of immunological functions, such as antibody production, inflammation, the activity of scavenger white blood cells, and even the production of new red blood cells. Because of its molecular structure, HIV can infect all T-helper cells, and infected T-helpers thus become reservoirs for replicating the virus—vehicles for its spread to millions of other T-helpers. As the virus spreads through the bloodstream, antibodies—which are effective only outside cells—can shield additional T-helpers from becoming infected. Unfortunately, HIV evolves so rapidly within a single individual that it evades the antibody responses that would protect new cells from infection (see the illustration on page 17). This cycle of antibody production and HIV escape occurs repeatedly over the course of an infection. In addition, HIV can also be passed directly from infected to uninfected T-helpers, thereby completely bypassing the protective shield of antibodies.

Therefore, controlling HIV infection requires eliminating the infected T-helper cells. This is the purview of cytotoxic T cells (CTLs), which recognize infected T-helpers by "seeing" small pieces of viral molecules (epitopes) presented by HLA-A, -B, and -C proteins on the infected T-helper's surface.

CTLs kill infected cells in most viral infections, but usually the cells that they kill are expendable—replaced by the cell division of still-healthy cells (for example, cells lining the digestive system). Unfortunately, in the case of HIV infection, the T-helper cells that are killed are crucial to the immune system's regulation, and, therefore, the actions of the CTLs have serious consequences. Ultimately, the combination of HIV-induced and CTL-induced cell death is not compensated by the production of new T-helpers, and over a period of years, this imbalance reduces the number of T-helpers enough to compromise the immune system's ability to respond to other infections. At this point, symptoms of AIDS ensue, with patients commonly succumbing to the secondary infections; hence, the viral nomenclature—human *immunodeficiency* virus.

A silhouette of the AIDS virus (arrow) is shown adjacent to a portion of a T-helper cell's surface to indicate approximate scale. Protrusions on the T-helper's surface are membrane-associated proteins, one variety of which (known as CD-4) represents the binding site for HIV before its internalization, the first step in its infection of the cell.

Receptor

HLA protein
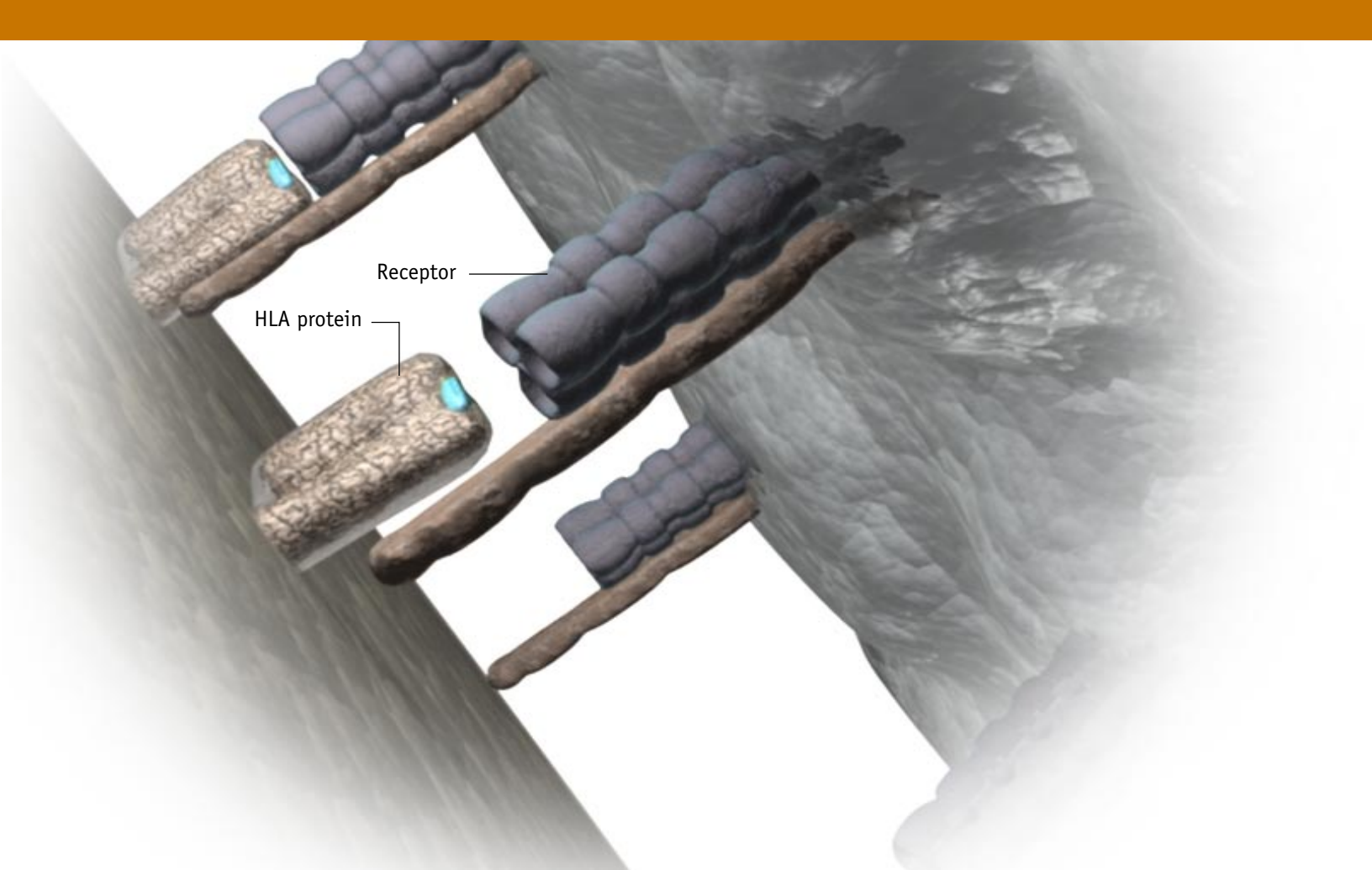
Simplified illustration of a cytotoxic T cell (upper right) identifying an HIV-infected T-helper cell (lower left). When a T-helper cell has been infected by the AIDS virus, small pieces of viral molecules, called epitopes (blue), become bonded to the HLA proteins and then transported to the infected cell's surface. After recognizing an HLA/viral-epitope combination with a receptor of complementary shape, the cytotoxic T cell will kill the infected helper. HLA proteins are thus indispensable in the process by which HIV-infected cells are identified and killed. Although only one viral epitope is recognized by a given cytotoxic-T-cell receptor, an individual's immune system potentially responds to dozens of different epitopes; in addition, different epitopes will be recognized by the immune systems of different individuals.

Specifically, the cytotoxic T cells of our immune system recognize these HLA proteins as molecular billboards, advertising that other body cells are virus-infected and should be killed.

Using a combination of viral molecular fragments and the HLA Class-I proteins, cytotoxic T cells can detect and attack HIV-infected T-helper cells, killing cells that normally support the immune system. For several years, biologists have observed that individuals with certain HLA variants progress more slowly to the point when symptoms of AIDS manifest (usually when their T-helper blood count falls below 200 cells per milliliter). But the significance of these observations remained uncertain until clarified by Korber's recent work.

HLA Supertypes

This work was done in the context of a decade-long study of HIV-infected gay men, known as Chicago's Multi-

center AIDS Cohort. Korber and her Los Alamos colleagues teamed with researchers from Northwestern and Duke Universities and the Oakland Children's Hospital to investigate the association between HLA types and two measures of the progression from initial HIV infection to the symptoms of AIDS.

To facilitate this analysis, the team grouped the many different HLA types into a smaller sets of "supertypes," where each supertype comprised functionally similar HLA proteins. Each supertype can be thought of as defining the specific HIV molecular fragments to which the immune system will respond. Individuals with different supertypes "see" the virus differently (in an immunological sense).

The team found statistically significant evidence that certain HLA supertypes could be associated with two benchmarks of AIDS progression. The first benchmark was viral load—the amount of virus contained within a unit

of an infected individual's blood; the second was the rate of T-helper-cell decline—the rapidity with which these immunologically crucial cells are killed. In the first case, the researchers were able to parse the test population into high, medium, and low viral-load categories that were correlated with specific HLA supertypes; logically, the higher the viral load, the higher the probability of rapid progression to full-blown AIDS.

By ascertaining an infected individual's HLA supertype, the researchers were able to reliably predict that individual's viral load. And more important, those with a low viral load—predictive of slow progression—were also those with the HLA supertypes that occur most rarely in the population at large. For example, supertypes occurring in only 1 to 2 percent of the population tend to have the lowest viral loads; conversely, those occurring in 20 percent or more have the highest.

Advantage of Genetic Rarity

These findings can be extended to the problem of viral escape mutants—genetically altered viruses in each infected person that have avoided recognition and elimination by the immune system. In this context, the researchers posit what is known as a "rare-allele advantage," referring to the low viral-load advantage of rare HLA supertypes. In essence, HIV escape mutants that have evolved in the context of a common HLA-supertype immune system will more likely be effectively combated by the immune system of a newly infected individual who possesses a rare HLA supertype. The rare-supertype immune system can see HIV

molecular nuances different from what the common-supertype immune system sees and, therefore, can recognize and better control viruses that have already escaped immune responses of more-common HLA-supertype individuals (see the sidebar on page 18).

The rare-allele advantage is relevant because simply on the basis of population frequency, rare-supertype individuals will, in fact, most likely be infected by sexual partners with one of the more-common HLA supertypes. Overall, this means that individuals with rare HLA supertypes should progress more slowly after infection. Their ability to combat the viral escape mutants of their common-supertype

Conceptual illustration of how an immune system response against one mutant of the AIDS virus is not necessarily effective against other HIV mutants. An antibody (top) binds to the surface glycoprotein (gp120) of an HIV mutant (middle), much as a socket wrench fits a bolt head. However, because of slight differences in molecular shape (conformation), this same antibody will fail to bind to the gp120 structure of a different viral mutant (bottom). Both arms of an antibody are identical in conformation, but in this illustration, one arm has been cut away to facilitate viewing of the antibody's binding with gp120.

HIV and Natural Selection

Natural selection—Darwin's theory of evolutionary change—normally operates slowly, over decades, centuries, or millennia. Mutations occur in genes as a consequence of both environmental conditions and slightly error-prone processes that replicate DNA. Some of these mutations may be advantageous to an organism's survival and reproduction in the face of local environmental circumstances (the selective force). This genetic advantage results in preferential reproduction of the "fittest," which alters the makeup of the organism's local population, creating an altered or even a new species over time.

In the microbial world, the evolutionary process generally proceeds at a greatly accelerated pace, given the rapidity with which bacteria and viruses reproduce. Hence, in HIV infection, this evolutionary process occurs over the time span of months to years, rapidly altering the makeup of the viral population within a single individual. Viral mutations occur continually, the consequence of a massively error-prone RNA/DNA replication process. These viral mutants differ in the structure of the various proteins that mediate their exact form and function. The local selective environment is represented by the infected person's immune system—individualized by his or her HLA (and other) genetic makeup. It is that makeup that determines the ability of the person's antibodies and cytotoxic T cells to recognize and eliminate viral mutants.

But with HIV infections, so many new forms—so many viral mutants—arise that many cannot be eliminated. In a grim example of natural selection, the immune system "selects" those mutants that escape detection and elimination—favoring the survival of the fittest viral mutants at the expense of their human host. Each infected person thus accumulates a collection of "escape mutants" that differ from those of other infected individuals, an ironic outcome when we consider that the immune system generally protects us from succumbing to viral infections.

The research of Korber and others has supported the view that since an individual's HLA genetic makeup defines the viral mutants that he or she attacks—either vigorously, moderately, or weakly—that HLA supertype is a prime contributor to defining the population of escape mutants found in individuals. These surviving viral mutants are the "fittest" for aggressively infecting individuals with the same HLA supertype. They are less effective at infecting individuals with a different HLA supertype.

partner leads to a lower viral load early in infection. A rough analogy can be found in the realm of computer viruses, where viruses targeted to disrupt code in the more-common Windows operating system are often benign in the less-common Macintosh operating system.

This hypothesis has several crucial implications for further work that must be validated in a population with different overall HLA frequencies. Such a study in a native African population in South Africa—with different HLA frequencies and from a region where HIV infection rates top 30 percent—is already in progress, under the aegis of the National Institutes of Health.
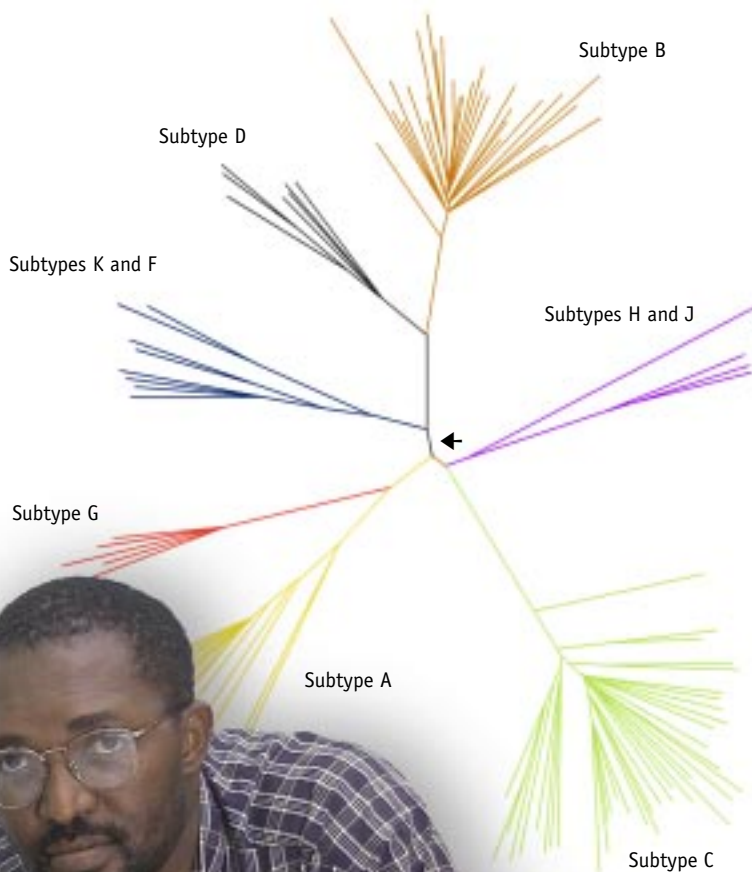
A Creative Leap

The idea to investigate the role of genetic rarity in HIV infection came to Korber from another AIDS study. Researchers and clinicians have long known that the newborns of HIV-infected mothers invariably become infected by their mother's blood and, moreover, that without immediate antiviral drug therapy, these infants progress to AIDS very rapidly. Since a large body of research has pointed to the cytotoxic T cells of the immune system as extremely important in antiviral immunity, the weak cytotoxic-T-cell response in these infants was suspected as contributing to this rapid disease progression. A multiuniversity study—in which Korber participated—of a South African maternity clinic shed some light on this issue.

By the laws of inheritance, and ignoring rare chromosomal events, an infant must share half its HLA supertype with its mother. Since that supertype defines the HIV mutants to
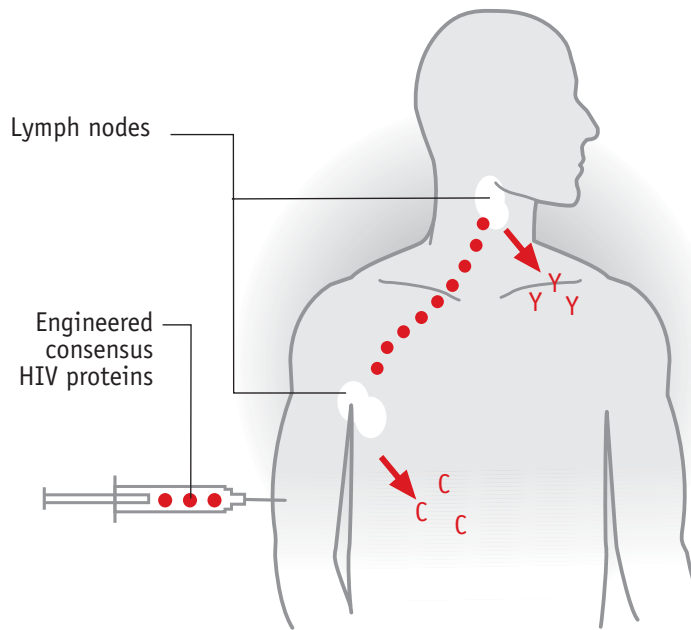
which the child's immune system responds strongly, weakly, or not at all (i.e., escape mutants), the infected child begins life in a double bind. Armed with an immune system quite similar to its mother's, it also receives, at birth, precisely the viral mutants that have already escaped its mother's immune system. The child thus begins life with an AIDS virus that has already evaded some of the potential immune responses that could otherwise help control the viral infection.

More than simply advancing the understanding of the AIDS epidemic, this study served as a creative springboard for Korber's pursuit of the HLA-related analysis in adults. As she frames her creative insight, "It seemed like if it could happen in mother-infant transmission, then it might also happen with gay partners." In other words, if the commonality of mother-infant HLA type made the infant more susceptible to its mother's escape mutants, then that same phenomenon should be observable in a large enough population of adults who had transmitted the virus through sexual contact. Greater HLA commonality should correlate with faster AIDS progression, precisely what the research with the Chicago Multicenter AIDS Cohort demonstrated. The leap from mother-infant to sexual-partner HIV transmission illustrates how the scientific mind latches onto evidence to create new hypotheses: it is the creative process at the heart of scientific progress.

Bette Korber, postdoctoral research fellow John Mokili, and their Los Alamos colleagues have worked to clarify the evolutionary tree of HIV subtypes and strains. The evolutionary tree shows the genetic relationship among different viral strains, maps the genetic distance between several of the different subtypes of HIV (different colors), and indicates different strains within each subtype. By comparing the genomes (genetic information) of these viruses, Korber and others have been able to reconstruct a genome that would represent a consensus copy (arrow) of this genetic information. This genetic information can then be used to synthesize proteins that can, in turn, become the components of an AIDS vaccine. While at the Lab, Mokili has developed an HIV vaccine database, and he serves as advisor to vaccine developers in his native Democratic Republic of the Congo.

Subtype B

Subtype D

Subtypes K and F

Subtypes H and J

Subtype G

Subtype A

Subtype C

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Lymph nodes

Engineered consensus HIV proteins

To fully protect against HIV infection, a vaccine should contain a viral protein or proteins that can evoke protection against all viral strains—at least in a given locality. In addition, both arms of the immune system must be activated by this engineered consensus vaccine, resulting in the production of antibodies (Y) and cytotoxic T cells (C) capable of attacking the virus when it is transmitted to an uninfected vaccinated individual. Antibodies attack the virus in the blood and other tissue fluids (and by other mechanisms), while cytotoxic T cells attack a virus that has already invaded cells—by killing those infected cells. This infected-cell killing is crucial because even if all detectable virus could be removed from the blood by antibodies (or by drug therapy), the persistence of infected cells would mean that the individual was still actively infected— and viruses would likely reappear in the blood at some later time.

Toward an Effective Vaccine

Among other issues, two considerations are crucial in designing any vaccine: first, stimulating the appropriate part of the immune system and second, including the most-effective molecular components of the target organism. Not surprisingly, Korber's research has implications for both aspects of vaccine design.

Antibody-mediated versus cell-mediated immunity: this quandary has confronted vaccine developers since the 1970s. Originally, the potency of a vaccine was judged by its ability to elicit the production of high concentrations of antibodies in the blood of those immunized. Although this strategy was remarkably effective in protecting against bacterial toxins such as tetanus, immunologists came to appreciate that it was not so effective against certain viruses.

The problem is that viruses spend a portion of their life cycle reproducing inside infected cells, and although antibodies can summon ancillary cell-killing (cytotoxic) mechanisms, these mechanisms may not always be effective at eliminating all infected cells. Even if all virus particles can be removed from the blood by antibodies, the persistence of infected cells means that viruses are still surviving and replicating, that is,

that the infection persists. Without stimulating cytotoxic T cells to kill infected cells, the vaccine often cannot eliminate the infection.

For some time, biologists have known that, in the early stages of HIV infection, an initially severe viremia (high blood levels of the virus) attenuates shortly after cytotoxic T cells respond. Although this finding supports the importance of cellular defenses in AIDS, Korber's research linking HLA supertypes to AIDS progression strengthens the significance of that observation for vaccine development, since HLA proteins help cytotoxic T cells recognize and kill HIV-infected cells. This insight has recently been underscored by the failure of the first large-scale AIDS vaccine trial, which used a vaccine designed primarily to elicit antibody rather than cytotoxic-T-cell immunity.

The goal of including the most-effective immune system stimulants in a vaccine is complicated by HIV's viral variability. Recall that within the same HIV genetic subtype—for example, the B subtype found in the United States— the average genetic variation between the viruses found in any two infected individuals is about 15 percent. As a result, a vaccine-stimulated immune response directed specifically against the virus of one person would likely not recognize the virus in the other person—in other words, the vaccine would be useless. By analogy, a socket wrench forged specifically to loosen bolts on one manufacturer's products would not necessarily fit the bolts of another manufacturer that differ in size or shape.

Although this variability is less than the average 30 percent genetic

difference between HIV subtypes (such as the U.S. B subtype and the African/Asian C subtype), it is nonetheless a daunting challenge to vaccine developers. To help contend with this viral diversity, Korber and others in the field have designed both consensus and ancestral strains; an ancestral sequence is a model of the historic root (origin) of the epidemic strains. Both of these strategies produce artificial strains that are genetically more similar to modern circulating strains than the strains are to each other. These made-to-order genetic sequences would then be used to synthesize a consensus viral protein or proteins that could be used as the immune system stimulants in a vaccine.

Korber and her colleagues at Duke University have constructed a consensus genetic sequence that is central to all HIV strains found globally and is a model of the most recent common ancestor of HIV-1 strains. In preliminary tests (not involving actual vaccination), they found that antibodies from both B- and C-subtype HIV-infected individuals recognized the consensus viral protein synthesized from this consensus sequence as well or better than they recognized viral proteins from within their own subtype. The implication is that such an engineered protein or group of proteins might ultimately help surmount the problem of extreme viral variation among infected individuals—one of the most challenging obstacles to the development of an effective AIDS vaccine.

Korber is excited about the consensus HIV protein. "So far, it has worked a lot better than I ever thought it would," she notes. "Vaccines are the way to get at preventing the spread of AIDS in the developing world, because [drug] therapy is so inordinately expensive."

Reflecting on a current study in Africa and another planned for China, Korber reasserts her world-health-activist persona: "Can you imagine living in a community and walking down the street where 50 percent of the

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young men and women are infected? Vaccines are useful in that setting, because it's one, two, three shots, and it's done; and you can afford to do that." There can be no doubt that both HIV-infected individuals and healthcare professionals waging war against the AIDS pandemic sincerely hope that Korber's remarks will prove prophetic. ■

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The Researchers

Bette Korber earned a Ph.D. in immunology at the California Institute of Technology. She was a postdoctoral fellow at Harvard University, Los Alamos, and the Santa Fe Institute before joining the Laboratory as a technical staff member in 1993. She serves on the editorial boards of three AIDS and viral research journals, is a member of the AIDS Vaccine Research Working Group, and has helped organize several meetings on HIV and microbial biology. She is also an adjunct faculty member at the Santa Fe Institute and the University of New Mexico Medical School.

Other Los Alamos researchers who contribute to this research include Thomas Leitner, Carla Kuiken, Brian Foley, Brian Gaschen, James Szinger, John Mokili, Robert Funkhouser, Karina Yusim, Dorothy Lang, Kristina Kommander, Ming Zhang, and Una Smith.