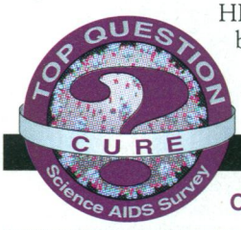
AIDS Research: The Mood Is Uncertain

Science surveyed 150 top AIDS researchers and found that the field is learning rapidly—and that the new knowledge is undermining assumptions held with confidence just a year ago

"The more we learn, the less certain we are." That was the message *Science* heard when we surveyed the world's leading AIDS researchers to find the key questions that must be answered before there is a cure for AIDS or a vaccine to prevent it. And as certainties that were accepted in the early days of the epidemic collapse, odd things happen. Researchers who were once hostile find they have much in common. Scientists who laughed at alternative theories about how HIV causes AIDS are beginning to consider them seriously. Treatments that seemed reckless now become, at the very least, intriguing. The definition of a preventive vaccine has been thrown open.



A CURE: THE TOP 10 QUESTIONS

Question	Number who mentioned	Mentions in top three
1. What causes the immune system collapse seen in AIDS?	46	38
2. How can HIV replication be controlled?	37	32
3. What are the correlates of protection?	36	23
4. Can combination therapy overcome drug resistance?	33	21
5. What are the best targets in the viral life cycle for therapy?	26	13
6. Will immunotherapies like vaccines and cytokine treatments work?	25	11
7. Can drugs target HIV in reservoirs like the lymph nodes?	20	13
8. How is HIV transmitted sexually, maternally, and intravenously?	21	11
9. Can the immune system be reconstituted after infection?	27	7
10. What are the best surrogate markers for evaluating therapies?	20	7

The reason for this new open-mindedness is simple: No cure or vaccine exists. After more than a decade of struggling in frustration as the epidemic gallops on, researchers are being forced to reexamine assumptions they once held without question. "This disease has been trying to tell us for a long time that it's complex," says Anthony Fauci, head of the National Institute of Allergy and Infectious Diseases. "People are finally coming to the point where they're realizing that it isn't one way or another. And that means people are starting to listen—not necessarily swallow—but listen to the data others have that they disagree with." Jay Levy, the University of California, San Francisco, researcher who was one of the first to isolate HIV, also sees attitudes of militant certainty softening into a less adamant posture. "The idea now is, 'Let's sit back and see,'" says Levy.

The reasons for this sea change aren't hard to find. Consider the following collapsing certainties that dot the recent past in AIDS research:

- AZT, the main anti-HIV drug now in clinical use, was once assumed to be helpful for infected people before they show AIDS symptoms. New data suggest the drug is probably of little help to that group.
- Many researchers who once believed almost all the damage caused by HIV could be explained by the virus's direct killing of cells now think indirect mechanisms must also be at work.
- The rise or fall of immune system cells known as CD4s was until recently considered the chief "surrogate marker" for evaluating AIDS therapies. Now its value as a marker is in question.
- Researchers who had striking success with experimental AIDS vaccines in monkey trials have found that their success could not be duplicated—and may even be due to artifacts.

Blows like that are more than enough to account for the "wait and see" attitude Levy describes. In fact, this should be a time for science to reexamine assumptions and catch its breath before the next assault on the disease. Yet the new uncertainty in AIDS research comes as politicians and AIDS activists are demanding results immediately. With the scientific community caught in a vise between escalating political demands and a lack of agreement about how HIV does its lethal work, rumblings are beginning to be heard among researchers that it's time for scientists to set the research agenda before the agenda is set for them.

But is there consensus on what that agenda ought to be? That's the question *Science* sought to answer in its AIDS Survey. The 150 researchers were asked to list, in order of priority, the top 10 questions that need to be answered to come up with a cure or, separately, a preventive vaccine for AIDS. The 74 who responded had lots to say—which isn't necessarily a good sign, as Arthur Ammann of the Pediatric AIDS Foundation said in a telling note on his survey reply: "I am curious if everyone will actually come up with 10 items in each category. I wish there would only be two or three. It would indicate that we've made progress."

In fact, most did come up with 10 responses—some even more. To tabulate the top 10 questions overall in each category we took into account both the number of researchers who mentioned a question and the number of them who put it in the top three. The top 10 in each category are presented in tables on these pages. Six stories on the following pages detail the top three questions under each heading.

Beware of dogma

It's difficult to imagine needing such a survey in any other field. Yet the lessons to be drawn from four recent episodes make it clear that in AIDS research it may be time for a concerted effort to sharpen the field's focus.

The first lesson is that even the best current treatments are very limited. Some limits of the anti-HIV drug AZT have long been known. In people with AIDS, the drug staves off death for maybe a year—not exactly what you would call a cure. In healthy, uninfected people, the effects have been more uncertain, though a large, 1-year study in the United States suggested the drug could delay the onset of disease. This study led the Food and Drug Administration (FDA) to approve use of AZT in healthy people whose immune systems have begun to show damage.

"Mediocre, but better than nothing." That was AZT's status until April, when results from a European study published in a letter to the *Lancet* suggested AZT offers no "significant benefit" to infected, healthy people in slowing the disease or prolonging life. Staged by researchers in the United Kingdom and France, the "Concorde" trial ran 3 years and

included 1749 patients— enough to put serious statistical muscle behind its conclusions.

Many researchers stressed that the Concorde results simply reinforced the belief that no drug is going to knock out HIV by itself. "This doesn't come as a surprise," FDA commissioner David Kessler told *Science* when the study was published. "There's a limited but real benefit to AZT. It's not the home run, and no one ever said it was the home run. That's why we're working so hard on combinations [of drugs] and other therapies."

But regardless of whether the Concorde results came as a surprise to a handful of insiders, they changed the landscape for infected people and their physicians. When it comes to early treatment, "mediocre, but better than nothing" may no longer hold true. Expect vigorous debates on this point over the next few months.

That's a hard lesson, but it's followed by others just as tough. Lesson two is that science's understanding of what causes the immune system collapse seen in AIDS is far from secure. Ranked as the leading question in the "cure" category of our survey, this mystery has given birth to two main theories. One is that HIV directly kills immune system cells. The other is that the virus does its damage through intermediaries.

Though these theories are not mutually exclusive, for years many researchers have insisted direct killing was sufficient; they dismissed the indirect school. Most prominent among the proponents of direct killing was Robert Gallo, the National Cancer Institute researcher whose lab first published conclusive evidence that HIV causes AIDS. Now Gallo—with many others—has had something of a scientific conversion. He says his insistence on direct mechanisms was partly a reaction to those who argue HIV isn't the cause of AIDS. And he is now convinced indirect killing has a critical role. It's "time to consider that alternative pathogenic mechanisms are close to being on target," says Gallo. "It's clear you don't need a lot of viral load to get disease."

As further testimony to Gallo's conversion, he is finding common cause with Joseph Sonnabend, a New York AIDS clinician with whom Gallo has clashed in scientific debate. Sonnabend has long argued that the immune system signal alpha interferon plays a key role in AIDS. Gallo paid little attention. But recently, at Gallo's invitation, Sonnabend visited Gallo's lab to give a talk. "Strange as it may seem, I have a linkage to Sonnabend's early notions," says Gallo. "I believe he was on to something very important."

It might have been hoped that, even in the absence of a clear scientific understanding of the disease, treatments could be developed. After all, physicians can cure other illnesses without understanding every aspect of the disease process. Yet the third recent lesson is that the search for effective drugs is also in an uncertain state, partly as a result of confusion over surrogate markers.

Surrogate markers are indicators, revealed by laboratory tests, that help researchers predict whether a treatment is actually preventing the progression of a disease or death. In the case of a disease such as AIDS, which has a long clinical latency, such markers are crucial, because without them, evaluating therapies could take years, even decades.

Because a reduction in the body's complement of white blood cells bearing the marker known as CD4 is the hallmark of AIDS, it makes intuitive sense that if a drug can prevent that loss, the drug is working. That logic helped convince the FDA conditionally to approve the anti-HIV drugs ddI and ddC. But however logical it seems that CD4 counts should be a good surrogate marker, it may not be true—an unsettling message that was reinforced by the Concorde trial. In that study, researchers reported that people receiving AZT had 30 more CD4 cells, on average, than the group that did not receive treatment. Yet the treated group was no healthier at study's end. "It throws open the whole question of using this small degree of change as a marker," says Ian Weller, Concorde's principal investigator in the United Kingdom. "It also questions the licensing of drugs based on these sorts of changes."

The fourth recent lesson may be the most disconcerting of all: the conclusion that even in areas where AIDS research may seem to be moving ahead quickly, time can undo that impression. For example, the most intensive soul-searching in AIDS research right now is probably going on among among vaccine developers who "probably know less today than we thought we did 2 years ago," according to Shiu-Lok Hu of the Oncogen branch of Bristol-Myers Squibb, developer of one of the first HIV vaccines tested in humans.

Primate researcher James Stott of England's National Institute for Biological Standards & Control, offers a similar assessment. Stott, who threw the AIDS vaccine world into a state of shock 2 years ago by revealing that a laboratory artifact likely was responsible for many "successful" monkey vaccine experiments, says: "There was a stage where we just seemed to be romping along, and now it's just so painfully slow. Things are not as simple as we thought."

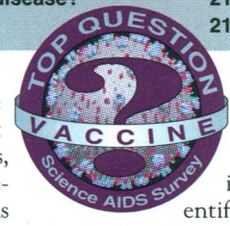
But Hu, who had success in a monkey experiment others failed to replicate, is not despairing. He says the new attitude simply means "we're being more realistic about what the problem is." For vaccine makers, a key part of the problem is simply figuring out where the finish line lies. The leading question in the vaccine part of *Science*'s survey was: What are the correlates of protection against HIV that a vaccine must imitate?

The summation of those hard lessons may be that it's time for an overall AIDS research agenda. Many researchers balk at the idea of setting AIDS research priorities. A significant group maintains that attempts to organize research contradict the process of science, which relies on individual investigators following scientific intuition wherever it leads. But the political pressure to set research priorities seems to be rising. The past year has seen (then-candidate) Bill Clinton call for an AIDS "Manhattan Project" and Congress pass legislation to centralize the spending of AIDS funds by the National Institutes of Health. In the years to come, it may be impossible to avoid setting research priorities. As the survey demonstrates, the community has no trouble coming up with the questions that need to be answered first. The challenge now is to start answering them.

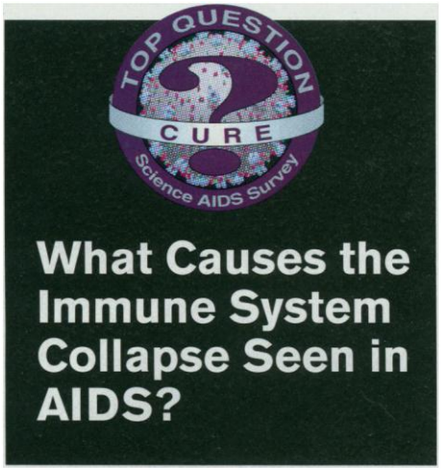
–Jon Cohen

A VACCINE: THE TOP 10 QUESTIONS

Question	Number who mentioned	Mentions in top three
1. What are the correlates of human protection?	62	60
2. How can viral variation be overcome?	44	24
3. What is the best way to present viral antigens to the immune system?	32	15
4. What are the key viral antigens that confer protection?	28	17
5. Are "old fashioned" attenuated and killed virus approaches better?	32	14
6. How is HIV transmitted sexually, maternally, and intravenously?	20	15
7. Is mucosal immunity critical to preventing infection?	27	10
8. What is the pathogenesis of HIV?	16	12
9. Should AIDS vaccines aim to prevent infection or disease?	21	8
10. Can better animal models be developed?	21	7



Anyone interested in obtaining a list of the 74 researchers who responded to our survey and the methods by which we determined the final ranking of responses may write to Jon Cohen, *Science* News Department, 1333 H St. NW, Washington, D.C., 20005



Among researchers working on an AIDS cure, this question was cited as far and away the most important: It's both fundamental and vexing. By now, it's well known that the hallmark of AIDS is a reduction in the number of T lymphocytes known as CD4s, which orchestrate the immune system. But there is no agreement about how HIV leads to the depletion of these critical cells.

Indeed, as each year passes, a new theory is hatched. The virus itself may kill the cells, say some. Alternatively, HIV may call in other elements of the immune system to do the job. Yet again, the virus may somehow trigger the cells of the immune system to commit suicide. Each of these scenarios can claim some supporting evidence, but there's no clear front-runner. And that's not all: In a sign of the disease's complexity, any one could be true without canceling out the others.

The simplest explanation for the loss of CD4 cells is direct killing by HIV. The virus might induce "lysis" (causing infected cells to implode or burst) or it might cause cells to fuse together into clumps called syncytia. In fact, both may be true, since HIV causes lysis and syncytia formation in the test tube. For years, however, the direct-killing scenario had a serious flaw: Little HIV could be detected in the blood during the time when damage was occurring to CD4 populations. That picture is changing as researchers develop more sensitive techniques for ferreting out HIV and begin to appreciate the amount of virus in organs such as the lymph nodes. The result of these advances is the understanding that infected people carry far more virus than was thought. Hence the direct-killing hypothesis is gaining currency.

Virologist Joseph Sodroski of Harvard's Dana-Farber Cancer Institute has been teasing out how HIV might lyse the cells it infects. Sodroski's work focuses on the virus's "envelope protein," gp120, and its neighbor, gp41, both of which are cleaved from a larger protein, gp160. Inside infected cells, that precursor binds to newly minted CD4 molecules (the receptor that identifies the cells HIV depletes). The Dana-Farber researcher is finding test-tube evidence that infected cells are killed when the complex of gp160 and CD4 fuses with cellular organelles (such as the Golgi apparatus) and destroys them.

Sodroski and others argue that this method of cell killing is aided by syncytia formation, a process that, at least in the test tube, can have a devastating effect on human cells. As in lysis, gp120 is thought to have a significant role in syncytia formation. When a cell is infected, newly produced gp120s bud through the membrane. Owing to that protein's strong affinity for the CD4 receptor, the infected cell can hook onto healthy, uninfected CD4-bearing T cells. In this way the infected cell may fuse with "innocent bystanders" and take them out of commission. Together, says Sodroski, these direct mechanisms can account for the cell loss seen in AIDS. "My hunch is that if you didn't have direct killing by the virus, you wouldn't get CD4 depletion," he says.

But many researchers think that direct mechanisms, even if they play a role, are inadequate to explain all of AIDS' devastation. One investigator who has long argued for that point of view is Jay Levy of the University of California, San Francisco. In 1988, Levy reported that people developed AIDS even though the variant of HIV isolated from them failed to kill CD4 cells directly in test tubes. Levy's inference: This "noncytopathic" virus must have indirect means of knocking out immune system cells.

Now Levy, in conjunction with Donald Mosiers of the Scripps Research Institute in La Jolla, California, is bolstering his argument with experiments in which human immune-system cells are transplanted into mice. The latest work (*Science*, 30 April, p. 689), shows that two noncytopathic HIV strains deplete human CD4 cells in these mice *faster* than three other strains known to kill CD4s directly in the test tube. What is more, the rate of replication of the different strains did not correlate with CD4 loss—suggesting, again, that the virus isn't doing the job on its own. "There has to be another process [than direct killing]," concludes Levy.

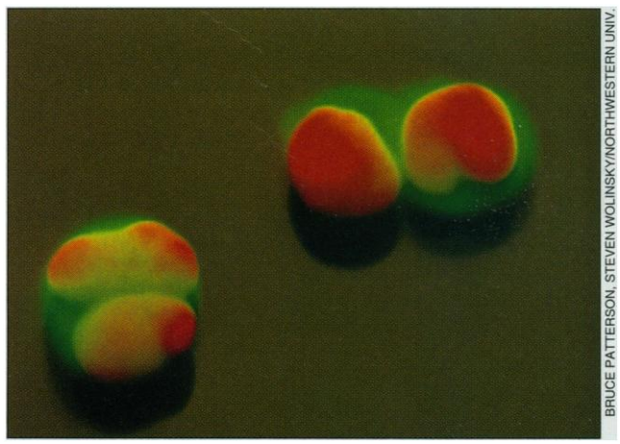
What might the indirect mechanism (or mechanisms) be? Perhaps infection somehow causes another set of immune cells known as killer cells to go haywire and eliminate *un*infected CD4s. It is possible that even more exotic destructive immunologic cascades are unleashed because parts of HIV mimic parts of immune-cell molecules, leading the body to see its own immune cells as foreign. Most firmly in the spotlight at the moment is a third elaborate notion: apoptosis (see Marie-Lise Gougeon and Luc Montagnier's Perspective, p. 1269). Apoptosis is a word coined to describe programmed death in cells, and if the apoptosis theory holds true, HIV can cause CD4 cells to destroy themselves.

Jean-Claude Ameisen of the Pasteur Institute in Lille, who first proposed this hypothesis in *Immunology Today* 2 years ago, has shown that when he uses foreign pathogens to "activate" $CD4^+$ T cells taken from HIV-infected people (a process needed to prime them for action), the cells die by apoptosis. The same holds true when $CD4^+$ T cells are taken from rhesus macaques infected with SIV, a relative of HIV-1 that causes AIDS in that species. In contrast, when Ameisen looked at $CD4^+$ T cells from HIV-infected chimpanzees—who do not develop AIDS—the cells did not go through apoptosis, suggesting the absence of that phenomenon was correlated with health.

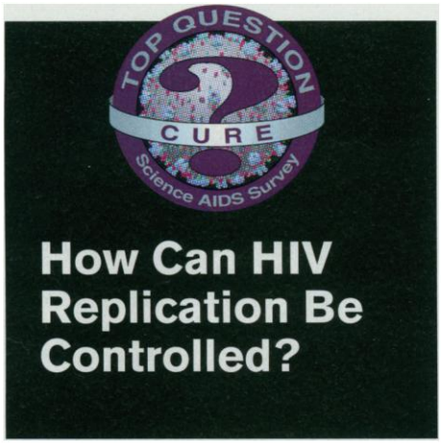
Terri Finkel of the National Jewish Center for Immunology in Denver is among those investigating what the mechanism for apoptosis in AIDS might be. A molecule that may be involved is the ubiquitous gp120. It is well established that in the blood HIV sheds gp120, which can bind to CD4 receptors. Finkel has evidence that the apoptosis switch might be thrown when two gp120-CD4 complexes are "crosslinked" by an antibody that binds the two gp120s. The crosslinking, Finkel believes, sends an aberrant chemical message to the CD4 cell, priming it to commit suicide the next time it meets a foreign pathogen.

There are plenty of reasons to doubt that apoptosis is really at the center of AIDS. But the same could be said of all the other leading theories as well, which gives some indication of the state of the field in 1993.

–J.C.


BRUCE PATTERSON, STEVEN WOLINSKY/NORTHWESTERN UNIV.

More than before. New techniques show more HIV in infected people than was thought to be present. Here, PCR-amplified HIV is revealed in blood cells by fluorescence staining.



Our AIDS survey purposely aimed high—asking respondents to list the questions that must be answered before there is a cure for AIDS. But that's the wrong way to frame the issue, objected many researchers. They stressed—with underlining and exclamation points—that "curing" HIV infection is an unrealistic goal. HIV, after all, can integrate itself into the host cell's genome and undergo a long latency during which it is virtually undetectable. To truly cure AIDS, researchers would have to devise a way to eliminate every latently infected cell, a level of scientific wizardry that is currently nowhere near the clinic.

At the same time, many of the same respondents were optimistic that ways will be found to delay AIDS symptoms significantly. And the main strategy they offered is to keep HIV from proliferating in the system of an infected person. They argued that regardless of whether HIV cripples the immune system by direct or indirect mechanisms (see page 1256), the less virus there is in an infected person's system, the less damage will be inflicted by the virus.

But beyond that general principle, there is little agreement on the best way to control viral replication. The HIV life cycle consists of more than a dozen steps; interrupting any one of them could prevent the virus from reproducing itself. While many strategies do that perfectly well in the test tube, success in the clinic has been elusive.

Until now, most clinical success has come from focusing on one step in the life cycle: the point where HIV's genetic material (RNA) is "reverse transcribed" into DNA, which then infiltrates the host cell's genes. All the anti-HIV drugs so far licensed for use in the United States—AZT, ddI, and ddC—attempt to cripple HIV here.

Methods for halting the cycle at other points, however, are in the works. One potential target is an enzyme called the HIV protease. When the host cell, under HIV's direction, makes the components of a new virus, it begins by turning out several large proteins that must be cut in pieces before a new virus particle assembles itself. The protease does the cutting, and without that enzyme, the new virus particle is malformed and noninfectious. Hoffmann-La Roche and Merck are trying to interrupt this step using drugs that inhibit the viral protease.

Other promising targets are HIV's regulatory proteins, which govern replication. When the "provirus" (the latent form of HIV DNA in the host's genes) is transcribed into messenger RNA, which is an early step in making new virus, a protein called Tat gives a boost to the process. A drug that could block Tat's action would trip up viral replication, and Hoffmann-La Roche (in collaboration with the National Institute of Allergy and Infectious Diseases, NIAID) is testing an anti-Tat compound in infected people.

A second viral regulatory protein, known as Rev, works downstream from Tat to help transport RNA from nucleus to cytoplasm, where it is packaged as viral genetic material in new HIV particles. Rev and Tat are both potential Achilles' heels for the virus, but Rev may be a meatier target, because while HIV can replicate with little Tat, the virus seems to need much higher levels of Rev to copy itself. Hence, in theory, a drug would only have to lower Rev production slightly to reduce replication. Flossie Wong-Staal of the University of California, San Diego, says, unequivocally, "Rev is the best target."

So far, however, no anti-Rev strategy has entered clinical trials. Many researchers said they would like pharmaceutical companies to conduct massive screenings of drugs already on the shelf to see if any can inhibit Rev's action.

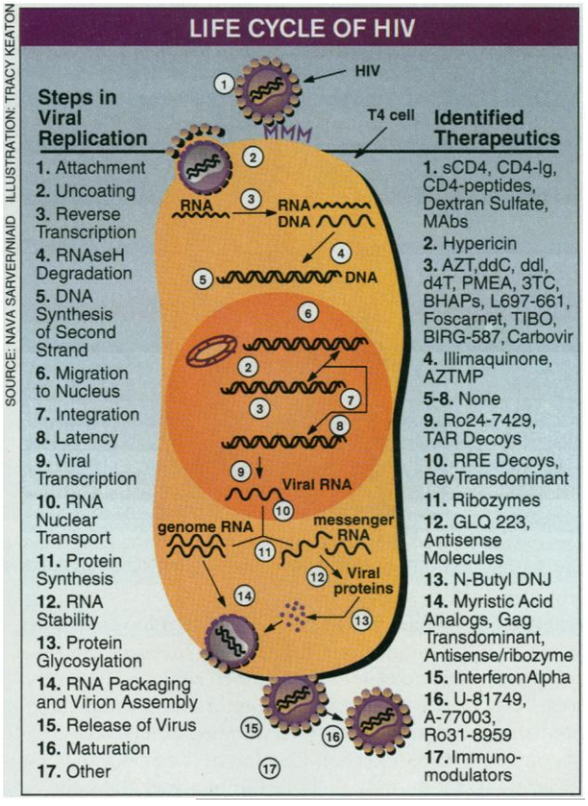
Another approach to "Revving down" the virus is to design anti-Rev strategies from scratch—something that's already being attempted. For instance, Gary Nabel and his colleagues at the University of Michigan hope to have a gene therapy approach in the clinic as early as next fall that specifically homes in on Rev. Nabel, a Howard Hughes Medical Institute researcher working with Duke University's Bryan Cullen, has a protocol now before regulatory committees that calls for removing CD4 cells from the blood of infected people and transfecting those cells with a mouse retrovirus carrying a mutant gene called *rev M10*. The cells would then be put back into the person's blood, and, theoretically, when HIV begins to replicate, the defective protein Rev M10 will be expressed and compete with normal Rev. "It's like trying to open a car door with a key when there's already a broken-off key in the lock," explains Nabel.

That sounds like an exciting idea, and many agree that Rev is an ideal target, but to some researchers, focusing on one or two viral proteins would be a mistake. Malcolm Martin, head of NIAID's Laboratory of Molecular Microbiology, believes that interfering with any of the viral proteins will slow HIV. This, he contends, holds true even for HIV's "accessory" proteins—so-called because they were originally assumed not to be essential for replication. "They've survived the test of time so they are required by HIV," Martin argues.

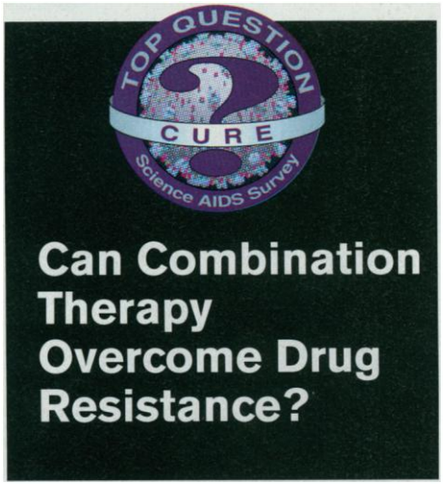
Martin thinks the bottleneck in drug development is the lack of assays for screening drugs against specific viral targets. In some instances assays exist, but they aren't being used because researchers are concentrating on only a few targets—like Tat and protease. For example, he says, it is a pity that the existing assay for drugs that derail integrase (an enzyme that allows the HIV provirus to integrate with the host cell) is not being utilized by many laboratories.

Martin may be right. Nonetheless, a variety of points in the viral life cycle are being considered as targets. And as the armamentarium of anti-HIV drugs increases, the prospect of converting AIDS into a manageable, chronic disease may move within reach.

–J.C.



Threatening life. Each step in HIV's life cycle is a potential target for interruption by therapeutic drugs.



Can Combination Therapy Overcome Drug Resistance?

In 1984, when HIV was definitively shown to be the cause of AIDS, it didn't take long to find drugs that devastated the virus, at least in the test tube. Some of those drugs quickly made their way from the lab bench to clinical trials. AZT, for instance, was first tried in infected humans in 1985, and the results were promising enough that the drug was hurried onto the market. But that initial burst of enthusiasm was soon spent. Eight years later, it is known that AZT's modest benefits typically fade within a year, probably because the virus quickly mutates into new forms that are resistant to the drug's action.

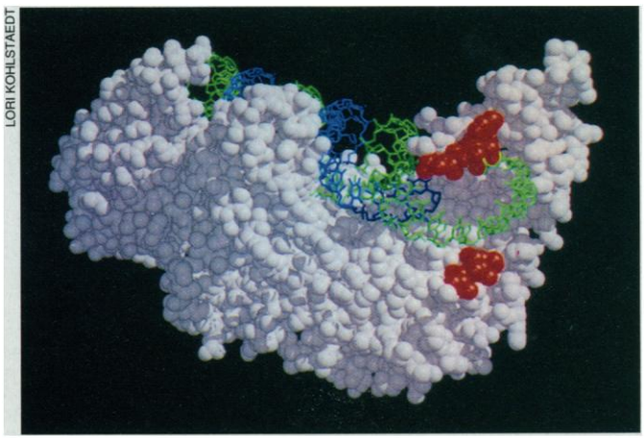
The most depressing part of this story is that HIV has been able to mutate into forms that evade every other antiretroviral drug so far tested in people. As a result, researchers have concluded that in order to beat HIV, they will have to bombard the virus with several drugs at once, since even HIV may not be able to mutate fast enough to become resistant to a multidrug combination. "Ultimately, no single drug is going to work," asserts Douglas Richman, an AIDS researcher at the University of California, San Diego (UCSD), who has done extensive studies of resistance in anti-HIV drug trials. Richman says his conclusion is backed up by experience with other diseases: "Every chronic process, be it an infectious agent or a malignancy, has required combinations of drugs."

Researchers' hopes for combined treatments are high enough that 15 pharmaceutical companies in Europe and the United States recently announced a unique collaboration to speed trials of therapeutic anti-HIV drugs in combination. That's a promising start, but deciding which drugs to use together is currently a trial-and-error proposition, since there are few firm leads about how to formulate these salvos effectively. Most data showing which way to go are based on tenuous, often contradictory, test-tube data, and are therefore vulnerable to hype—as recent experience has shown.

Since there isn't a clearly marked path toward combination therapies, some researchers, such as Simon Wain-Hobson of the Pasteur Institute, are calling for a shotgun approach: Find a handful of promising drugs that have relatively low toxicity and throw them together to see whether they help. That untested approach has a downside, however, say some researchers, a downside directly related to resistance itself.

Brendan Larder, who heads the antiviral therapeutic unit of England's Wellcome Research Laboratories (a division of Burroughs Wellcome, manufacturer of AZT), argues that "unless you have a rationale" for a specific combination of drugs "what you're likely to start seeing is multiple resistance," as viral strains that can shrug off the effects of all the drugs are selected for in the mix.

The brute force method isn't the only possibility, though. Another popular idea is mixing drugs that target different steps in HIV's life cycle. The rationale for this approach is that the combination will lower the replication rate more than any single drug could, which should lead to a lower number of mutations and thereby increase the time it takes for resistance to surface. Currently, the National Institute of Allergy and Infectious Diseases (NIAID) and others have combination trials like that in the works; the new industry collaboration promises the same.



LORI KOHLSTAEDT

Red alert. A computer model of the HIV enzyme called reverse transcriptase shows areas affected by mutations in red.

Larder himself is opting for a still more focused attack. He has been studying the effects of combining AZT with other drugs that aim at precisely the same target: the viral enzyme reverse transcriptase (RT), which copies viral RNA into the DNA that inserts itself among the cell's genes. Larder reasons that when HIV has developed resistance to one drug and a new drug with the same target is added to the mix, the virus will mutate so as to resist the second drug—and somehow, by a mechanism that is not fully understood, the presence of the second mutation make the virus again susceptible to the action of the original drug.

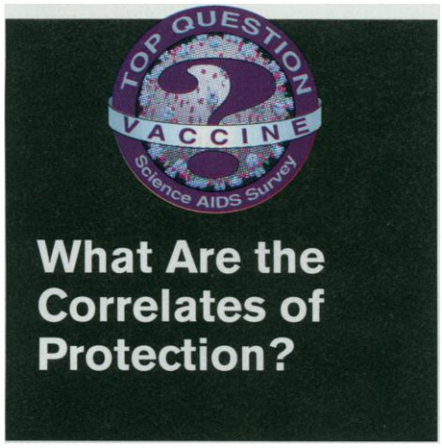
Larder has some evidence backing up this idea. In the test tube, resistance is caused by "point mutations" in the gene for a viral protein. Five different types of point mutations, leading to changes in the amino acid sequence of the RT, are known to defang AZT and protect the RT protein. Additional point mutations protect the RT against other drugs. But, these mutations cannot all be effective at once, as the Wellcome group showed by making HIVs containing mutations known to cause resistance to several drugs: AZT, Bristol-Myers Squibb's ddI, Glaxo's 3TC, and Boehringer-Ingelheim's nevirapine. The resulting HIVs remained susceptible to AZT, suggesting that the combined mutations somehow "mask" the effect of the mutations that cause AZT resistance.

Larder's group isn't the only one drawing a bead on the RT with several different agents. Yung-Kang Chow, Martin Hirsch, and Richard D'Aquila at Harvard University have been testing a strategy called "convergent combination therapy." As spelled out in the 18 February *Nature*, the Harvard group built an HIV that contained several RT mutations. These mutations should have rendered this HIV resistant to AZT, ddI, and a third class of RT inhibitors that includes nevirapine. Instead, the mutant HIV apparently had too many mutations for the RT to function properly, leaving the virus noninfectious. By itself, the result hinted that a viral mutant may not be able to develop resistance to all of those drugs at once and still retain a working enzyme.

For reasons that mystify many AIDS researchers, the lay media went wild over the convergent combination therapy story, *The New York Post* going so far as to print a picture of Chow on its front page. NIAID announced it would double the size of a planned trial to test AZT, ddI, and nevirapine together. But UCSD's Richman, who wrote a guarded editorial accompanying the *Nature* article, was incensed at what he calls "a case study in journalistic irresponsibility." Since the data may well fizzle—like many other test-tube studies—"the response to it was inappropriate and destructive."

The fact that so much hype and so much hope can coexist in a single episode shows just how crucial the field of combination therapy is today—but also how little solid ground there is for researchers to tread on as they search out the right combinations.

–J.C.



Nothing in AIDS vaccine research has proved more difficult than puzzling out the specific responses a vaccine must evoke from the immune system in order to protect a person from the ravages of HIV infection. And that quandary is reflected in our survey results: Respondents roundly agreed that finding the "correlates of protection" is topic Number One for vaccinologists.

As in other areas of AIDS research, in the search for the correlates of protection the picture is changing rapidly. At the start of the vaccine quest, researchers focused on stimulating production of a specific type of antibodies that attach themselves to the virus and prevent it from infecting cells. The logic of that approach was that most vaccines against viral diseases work by eliciting production of such "neutralizing" antibodies.

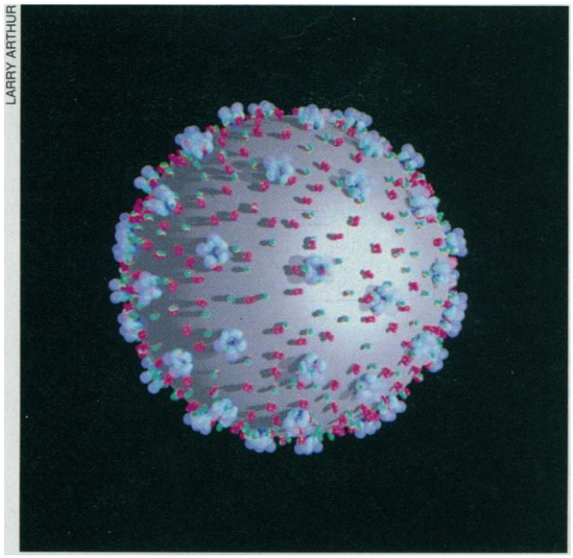
But neutralizing antibodies didn't hold the stage alone for long. In addition to its antibody-producing (humoral) arm, the immune system has a "cell-mediated" arm that relies on killer T cells (cytotoxic T cells, or CTLs) to destroy virus-infected cells. Things got complicated quickly as some animal studies suggested humoral immunity could protect against HIV infection, while others suggested cell-mediated immunity was more important—a conclusion reinforced by intriguing new data from human beings. As a result of this changing picture, the consensus now seems to be that a one-two punch of cellular and humoral immunity would be the best of all possible worlds.

Early faith in neutralizing antibodies was backed by experiments with chimpanzees. Researchers found that when HIV vaccines raised high levels of neutralizing antibodies in chimps, the animals could resist a subsequent "challenge" with an intravenous dose of live virus. But no one knows whether these antibodies could offer protection from the same virus transmitted via vagina or rectum, as HIV commonly is in humans. Furthermore, all the chimp tests were designed for success: Animals were challenged with small doses of virus just at the moment when their antibody levels were peaking. To cap things off, no vaccine made from one viral strain has protected a chimp from a challenge with a different strain—which is surely what will happen in real life (see page 1260).

Those caveats have caused considerable skepticism about whether the vaccine strategies tested in chimps will ever work in humans. Meanwhile, other vaccine trials in monkeys suggest that protection may not stem from neutralizing antibodies at all. Ronald Desrosiers of Harvard's New England Regional Primate Research Center has done the most convincing experiment reported so far, giving monkeys a vaccine consisting of a live, weakened strain of SIV, HIV-1's simian cousin (see page 1261). More than 2 years later, he challenged them with an enormous dose of virus. All the inoculated monkeys remained healthy, while control animals became ill or died.

Desrosiers does not believe neutralizing antibodies played a significant role in protecting his animals, because other vaccines he has tested have triggered even higher levels of neutralizing antibodies and still failed. Still, Desrosiers doesn't claim that he knows precisely why his monkeys were protected. In fact, he says, "we don't know anything about the correlates of protection." But other researchers think his data suggest—by elimination—that cell-mediated immunity is the key to protection, and they are eager for him to evaluate that possibility in his monkeys.

The conflict evoked by these two sets of data—from chimps and from monkeys—is acute. "If you believe the [monkey model], you'd say cellular immunity is more important," says the Pasteur Institute's Marc Girard, who studies AIDS vaccines in chimps. "If you believe the chimp model, you'd say humoral is more important. I don't think anybody has a clue."


LARRY ARTHUR

Ball of confusion. What will it take to stop HIV (shown in a supercomputer image)? Antibodies? Killer cells? Both?

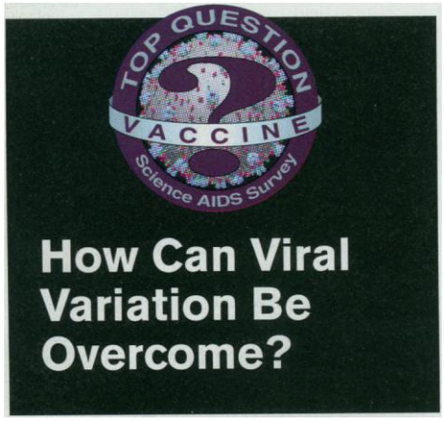
There is at least one clue, though: new data from human studies that come down on the side of cell-mediated immunity. Evidence has been piling up that some infected people remain healthy because their cell-mediated immune response produces an as yet unidentified "soluble factor" that suppresses HIV replication. Several labs have also shown that CTLs appear to reduce the amount of virus in recently infected people long before neutralizing antibodies kick in. Still more evidence comes from people who were probably exposed to HIV but are now uninfected. Though these people have no antibodies against HIV, they show immunologic signs of having once mounted a cell-mediated response.

These observations, combined with the fact that infected people can become ill despite having high levels of neutralizing antibodies, lead Jonas Salk of the Salk Institute for Biological Studies, Peter Bretscher of the University of Saskatchewan, and Gene Shearer of the National Cancer Institute to argue that cell-mediated immunity is the key to protection. As they and their coauthors explain in a Perspective on page 1270 of this issue, cell-mediated and humoral immune responses are cross-regulated by chemical messengers called cytokines. They conclude that protection against the ravages of AIDS corresponds to "locking in" the immune system to a cell-mediated state—a phenomenon that theoretically can follow exposure to low doses of a pathogen like HIV.

Though these investigators are taking a clear stand on one side of the debate, most researchers occupy a middle ground: holding that both cellular and humoral immunity are needed. "I suspect that ultimately everything contributes to protection," says David Ho, head of the Aaron Diamond AIDS Research Center. Primate researcher Murray Gardner of the University of California, Davis, agrees: "We're not going to have a good vaccine until we have both wings of the immune system in full force."

The bad news is it will take time to deduce whether both wings are necessary. The good news—which many researchers overlook —is that it may be possible to make a successful vaccine without the answer. "It's important to figure out which vaccine works for humans independent of understanding how it works," contends Desrosiers. History backs him up. One of science's most successful vaccine makers—England's Edward Jenner—discovered the smallpox vaccine without even knowing what a virus was, let alone an immune response.

–J.C.



The precision of the immune system is both a blessing and a curse. On the credit side of the ledger, this bodily system is so fine-tuned it can distinguish one strain of virus from another, slightly different strain of the same virus—and mount a tailor-made attack on each one. Yet as a result of that precision, a vaccine that protects against one viral strain may be useless against all others. Because HIV shows remarkable genetic variability, this problem has long been a keen worry of AIDS vaccine developers, who have watched in horror as branch after branch was added to the phylogenetic trees that track HIV. Indeed, the variability of HIV has led some researchers to conclude that no AIDS vaccine will ever stand up to real-world challenges. Recently, however, a few signs of optimism have appeared.

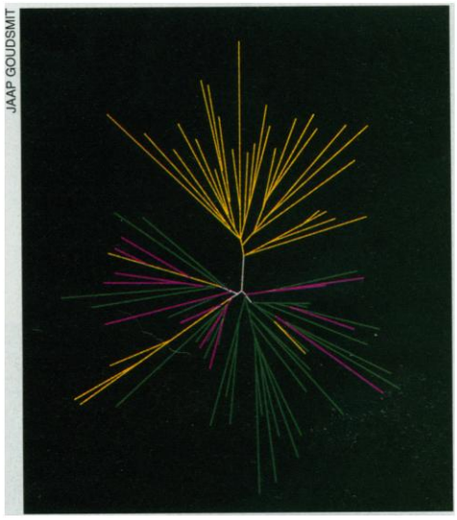
As *Science*'s survey shows, AIDS vaccine researchers believe variability is a significant hurdle; in the vaccine category, the question of how to overcome variability was ranked Number Two. But unlike the fairly pessimistic attitudes expressed about some other key questions, many researchers focusing on variability are confident that this is one hurdle that can be cleared. "Variation might not be as big a problem as we now think," says Jaap Goudsmit of the University of Amsterdam, who's been tracking the spread of the virus in that city for nearly a decade. Francine McCutchan, a leading authority on viral variation, echoes Goudsmit's confidence. "I don't really see [variation] as an insurmountable obstacle for an AIDS vaccine," says McCutchan, head of vaccine development at Maryland's Henry M. Jackson Foundation, which works closely with the Walter Reed Army Institute of Research.

That attitude is quite different from the mindset among vaccine researchers only a short while ago, when variability looked like a hurdle too tall for even the most athletic science. To understand why the mood is becoming more upbeat, it helps to turn the clock back 5 years, to a time when many investigators believed stimulating neutralizing antibodies would be the key to a vaccine (see p. 1259). And that led directly to worries about variability, since the part of HIV that best stimulates neutralizing-antibody production is one of the virus's most variable regions: a 25-amino acid section of HIV's surface protein called the V3 loop. Common wisdom held that a vaccine would have to trigger many types of neutralizing antibodies against a myriad of V3 loops.

Today, different immune system weapons—the so-called killer cells—share the limelight with, and possibly even eclipse, neutralizing antibodies as possible correlates of protection. In contrast to antibodies, killer cells are produced largely in response to the inner proteins of HIV, which vary little from one viral strain to the next.

The shift away from concentrating exclusively on antibodies has lessened some worries about variability. And that shift has been complemented by a move away from measuring variability based solely on viral genetic sequences and toward a structural system of classification.

Most attempts at classifying HIV strains so far have relied on comparing their genetic sequences, but variation among viral genes isn't necessarily significant for vaccine developers. The reason is that the immune system responds to three-dimensional molecules, not to the linear DNA or RNA sequences that code for those molecules. The essential question for a vaccine developer is not how many different varieties of viral genes, or genotypes, there are, but how many different families of three-dimensional shapes, or conformations, those genotypes give rise to. "We're beginning to appreciate a lot more that conformation is important," says Duke University's Dani Bolognesi, a leading AIDS vaccine developer.



Branches of a deadly tree. HIV phylogenetic tree traces viral variation in Amsterdam among IV drug users *(yellow)*, homosexuals *(green)*, and hemophiliacs *(purple)*.

Now, rather than shaking their heads at the multiplicity of genetic sequences, researchers are trying to see how a given genotype corresponds to the three-dimensional configuration of a specific protein, which is known in the trade as the protein's "immunotype." "We really don't know whether genotypes will correspond one-to-one to immunotypes," says the Jackson Foundation's McCutchan, "but we see the genetic data as a way to organize our thinking."

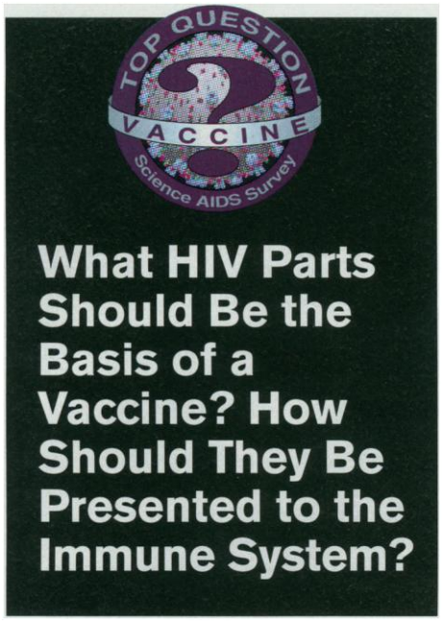
Gerald Myers of the Los Alamos National Laboratory who, like McCutchan, has grouped HIVs into families called clades, is moving away from using clades classified by genetic sequences and toward "phenetics"—a system based on amino acids, which, far more than the genetic sequence, determine the molecule's final shape. "We're trying to move sequence information toward bench information that is clinical," says Myers.

Echoing the same theme, the World Health Organization (WHO) has launched a project to analyze the genetics of viral strains from around the world and correlate them with their three-dimensional conformations. This work is in preparation for AIDS vaccine trials in Rwanda, Brazil, Thailand, and Uganda that are set to begin in the next few years. Genotypes and immunotypes are only two-thirds of the WHO project, however. The final component is "phenotyping"—classifying a particular viral strain by its specific type of activity in the patient.

As Matthijs Tersmette and Frank Miedema of the Central Laboratory of the Netherlands and Eva Maria Fenyö and Birgitta Asjö of Sweden's Karolinska Institute have shown, HIVs can be divided into two phenotypes: those that induce immune-system cells to form syncytia (useless clumps), SIs, and those that don't, the non-SIs, or NSIs. SIs easily infect T cells, replicate at high levels, and speed the course of disease. NSIs, on the other hand, favor cells called macrophages, grow slowly, and are not associated with disease progression.

Several labs have found that people recently infected with HIV have NSIs almost exclusively. Goudsmit and colleagues recently published in the journal *AIDS* that 94 of 96 newly infected people they studied were infected exclusively with NSIs, suggesting that this phenotype may be the more infectious one. Those findings indicate to Goudsmit that the real issue is not how much variation there is among HIV strains overall, but how much variation there is in the strains that are actually transmitted in infection. Whether or not that turns out to be true, the optimism among those studying variation seems merited, because work like Goudsmit's shows that he and many other researchers are beginning to look at HIV variation through the eyes of the immune system. And that's the perspective that will be needed to beat the virus.

–J.C.



Fooling the immune system is not a simple matter, but that is what a successful vaccine must do. Vaccines are impostors, harmless foes intended to be perceived by the body as vicious enemies. When the deception works, the harmless mimic offers the immune system a crash course in self-defense that is remembered for years to come—a lesson that comes in handy when the real thing comes along. Several key questions for vaccine researchers have to do with which elements of the real enemy should be included in the artificial concoction that is a vaccine and just how those components should be presented to the immune system.

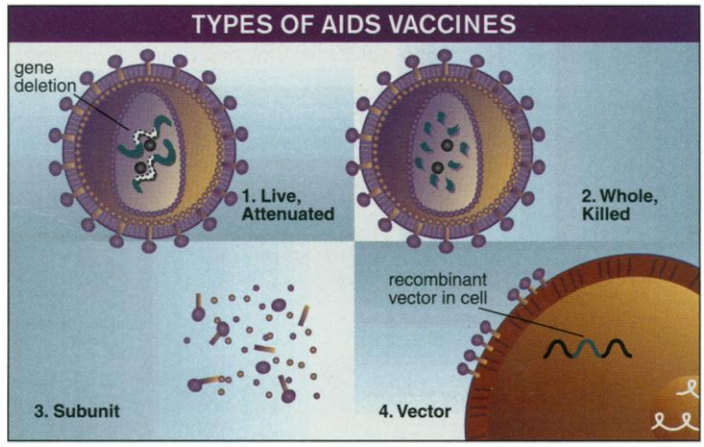
In discussing this subject, we've combined three questions our respondents raised almost in the same breath and gave much the same weight to: What is the best way to present parts of the virus to the immune system? Which viral components hold the key to protection? Are "old-fashioned" attenuated and whole, killed approaches better than newfangled genetic ones?

Almost all effective antiviral vaccines are based on one of two approaches. The "live, attenuated" strategy takes the entire virus and weakens it until it is innocuous but still causes what the immune system sees as an infection. The "whole, killed" approach attacks the viral genetic material with chemicals, heat, or irradiation, then mixes this benign preparation with an adjuvant, a chemical mixture that boosts the immune response. Because a live vaccine causes an actual infection, it presents virus to the immune system in what some believe is an intrinsically more powerful way than other approaches.

When it came to making an AIDS vaccine, almost all researchers ruled out these classical approaches for safety reasons. The live, attenuated approach was dismissed because viral genetic material in the vaccine could integrate into the host's genes, which theoretically might later cause cancer. The whole, killed strategy was nixed because if some HIV was not killed, the vaccine could cause AIDS.

At the time, it made sense to many researchers to reject these time-tested strategies, because there were some high-tech alternatives that appeared to be safer. The era of genetic engineering offers vaccinologists a new bag of tricks. Instead of throwing an entire virus at the immune system, vaccine makers can clone or synthesize just the necessary pieces of the virus, omitting the disease-causing genetic material. These viral pieces, or "antigens," can be presented with novel adjuvants, making, in effect, a killed vaccine. Alternatively, the genes that code for the antigens can be stitched into harmless viruses or bacteria and delivered to the body. These vaccines behave something like the attenuated live products of yore.

Since 1986, more than 15 AIDS vaccines based on these biotech concepts have been tested in humans to assess their safety and capacity to stimulate the immune system. In keeping with the precise, genetic engineering ethos, most of these vaccines are based on only a single viral antigen: HIV's surface protein, gp160 (or even just some subunit of that single protein).



TRACY KEATON

None of these candidate preparations has yet been tested in large groups of people at high risk of becoming infected. Nonetheless, clues to their effects are emerging. A mere 6 months ago, says AIDS vaccine developer Dani Bolognesi of Duke University, vaccines only stimulated low levels of antibodies that quickly disappeared and did not work against many strains of HIV. But new data from vaccines containing surface proteins that more closely resemble the shape of real proteins are showing promise. "The situation has gotten a lot better," says Bolognesi. "We've graduated from elementary school to possibly even high school."

But Bolognesi's enthusiasm is checked by several disturbing facts. For starters, though they stimulate antibody production, genetically engineered AIDS vaccines have produced only ephemeral effects on the second arm of the immune system: the one that rids the body of cells infected by virus through the action of killer cells. In addition, all vaccines tested to date are made from "syncytium-inducing" HIV isolates, which cause infected cells to fuse together and die (see opposite page). Yet there is evidence that non-syncytium-inducing isolates are preferentially transmitted, suggesting that vaccines should be based on those strains. The difference between those two types of virus "is a morass," acknowledges Bolognesi, "probably the most confusing picture that exists."

Partly because results from the genetically engineered approaches are so confused, the classical approaches are being reconsidered. The first time the past reared its head was in 1989, when two groups used a whole, killed vaccine in monkeys and showed the first AIDS vaccine protection ever. For 2 years, lab after lab confirmed these results, until it was discovered that most of these protections were due to a lab artifact (see page 1265), throwing the field back into confusion.

Then last December, the past took center stage again when Ronald Desrosiers at Harvard's New England Regional Primate Research Center reported dramatic success in monkey experiments with a live vaccine that had been attenuated by deleting a key viral gene (see page 1259). Desrosiers isn't sure of the mechanism by which his vaccine works, but to him that's almost beside the point. "You think you're going to find some other way to produce this effect?" he asks. "Dream on. It's not going to happen."

Though Desrosiers believes safety concerns about an attenuated HIV vaccine are real, he thinks that by strategically deleting viral genes, the risk can be all but eliminated. Jose Esparza, chief of AIDS vaccine development for the World Health Organization, has scheduled a meeting next week with 20 experts to discuss the issues. "My personal agenda is to give a chance to the live, attenuated vaccine as a possibility," says Esparza. And so it looks as though the lessons from the past are going to get a reexamination in the field of AIDS vaccines.

–J.C.