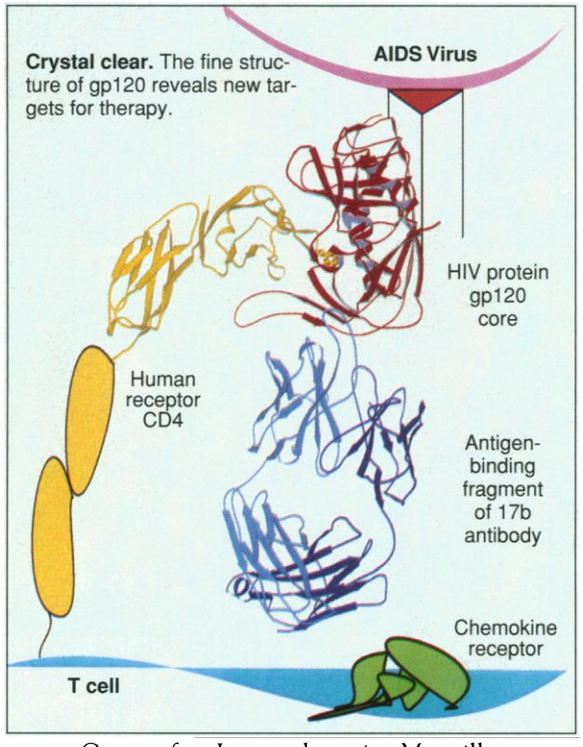
Revealing HIV's T Cell Passkey

Crystallographers have given AIDS research a boost with the long-sought structure of the protein HIV uses to access T lymphocytes. A special issue on AIDS research follows on page 1855

In any war, it is vital to know your enemy. During the battle against AIDS, researchers have gathered as much intelligence as possible about HIV, the virus that causes the disease, seeking out weaknesses in its defenses that could be breached with antiviral drugs or a vaccine. One key target that researchers have put under intense surveillance is an HIV protein called gp120, which studs the virus's outer coat. By latching onto receptors on the surface of T lymphocytes, the immune cells that are HIV's primary target, gp120 allows the virus to enter the cells and reproduce. But intelligence about gp120 has been limited. Researchers have had to use indirect techniques to study the protein, which provided only a blurry picture of gp120's structure—not enough to design well-targeted drugs or mount an effective vaccine strategy. Now, however, a powerful searchlight has been focused on gp120.

In this week's issue of *Nature*, a team led by x-ray crystallographer Wayne Hendrickson at Columbia University in New York City and molecular virologist Joseph Sodroski at the Dana-Farber Cancer Institute in Boston reports that it has determined, for the first time, gp120's atomic structure to a resolution of 2.5 angstroms. This achievement has brought the protein into sharper focus than ever before. Moreover, as evidenced by two companion papers from the Hendrickson-Sodroski partnership—one in *Nature* and one on page 1949 of this issue of *Science*—the new closeup view of gp120 is already helping researchers understand why the antibodies that HIV-infected patients produce against the virus fail to knock it out, as well as revealing weak spots on the protein where a drug or an antibody might be able to slip in and gum up its molecular works.

"This is a big deal," says Nobel laureate David Baltimore, president of the California Institute of Technology and head of a U.S. government advisory panel on AIDS vaccines. Immunovirologist John Moore of the Aaron Diamond AIDS Research Center in New York City agrees, calling the new findings "a major advance in our knowledge of the virology and immunology of HIV infection." Such superlatives are all the more deserved, researchers say, because the structural peculiarities of gp120 had, over the past decade, frustrated the efforts of several competing labs to crystallize it—a prerequisite to determining its structure by x-ray crystallography. "A lot of people thought that to tackle gp120 would not be possible with current technology," says Quentin Sattentau, an immunologist at the Center for Immunology in Marseilles, France. "Finding the structure was an extraordinary feat of perseverance."



Crystal clear. The fine structure of gp120 reveals new targets for therapy.

SOURCE: W. A. HENDRICKSON, P. D. KWONG & E. MARTINEZ-HACKERT KWONG *ET AL.*, *NATURE* **393**, 648 (1998)

Much of the tough job of persevering with gp120 fell to crystallographer Peter Kwong, who joined Hendrickson's lab in 1987 as a graduate student. Kwong's first project was to team up with other colleagues to crystallize the protein CD4, which HIV uses as its primary receptor to gain entry into cells. In 1990, the group determined the structure of those segments, or domains, of CD4 that bind to gp120, an accomplishment simultaneously published by Stephen Harrison's group at Harvard University. But the next step, crystallizing a complex of CD4 and gp120 bound together, which could provide valuable information on how these two molecules interact, proved much more difficult.

Certain of gp120's features, including carbohydrate groups on its outer surface and its so-called variable (V) loops, make it flexible and irregular in shape, and hence nearly impossible to form into a crystal. "It was a tough project, and we were really stuck," Kwong told *Science*. But before long, help was on its way. This came from Sodroski's group, which, like other research teams, had created mutant versions of gp120 as a way of probing which parts of the molecule are most critical to its role of leading the viral penetration of T cells (*Science*, 25 October 1996, p. 502). One day in 1993, Sodroski called Hendrickson to ask if his lab would be interested in trying to crystallize some of the altered gp120 molecules that Sodroski's lab was producing.

Over the next several years, Sodroski and Richard Wyatt, another molecular virologist at Dana-Farber, kept in constant touch with Hendrickson and Kwong, discussing strategy and designing one gp120 variant after another. The team was also aided by immunologists James Robinson at the Tulane University Medical Center in New Orleans and Raymond Sweet at SmithKline Beecham Pharmaceuticals in King of Prussia, Pennsylvania, who provided antibodies and CD4 molecules.

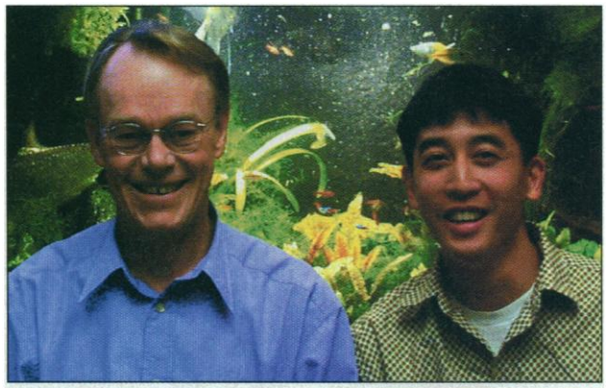
Kwong put the gp120s together in various combinations with CD4 and antibodies, looking for a molecular complex that was rigid enough and regular enough in shape to crystallize. Finally, he hit on the right combination: When he mixed the gp120-binding domains of CD4 with a truncated version of gp120—missing most of its carbohydrate groups, some of its V loops, and a few other bits—and then added in a fragment of an antibody called 17b that only binds tightly to gp120 when it has already bound CD4, this complex produced crystals up to 50 micrometers across. This size was just adequate for x-ray crystallography. Because the complex contained the regions of both gp120 and CD4 that previous studies had shown were critical for HIV entry into cells, the crystal structure was guaranteed to contain a gold mine of information.

"For the last 10 years, crystallographers have wanted to get this structure," says Baltimore. "It took deep insight to know how to shave down the molecule to get a core that would crystallize." Hendrickson adds that he is reasonably confident that the structure of the gp120-CD4-17b complex, despite its abbreviated form, accurately reflects the relationships among its three partners. He notes, for example, that the strength of the binding between the CD4 domains and the gp120 core is "quite similar" to that of the native proteins. Moore, too, says that he has "no concerns about the modifications necessary to crystallize this molecule," adding that "whatever minor limitations exist are massively outweighed by what has been learned."

The structure obtained from those crystals immediately began to reveal new insights. It shows, for example, that gp120 is divided into two principal domains, which are connected by four polypeptide strands that form a "bridging sheet" between them. Moreover, although most of the carbohydrate groups and V loops have been removed, these parts of the protein—whose positions can be deduced from the remaining core structure—appear to partly cover and obscure the binding sites for both CD4 and another receptor, called CCR5, that HIV-1 must also attach to in order to infect cells. This information, elaborated further in the companion paper in *Nature*, helps explain why HIV-infected patients produce so few antibodies that are effective in blocking these regions. The immune system essentially cannot "see" these prime targets and generate a response to them.

Another obstacle to immune attack is revealed by the detailed structure of the CD4-binding site itself. CD4 fits into a recessed pocket in gp120, which, the authors say, may simply be too deep to be easily accessed by antibodies. Moreover, the surfaces of gp120 and CD4 make only partial contact, leaving two relatively large cavities between the molecules. One of these cavities is filled with water molecules, which may provide another mechanism for HIV to escape antibody attack: Because the region of gp120 facing this cavity does not make intimate contact with CD4, the amino acids that make it up are freer to undergo mutation and avoid recognition by antibodies. "The mechanisms used by gp120 to resist [the immune system] are nicely revealed" by the new structure, says Moore.

An additional key insight gleaned from the structure, described in the *Science* paper, is that the bridging sheet—which is partially bound by the 17b antibody—also helps make up part of gp120's binding site to CCR5. This finding solves a mystery about how HIV recognizes CCR5, whose normal function is to bind immune regulatory molecules called chemokines. The virus had been thought to attach to CCR5 primarily through one of the V loops, called V3. But the amino acid sequence of V3 varies greatly from one HIV strain to another. In fact, it mutates so readily that the changes enable HIV to switch, after the early stages of infection, from using CCR5 to infect cells to using another chemokine receptor called CXCR4 in later stages. But that high degree of variability caused researchers to wonder how all the different variants could bind so tightly and specifically to the genetically conserved chemokine receptors in the first place.



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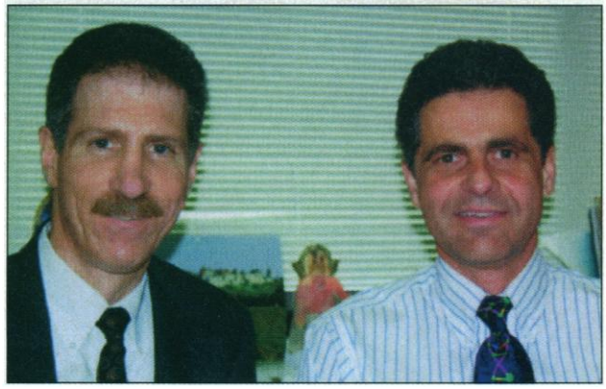

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Feat of perseverance. Top, Hendrickson *(left)* and Kwong *(right)*; bottom, Sodroski *(left)* and Wyatt *(right)*.

Sodroski and Wyatt provide the answer. Using the crystal structure as a guide, they designed a series of gp120 mutants that contained alterations in the polypeptides implicated in this binding. They discovered that the chemokine receptor binding site on gp120 includes elements of both the highly variable V3 loop and the bridging sheet, which is highly conserved. This new finding explains how this site can have both the specificity required for tight binding to one chemokine receptor and still be variable enough to allow the virus to switch later to another.

Researchers are confident that the insights they are gleaning from the structure will open new doors to drug and vaccine intervention against HIV. For example, Sodroski points out that the conserved region of the CCR5-binding site on gp120 "gives people a [drug intervention] target that looks much more attractive" than either the variable V3 loop or the chemokine receptors themselves. Targeting the receptors, for example, could end up disrupting the immune systems of already immune-compromised patients. In contrast, blocking the binding site on the viral protein might be less likely to have harmful side effects. Other possible drug targets, team members say, are the cavities formed when CD4 binds to gp120. "That is where you really want to put in some therapeutic compound" to disrupt the binding, says Kwong.

Vaccine strategists will find much to mull over as well. For some researchers, the finding that the CD4- and CCR5-binding sites are essentially invisible to the immune system constitutes additional evidence that basing vaccines on whole gp120 molecules is doomed to failure (*Science*, 30 January, p. 650). "This plays right into HIV's defenses," Moore says. "Knowing what not to attack is sometimes extremely valuable."

As for what would be effective, Marseilles's Sattentau suggests that one way to get around gp120's defensive shroud might be to use a vaccine equivalent of a one-two punch. Thus a vaccine might be designed to stimulate antibodies in high enough concentrations that they could penetrate the shroud and attach to CD4-binding sites, possibly triggering a change in gp120's shape and exposing the CCR5-binding sites—which would then be vulnerable to attack by a second antibody. But whatever the winning strategy—or strategies—turn out to be, Sodroski says that the gp120 structure should give a big boost to vaccine research. "Over the next year or so, we are going to see a lot of people doing all kinds of modifications to gp120," with the aim of finding ways to induce stronger antibody responses.

It may take some time before AIDS researchers figure out how to take maximum advantage of the weaknesses now revealed in gp120's structure. But they are confident that this intimate new knowledge of the protein will lead to a whole new battle plan and—with luck—new weapons that could deliver the knockout blow.

–Michael Balter