Close-Up of a Killer

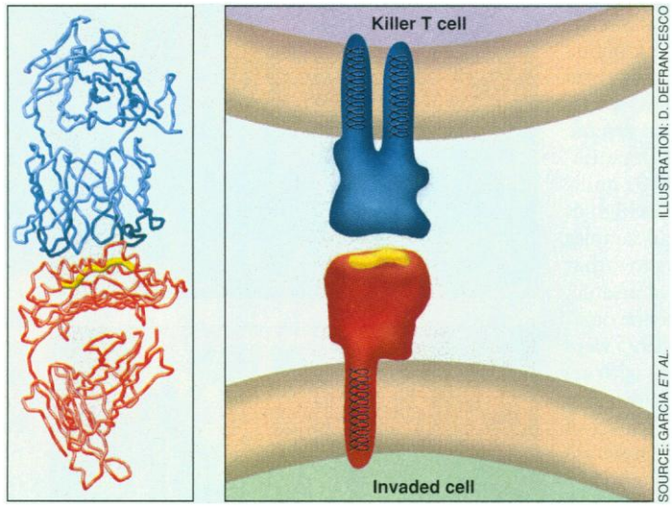
New atomic-scale images of the receptor of a killer T cell detail how the immune protein recognizes infected cells

In 1984, Stanford University immunologist Mark Davis and his colleagues cloned a snippet of DNA that sounded a starting gun in a race for one of immunology's greatest prizes: the precise atomic structure of the business end of T cells. These cells come in two forms, killer and helper cells, and are the immune system's border police, responsible for finding infected or cancerous cells. The Davis group had found the code for the part of the molecule that locks onto these undesirables and spurs an immune attack. Over the next several years, a host of research teams jumped into the contest, which many hoped would be the scientific equivalent of a 100-meter dash, fleet and sweet. Most dropped out as obstacles turned the race into a grueling marathon. But now, a group of California researchers has finally broken the tape.

On page 209, Ian Wilson at The Scripps Research Institute and Per Peterson at the R. W. Johnson Pharmaceutical Research Institute in La Jolla, California, and colleagues report completing the first-ever x-ray crystal structure of a T cell receptor (TCR), a closeup image that details the molecule's atomic structure. "It's a beautiful scientific result," says Don Wiley, a crystallographer at Harvard University, whose team is on the verge of completing its own three-dimensional model of the receptor. Adding to the beauty of the result, the researchers also managed to sneak a less detailed glimpse of how the receptor, in this case from a killer cell, binds to an infected cell's distress signal: a combination of one of the cell's own proteins and a tiny fragment of the invader. The glimpse "is a big milestone on the home stretch to completely understanding how the T cell receptor ... controls the function of T cells," says John Kappler, an immunologist at the National Jewish Center for Immunology and Respiratory Medicine in Denver.

The new finding should also pique the interest of medical researchers. "Malfunctions of this recognition system are thought to be important in many [autoimmune] diseases," says Richard Lerner, an immunochemist and the president of Scripps. In lupus, for instance, the TCRs bind to fragments of the body's own proteins, triggering the destruction of healthy cells. Equipped with an atomic-scale image of how this interaction takes place, researchers may be able to design drugs to inhibit overactive T cells—although, by all accounts, realizing such goals remains years away.

Over the past 3 decades, many research teams have uncovered pieces to the puzzle of how T cells manage to recognize once-healthy cells that have gone bad. In work that won this year's Nobel Prize in physiology or medicine, Peter Doherty and Rolf Zinkernagel—then at the John Curtin School of Medical Research in Canberra, Australia—learned that T cells will not react to fragments of foreign proteins alone. Rather, special proteins coded for by genes of the major histocompatibility complex (MHC) must be present as well. The bits of foreign protein, researchers later determined, are made with the help of enzymes inside the invaded cell that chew up the pathogens into protein fragments, or peptides, which are then scooped up by the MHC proteins and carted through the cell membrane. More recently, other researchers have produced x-ray crystal images of substantial chunks of the TCR—although not the entire structure. And they have also found that just six short segments of a TCR—the so-called complementary determining regions (CDRs)—actually come into contact with peptide-MHC complex, and just two of the CDRs are involved in identifying the foreign peptide sitting wedged in a groove of the MHC protein.



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SOURCE: GARCIA *ET AL.*

Close encounters. At right, T cell receptor *(blue)* recognizes complex of foreign peptide *(yellow)* and MHC protein *(red)* embedded in cell membrane. Detail of structures is at left.

Still, the receptor's complete atomic structure eluded researchers until a month ago. Determining a molecule's structure is rarely easy. Scientists must generate millions of copies of the molecule, coax them into a crystal, and bombard it with x-rays. By analyzing how the x-rays scatter off atoms in the crystal, researchers can reconstruct the precise location of every atom in the molecule and also determine their identities, because different-sized atoms deflect x-rays differently.

But creating good crystal images of the TCR was harder than anyone had imagined. The first phase alone—generating millions of copies of the molecules—stymied researchers for years. Such copies are usually produced by splicing DNA coding for a protein of interest into bacteria or other cells that can be grown in the laboratory. The cells' own machinery then churns out copies of the protein.

But when researchers first tried this with the TCR they had little luck. The TCR, explains Wilson, consists of two protein subunits, known as the α and β chains, which must fold together in a precise arrangement. But although bacteria such as *Escherichia coli* could be coaxed into producing the subunits, the proteins remained a jumbled mess instead of folding neatly into the receptors.

Hoping to get around the problem, in 1992 Wilson's team began collaborating with Peterson and his R. W. Johnson colleagues, who had successfully produced other recalcitrant proteins in cultured cells from the fruit fly *Drosophila melanogaster*. Unlike *E. coli*, *Drosophila* cells have biochemical accessories, such as cellular machinery that adds sugar molecules to the protein subunits, which promote proper protein folding. After more than a year of effort, the team got a break. By adding to the DNA code for a TCR a tiny addendum that specifies a short amino acid tail on the protein, the researchers were able to persuade the fly cells to express and release large amounts of the receptor. "We don't know why it works," says team member Luc Teyton, but

life became much easier when it did.

The tails caused their own share of problems, however. The receptor proteins are shaped somewhat like rectangular boxes; to form a crystal they must pack together tightly like bricks in a wall, says Scripps team member Christopher Garcia. But the amino acid tails kept waggling around, preventing the molecules from adopting a uniform crystalline arrangement. To surmount this hurdle, the researchers used protease enzymes to chew off the amino acid tails. Shortly after that, they started getting small crystals.

The early crystals were good enough to help the researchers complete most of their model, by confirming the location of atoms in the chunks of TCR that they had crystallized earlier. But the crystals were too small to provide the high-resolution data needed to resolve the portion of the TCR—a region of the α chain—that had never before been glimpsed. The small crystals degraded rapidly when bombarded by x-rays, limiting the amount of data the researchers could collect. So the researchers tried freezing the crystals, which typically stabilizes them. But because a deep freeze can also damage the crystal lattice, Garcia spent much of this summer trying to get the TCR crystals to freeze in the x-ray machine without being damaged.

Finally, in late July, after some 50 attempts to find the right combination of protective compounds, Garcia hit upon a promising recipe. He launched the 2-week data-collection run, then he waited to see if the data would have a high enough resolution to allow the researchers to determine the TCR's atomic structure. "My God, it was nerve-wracking," says Garcia. Once the data started coming in, however, "it was clear we were going to get the structure," he says. He adds simply, "I was very happy."

At the same time that the group was crystallizing and studying the TCR alone, it was also trying to get an x-ray picture of the TCR in action: bound to a peptide-MHC complex. Doing so meant creating crystals of the entire, three-molecule complex, stabilizing it, and analyzing it. But to Garcia's surprise, the added complexity didn't bring new obstacles. "The crystallization of the complex was much easier than the TCR alone," he says. "It's sort of a Zen truism. What you imagine to be the most difficult thing turns out to be the easiest."

The completed structures confirm some earlier notions about the TCR's structure and how it works. As soon as Davis and his colleagues sequenced the DNA for the TCR back in 1984, they saw that its amino acid sequence closely matched that of antibodies. Davis and others suspected that the TCR would resemble an antibody in shape, a suspicion the new TCR structure supports. The structures also bolster the idea advanced by several labs that just one CDR segment on both the α and β chains of the receptor—the so-called CDR 3 regions—is primarily responsible for binding to the peptide.

But the larger picture of how the TCR binds to the peptide-MHC complex holds some surprises. The details of this binding have long intrigued biochemists, because they reveal precisely how different T cells manage to recognize trillions of different foreign peptides in conjunction with just a dozen or so MHC presenters. And attention has focused on the central role of the peptide-binding CDR 3 regions, because they seem to hold the key to the T cells' ability to discriminate between an enormous array of potential targets.

Most researchers agree that the two CDR 3 regions bind primarily to the peptide. But researchers differ about the orientation of the structures as they bind and the precise role of the other CDR regions. Peptides, MHC molecules, and CDRs all tend to have an oblong shape. And one model, put forward by Davis and his colleagues in 1992, suggests that the long axes of CDR 3s on the receptor are positioned perpendicularly to the long axis of a peptide-MHC complex as the two structures interlock. It also predicts that the other CDR segments, known as the 1 and 2 regions, are bound mainly to the MHC molecule. Another model, offered earlier this year by Charles Janeway and his colleagues at Yale University, postulates that the long axes of the two complexes are parallel and that all the CDRs interact with both the MHC and peptide.

As it turns out, neither model was right on, because the CDR 3's bind to the peptide at an intermediate angle. But the way in which the CDRs 1 and 2 bind to both the peptide and MHC molecules appears to be more in line with the Yale model's prediction, says Wilson. Harvard's Wiley, when asked whether his group sees this same orientation in its emerging structure, says that it's still difficult to tell. However, he adds, "it's obvious from both our labs that it's not 90 degrees." While Yale's Janeway says he feels "vindicated" by the result, he cautions that other TCRs could bind to the peptide-MHC complex with a different orientation. "We need a lot more crystal structures of other TCR complexes to say we know it always works like that," says Janeway.

Wilson also emphasizes that their lower resolution TCR-peptide-MHC crystal only offers a preliminary look. "It doesn't tell us the fine details of the docking," says Wilson. "There's more to the story," says Wiley, who says his team has completed a high-resolution look at the three-way binding, but they are waiting to publish their results until they too have resolved the α chain segment. Such details will be vital in helping drug designers tailor molecules to either block or promote the binding between TCRs and their peptide-MHC targets, says Scripps's Lerner. So while the marathon run for the T cell receptor may be over, the sprint for seeing the fine details of how the receptor recognizes its target is just heating up.

–Robert F. Service

NEUROBIOLOGY

New 'Alzheimer's Mouse' Produced

For researchers seeking to understand Alzheimer's disease, one item has long topped their wish list: a small-animal model that exhibits both the brain degeneration and the memory deficits characteristic of the disease. Last week's announcement of a new genetically engineered strain of mice that appears to suffer these dual symptoms brought them a big step closer to that goal. And further work with the animals has demonstrated how valuable an animal model of Alzheimer's could prove to be: The researchers who developed the mice have already found encouraging signs that the animals will be useful for testing current ideas about the biochemical pathways responsible for the so-called amyloid plaques, which stud the brains of Alzheimer's patients and may contribute to the neuronal damage they suffer.

As neurobiologist Karen Hsiao of the University of Minnesota, Minneapolis, and her colleagues reported in last week's issue of *Science* (4 October, p. 99), they produced the new mouse strain by introducing into the animals a mutant version of the human gene encoding the amyloid precursor protein (APP). Previous genetic studies had linked the mutant APP gene to some cases of hereditary Alzheimer's, and in the mice it triggered typical Alzheimer's symptoms, including both plaques containing β amyloid, a protein released from APP, and learning and memory impairments. It's not a perfect model—conspicuously missing so far, for example, is another major feature of Alzheimer's pathology, the so-called neurofibrillary tangles that consist mainly of an abnormal form of another neuronal protein called tau. However, Alzheimer's researchers say it is a marked improvement over other previous models made by introducing mutant APP genes into mice. At least in the results currently published, those animals had either plaques or cognitive impairment, but not both.

"What's so special about this [Hsiao] paper is that it does go the extra distance and show the learning impairment," says neurogeneti-