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-

cist Rudy Tanzi of Harvard Medical School in Boston. "It's clear that we're waiting for such a model," agrees neurologist John Morris of Washington University School of Medicine in St. Louis. In particular, researchers want to test an idea that has long been controversial in Alzheimer's research: that amyloid deposition is a cause, not just a byproduct, of the brain changes that lead to memory loss in the disease. The fact that the altered mice show a particular biochemical abnormality—an increase in a "sticky" β-amyloid fragment that other recent results have implicated as a villain in the genetic forms of the disease—suggests that the animals will be well suited to the task.

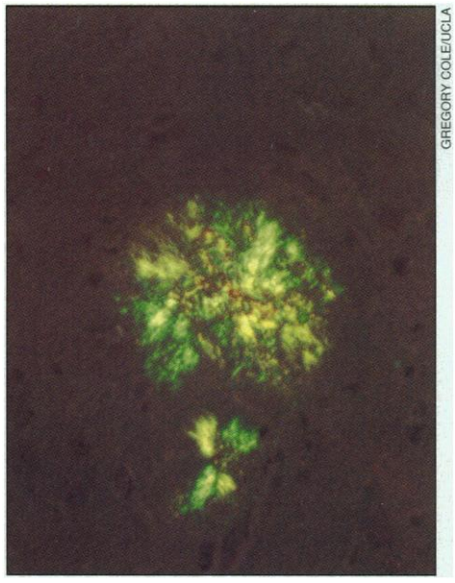
Hsiao says she does not know exactly why her team succeeded in getting both plaques and memory impairment in the same animals, but her hunch is that they were lucky in choosing the right mouse strain. Indeed, in the first strain Hsiao and her colleagues worked with, designated FVB/N, expression of the mutant APP gene did not lead to plaque formation, although it did produce a behavioral change. Unlike normal mice, the transgenic animals didn't set out to explore when they were placed in an unfamiliar cage, an indication that they were fearful of novel environments. They also died earlier than did normal controls. "We developed an animal with a very striking phenotype," Hsiao says, "but not the classic Alzheimer's pathology." (The group reported these results in the November 1995 issue of *Neuron*.)

However, a different mouse strain (C57B6/SJL) that overexpresses the mutant gene did develop typical plaques in their brains as well as difficulties in learning. And both abnormalities were most apparent in older animals—a situation that mimics what happens in human Alzheimer's. For example, 2-month-old and 6-month-old transgenic animals did about as well as controls did in learning to locate a submerged platform in the water-maze test, but 9- to 10-month-old transgenic animals were unable to learn the platform's location.

Why the mutant APP transgene had such different effects in the two mouse strains is unclear. Presumably, other genes influence how the animals handle APP or its β-amyloid byproducts, Hsiao says, adding that comparing the two strains might reveal the crucial genetic differences. However, in the strain that does develop the plaques, the researchers have already found what may be a clue to the biochemistry that leads to their formation.

The β-amyloid peptides released from APP vary slightly in length from 39 to 42 amino acids. And the Hsiao team found that expression of the mutant APP led to a 14-fold increase in the 42-amino acid β-amyloid variant (Aβ42), compared to only a fivefold increase in the 40-amino acid version. That observation dovetails with recent work by several labs suggesting that Aβ42 may be especially prone to forming plaques.

Not only does that peptide have a greater tendency than its smaller cousins to clump into insoluble aggregates in the test tube, but it is also the predominant β-amyloid species in the plaques in Alzheimer's brains. What is more, work reported in the August issue of *Nature Medicine* by a large multiinstitutional team suggests that all the known mutant genes that cause Alzheimer's increase Aβ42 concentrations.



GREGORY COLE/UCLA

Alzheimer's mimic. In mice, the mutant APP gene leads to plaques, like this one, resembling those in Alzheimer's brains.

In addition to the APP gene, these mutant genes include two that encode structurally related proteins called presenilin 1 and 2. Together, mutations in the three genes account for 50% of all inherited Alzheimer's cases. In the *Nature Medicine* paper, the team, led by Steve Younkin of the Mayo Clinic in Jacksonville, Florida, reported that people who have mutations in either the APP or presenilin 1 or 2 genes have higher concentrations of Aβ42 in their blood plasmas than do normal controls of comparable ages or people with so-called "sporadic" Alzheimer's—cases that do not appear to be hereditary. In addition, the researchers found that skin cells from people with the mutations secrete more Aβ42 into the culture medium than do cells from controls.

And in as yet unpublished work, several teams have introduced mutant presenilin genes into mice and are also seeing increased Aβ42 concentrations in the animals' brains as a result. "There are good data from several labs that mutated presenilins increase Aβ42," says Sam Sisodia, a molecular neurobiologist whose lab at Johns Hopkins University School of Medicine is one of those doing the work. "These data support the idea that it is the culprit." Accumulation of this sticky Aβ42 might then lead to nerve damage in the brain. Other work has shown that aggregated β amyloid is toxic to neurons.

Sisodia, who presented his group's data in July at the Fifth International Conference on Alzheimer's Disease and Related Disorders in Osaka, Japan, cautions, however, that no one has yet shown that the presenilin transgenes lead to either plaque formation or memory impairments in the mice. "We probably have some of the oldest mice around, but we don't have any pathology yet," he says. "It's just a matter of wait and see."

In spite of the intriguing correlation between plaques and Aβ42 elevation in her own mice, Hsiao is also cautious about what her team's findings might mean for the amyloid hypothesis. "We found a correlation between impairment in learning and memory and increased β amyloid and senile plaque formation, but this doesn't prove that the mice's amyloid problems cause the behavioral problems," she says.

But researchers should soon be able to make a more direct test of the amyloid hypothesis in the new mouse strain. Researchers in industry and academia are working to develop drugs that inhibit amyloid deposition in plaques. If any of these can be shown to inhibit both plaque formation and the memory impairments in the transgenic mice, it would be the best evidence yet that the two are causally related.

And the Hsiao team's mouse may not be the only one out there suitable for exploring that question. In the 9 February 1995 issue of *Nature*, a team led by Ivan Lieberburg of Athena Neurosciences Inc. in South San Francisco, California, described its own Alzheimer's mouse, also made by genetically engineering the animals with a mutant APP gene. Those animals developed typical plaques, but in their original paper the Athena team did not report any cognitive deficits. Lieberburg now says the group has unpublished results showing that the animals are "behaviorally disturbed," with memory impairments.

Hsiao and her colleagues, meanwhile, are doing their best to ensure that the wait for a verdict on the amyloid hypothesis will be as short as possible. They have decided to supply their mice to academic researchers without restrictions on their use. "The best news about this is that they're making [the new mouse strain] freely available to academia," says Harvard's Tanzi. Researchers around the world should soon discover for themselves just how valuable this particular mouse model will turn out to be.

–Jean Marx