The T-Cell Receptor—the Genes and Beyond

With the T-cell receptor for antigen in hand, immunologists tackle problems in T-cell function that were not previously approachable

For many years immunologists were hampered in their studies of T cells by a crucial gap in their knowledge. They had been able to obtain almost no direct information about the T-cell receptor, the cell surface molecule that recognizes and binds foreign antigens, thus triggering the chain of events that culminates in T-cell activation and proliferation. Without this activation a normal immune response could not be mounted. T cells not only serve as killers of cells that they recognize as foreign (virus-infected cells, for example) but they also play an important role in regulating the activities of other immune cells, including the antibody-producing B cells.

About 2 years ago, investigators finally identified and isolated the T-cell receptor molecule. Since then they have cloned the genes coding for the two protein chains of which it is composed (*Science*, 25 May 1984, p. 859; 30 November 1984, p. 1065). These accomplishments have enabled them to tackle some previously unapproachable questions concerning the nature of the receptor, the development of T cells, their interactions with other cells, and the biochemical changes produced during activation. A recent meeting, "The 24th Midwinter Conference of Immunologists," which was held on 19 to 22 January in Pacific Grove, California, focused on the early results of these studies. Although all T-cell problems have by no means been solved, it is clear that the research is off to a running start.

Not surprisingly, the most progress so far has been in determining the structures of the receptor genes and thus of the proteins themselves. The receptor genes are structurally related to antibody genes. They all belong to the same large "supergene" family that codes for a variety of proteins that are required for normal immune responses.

T-cell receptors face the same challenges as antibodies do. For example, they both must be able to recognize large numbers of foreign antigens. Antibody genes have evolved a variety of means of generating a repertoire of proteins with the needed diversity of structure. Studies of the receptor genes now show that they have adopted some of the same strategies but that there are also significant differences in diversity generation by the two sets of genes.

One strategy used by both is the assembly of complete genes from smaller coding segments. The genes for the larger of the two protein chains of which antibodies are composed and for the β-chain of the T-cell receptor are assembled from four such segments, which are designated V (for variable), D (for diversity), J (for joining), and C (for constant). A complete gene for an antibody light chain requires only V, J, and C segments. At present, it is not clear whether the α-chain gene, which is the more recently cloned of the two receptor genes, has a D segment, but it does contain the other three.

During the development of mature B and T cells, the DNA rearranges to bring together the V, D, and J, or V and J, segments. When joined, these encode the antigen-binding portions of the antibody and receptor proteins, which vary from one molecule to the next. The greater the number of different V, D, and J segments, the greater the diversity of the proteins that can be made from them. On this point the T-cell receptor genes appear to differ from those of antibodies. There are approximately 100 V segments for antibody heavy chain genes and perhaps 200 to 300 for antibody light chain genes. But according to Leroy Hood of the California Institute of Technology, "The repertoire of V segments is going to be incredibly limited for the β-chain of the T-cell receptor family."

This conclusion is based on a statistical analysis of the frequency with which particular V segments appear in the 19 β-chains sequenced thus far in the various laboratories. One V region turned up in six different β-chains, another in three, and three more appeared in two proteins each. From this it was calculated that there are between 15 and 30 V gene segments for β-chains, with 15 being the more probable number.

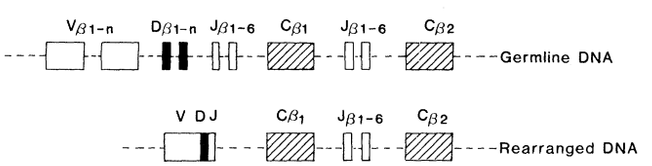
Fewer α-chain sequences have been determined and a comparable analysis is not yet possible. Mark Davis of Stanford University School of Medicine suggests that there are at least 50 V sequences for this chain and perhaps many more.

Antibody genes are prone to undergo mutation in dividing B cells, which provides them with another means of generating diversity. In contrast, the sequence data for the receptor β-chains indicate that analogous mutations are rare.

The question then is how is the required diversity generated in T-cell receptor proteins if not by mutation or by using a large number of V-gene segments? At least for the β-chain, part of the answer seems to lie in the flexible way in which the D segment is joined to the V and J segments, Hood suggests. In particular, the joining may result in the use of any of the three possible reading frames of the D-segment codons, each of which would give a different amino sequence in the resulting protein. "The D regions of heavy chains can be used only in one reading frame," Hood points out.

In addition, the attachment of sugars to the receptor proteins (glycosylation) may influence their antigen specificity, according to James Allison of the University of California at Berkeley. He finds that monoclonal antibodies directed against the antigen-binding sites of receptor molecules do not recognize the proteins unless they are glycosylated. This suggests that the sugar addition, a normal part of receptor protein synthesis, influences the shape of the binding site and consequently its specificity for antigen. Usually, he notes, antibodies to antibodies bind to their targets even if those targets are not glycosylated.

The sequence studies may also provide some clues to another puzzle re-



In germline cells, the gene sequences coding for the four portions of the β-chain of the T-cell receptor are separate. During T-cell maturation, the DNA rearranges, joining the V, D, and J segments. Attachment of this combined sequence to the C-region segment occurs in the messenger RNA when the unnecessary sequences are spliced out.

What's Going on at Neptune?

Just when astronomers thought they might have shown that Neptune has no rings detectable from Earth, along comes last summer's occultation of a star by an apparent ring. Apparent because the evidence is so strong but the implications so odd—even for the bizarre world of planetary rings. If a ring did briefly obscure that star, it was like no other ring known or imagined.

Last summer's occultation has met all the requirements for credibility demanded of it by astronomers. The key is that the observations were made at different sites through different telescopes having filters of different colors. On 22 July, Patrice Bouchet of the European Southern Observatory in Chile and colleagues were using both the observatory's 1- and 0.5-meter telescopes to observe the star for André Brahic of the University of Paris. The star dimmed in both telescopes. That ruled out instrument error.

One hundred kilometers to the south, an observing team headed by William Hubbard of the University of Arizona also recorded the dimming through the 0.5-meter telescope at the Cerro Tololo Inter-American Observatory. That ruled out a cloud or another relatively nearby obstruction. Hubbard's group was observing the bluish planet and the reddish star at three different wavelengths so that Hubbard can state with confidence that only the star was occulted. Only something in the vicinity of Neptune would be likely to block the light from the star and not the light from the planet.

Other characteristics of the occultation leave little doubt that both groups of observers saw the same, real event. It occurred about 0.1 second later in the south than in the north, as would be expected for a true ring occultation. When Hubbard and Brahic first compared their occultation records in detail last month, they found that their profiles of varying light intensity are "virtually identical." Both are V-shaped, about 0.8 second wide at half depth, and equivalent to a loss of about 35 percent of the star's light. That would correspond to a ring about 10 to 15 kilometers wide and about 70,000 to 80,000 kilometers (3 Neptune radii) from the planet's center.

But then there are the oddities. Where both groups should have seen a second occultation as the star passed to the outside of the ring farther around its circumference, they saw nothing like the earlier event. Conceivably, only a fragment of a ring might have been responsible. And the occultation places the apparent ring so far from the planet that only the most accommodating theory would allow ring particles to avoid agglomerating into a satellite.

"I'm willing to accept that they saw part of a ring," says Harold Reitsema of the Ball Corporation in Boulder who is an experienced occultation observer, "but that creates real problems because I don't understand how you get parts of rings." He is not alone. Theory requires that the particles spread to form a complete ring or coalesce into a satellite. Two narrow rings in Saturn's Encke gap do seem to be undetectable in about half of the close-up Voyager images, but in view of all of the negative reports from Neptune observations of the past few years (*Science*, 21 October 1983, p. 311), Hubbard estimates that this "ring" must be undetectable nine times out of ten that a star passes behind it. Perhaps a ring could broaden and thus thin out rapidly enough to render it undetectable a short distance farther around its circumference, but everyone finds that hard to accept.

There may be other strange things going on. In 1981 Reitsema and his colleagues observed another solitary occultation near Neptune, also at a distance of roughly 3 Neptune radii. Because the star's obstruction appeared to be complete and two nearby telescopes recorded it while a third did not, it seemed reasonable that a satellite at least 180 kilometers in diameter had blocked the star. A small satellite is not so odd, but catching one occulting a star was a 1000 to 1 shot, unless there is a whole belt of them. Elsewhere small, inner satellites and rings go hand in hand.

The latest occultation has reinvigorated the search for Neptunian rings, but astronomers are still mostly mystified—such strong evidence and such confusing implications. Galileo must have felt the same way. He died decades before Huygens figured out that Galileo's discovery of Saturnian "companions" is actually a system of rings.—**Richard A. Kerr**

garding T-cell recognition of antigen. The T-cell receptor can only be activated by a foreign antigen when it is presented on a cell surface in conjunction with an appropriate histocompatibility molecule. After arguing for many years, most immunologists have come to the view that the same T-cell receptor recognizes the foreign antigen and the histocompatibility molecule together. (The opposing view is that there is one receptor for the antigen and a separate one for the histocompatibility molecule.)

Davis proposes that the β-chain may be the one that interacts with the histocompatibility molecule. He finds them to be distinctly more variable in amino acid sequence than are α-chains. "There is quite a lot of variability," he says of the β-chains. "Moreover, you find it in places where you don't find it in immunoglobulins [antibodies]." In his view, the extra regions of high variability might form the recognition site for the histocompatibility molecule. The remaining hypervariable regions, which are comparable to those of antibody proteins, would then bind antigen.

Hood, who has also compared amino acid differences among β-chains, does not think that the data provide convincing evidence for the existence of the extra hypervariable regions, however. In addition, he points out that recent studies have shown that antigen contacts with antibody may extend beyond the antibody's three hypervariable regions. If the T-cell receptor is similar, then its antigen-binding region might extend into the postulated histocompatibility-recognizing areas.

However the T-cell receptor binds antigen and histocompatibility molecule, one immediate consequence, according to Lawrence Samelson, who works with Ronald Schwartz at the National Institute of Allergy and Infectious Diseases, is the addition of a phosphate group to a 20-kilodalton protein that is closely associated with the receptor in mouse T cells. The finding suggests that the T-cell receptor may be on the same footing as numerous other receptors, including those for growth factors, hormones, and neurotransmitters, in which activation results in a phosphorylation event. Moreover, the 20-kilodalton protein may be the murine analog of the "T3" molecule that participates in the activity of the human T-cell receptor.

Another consequence of T-cell receptor activation, as shown in several laboratories, is a flow of calcium ions into the cell and a stimulation of the production of interleukin-2, which is also called T-cell growth factor, and its receptor. As a

result of these changes the activated cell is stimulated to divide, thus expanding the population of cells with the desired specificity for antigen.

The new ability to study T-cell receptor function may also help to clarify the role of the thymus gland in T-cell development. Newly formed T cells enter the thymus and there acquire the surface molecules characteristic of T cells in general and of the various subgroups. Several participants in the Pacific Grove meeting reported that formation of the antigen receptor at least begins during the cells' sojourn in the gland. They found that the gene rearrangements needed to join the V, D, and J segments of the β-chain gene occur in thymic T cells. "The rearrangement starts in the thymus," says Tak Mak of the Ontario Cancer Institute in Toronto. "This suggests an inductive role of the thymus on the gene." The α-chain gene apparently rearranges later, however.

One of the many mysteries of T-cell development relates to the requirement that the receptor recognize foreign antigen in conjunction with a histocompatibility molecule, which is essentially a marker that distinguishes "self" from "nonself." The T cell can be activated only by an antigen-presenting cell of the same histocompatibility type but it must not be triggered by the self-marking histocompatibility molecule alone because the result would be an immune attack on the body's own tissues. This implies that there is a way of eliminating, presumably in the thymus, any T cell with a receptor having a high affinity for the self marker. Ellis Reinherz of Harvard's Dana-Farber Cancer Institute has proposed a possible means of achieving this.

He and his colleagues have found that there are two distinct ways of activating T cells, one through the specific antigen receptor and the second through another surface molecule, which is found on almost all T cells and has been given the designation T11. Activation through T11, unlike that through the specific receptor, does not require antigen-presenting cells or interleukin-1, although triggering cells by either pathway induces the calcium ion influx and interleukin-2 production. The T11 pathway is regulated by the specific receptor. Occupation of the antigen receptor makes the cells refractory to activation through T11.

The T11 molecule may be the evolutionary ancestor of the specific receptor, Reinherz speculates. It appears on T-cell surfaces early in the thymic portion of development, before the receptor is formed, and may be important in stimulating expansion of the immature T-cell population. Antigen-specific receptors appear later. Reinherz suggests that if any of these receptors binds well to the self histocompatibility molecules in the thymus, the cells bearing them will be unable to divide because the thymus lacks interleukin-1, which is needed for activation by the receptor pathway. Moreover, they will also become refractory to activation through T11 and will effectively be removed from further action. "This may explain how you can select out T cells with high affinity for self," he says.—**JEAN L. MARX**

Virus-Heart Link Studied

Viruses can produce atherosclerosis in experimental animals and can cause human cells to accumulate cholesterol in tissue culture. Viruses also have been found in human arterial tissue removed during bypass surgery. On the basis of this information, some investigators are persuaded that viruses may be the underlying cause of heart disease in people.

The story of viruses and heart disease began about a decade ago with the discovery by Catherine Fabricant of Cornell University that a herpesvirus that infects cats causes lipids and cholesterol to accumulate in cultured cat kidney cells. "The idea that viruses could induce cholesterol crystals in cell culture made me hypothesize that similar viruses might do the same thing in human cells and might possibly cause atherosclerosis," she says.

Fabricant and her colleagues, including Julius Fabricant of Cornell and C. Richard Minick of New York Hospital–Cornell Medical Center, went on to show that the herpesvirus that causes Marek's disease in chickens causes atherosclerosis in those animals. The virus also alters lipid metabolism in cultured chicken arterial cells, causing an accumulation of cholesterol and cholesterol esters, Fabricant says. More recently, Fabricant found that cytomegalovirus, a human herpesvirus, causes human arterial cells to proliferate and accumulate cholesterol in culture.

For years, however, Fabricant's group worked nearly alone. "I've been considered a crackpot," she remarks, "but now I've gained a little more acceptance." Among the investigators who are looking at her work with renewed interest are Michael DeBakey, Joseph Melnick, and their associates at Baylor College of Medicine and Earl Benditt and his colleagues at the University of Washington School of Medicine.

For the past few years, the group at Baylor has been examining arterial tissue from bypass surgery patients for evidence of herpesviruses. Using electron microscopy, they found that about one-third of the more than 200 patients had cytomegalovirus in their diseased vessels.

Benditt, working with Thomas Barrett and James K. McDougall, also looked for herpesviruses in artery tissue using molecular probes of viral messenger RNA. With that technique he found herpes simplex viruses.

It is not entirely clear what these associations of herpesviruses with atherosclerotic plaques mean. Nearly everyone, at some time, is infected with at least one of the herpesviruses and there is no evidence that heart patients are any more likely to have had a herpes infection than anyone else. But herpesviruses are attractive as a cause of heart disease for several reasons. First, they cause cells to proliferate, "like micro tumors," as Melnick puts it. Then, there is the cell culture work showing that human cells accumulate cholesterol and lipids when they are infected.

In addition, the viruses remain latent in cells after a person has been infected, leaving open the possibility that they can take years to produce disease. Douglas Cines of the University of Pennsylvania, who is intrigued by the viral hypothesis, remarks, "Atherosclerosis begins in young people and develops over decades. Herpesviruses persist in cells in a latent state and are activated periodically. It is possible to imagine that from time to time the virus breaks out and does its thing."

But since herpesviruses are so ubiquitous, it is very difficult to go from an association of the viruses with atherosclerotic plaques to a demonstration that the viruses actually caused the plaques. Still, as Melnick remarks, "We are continuing to put a lot of effort in [to studies of the viral hypothesis]. If we thought it was a blind alley, we would stop."—**GINA KOLATA**