What T Cells See and How They See It

Studies on pattern recognition by T cells are resolving some of the most fundamental issues of immunology. The information may aid vaccine development

AT THE END OF SEPTEMBER, some 30 immunologists gathered in New York City for a workshop* on "T Cell Pattern Recognition." Their goal was to assess the current state of knowledge about what the T cells of the immune system "see" when they recognize foreign antigens. A better understanding of how T cells interact with their target antigens—a necessary step for mounting many immune responses—may be helpful for developing vaccines to protect against diseases such as AIDS (acquired immunodeficiency syndrome) and malaria.

The current work is also beginning to provide answers for some long-standing immunological questions, such as what causes the rejection of foreign tissue grafts and how does the immune system refrain from attacking the tissues of its own body? Both graft rejection and tolerance for self-tissues appear to depend on the same types of T cell recognition events as those that initiate ordinary immune responses.

Much of the discussion at the workshop concerned the nature of immunodominance. One of the peculiarities of T cell responses is that the cells actually see just a few short segments, usually containing some 10 to 20 amino acid residues each, of a protein antigen. These segments are said to be immunodominant.

Most of the protein remains invisible to T cells, and therefore incapable of triggering immune responses that are dependent on the cells' participation. "The question is," says workshop cochairman Stanley Nathenson of Albert Einstein College of Medicine in New York, "how does the immune system pick out a particular peptide to be immunodominant?"

Peptide structure is undoubtedly one of the factors that help to determine immunodominance. The protein antigens to which T cells respond are cut into peptides within cells. Some of these peptides are picked up by one or another of the major histocompatibility (MHC) proteins and displayed on the cell surface for presentation to T cells. A T cell recognizes the peptide-MHC protein complex, not the peptide by itself. An immunodominant peptide must have a structure that permits it to form a complex that can be detected by a T cell.

At the meeting, two researchers, Jay Berzofsky of the National Cancer Institute (NCI) in Bethesda, Maryland, and Jonathan Rothbard of the Imperial Cancer Research Fund in London, described structurally based models they have devised for predicting which segments of a protein antigen are likely to be immunodominant. Neither model is infallible, but the information may nonetheless aid in vaccine design.

"How does the immune system pick out a particular peptide to be immunodominant?"

One approach to vaccine development that is now under active exploration involves vaccinating not with a whole virus but with peptides corresponding to short segments of the viral proteins. Some viruses—the AIDS virus is a case in point—are considered too dangerous to use whole, even if killed or attenuated. If a peptide vaccine could be made to work it would not entail the same dangers as use of a whole virus, with its genetic material. The question is which peptides to use in view of the fact that T cells are not going to be able to recognize most of them in the great bulk of a protein sequence.

Berzofsky, with Charles DeLisi, also of NCI, proposed a few years ago that immunodominant peptides are those that can assume the three-dimensional structure of an amphipathic helix, that is, a helix in which the uncharged, hydrophobic amino acids cluster on one side with the charged, hydrophilic amino acids on the other. The researchers postulated that such a helix would be well suited for binding first to the MHC protein and then to the responding T cell.

Since then they have tested this model by examining the amino acid sequences of the protein sites found to be recognized by T cells in a variety of laboratories around the world. About 70% of these have structures with the potential of forming an amphipathic helix, Berzofsky says.

Moreover, the model successfully identified regions of the envelop protein of the AIDS virus and of a malaria parasite protein that are capable of eliciting T cell responses. "If the goal is to find a few T cell sites by using a predictive approach like this, you have a good chance of finding them," Berzofsky says, although he cautions that so far at least the models do not identify all possible T cell recognition sites. Without predictive information, however, a large number of peptides—perhaps 200 for the envelop protein of the AIDS virus—would have to be screened to find the few that are capable of inducing T cell responses to the protein.

Rothbard and his colleagues derived their model by surveying the known T cell recognition sites for a common structural motif. They found that about 85% of them carry a sequence of four or five amino acids in which the first is either glycine or a charged amino acid, the next two or three are hydrophobic amino acids, and the last is another glycine or charged amino acid. This motif has also successfully predicted which protein sites will be recognized by T cells.

Although the Berzofsky and Rothbard groups took different routes to their models, there is a considerable overlap between the sites that each identifies. This is perhaps not surprising as the four or five amino acid sequence of the Rothbard motif could form one turn of an amphipathic α-helix. Rothbard finds, moreover, that stabilizing the helical structure of a peptide makes it a better stimulator of T cell activities.

Neither the Berzofsky nor the Rothbard model is 100% effective at identifying T cell recognition sites. At the current state of knowledge then, researchers have some good clues to immunodominance, but the whole story is not yet in.

A number of other factors in addition to peptide structure apparently help to determine whether a particular protein segment is recognized by T cells. The processing step, by which large proteins are cut into smaller peptides for complexing with MHC molecules and eventual presentation to T cells, is apparently one of them.

For example, at the workshop Eli Sercarz

*The workshop, which was sponsored by the Cancer Research Institute, was held on 26 and 27 September.

of the University of California School of Medicine at Los Angeles described work indicating that amino acid sequences that are located far away from the immunodominant sites on a protein influence the choice of those sites. "This suggests," Sercarz says, "that the protein was not cut up before it made contact with the [MHC] antigen."

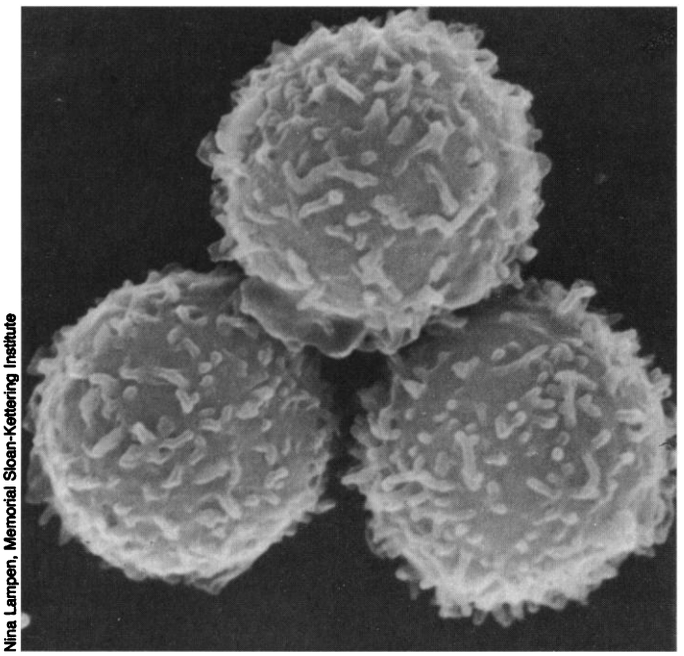
He proposes that different segments of a protein antigen compete for binding to an MHC molecule, with the one that wins becoming immunodominant. Only after that binding occurs is the protein antigen cut up into pieces. "It is oversimplified to think that these molecules are unfolded and chopped up first," Sercarz maintains, although that is currently the prevailing view of what is happening.

Moreover, the peptides bound to an MHC protein may be somewhat bigger than expected, and this could influence their interactions both with the protein and with T cells. According to Howard Grey of the Cytel Corporation in San Diego, the molecular weights of peptides bound to one type of MHC molecule range from 2,000 to 10,000, which is about five times heavier than the molecular weight of a 20–amino acid peptide. "At the high end, a lot of peptide isn't in the site, but is oozing over the side, so to speak," Grey says. Berzofsky's group also has evidence that MHC proteins bind peptides that are larger than 10 to 20 amino acids.

Immunologists generally do not yet know very much about the details of antigen processing, although recent research indicates that there are two major processing pathways, which result in the activation of two different types of T cells. "The predominant paths of presentation to T helpers and T killers are different," says Thomas Braciale of Washington University School of Medicine.

Helper T cells, which are so called because they help the B cells make antibodies, recognize antigens in combination with MHC proteins of the class II type. Antigens that complex with the class II proteins are generally taken up from outside the cell by being engulfed into small membrane-bound vesicles called endosomes. Processing of the antigens and complexing with the class II MHC molecules apparently occurs in the endosomes.

Killer T cells, which seek out and destroy virus-infected cells, recognize antigens in combination with class I MHC proteins. These antigens, in contrast to those presented by class II MHC proteins, are generally made within the presenting cells, although recent work by Jonathan Yewdell of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda and his colleagues and by Michael Bevan's group at the Research Institute of Scripps Clinic in La Jolla, California, indicates that a cytoplasmic location, not internal synthesis itself, is what puts a protein into the class I pathway.



Nina Lampen, Memorial Sloan-Kettering Institute

Blood lymphocytes. *The T lymphocytes are activated when their receptors detect fragments of foreign proteins that have complexed with MHC proteins on the surface of antigen-presenting cells.*

The work suggests that even soluble proteins from outside the cell, which might otherwise elicit only helper T cell and antibody responses, can trigger killer T cells if the proteins can be maneuvered into the cytoplasmic class I pathway. This may be useful for developing vaccines made from killed, nonreplicating viruses or individual viral proteins.

No matter how an antigen is processed, however, the peptides produced will not be presented to T cells unless they first complex with appropriate MHC molecules. Accordingly, another major focus of the workshop concerned the structural features of the MHC proteins that allow them to interact first with the peptides and then with the T cell receptor for antigen.

Identification of these features was greatly aided about a year ago when researchers from the Harvard laboratories of immunologist Jack Strominger and crystallographer Don Wiley determined the three-dimensional structure of a class I MHC protein (*Science*, 30 October 1987, p. 613). The top of the molecule features a deep groove that seems ideally designed for binding and presenting antigen peptides to responding T cells.

Currently immunologists are making systematic mutations in the antigen-binding regions of MHC proteins to see how this affects their ability to interact with antigen peptides and T cells. Several workshop participants† described the results of such analyses.

In general, the researchers are finding that mutations of amino acid residues at the bottom and inner sides of the groove affect antigen binding, whereas mutations along the upper and outer edges of the two α helices that bound the groove affect recognition by T cells. These results are not surprising but they do provide confirmatory evidence for the current model of antigen presentation. "If the results didn't fit expectations, we would have had to question the model," Strominger says, "but they did fit expectations."

Although antigen peptides must bind to an MHC protein to be presented to T cells, their binding does not automatically insure an immune response. "No peptide that fails to bind is immunogenic, but some peptides that do bind are not," Grey says. In his experiments about 40% of the peptides that bound failed to elicit T cell responses. Apparently there are "holes" in the T cell repertoire for antigen recognition.

Why T cells should fail to recognize some antigen-MHC protein combinations is unclear, but one possibility is that the failure is a reflection of the immunological phenomenon known as tolerance. The immune system must learn not to attack the constituents of the body in which it resides. In some cases, says Paul Allen of Washington University School of Medicine, this tolerance for self-antigens occurs simply because the self-antigens do not produce peptides that

†They include James Forman of the Howard Hughes Medical Institute at the University of Texas Southwestern Medical Center in Dallas; Ronald Germain of the NIAID; Janet Maryanski of the Ludwig Institute for Cancer Research in Lausanne, Switzerland; Stanley Nathenson of Albert Einstein College of Medicine in New York; James Sheil of West Virginia University Health Sciences Center in Morgantown; and Jack Strominger of Harvard University in Cambridge.

are capable of binding to MHC proteins.

For others, however, binding of antigen peptides occurs but the complex is not recognized by T cells. Complexes of foreign peptides and MHC proteins may also escape recognition if they happen to look like the self-complexes.

Immunologists think that T cells with a strong predilection for recognizing self-antigens are somehow eliminated in the thymus gland, the site of T cell maturation. Allen suggests that the cells get deleted if they encounter a self-peptide complexed with an MHC protein. "Conceptually, it's an appealing hypothesis, but we have to go and show it," he remarks.

His own work demonstrates that complexes of self-peptides and MHC proteins normally exist in the body. As Strominger points out, "The proteins of a cell are being degraded and resynthesized all the time." Cell peptides would therefore be available for complexing with MHC antigens, just as are the viral peptides that are synthesized in infected cells.

Complexes between an MHC protein and a self-peptide may also be at the root of graft rejection, according to Strominger. His group has found that mutations in an MHC protein affect its ability to participate in a graft rejection reaction the same way as they affect its ability to participate in a response to a viral antigen. The recognition event therefore appears to be the same in both responses, that is, a T cell interacting with a peptide bound to an MHC molecule.

What researchers would like to see next is a clear view of an antigen peptide in the MHC protein groove. The MHC protein analyzed by the Strominger-Wiley group contained something in its antigen-binding groove, but the resolution of the x-ray structure was not good enough to see what it was. A clear view could help resolve some of the issues about the structures of immunodominant peptides. Immunologists could see, for example, whether they bind in a helical configuration or in a more extended conformation.

Harden McConnell of Stanford University notes, however, that even this will not necessarily reveal the structural requirements for forming the complex between an antigen peptide and an MHC molecule. His work indicates that this complex undergoes a significant structural change after the binding event. The structure captured by x-ray crystallography will be final one, not the initial one.

Nevertheless, the studies of T cell pattern recognition are beginning to resolve some of the most fundamental issues of immunology, including immunodominance, tolerance, and graft rejection. ■ JEAN L. MARX

Huge Impact Is Favored K-T Boundary Killer

A large impact rather than a volcano is widely taken to be the primary agent of destruction at the end of the dinosaur age

Snowbird, Utah

NO ONE ASKED FOR A SHOW OF HANDS, but a vote among those attending the conference on Global Catastrophes in Earth History* here would have given a clear-cut victory to an asteroid or comet impact as the most likely explanation of the mass extinction 66 million years ago. That was when the last of the dinosaurs died out.

For several years a small group of researchers has been advocating millennia of volcanic eruptions of previously unimagined power as an alternative agent of destruction. The geologic record is being misread, this group claims, by those insisting that a large impact instantly laid down the thin layer of exotic sediment found in the late 1970s sandwiched between sedimentary rock of the Cretaceous period and the younger rock of the Tertiary period. The debate looked like it could continue indefinitely.

After this Snowbird conference, the second of its kind (*Science*, 20 November 1981, p. 896), the end seems to be in sight. The evidence for an impact continues to mount. The volcanic hypothesis, which has consisted of a hotly contested plausibility argument and claims of inconsistencies in the evidence for an impact, made a poor showing. And the detection of the mineral stishovite, a form of quartz formed only by the extreme pressure of an intense shock, was announced; if confirmed, this evidence would be widely regarded as definitive proof of an impact. Perhaps most encouraging were the frequent agreements between feuding partisans to cooperate finally in sampling, intercalibration, and analysis.

One advantage held by the theory that a large impact killed off more than 70% of the species living at the end of the age of the dinosaurs is the inevitability of such impacts, given the existence of asteroids and comets that cross Earth's orbit. Globally disastrous eruptions remain hypothetical. Eugene Shoemaker of the U.S. Geological Survey (USGS) in Flagstaff told the conference about his latest estimates of the frequency of large impacts based on discoveries of Earth-crossing asteroids and comets. About every 100 million years on average, Shoemaker concludes, an object 10 kilometers in diameter slams into Earth at perhaps 20 kilometers per second or more, releasing 60 million megatons of energy and creating a 150-kilometer-wide crater. That is the size impact thought necessary to explain the chemical composition of the layer at the boundary between the Cretaceous and Tertiary periods. (K-T boundary is used to denote this moment in geological time.)

Bruce Bohor of the USGS in Denver soon followed with a recounting of how the K-T boundary seems to be littered with the debris of such an impact. There are the shocked quartz grains shot through with intersecting lamellae characteristic of the extreme pressure generated by intense shocks. Multiple lamellae have been found only in minerals from known impact sites, nuclear test sites, laboratory shock experiments, and the K-T (*Science*, 25 May 1984, p. 858). And there are spinel crystals, hollow spherules, and vitric clasts unlike anything spewed from volcanoes. All this evidence is consistent with an impact, excludes a volcanic eruption, and is consistent with most of the geochemical evidence, Bohor said.

The volcanologists invited to the conference, who had not as yet been drawn into the volcano versus impact controversy, provided little moral support for the eruption advocates. There have been huge eruptions, the volcanologists noted, far larger than any experienced by humans. About 16 million years ago, eruptions in eastern Oregon and Washington over a period of days spewed forth batches of lava as large as 5,000 cubic kilometers to form lava lakes up to 700 kilometers across. The eruption of Long Valley caldera in California 700,000 years ago dumped 5 centimeters of ash on much of the central United States. And no one can say that even larger eruptions have not occurred.

Even with this daunting record of eruptions, the volcanologists could not offer

*Global Catastrophes in Earth History: An interdisciplinary conference on impacts, volcanism, and mass mortality, held 20 to 23 October at Snowbird, Utah. Sponsored by the Lunar and Planetary Institute and the National Academy of Sciences.