Structure of MHC Protein Solved

The three-dimensional structure of a major histocompatibility protein gives the clearest picture yet of how the proteins perform their immune functions

Now, for the first time, immunologists can see exactly what a major histocompatibility protein looks like. A team of researchers from Harvard University, using x-ray crystallography, has determined the three-dimensional structure of one of the proteins to a resolution sufficient to see the position of every amino acid that it contains. "Everybody's been waiting a long time for this structure—and it's beautiful," says Ronald Schwartz of the National Institute of Allergy and Infectious Diseases.

Having the structure means that the functions of the major histocompatibility proteins, which play a critical role in the mounting of immune responses, can be understood to a degree not previously possible. "If you didn't know anything about the immune system and you saw this structure, you would know how it works," says Jack Strominger of the Harvard group.

Taken together with other research on histocompatibility protein activities, the structure helps to explain how the ability to mount immune responses may vary from one individual to another, thereby making some of us more susceptible to certain diseases than others. Moreover, the work puts the final nail in the coffin of a longstanding controversy about histocompatibility molecule function.

The proteins were first discovered 50 years ago as "transplantation antigens" that trigger the rejection of tissues grafted from one individual to another. Then, beginning in the early 1970s, immunologists learned that the molecules, which are located on immune cell surfaces, mediate the myriad cellular interactions that are required for recognizing foreign antigens and mounting an attack on them.

In particular, the activation of the T lymphocytes of the immune system depends on histocompatibility protein function. T cells recognize foreign antigens and become active only when the antigens are presented in conjunction with a major histocompatibility protein, which must usually be of the same type as that carried by the T cell itself.

The discovery of restriction, as the need to recognize antigen in conjunction with a major histocompatibility molecule is called, raised a controversy over what T cells "see." Do they see the foreign antigen and histocompatibility molecule together as a single structure or as two separate entities?

Indirect evidence of the past few years has favored the first possibility, that the histocompatibility molecule and foreign antigen are recognized as a single unit. The three-dimensional structure leaves little doubt that this is the case. "It solves a major question that has plagued us from the first demonstration by Zinkernagel and Doherty* of MHC [major histocompatibility complex] restriction," says Stanley Nathenson of Albert Einstein College of Medicine in New York City.

The structure is the culmination of an 8-year collaborative effort by the groups of immunologist Strominger and x-ray crystallographer Don Wiley of Harvard. The researchers performed the x-ray crystallographic analysis on a human class I histocompatibility protein that was originally crystallized by Pamela Bjorkman, who has since moved to Stanford University. The other members of the group that solved the structure are Mark Saper, Boudjema Samraoui, and William Bennett.

The class I molecules are the targets of the killer T cells, which destroy cells detected as foreign. This includes, in addition to those of transplanted tissue, cells that have been infected by viruses and are carrying viral antigens on their surfaces. In the latter case, the viral antigen and histocompatibility molecule on the target must both be recognized by a killer cell, as required by restriction.

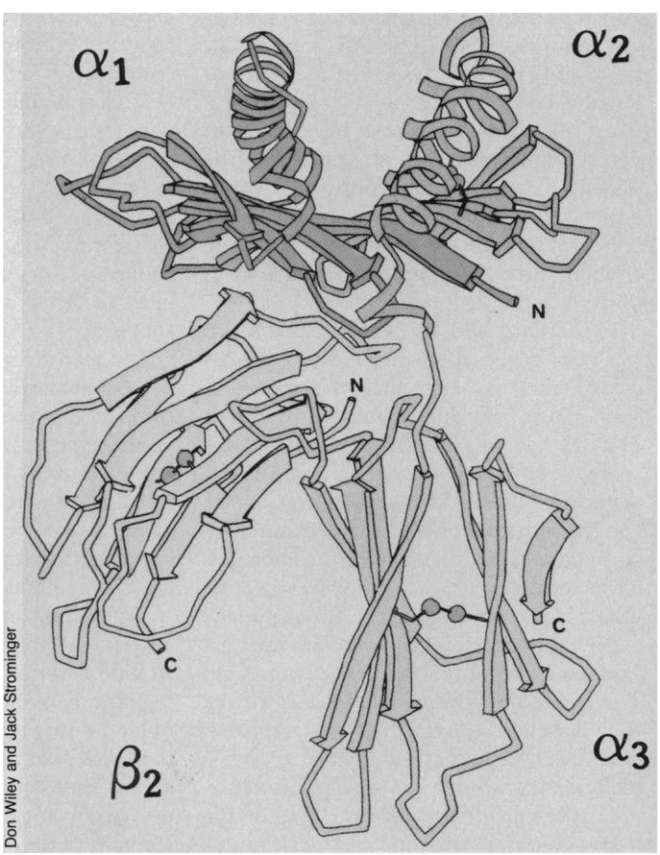
The structure determined by the Harvard workers for the histocompatibility protein shows that it has a binding pocket for the antigen. A class I molecule consists of a complex of two protein chains, one with a molecular weight of 45,000 and the other with a molecular weight of 12,000. The smaller protein is called β_2-microglobulin.

A short segment of the heavy chain anchors a histocompatibility molecule to the

*Rolf Zinkernagel of the University of Zurich, Switzerland, and Peter Doherty of the Australian National University in Canberra.

Histocompatibility protein structure

This side view of the molecule shows the arrangement of its four regions. The β_2-microglobulin protein and α_3 segment of the heavy chain are associated with one another and are close to the membrane. The heavy chain would be attached to the membrane by a short amino acid sequence that was removed from the molecule before the crystals were prepared for x-ray analysis. The α_1 and α_2 regions of the heavy chain together form the antigen binding pocket. [Nature (London) *329, 590 (1987)*]



cell membrane and extends into the cell interior, but the rest of the protein structure protrudes to the outside. The outer segment of the heavy chain can be divided into three functional domains, designated α_1, α_2, and α_3.

The crystal structure reveals that the β_2-microglobulin and the α_3 portion of the heavy chain are associated with one another, next to the membrane. The α_1 and α_2 domains sit atop them.

Most strikingly, eight segments of these two α domains combine to form a flat β sheet, "like a tabletop parallel to the plane of the membrane," Wiley explains. On top of the eight strands are two long helices with a deep groove between them. "When you just see the structure, you say this is a place to put something," Wiley declares.

In fact, something is already there, possibly a segment of peptide antigen that crystallized out with the histocompatibility molecule. The resolution of the structure is not yet good enough to say this with certainty, however. The amino acid sequence of the histocompatibility protein guided the Harvard group in working out that three-dimensional structure, but sequence information is obviously not available for the unidentified substance in the binding groove.

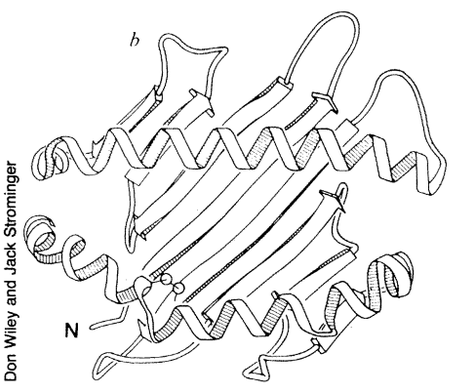
There is growing agreement that protein antigens are not presented intact to T cells but are first broken down in the presenting cell into peptide fragments containing 10 to 20 amino acids. Emil Unanue of Washington University School of Medicine in St. Louis and Howard M. Grey of the National Jewish Center for Immunology and Respiratory Medicine in Denver have independently shown that this is the case for the class II histocompatibility proteins, which present antigens to helper T cells. The helper cells are so called because they stimulate antibody production by the B lymphocytes of the immune system.

In addition, work by Grey, some of which was carried out in collaboration with Malcolm Gefter of Massachusetts Institute of Technology and John Smith of Harvard Medical School, and by Unanue and his colleagues has shown that binding of the peptides to class II molecules is necessary for antigen presentation and the generation of an immune response. The histocompatibility molecules in effect behave like receptors with a single binding site for the peptides.

Although comparable work with the class I proteins has not yet been carried out, Alain Townsend and Andrew McMichael of the John Radcliffe Hospital in Oxford, England, have evidence indicating that the class I molecules may work in a similar fashion.

That certainly is the inference to be drawn from the class I structure just determined. "There is clear confirmation of the single binding site for peptides," Gefter says.

The groove can readily accommodate a peptide containing 12 to 20 amino acids. The number that will fit depends on whether the peptide is in an extended, linear configuration or is coiled in a helix. There is currently some disagreement among immunologists about the conformation in which peptide antigens bind to the presenting histocompatibility protein, and this issue has not been resolved by the current structure.



Don Wiley and Jack Strominger

The antigen-binding pocket *has a floor consisting of eight strands of* β *sheet and is bounded by two helical protein segments.* [Nature (London) *329, 590 (1987)*]

Further support for the idea that the groove is the binding site for peptide antigens comes from an analysis of the amino acids located in and around the groove. Histocompatibility proteins are polymorphic, that is, they show variations in amino acid sequence among individuals that are related to the individual's ability to make immune responses to various antigens. T cells, for example, can recognize a particular antigen in conjunction with a histocompatibility molecule with one sequence, but not another.

The Harvard workers find that almost all of the polymorphic amino acids of the histocompatibility protein are located in the area of the groove. Most of them are on the bottom or sides, but a few are located on the top faces of the helices. Those inside the groove would be prime candidates for making contacts with a peptide antigen. Changing them might well alter the molecule's ability to bind and present antigens to killer T cells. Without the presentation there would be no response.

The amino acids on the outer helical faces might be important for making contact with the killer cells themselves. Changing those amino acids could also influence the ability of a histocompatibility molecule to present antigens to the T cells.

In a similar vein, Nathenson and his colleagues have identified a series of mutations in a mouse class I histocompatibility molecule that abolishes its ability to be recognized by killer T cells. Many of these mutations also affect amino acids in the groove or on the outer surfaces of the helices, and the same is true for a large number of human histocompatibility variants. All in all, the results provide firm support for the hypothesis that histocompatibility proteins bind peptide antigens and that the complex is what is recognized by killer T cells.

Whether class II histocompatibility proteins have a similar binding site remains to be established, although, as mentioned previously, the biological evidence already points strongly in that direction. Models currently being constructed by the Harvard group from the known amino acid sequences of class II proteins, which resemble those of the class I proteins, also lead to the same conclusion.

A more direct answer may soon be available. Jerry Brown of the Wiley group and Joan Gorga of the Strominger laboratory have obtained crystals of a class II histocompatibility protein, although these are not yet of quality good enough for x-ray crystallographic analysis.

From the practical point of view, having a clear idea of how histocompatibility molecules work could increase the ability of researchers to manipulate immune responses. "You always gain information from the structure," Schwartz explains. "Now you can go in and change a particular residue to see how it changes peptide binding or T cell recognition."

The information gained from such experiments will help to explain why an individual who carries a particular histocompatibility protein variant is more, or less as the case may be, susceptible to a disease than others. Such information may help, for example, in the design of vaccines for stimulating immunity, or in the development of immunosuppressive agents for preventing the rejection of transplanted organs or treating autoimmune diseases. ■ **JEAN L. MARX**

ADDITIONAL READING

P. J. Bjorkman *et al.*, "Structure of the human class I histocompatibility antigen, HLA-A2," *Nature (London)* **329**, 506 (1987).

P. J. Bjorkman *et al.*, "The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens," *ibid.*, p. 512.

S. Buus *et al.*, "The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides," *Science* **235**, 1353 (1987).

J.-G. Guillet *et al.*, "Immunological self, nonself discrimination," *ibid.*, p. 865.

E. R. Unanue and P. M. Allen, "The basis for the immunoregulatory role of macrophages and other accessory cells," *ibid.* **236**, 551 (1987).