Invertebrate paleontologist Karl Flessa of the University of Arizona is at one end of the spectrum: "The evidence is overwhelming for an impact at the K-T and for it as the cause of the extinctions," he says. But the prevailing opinion seems to be that the impact must share the blame with more mundane perpetrators.

Anthony Hallam of the University of Birmingham speaks for most paleontologists when he says, "I may accept the story of the impact, but I think it was at most a coup de grâce. I believe a mass extinction would have taken place in the marine realm even without an impact. I like the idea of Earth-induced mass extinctions." His preferred agents of extinction are the change in sea level that took place at the K-T boundary or shortly before it, volcanism—the 2-million-year eruption of the Deccan Traps in India is centered on the K-T—and bouts of asphyxiating anoxia in the ocean brought on by changes in ocean circulation.

Sorting out just how much the impact contributed will require identifying plausible killing mechanisms for specific fossil groups: finding evidence, for example, that the impact produced abundant acid fallout, which could have killed off marine plankton by dissolving their carbonate skeletons. Blaming the impact for a group's disappearance will also take a confirmed coincidence in time between the supposed death blow and the last glimpse of the species.

So far, the evidence is uncontested in perhaps only two instances. The impact's dust-induced darkness and cold, not to mention continent-wide fires, may well have done in the plants in the western United States, given the exact coincidence of the impact debris and an abrupt shift in the flora. And the crash of the marine food chain recorded in sediments at the boundary probably cut off the spiral-shelled creatures called ammonites. As Peter Ward of the University of Washington, Seattle, has shown, these creatures—previously believed to have faded gradually—actually thrived right up to the K-T boundary and then vanished. Says Ward: "I'm convinced a meteorite ripped into the earth. It certainly, I think, killed off my beautiful ammonites." That's not the case, he hastens to add, for another extinct group he studies, the inoceramids, a group of large clams. They disappeared 2 million years before the impact, he says. "Something phenomenal happened" then, Ward says, "but it's not the impact."

To build more cases for the impact as a cause of extinctions, paleontologists and geologists will continue their detailed dissection of the millennia immediately around the K-T boundary. Most convincing of all would be the discovery of a second bona fide impact in the midst of another mass extinction. For the time being, the greatest obstacle to understanding—and accepting—the K-T event may be its uniqueness.

–Richard A. Kerr

IMMUNOLOGY

Getting Some "Backbone": How MHC Binds Peptides

The immune system is always at war, fighting viruses, bacteria, and other pathogens that try to invade the body. In that war the class I proteins of the Major Histocompatibility Complex (MHC) play the role of informer, first having intimate contact with the enemy and then revealing the enemy's location. The MHC molecules display on the surfaces of all cells pieces of the proteins made inside the cells. If the cell is foreign or harbors a virus, some of those protein fragments, or peptides, will be foreign. They mark the cell for destruction by "killer" T cells, the immune system's hand-to-hand combat troops. By this process, the body not only fights off infection but also rejects tissue grafts, and, in cases where confused T cells take the body's proteins for foreigners, triggers the tissue destruction common to autoimmune diseases.

Researchers who study the MHC proteins have long wondered how these informers can master so many different types of military intelligence. The problem: Hundreds of different peptides are displayed on the cell surface, but each person has at most six different MHC proteins. Each protein must therefore be able to display many different peptides. What's more, the MHC proteins bind peptides tightly, and when proteins bind tightly, that usually means the fit is very specific. "The question that has been on everybody's mind for so long," says Pamela Bjorkman, a Caltech immunologist who studies MHC protein structure, "is how it is that MHC molecules bind with high affinity to peptides, and yet can bind such a wide variety."

Now, thanks to a wave of new findings from three research teams, an answer to the puzzle is at hand. And the answer is more than academic, since a better understanding of MHC-peptide binding could eventually lead to new drugs that, by blocking some MHC binding sites, could combat transplant rejection or autoimmune disease. The first team to publish its new results is that of Ian Wilson, Per Peterson, and co-workers at the Scripps Research Institute in San Diego, whose pair of papers appear on pages 919 and 927 of this issue of *Science*. Groups with similar work in press or in preparation are headed by Stanley Nathenson and James Sacchettini at Albert Einstein College of Medicine and Don Wiley and Jack Strominger of Harvard.

All three groups have independently reached the same conclusion: MHC molecules can bind a variety of peptides because they concentrate on what all peptides have in common. That is, they bind tightly to the backbone structure shared by peptides and don't bother as much with the amino acid side chains that differ from peptide to peptide. In an additional report on page 000 of this issue of *Science*, Strominger, his Oxford collaborator Andrew McMichael, and their colleagues report that they have created specific mutations in the MHC molecule and used them to confirm the importance of some of the bonds to the backbone. "It's a very pretty story," says Stanford University immunologist Hugh McDevitt of the whole collection of work. "You can really begin to see the nature of the class I binding site."

First glimpse. The first glimpse of that binding site came in 1987, when a team led by Wiley and Strominger at Harvard published the first structure of a class I MHC protein, as determined by x-ray crystallography. That structure revealed a groove in the protein that somehow holds the peptide, although how it holds it wasn't clear. "You could see the peptide there, but you couldn't see where the individual side chains pointed," says Caltech's Bjorkman, who was the first author on the pathbreaking paper. The peptide position was so indistinct partly because even though the MHC molecules in the crystal themselves were chemically identical, they held different peptides in their grooves. Since the structure was computed from an average of all the various MHC-peptide combinations, it was clear for the MHC molecule itself, but the peptide was a blur.

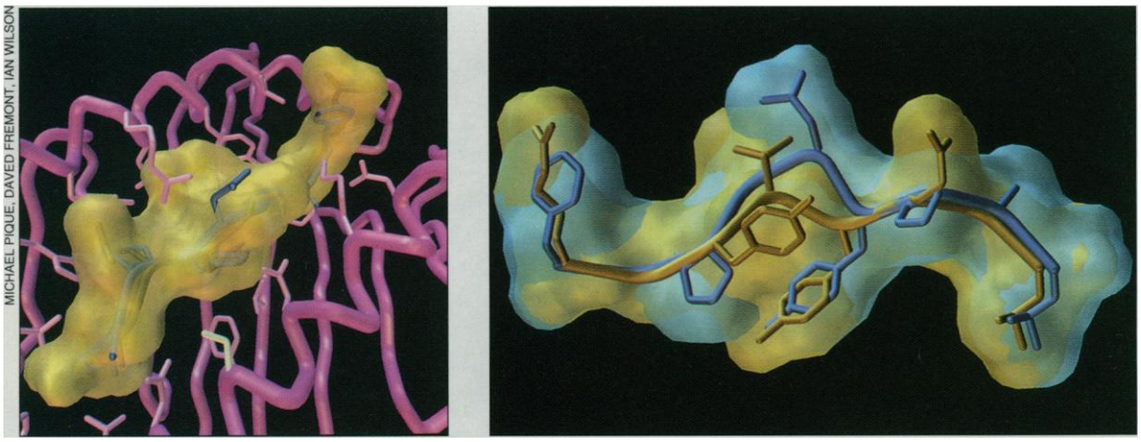
Over the next few years, several groups pushed the story further. With higher resolution structures, the Harvard group discovered pockets inside the groove, two of which seemed to tether the ends of the peptide, while others looked as if they could accommodate some of the peptide's amino acid side chains. Meanwhile, several groups found that, while MHC molecules are not terribly choosy about the peptides they bind, each one has a few requirements: for a specific amino acid, or one of a certain general size or shape, at certain positions along the peptide chain. It began to look as if the side chains of these "anchor" amino acids might sit in the pockets in the groove.

But how tightly bound were the anchor side chains in the pockets? Did they form bonds with the pocket that would help hold the peptide in place? As long as the crystals contained a mixture of peptides, these questions were difficult to answer, says Dean Madden, a graduate student who works with

Wiley. "Wherever you have a highly variable amino acid position, the side chain is blurred," Madden says. To see clearly the positions and bonds of those side chains, he adds, "you need to go to a single-peptide structure."

That's just what Wilson, Peterson, and colleagues Daved Fremont, Masazumi Matsumura, and Enrico Stura did. Their papers present the first two structures of MHC molecules crystallized with single peptides. The new work, says Stanford immunologist Mark Davis, "certainly tells you what sort of specific contacts are made."

The structures reveal that the peptides are held snugly in the groove by hydrogen bonds between the MHC protein and the peptide backbone. "It appears that...the backbone interaction must contribute the large majority of the binding energy," Wilson says.



MICHAEL PIQUE, DAVED FREMONT, IAN WILSON

In the groove. A viral peptide (protein fragment) eight amino acids long fits into a groove in the mouse MHC Class I molecule *(left)*. The MHC molecule is shown in pink, the peptide in yellow.The right panel shows how that peptide, and a second one, eight amino acids long, shown in blue, are configured when they fit into the MHC binding groove.

The side chains of the amino acids may fit into pockets or protrude from the groove, but in either case the fit is not precise, and any bonds formed are weak. In that respect "it's a relatively sloppy groove," says Wilson. "Water molecules can fill in and help provide the fit."

Even before the new structures were obtained, some of Madden's earlier structural work suggested that the key bonds were those between the groove and the backbone, Strominger says, so he, McMichael, and their co-workers decided to test the importance of those bonds by changing key amino acids in the MHC protein and checking to see how the changes affect peptide binding. Based on what was known about the MHC structure, it appeared that some of the most important bonds were in the pockets that tack down the backbone of the peptide at its two ends, so that's where they focused their attention.

At one end of the peptide, Strominger says, there is a positively charged amino group, which seemed to be held in place by hydrogen bonds it formed with the side chains of two of the MHC amino acids. To find out whether those bonds are indeed critical, the group made two mutant MHC molecules, each of which had one of the amino acids replaced by one that can't form hydrogen bonds. Neither mutant was able to bind the peptide. "You can see [this finding] as an experimental test of the predictions" made from the structure, comments Massachusetts Institute of Technology immunologist Hidde Ploegh. "This report confirms that the predictions were valid." Strominger says his group will make more mutations to test the importance of bonds in other parts of the groove.

Out of pocket. With an understanding of the MHC-peptide bonds in hand, other questions remain, and the new work addresses some of these as well. The Scripps team's comparison of two peptides bound to the class I MHC protein has provided the first clear illustration of how an MHC molecule accommodates peptides of different lengths, although their ends must be tethered in the same spots. The group's structures show the longer peptide simply bulging out of the groove in the middle. "It's like taking a rubber band and fixing two points and leaving a bump in the middle," Strominger marvels. "That's a point you don't get from the earlier papers." The Wiley lab, he says, has similar unpublished findings.

The new work also sheds light on another major issue in immunology: What the T cell receptor sees when it recognizes an MHC-bound peptide. Whether or not a peptide bulges like a tacked-down rubberband, some of its surface will certainly protrude from the groove. And that, presumably, is the part of the peptide detected by the receptors on T cells. But since no one has yet crystallized a T cell receptor molecule in contact with an MHC-peptide complex, questions remain about exactly what parts of the complex the T cell receptor surveys. Must variable parts of a peptide be exposed to be recognized by the receptor? Or are there indirect ways of drawing the receptor's attention, perhaps through a change in shape of the MHC molecule itself, induced by a buried or partly buried side chain of the binding peptide?

Nathenson, Sacchettini, and their coworkers at Einstein addressed this question in their work, which is in press in the *Proceedings of the National Academy of Sciences*. They determined the structure of the same MHC molecule as the Wilson group, but they focused on just one peptide, relating its position in the groove to what they knew about parts of the peptide that are important for T cell recognition. In earlier work, they had changed each amino acid in the peptide and found that four out of eight are critical for T cell recognition. In their structure, Nathenson says, those amino acids protrude from the groove. "They are available for direct contact," he says. "It sounds trivial, but you might have found something different." They might have been buried in the groove, he says, distorting the MHC molecule's shape from within, like the shape of a baseball glove is changed by a ball in the pocket.

While the ball-in-the-mitt model can't be the whole story, a finding by the Scripps group suggests that it may play a role. They compared the shape of the same MHC protein bound to two different peptides and found the first evidence that the binding of different peptides can alter the protein's shape. "That is important," says Stanford's Davis. "It's likely that it will have an influence on T cell recognition."

But just how it has that influence is an open question, one that defines the next goal for those studying the structure of class I MHC proteins: solving the structure of the T cell receptor bound to an MHC protein-peptide complex, to see just how MHC informers pass their messages to their T cell colleagues.

–Marcia Barinaga

Additional Readings

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