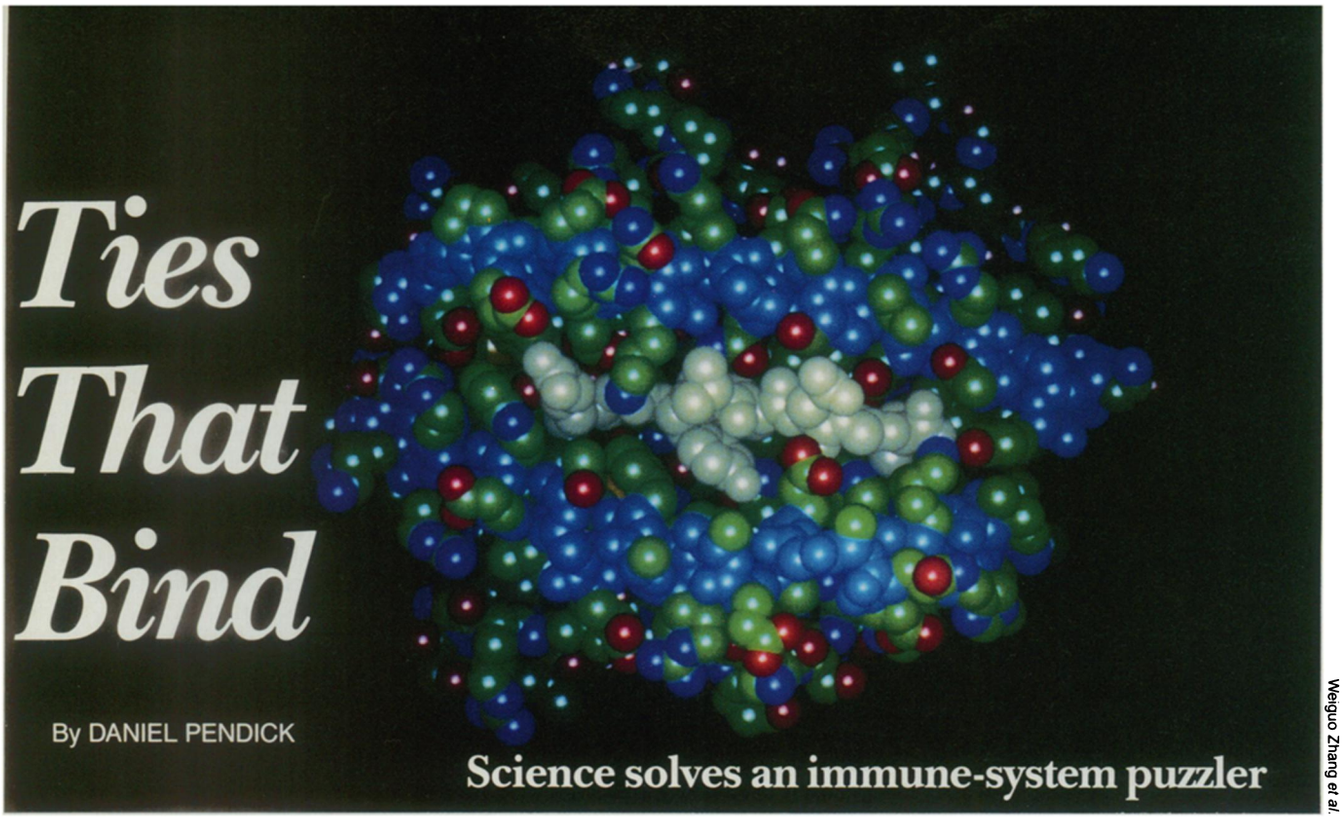
Ties That Bind



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By DANIEL PENDICK

Science solves an immune-system puzzler

Faced with a relentlessly hostile environment, invaded by airborne bacteria, marauding viruses, and other foes, the human body has evolved a single-minded defensive posture: If you're not with us, you're against us. Through an interplay of biology and chemistry, the immune system "knows" the difference between things that belong inside the body and things that don't.

Starting with studies 50 years ago of why severely burned British fighter pilots rejected skin grafts from genetically unrelated donors, scientists have probed in increasing detail the immune system's ability to distinguish between "self" and "nonself."

Late last year, researchers reported findings that significantly advance scientists' understanding of how the immune system distinguishes among the millions of different proteins, both friendly natives and hostile invaders, that coexist in our bodies from day to day. These advances, they say, may lead to more effective vaccines or to new ways of combating diseases that pit the body's immune system against itself.

The body's ability to recognize and respond to foreign substances traces eventually to a group of genes called the major histocompatibility complex (MHC), which collectively holds the construction plans for several kinds of large protein molecules. These molecules inform the immune system of potentially harmful activity, such as viral infection, occurring deep inside individual cells.

The body depends on class I MHC molecules (MHC-I) in its defense against viruses. This is because a virus — a protein shell containing just enough genetic code to reproduce — carries out much of its mischief in the nuclei of cells, beyond the direct reach of killer T-cells and other immune-system components that dispose of undesirable foreign material.

During infection, a virus enters cells and puts into effect its genetic program, hijacking the cells' protein-making machinery to make multiple copies of itself. Unchecked, this viral replication can cause sickness or death.

In response to a viral infection, MHC-I molecules collect and "present" viral protein fragments, or peptides, on the surfaces of infected cells. These foreign peptides betray the invader to immune-system agents on patrol in the body, inviting them to attack the nonself cells. When a sufficient number of MHC-I molecules has decorated the outside of a cell with foreign peptides — in this case, parts of viral proteins — the immune system's killer T-cells recognize the infection, latch onto the cell, and destroy it.

MHC-I molecules also present bits of the proteins produced by cells during their normal lives. These self peptides signal the immune system, *Leave this cell alone. It's one of us!*

Scientists understood in a general way what the MHC-I molecules do, but until recently they did not know exactly how the molecules went about it. In the closing months of 1992, researchers published a series of atomic-scale portraits of MHC-I molecules joined to peptides. These structural studies explain for the first time the precise nature of the chemical bonds that hold peptide-MHC-I complexes together, confirming ideas postulated but not previously observed.

What a T-cell sees: a mouse MHC-I molecule, viewed from above its grooved binding site, as it "presents" a protein fragment, or peptide, on the outside surface of a cell. When a cell displays a sufficient number of these MHC-I molecules bound to foreign peptides — a sure sign of viral infection — immune-system assassins called T-cells recognize the cell as "nonself" and destroy it. The colored balls in this diagram represent the total space occupied by various atoms and their encircling electron clouds.

"Our understanding of the MHC molecule has advanced enormously from this set of papers," says Ian A. Wilson, a crystallographer at the Scripps Research Institute in La Jolla, Calif., who coauthored an exhaustive study of mouse peptide-MHC-I complexes in the Aug. 14, 1992 SCIENCE. "The rules for how MHC binds peptides are very clear now."

These studies "enable us to figure out what the requirements are for a peptide to bind to a particular class of MHC molecule," says Dean R. Madden, a member of the Harvard University team whose most recent work appears in the Nov. 26, 1992 NATURE.

By combining these binding rules with more detailed knowledge of MHC-I biology, "you could actually start to predict which peptides from a virus would be presented to the immune system [on the surface of infected cells]," Madden suggests. Ultimately, understanding the MHC on this level might prove useful in the design of peptide-based vaccines.

This more detailed comprehension of the immune system emerged while scien-

tists were reconciling two seemingly contradictory aspects of MHC-I molecules. Every person carries genetic blueprints for only six, slightly different versions of these intracellular ferry boats, each of which contains a groove in its surface to carry a single peptide. Yet each of the six MHC-I molecules binds quite strongly to an enormous number of different peptides.

Strong chemical bonds imply a custom fit between molecules, much like the close match of key and tumbler that opens a lock. So researchers embarked on crystallographic studies to learn how thousands of unique peptide keys, each with its own shape and chemical makeup, could fit so snugly into a particular MHC lock.

"How is it that the MHC [molecule] can bind all these different peptides tightly?" asks Madden, repeating the question that captivated his colleagues. "The answer is the main chain, because the peptide's main chain is a constant feature that's always available, no matter what peptide you're binding."

The main chain is a repeating pattern of atoms that all peptides have in common. This structural similarity among peptides stems from the fact that they are made of amino acids. These peptide building blocks are composed of two atomic patterns. The first, the main chain, does not change from one amino acid to the next. But the second pattern, the side chain, is different in each of the 20 basic amino acids. Thus, when amino acids combine end to end, no matter in what combination, their main-chain backbones look exactly the same to an MHC-I molecule; only the type and number of side chains branching off the peptide vary.

This proved a critical fact in the explanation of why MHC-I molecules can bind to so many different peptides. Harnessing the fine-imaging technique of X-ray crystallography, scientists have discovered that clusters of side chains at either end of the MHC-I binding groove form strong bonds with the main-chain atoms at both ends of a peptide.

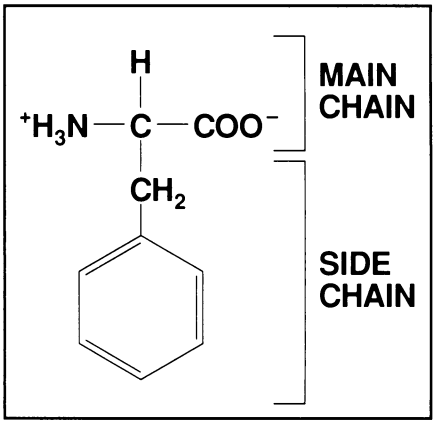
Significantly, these binding sites in the MHC-I groove change very little from one MHC-I type to another. This enables the molecules to bind securely to an impressive range of peptides, even though the peptides may have only one or two amino-acid positions in common. Madden compares this bond to getting one's foot and hand glued to the floor: "Then you have a little bit of wiggle left in the rest of you, but you're basically on the floor."

But just a *little* wiggle. For a peptide to remain locked into place on the tortuous journey to the cell surface, some of its protruding side chains must plug into depressions lining the inside surface of the groove, whose contours differ slightly from one MHC-I molecule to the next. In these depressions, peptide side chains form bonds weaker than those at the ends of the groove but still strong enough to anchor the peptide in place.

Researchers have also found that although MHC-I molecules seem to prefer binding to peptides containing nine amino acids, they can accommodate longer ones by forcing them to bulge in the middle. Hwai-Chen Guo and colleagues at Harvard University showed a dramatic example of this phenomenon – an 11-amino-acid peptide tethered to a human MHC-I molecule – in their November 1992 NATURE article.

Based on their understanding of these peptide-MHC-I binding rules, scientists can start thinking of ways to alter the peptide messages that MHC-I molecules relay to the immune system, says Ronald N. Germain, an immunologist at the National Institute of Allergy and Infectious Diseases in Bethesda, Md.

"These studies give chemical definition to principles that we generally understood from past functional studies," he explains. "This allows us to get a better picture of which parts of peptides contribute to T-cell recognition or MHC-I binding, and how that might be manipulated for useful purposes."



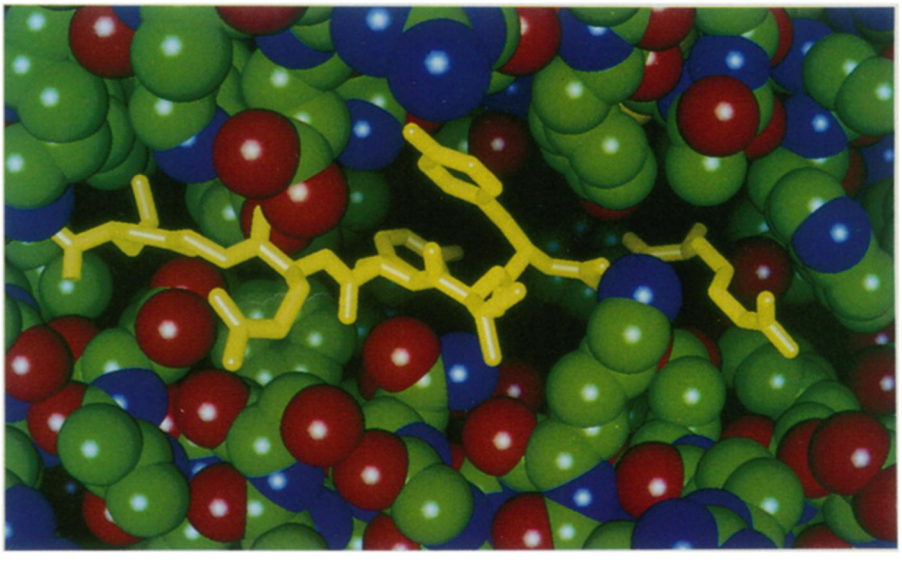
Like all of the 20 basic amino acids, phenylalanine has a backbone structure, or main chain. Cells string these amino acids together to form peptides and proteins. Each type of amino acid has the same main chain but a different group of atoms, called a side chain, that branches off the main chain. MHC-I molecules, scientists have discovered, form their strongest bonds with a peptide's main-chain atoms, primarily those at the ends.

A technical advance made possible the latest round of experiments, explains James C. Sacchettini, head crystallographer for the MHC-I researchers at the Albert Einstein College of Medicine in New York City. A group headed by Sacchettini and immunologist Stanley G. Nathenson published their study of a mouse MHC-I molecule in the Sept. 1, 1992 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

That advance was the ability to produce MHC-I molecules bound to identical peptides in sufficient quantity for X-ray studies. Before the advent of these single-peptide complexes, the researchers studied crystals of MHC-I molecules isolated from laboratory-grown tissue, which contained a number of different kinds of peptides.

Unfortunately, X-ray crystallography produces sharp images of only the most regular features of these crystals – in this case, the identical MHC-I molecules.

Close-up of a mouse MHC-I molecule presenting a fragment of protein from the vesicular stomatitis virus (VSV). In this diagram, crystallographers have depicted the peptide as an atomic Tinker-Toy model, showing the twisted peptide main chain and its various side chains. Ring-like side chains reach into the sides and floor of the groove, whereas Y-shaped chains protrude from it. These exposed side chains form part of a virally infected cell's self-destructive distress call to the immune system.



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Thus the details of the chemical bonds that hold the peptide-MHC-I complexes together remained obscure, recalls Sacchettini, so "we could never figure out how the peptide was bound."

Scientists solved the problem with biotechnology. The researchers at Albert Einstein, for example, harnessed bacteria to mass-produce the two different protein chains that make up a complete MHC-I molecule, then combined the pieces with a peptide made by a virus called VSV. Wilson and his Scripps colleagues used another approach: They coaxed lab-grown fruit fly cells into manufacturing empty MHC-I molecules, which the researchers bound to viral peptides. The Harvard researchers, including Guo, harvested MHC-I molecules from human cells, scrubbed off the mix of peptides present, and paired the molecules with peptides from the influenza virus.

Crystallized and bombarded with X-rays, these materials yielded information that computers translated into several kinds of colorful, three-dimensional structural diagrams. The computer can create "space-filling" diagrams, for example, which show the atoms' intersecting electron clouds. Or it can depict a peptide's main chain and side chains as a network of thin tubes resembling a twisted, Tinker-Toy inchworm. Based on these computer models, the crystallographers can deduce the types of chemical bonds holding the entire structure together.

Previously, low-resolution X-ray studies of MHC-I molecules had suggested the importance of the ends of the peptide main chain, Madden says. Also, a series of biochemical experiments had demonstrated the role of certain amino-acid side chains in strong peptide binding and immune-system response. But the latest round of papers powerfully confirmed the scientists' emerging ideas about the binding mechanisms of MHC-I molecules.

Most intriguing to immunologists is that the colorful computer portraits reveal what MHC-I molecules and their peptides "look like" to the body's T-cells. When MHC-I molecules on a cell display foreign peptides – called antigens – their outward-facing side chains constitute a large part of the cell's distress signal to the immune system. Evidence suggests that buried peptide side chains may warp the MHC-I molecule and that this warping also helps to bind T-cells, the body's first line of defense against nonself cells.

Immunologists would like to know which parts of an MHC-I molecule bound to an antigen activate T-cells, causing them to multiply and attack the infected cell. However, researchers have yet to crystallize and image peptide-MHC-I complexes bound to T-cells.

Such a feat would have important implications for immunology, notes Per A. Peterson, a senior researcher at the Scripps Research Institute. "If you understand the MHC molecule-peptide binding *and* its interaction with the T-cell receptor, you'll be better off in designing improved vaccines," he says.

Researchers offer various examples of how detailed structural knowledge of the MHC-I molecule and its interaction with T-cells might provide practical benefits. Germain, for example, explains that such knowledge might enable immunologists to engineer a vaccine that confers immunity to different strains of the same basic type of virus. Some researchers are now exploring this idea, he notes.

Another, more speculative scenario would require complete understanding of the MHC-I system – including processes that occur long before the MHC molecules reach the cell surface. Based on the way cells chop up and display viral proteins, researchers might predict which peptides MHC-I molecules would most likely display to the immune system. A vaccine based on these peptides, matched to a person's particular complement of MHC-I proteins, might confer immunity to specific viruses.

"That would certainly be quite a goal," says Scripps' Wilson, who remains skeptical of these speculations. He notes that a lot of poorly understood cellular processes work together to break proteins down into peptides and display them to T-cells. The recent structural studies, though impressive on their own, only explain the middle cogs in this machinery.

"Our understanding of the MHC molecule and how it works is really very good now," he says. "But we don't yet understand the presentation to T-cells, and we don't understand the processing of proteins inside the cell and how particular sequences end up being presented."

Despite these important gaps, understanding of the MHC-I molecule has advanced rapidly since 1987, when scientists at Harvard University first glimpsed its convoluted architecture.

"There's really been such an incredible progression from even four years ago to now, where we understand at the atomic level how peptides are presented by the MHC," says Madden. "We're just now seeing what the T-cells have been seeing for all these years."

And although crystallographic pictures of the MHC-I molecule don't lead directly to new vaccines, knowing the basic chemical rules of antigen presentation to the immune system is important.

"If you want to do things on a rational basis, you need the details," Germain maintains. "And the details come from structural studies." □

News of the Week continued from p.71

Life at other stars: A matter of climate

Among the glittering denizens of the heavens, which stars are most likely to support life? Researchers had previously concluded that stars at least 2 billion years old, with a surface temperature and mass similar to those of the sun, might form planets capable of fostering life. A new study suggests that a group of stars with slightly lower mass and surface temperature has an equally good chance of creating life-sustaining planets.

Results of the study, which uses a computer model to determine the climate of planets near a variety of stars, could help guide NASA's Search for Extraterrestrial Life (SETI) (SN: 11/7/92, p.317), says James F. Kasting of Pennsylvania State University in University Park. He and his colleagues, Daniel P. Whitmire of the University of Southwestern Louisiana in Lafayette and Ray T. Reynolds of NASA's Ames Research Center in Mountain View, Calif., report their work in the January ICARUS.

The researchers restricted their study to possible planets that would contain liquid water – an ingredient deemed essential for life – and that would have an atmosphere similar to Earth's. They also assumed that stars capable of forming planets would space those bodies logarithmically, as in the solar system.

In determining the "continuously habitable zone" around a particular star class – the region in which climate is temperate and stable long enough to sustain life – the team took into account the intensity and variation of radiation emitted by different star types. A planet forming too close to a given star loses water due to heating and photodisassociation, while a planet too far away will be frozen. Because more massive stars burn more intensely, their habitable zone begins farther out, notes Kasting.

The study supports previous findings that sun-like stars, classified as G stars, are good candidates for producing life. The team discovered that K stars, which have 70 percent of the sun's mass, may make equally good candidates. The team suggests that it may be wise, as SETI progresses, to look for telltale radio signals among nearby K stars rather than more distant G stars.

David R. Soderblom of the Space Telescope Science Institute in Baltimore says he has included some K stars in a list of stars for the SETI survey. But it is difficult to determine whether a given K star is old enough to have supported the evolution of multicellular organisms. Soderblom says that with improved star-dating techniques on the horizon, the new report may convince him to add more K stars to the SETI survey. – *R. Cowen*