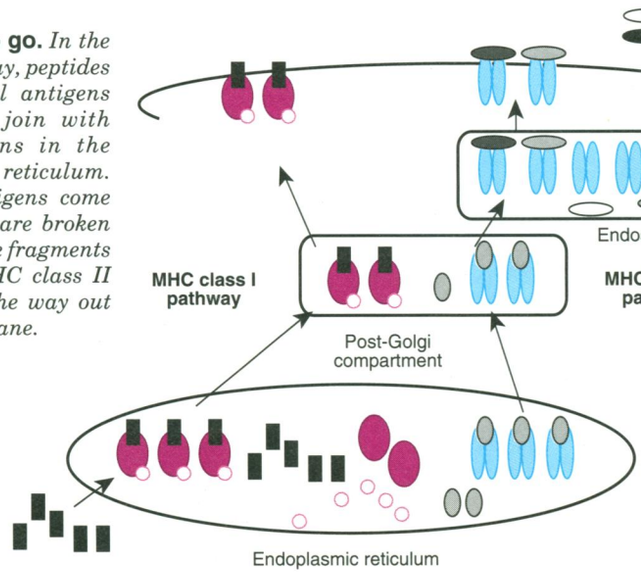
Determining What Immune Cells See

Unraveling the mechanisms by which cells prepare foreign antigens for display to the immune system may lead to better vaccines and ways of treating autoimmune diseases

EVERY SO OFTEN A PREVIOUSLY OBSCURE field suddenly takes off, with new findings popping up in scientific journals. And once in a while, such hot fields show great potential for understanding human disease. Such is the case with "antigen processing."

With the aim of understanding how the human body initiates immune responses, immunologists have been examining, for some time now, the way the individual cells chop up foreign proteins and then present them to the immune system. And gradually, they have been closing in on the cellular machinery that processes the antigens—indeed, researchers are beginning to understand just what an immune cell "sees" when it encounters a foreign protein and is triggered into activity. This in turn has raised hopes for more potent vaccines that are better at stimulating immune responses, or for novel ways of scaling down the abnormal immune system activity that causes autoimmune diseases such as multiple sclerosis and diabetes. Researchers at Harvard Medical School and Massachusetts General Hospital have recently suggested that a defect in antigen processing might even contribute to diabetes susceptibility, although that suggestion is highly controversial (see story on p. 532). With potential applications like these, no wonder antigen processing is getting so much attention.



Two ways to go. *In the class I pathway, peptides from internal antigens* (rectangles) *join with MHC proteins in the endoplasmic reticulum. External antigens come into the cell, are broken down, and the fragments join with MHC class II proteins on the way out to the membrane.*

SOURCE: DENISE FAUSTMAN ILLUSTRATION: J. CHERRY

But while antigen processing research has surged only recently, the story actually began more than 10 years ago, says immunologist Jack Strominger of Harvard University, with Emil Unanue's pioneering discovery that antigens are processed at all. In 1980, when Unanue, an immunologist who was then at Harvard, began his studies of the interactions between the different types of immune cells, he was laboring under a misconception that had been around since the 1960s. "There was a strong dogma then that immune cells recognized the entire protein in its native configuration," says Unanue.

Unanue's experiments soon had immunologists questioning that dogma. When he began, researchers knew that immune responses to extracellular antigens, such as bacteria and their toxins, get under way when certain immune cells, including B cells and macrophages, "present" the antigens to other immune cells, known as helper T cells. That activates the helper cells, which respond by secreting proteins that stimulate a full-scale immune attack on the invading bacteria. Unanue, who is now at Washington University School of Medicine in St. Louis, was particularly interested in learning how the antigen presenting cells worked, and he and his colleagues found that the cells had to take in the foreign antigen before they could activate T cells. That discovery raised two questions: Why must antigens be taken into the presenting cell interior when they would ultimately have to go back to the surface to be displayed to helper cells? And how did they get back to the surface?

Unanue got the first clue when he showed that once inside the cells the antigens end up in small membranous vesicles called lysosomes that contain enzymes that chop up proteins. If he introduced drugs to stop the protein degradation, antigen presentation also stopped. This finding didn't fit with the then common view that immune cells recognize intact antigens, Unanue recalls. A second clue came when Unanue and his colleagues realized that even though lysosomes are loaded with protein-degrading enzymes, the antigen proteins aren't dismantled completely into amino acids. Antigen processing instead produced protein fragments, or peptides. That meant, says Unanue, that "there must be some system that protected antigens from complete degradation and allowed them to emerge as peptides."

Enter the proteins encoded in the major histocompatibility complex (MHC), a large multigene complex long known to be involved in immune regulation. Beginning in the mid-1970s, immunologists learned that antigens are presented only in conjunction with appropriate MHC proteins. Those proteins come in two types, designated class I and class II. Bacterial and other extracellular proteins are presented with class II MHC proteins, and in 1985 Unanue showed that degradation of the antigen peptides stops when they bind to their MHC partners.

It would take a few more years, however, to flesh out the details of where the binding takes place and how the resulting complexes get back to the membrane. In fact, it was only last year that Unanue and others, including Francis Brodsky at the University of California, San Francisco, and Hidde Ploegh of the Netherlands Cancer Institute in Amsterdam put together the complete picture. They found that incoming lysosomes containing antigen peptides fuse with other vesicles carrying newly made MHC class II proteins out to the cell surface. The fused vesicles then continue on their journey to the cell membrane where the MHC II-peptide complexes are inserted into the membrane with the peptide pointed outward so that it's exposed. T cells can then recognize and bind to the peptide-MHC protein complex.

Unanue's work appeared to nail down how external antigens are processed, but these are only one type of invader that the immune system must deal with. Many pathogens—viruses, for example—cause disease only after they gain entry to host cells. Is processing of these antigens similar to that of the extracellular antigens? Recent research suggests that the answer is yes." Indeed, says Andrew McMichael, an immunologist at the John Radcliffe Hospital in Oxford, England, "The recent surge in research stems from the realization that the two pathways are so similar."

Just as in the case of the extracellular antigens, the realization that the intracellular antigens need processing was a long time coming. Until the mid-1980s immunologists thought that immune cells recognized viral external proteins, which become incorporated in the membranes of infected cells. In 1984, however, when researchers, including McMichael and his Radcliffe colleague Alain Townsend, began identifying the proteins that are actually recognized, they received what Townsend describes as "the biggest shock." They found that internal viral proteins were also recognized by attacking immune cells. What's more, Townsend's work showed that the cells were recognizing "not the protein in folded form, but protein fragments," he says.

At that point, the question facing Townsend and others was how the protein fragments were transported to the surface membrane. The best bet was again that they got there in combination with MHC proteins, although it's the class I type that presents viral antigens. The class I structure, determined in 1987 by Strominger's team in collaboration with Don Wiley's group, also at Harvard, turned out to have a neat cleft on its upper surface that's just the right size to hold a peptide. "When we saw the MHC I structure, it fitted beautifully," Townsend says. "You could see how the site [on the MHC I molecule] could bind peptide."

The researchers still didn't know, however, where in the cell the peptides were produced and how they managed to combine with the MHC proteins. Two years later, in 1989, experiments by Richard Klausner and his coworkers at the National Institutes of Health, and, independently, Jonathan Yewdell and Jack Bennink, also at NIH, shed some light on these issues. They showed that viral protein fragments, which Townsend and others

New Theory of Diabetes Etiology Riles Immunologists

Iconoclastic ideas in any field can trigger an eruption of excitement and controversy—and immunology is no exception. Take for example the furor stirred up by a recent study reported by Denise Faustman and colleagues at Harvard Medical School in the 20 December 1991 issue of *Science*. If the group's results hold up, they would provide the first evidence that an error in how antigens are prepared for presentation to the immune system may contribute to the development of a disease—type I diabetes. The paper is, says diabetes expert Arthur Rubenstein of the University of Chicago Medical School, "very, very original. Here's a new idea and maybe a new mechanism. It opens a new avenue for exploration." But the paper also runs counter to accepted dogma—held by some of the field's top experts.

Truth is, Faustman and her colleagues have challenged almost everything the immunological community thinks it knows about diabetes. And the stakes are higher still because of what the work may mean for diabetes therapy. Type I diabetes is an autoimmune condition that results when the immune system erroneously attacks and destroys the insulin-producing islet cells of the pancreas. Researchers have known this for several years, and have had a quasi-consensus on why it happens—one that seemed so solid that it had become the basis of efforts to develop improved ways of preventing and treating the disease. So if Faustman is right, those approaches may well not work. "It makes a real big difference," acknowledges immunogeneticist Linda Wicker of Merck, Sharp, and Dohme in Rahway, New Jersey. Faustman's findings, she says, "would change the drug target."

Some people, the thinking went, are more susceptible to the immune attack on their islet cells than others because they have a defect in one of the members of a particular family of immune regulatory molecules, known as the class II MHC proteins (where MHC stands for major histocompatibility complex, the large multigene complex where the genes for the proteins are located). The early evidence for that view had come from genetic studies linking diabetes to the part of the MHC that carries the class II genes. But most experts think that Stanford immunologist Hugh McDevitt and his colleagues produced the clincher in 1987 when they discovered that people with diabetes were more likely than a control group to sport a specific altered amino acid in a class II protein. Since the MHC II molecules hold pieces of foreign antigen on immune cell surfaces and induce other immune cells to attack anything with a similar configuration, McDevitt's finding suggested that the altered MHC II molecule might trick the immune system into destroying the individual's own tissues, in this case the insulin-producing pancreatic islet cells.

Now come Faustman and her colleagues with new evidence that says that a defect in the other family of MHC proteins, known simply as class I, may be involved. They find that cellular expression of the class I proteins is lower in 300 human diabetes patients than in normal controls. They made similar observations in NOD (for nonobese diabetic) mice, which spontaneously develop diabetes. And the researchers also found that mice from another strain that have been genetically engineered so that they can't express class I proteins on their cell surfaces develop mild diabetes late in life. These results led Faustman to suggest that decreased class I protein expression may contribute to diabetes susceptibility by making the immune system less able to distinguish the body's own tissues from foreign antigens. "If you don't have sufficient class I molecules, you can't present your own proteins to the immune system and the immune system never learns not to attack these proteins," says Faustman. As a result, she says, proteins like insulin and the islet cells that produce them are treated as foreign by an immune system that has never learned to recognize them as self.

So how does Faustman reconcile her theory with all the evidence linking class II molecules with diabetes? She doesn't precisely. "Maybe it is not only class II molecules themselves that are faulty. Maybe the mutation [that causes diabetes susceptibility] is actually in genes that lie in the class II region but code for class I-related molecules," she says.

And indeed, not only do such genes exist, but Faustman has found an alteration in one of them in NOD mice, further buttressing her case. The change is in a gene encoding a "transporter" protein that inserts antigen fragments into the cellular compartment containing MHC class I proteins. If the fragments and class I proteins don't get together and form a complex, the MHC class I proteins aren't displayed on the cell

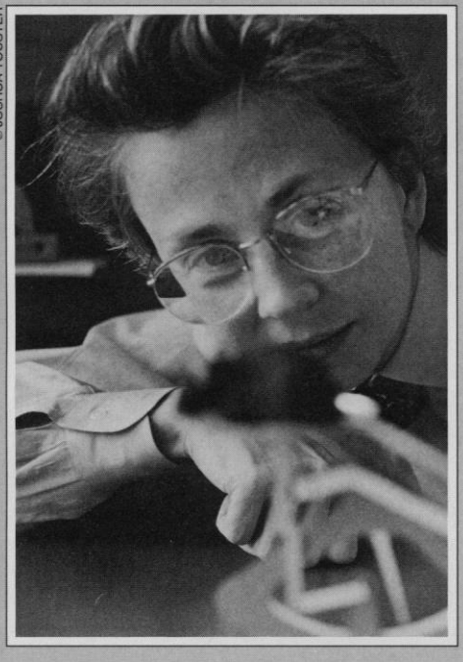
had found to be produced in the cell cytoplasm, were probably meeting up with newly synthesized class I proteins while they were traversing the membranous intracellular compartment known as the endoplasmic reticulum on their way to the cell membrane. But that finding was also puzzling.

To enter the endoplasmic reticulum, the peptides have to cross a membrane, a journey that usually requires that they have a signal sequence that will allow them to insert into the membrane. Yet Townsend could detect no sign that either viral proteins or the peptides derived from them used a signal sequence to cross the endoplasmic reticulum membrane. As a result, he proposed that some kind of protein might transport the peptides into the endoplasmic reticulum, although he then had no direct proof of that.

But about the same time, Townsend recalls, he learned about some new mutant cell lines that were eventually going to help provide the proof. A complete class I MHC protein contains two different protein chains, and in the mutant cells, which had been identified by Klaus Karre and George Klein at the Karolinska Institute in Stockholm, the two proteins were made but did not get together. As a result, the cells could not present internal viral antigens.

Subsequent work by Townsend's group pointed to an explanation for the failure to assemble class I proteins. The intact proteins are very unstable unless they bind antigen fragments, and in the mutants the fragments weren't transported into the endoplasmic reticulum. The result: The two chains of the class I proteins join briefly but fall apart again.

Then, barely a year ago, four research teams traced the mutant cells' inability to transport antigen peptides into the endoplasmic reticulum to deletions in genes that they postulated to code for the transporter proteins predicted by Townsend. (The teams were headed by John Monaco at Virginia Commonwealth University in Richmond, Virginia; John Trowsdale of the Imperial Cancer Research Fund in London; Jonathan Howard of Cambridge University; and Thomas Spies of the Dana-Farber Cancer Institute, in collaboration with Robert DeMars of the University of Wisconsin at Madison and Elizabeth Mellins and Donald Pious of the University of Washington.) Last spring, Spies and DeMars provided the best evidence that the genes encode the transport proteins when they found



Taking a new view. *Denise Faustman suggests that a defect in class I MHC protein display may contribute to diabetes susceptibility.*

surface. So a defect in the protein transporters, argues Faustman, could explain the decrease in MHC class I protein she sees in cells from diabetic mice and humans.

Many immunologists aren't persuaded by the theory, however. For example, Emil Unanue, an immunologist at Washington University School of Medicine in St. Louis, asserts that the Faustman theory disregards the weight of evidence that links diabetes with defects in class II MHC. But Faustman insists that while her data propose a new role for MHC class I proteins, they "do not add to or eliminate the role of MHC class II in disease pathogenesis."

But even if she eased the fears of the class II contingent, there are criticisms from researchers who work with the same strains of mice as Faustman and have not obtained the same results. Foremost among the issues they raise is the question of whether the transporter gene change seen in NOD mice by the Faustman group actually affects the expression of class I MHC proteins as she proposes. Edward Leiter and Rex Gaskins at the Jackson Laboratory in Bar Harbor, Maine, found the same genetic variation, but they found no structural change in the transporter protein that might affect its function. And Thomas Spies of the Dana-Farber Cancer Institute in Boston has identified a number of genetic variants in human transporter genes and reports that, "Our data do not support a significant association of any of these variants with human disease, including diabetes." Spies, however, does not rule out the possibility that other variants may exist that do correlate with disease.

Then there is yet another class of class I iconoclasts. Take Maarten Zijlstra of the Netherlands Cancer Institute in Amsterdam. He says that in his lab the genetically engineered "knockout" mice don't develop diabetes. Leiter says the same of knockout mice in his lab. And Wicker, who routinely assesses MHC I expression on the cells of NOD mice says, "We've never seen anything to make us think that class I expression on spleen cells in NOD mice is abnormal."

Faustman responds to these comments by pointing out that a lot of different factors can influence the incidence of diseases such as diabetes. Even among NOD mice, she says, the incidence of diabetes ranges from 0% to 85% in different colonies at 6 months of age. The animals studied by Zijlstra and Leiter may not have been old enough to show the changes Faustman detected. Viral exposures, which could vary from colony to colony, may also have affected the results. A great deal of evidence suggests that viral infections, as well as genetic susceptibilities, contribute to diabetes incidence. And then there's the possibility that Faustman may be right about a role for decreased class I expression, but wrong about what causes it since several genes are needed for normal assembly and display of the proteins.

As is always the case in such scientific debates, it will take some time to sort out who's right and who's wrong. All that can be safely said now is that Faustman has had the first, but by no means last, peer-reviewed word. While she waits for confirmatory data to crop us, Faustman says of the storm she engendered: "A totally new theory always causes a pause and brings re-evaluation of past and new data, especially when the concepts are novel and everyone was working in unison on a different mechanism." ■ M.H.

that they could restore class I expression to the mutant cells by replacing a mutant transporter gene with a good one.

In addition to finding the components for peptide transport, immunologists are also identifying the structures that produce the peptides in the first place. Just last fall, researchers in Monaco's and Trowsdale's laboratories independently produced evidence suggesting that the production occurs on a structure called a low-molecular mass polypeptide (LMP) complex, which resembles a proteasome, described by Monaco as a "big ball of degradative enzymes."

Now that immunologists have a good idea of how both intracellular and extracellular antigens are prepared for display on the cell membrane, they are moving ahead to try to understand why some antigens elicit stronger immune responses than others—information that could help produce more effective vaccines. And other immunologists, like McMichael, are exploring the connection between antigen processing and human disease. The story, says McMichael, is becoming remarkably clear. "Molecular structure is fitting together with cell biology and old fashioned immunology and virology. It's all coming together to give the same picture." ■ MICHELLE HOFFMAN

ADDITIONAL READING

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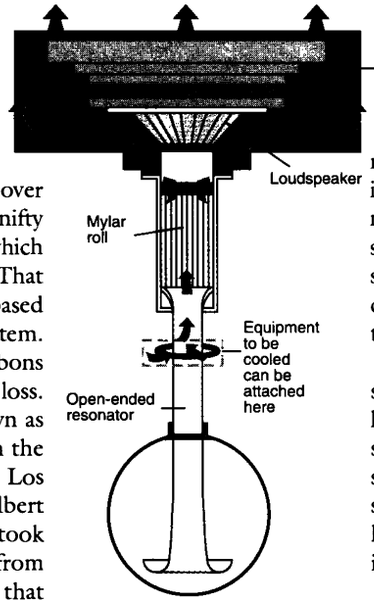
Acoustic Fridge Takes to Space

Rock and roll loudspeakers have always been used to make the coolest music around. Until now, however, they've never been thought of as actual cooling devices. But that's all changed, thanks to the work of Steven Garrett and his colleagues at the Naval Postgraduate School in Monterey, California. Building on work done over the past decade, Garrett's team has designed a nifty refrigerator powered by a standing sound wave, which flew this week on the space shuttle Discovery. That flight was a test, but in the future refrigerators based on this concept could become a hot commercial item. And, since they contain no chlorofluorocarbons (CFCs), they might even help slow global ozone loss.

The principle behind the space cooler, known as thermoacoustic refrigeration, was developed in the 1980s by a team that included Garrett and Los Alamos scientists Gregory Swift, Tom Hofler, Albert Migliori, and the late John Wheatley. They took their inspiration from the laws of acoustics and from the centuries-old observations of glass blowers that when they heat one end of a glass tube while keeping the other cool enough to touch with their lips, the temperature gradient sometimes sets up a sound wave, causing the tube to "sing." The thermoacoustic refrigerator does just the reverse: It exploits sound waves to create a temperature gradient.



It's a gas. *Expansion and compression of a helium-xenon mix* (pink) *causes heat flow* (arrows) *out of the acoustic fridge.*

SOURCE: STEVEN GARRETT ILLUSTRATION: J. CHERRY

The new fridge is decidedly low-tech—and true to the spirit of garage rock. Its 4-inch JBL loudspeaker plays one note—roughly concert A—very loud. The note is just the right frequency to set up a standing sound wave in a cylindrical tank filled with a mixture of helium and xenon at a pressure of 10 atmospheres. The sound wave causes the gas at each spot in the tank to go through cycles of compression and expansion. That's the key to the device, because gas heats up a bit when compressed and cools as it expands.

The refrigerator capitalizes on that heating and cooling cycle with a low-tech heat absorber: a rolled-up sheet of mylar that fills the upper end of the chamber that holds the gas. Spacers made of fishing line create gaps that allow the gas to permeate the mylar jellyroll. When a compression phase of the sound wave comes along, the gas molecules collide with the mylar and transfer some of their heat to it. The mylar in turn passes the heat to the speaker-casing, from which it radiates away. Then, when the gas expands, it cools further than it would otherwise, since some of its heat has been drawn off. The process causes a progressive cooling, which can be exploited for refrigeration.

The result is a fridge that uses no ozone-eating CFCs and has only one moving part (the speaker), which should boost its reliability. Remarkably enough, the one-note fridge is also quieter on the outside than standard models; although the noise level inside is 10,000 times that of a Rolling Stones concert, the high-frequency sound is easily contained by the chamber walls.

All that piqued NASA's interest, because the space agency's other refrigeration options are less than ideal. A freon-based refrigerator for storing biological samples failed on a shuttle life-science mission last year, and the refrigeration systems that keep the equipment cool on surveillance satellites are also problematic: They vibrate in a way that disturbs imaging equipment.

Because of these problems, General Electric, which provides NASA with its life-science refrigeration, and the Department of Defense have contracted with the Navy group to develop the thermoacoustic alternative. The model being tested for the first time on the Discovery is a cryo-cooler, designed to maintain equipment at very low temperatures such as those required on surveillance satellites. But the next generation—for which Garrett's group already has a G.E. contract and a future shuttle berth—will run at the temperatures of a home refrigerator.

Speaking of home refrigerators, the rock and roll fridge's advantages could play well on terra firma as well; Garrett says it could be less expensive to produce than today's models, and just as durable. In fact, he says the only thing keeping the acoustic fridge out of American homes is a lack of interdisciplinary talent: "The people who do refrigeration don't know acoustics."

Maybe that's the reason why there's been so little interest from terrestrial refrigerator manufacturers. Whatever the reason, Garrett thinks "it's a sin that it has to be tested in space. It should be tested in Whirlpool's home-economics lab." That's not to say that there are no cool companies: Volvo has inquired about the possibility of developing an acoustic car air conditioner. But Garrett and his team are, for the moment, spread too thin to give it much of their time. ■ MARCIA BARINAGA