Teetering on the Brink of Danger

New experiments undermine the idea that the immune system distinguishes self from nonself and open the door to a new theory—that it responds instead to danger signals

When immunologist Polly Matzinger arrived at the National Institute of Allergy and Infectious Diseases (NIAID) 6 years ago, she never expected to be trying to topple one of immunology's seemingly most solid pillars. But that's just what Matzinger and colleagues John Ridge and Ephraim Fuchs are now doing—with some help from two groups from other institutions.

For nearly 50 years, immunologists have thought that during embryonic development or early life the immune system undergoes a critical education in which it learns how to tolerate the body's own tissues while retaining the ability to mount an attack on bacteria, viruses, and any other foreign invaders. Indeed, the idea that the immune system learns to distinguish "self" from "nonself" was so persuasive that the Nobel Committee awarded the 1960 medicine prize to its chief architects: Australian virologist F. Macfarlane Burnet, who provided the theoretical underpinnings, and British biologist Peter B. Medawar, who shored up Burnet's theory with experimental support. In particular, Medawar demonstrated that while adult mice reject transplanted tissues from immunologically foreign animals, fetal and newborn mice can become tolerant to the foreign cells and do not reject them. Medawar and Burnet attributed this neonatal tolerance to the critical education period, during which the newborn animals' immune cells could learn to accept the foreign tissue as "self," just as they accept the body's own cells.

But in this week's issue of *Science*, Matzinger's group and those of Marcella Sarzotti of the Veterans Affairs Medical Center in Baltimore and Paul Lehmann of Case Western Reserve University in Cleveland report new results that undermine the experimental foundation Medawar had laid down for the self-nonself theory (see pp. 1723, 1726, and 1728). The work demonstrates that, contrary to Medawar's thinking, the neonatal immune system, like an adult's, can be primed to recognize and attack foreign antigens, as long as the antigen is introduced to the animals' T cells, which help get the immune attack under way, under the right conditions.

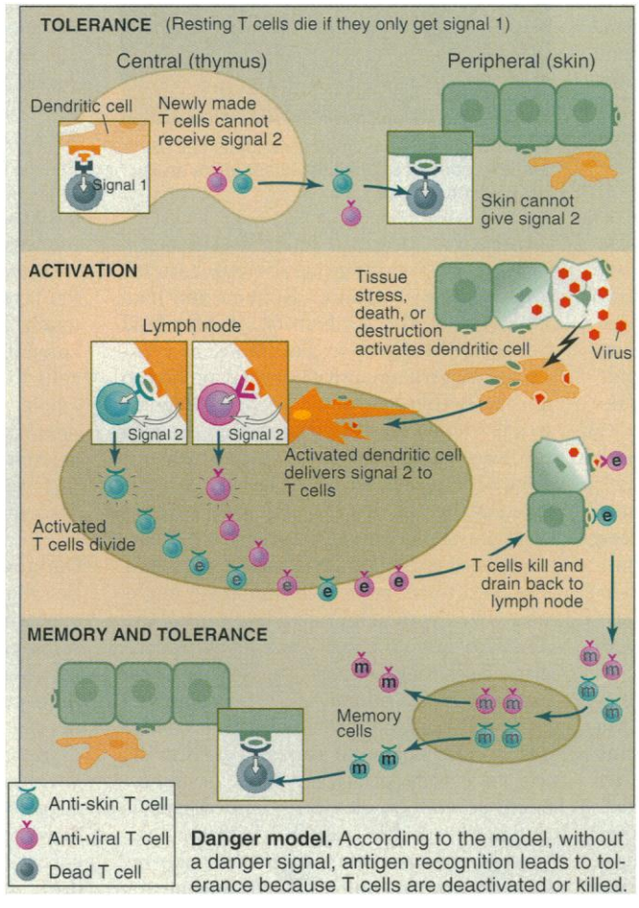
"All you have to do is put them [the T cells] in the right environment," says Matzinger. Conversely, the results also show that the appropriate conditions can lead to tolerance in adult animals.

It's not the first time that immunologists have observed an immune response when they expected tolerance or vice versa. But in the past, researchers tended to assume they had misjudged the window of neonatal tolerance and given the antigen at the wrong time, says Albert Bendelac, an immunologist at Princeton University: "They used that as a convenient way to hide the inconsistencies."

These new papers can't be seen that way, he adds: "These are three independent teams, using three different systems and doing three very carefully done [studies]. They really dissect out exactly what was going on." Alfred Singer of the National Cancer Institute agrees they clarify an important point. "These experiments clearly show that there is nothing special about the neonatal period. That's an important correction," he says.

Matzinger, however, sees the work not just as an important correction, but as a step toward challenging the whole notion that the immune system distinguishes self from nonself. She, Ridge, and Fuchs argue that if the immune system can learn to tolerate antigens at any time, and there is thus no critical period for learning to distinguish self antigens from nonself antigens, it must have another way of discerning when to respond. They propose instead that the immune system springs into action only when an antigen is associated with causing harm. With this danger model, as they call it, "we can build an immune system that's very simple and that really works," Matzinger says.

That's going too far for other immunologists, even some of the authors of the papers appearing with hers in this issue. But if Matzinger and her colleagues are right about the danger model, the immunology textbooks will need drastic revisions. But even if the researchers are wrong about the danger theory, the results reported in the three papers could still have important therapeutic implications. They could point the way, for example, to effective vaccines for infants, better success rates for organ transplants, and improved treatments for autoimmune diseases, in which the immune system turns on and attacks the body's own tissues.



Danger model. According to the model, without a danger signal, antigen recognition leads to tolerance because T cells are deactivated or killed.

SOURCE: MATZINGER *ET AL.* ILLUSTRATION: K. SUTLIFF

Nurturing immunity

Matzinger herself credits Fuchs, who was then also at NIAID, for reawakening her doubts about the observations underlying the self-nonself theory. In the late 1980s, he had been impressed by work showing that T cells need to be activated not just by the bits of antigen displayed on the surfaces of special "antigen-presenting cells," but also by a second signal from these presenting cells.

Indeed, if a T cell sees just an antigen without a second signal, it disappears. Some researchers think it dies; others argue that it becomes unresponsive, or anergic, or gets transformed into a cell that can only stimulate antibody production. No matter what, however, the

immune system becomes tolerant of that antigen thereafter. "I came to believe that the antigen-presenting cell was the critical determinant of how the T cell responded," Fuchs recalls. Still, even though a few other scientists were also questioning aspects of the self-nonself theory, it continued to reign as an unshakable paradigm, and it took Fuchs 2 years to convince Matzinger that it should be toppled.

He convinced Matzinger of this with one particular experiment that built on early findings that B cells, one type of antigen-presenting cell, were less effective at evoking an immune response than were dendritic cells, antigen-presenting cells that ferry antigens from various tissues to the lymph nodes. In this study, he showed that B cells could actually invoke tolerance.

As a result, he began to wonder if the newborn mice in Medawar's experiments tolerated foreign tissue simply because B cells disarmed the T cells before the foreign antigens could be presented by dendritic cells. In those experiments, Medawar had first injected the animals with spleen cells from unrelated mice, which had few dendritic cells relative to B cells. Newborn recipients would later accept skin grafts from those cell donors. In adults, however, the cell injections did not prevent the animals from later rejecting skin grafts.

Fuchs then decided to see whether he could get a litter of newborn mice to respond to a foreign antigen if he increased the number of dendritic cells in the first injections and reduced or eliminated the B cells. The antigen he chose to use is the H-Y antigen, which is carried only on the surfaces of male cells. This way Fuchs could expose newborn female mice to dendritic cells purified from the spleens of male mice and know that the female immune system had never seen the H-Y protein before. And in contrast to Medawar's results, when Fuchs later re-exposed his litter to the protein, they mounted an immune response to it. "Lo and behold," he says, "the female mice were primed by the male dendritic cells."

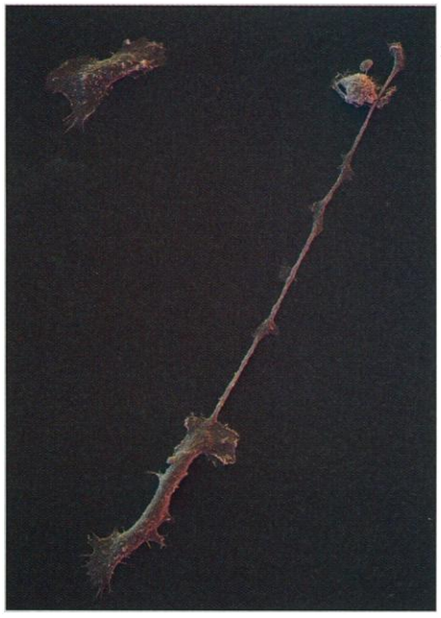
Fuchs then left for the Johns Hopkins Oncology Center in Baltimore. Ridge, newly arrived at NIAID, and Matzinger continued the work and went on to do the converse experiment: inducing tolerance in adult animals. Here again, they found that the relative numbers of cells injected were the key.

Medawar had injected newborns with some 50 million cells, plenty to make those immune systems tolerant but not enough to do the same for an adult immune system. But when Matzinger and Ridge upped the ante and injected 500 million into the adults, they found that the animals also became tolerant to subsequent transplants of donor tissue. Because adult mice have 10,000 times more T cells than do newborns, Ridge says, many more B cells were needed to disarm the T cells before they had a chance to run into the dendritic cells.

"This is terribly important," says Kevin Lafferty, an immunologist who works on organ transplantation at the John Curtin School of Medical Research in Canberra, Australia. "As transplanters, that's what we've been trying to do for years."

Less virus, different adjuvant

There may be other ways of breaking the expected pattern of neonatal tolerance and adult immunity besides tinkering with the ratio between dendritic cells or B cells, and T cells, as the Sarzotti group showed. Sarzotti, who works with Deanna Robbins and Paul Hoffman of the University of Maryland School of Medicine in Baltimore, had suspected that the amount of antigen might also affect whether an animal becomes tolerant to a particular antigen. They tested the idea using a mouse leukemia virus that newborn mice ordinarily learn to "tolerate," as indicated by the fact that they are unable to fend it off on subsequent exposure.



DAVID SCHARF

Reaching out. An activated dendritic cell extends to contact a T cell. At upper left is an inactive dendritic cell.

Sarzotti guessed that the viral doses others had used to try to induce immunity might simply be overwhelming and disarming the newborn mice's T cells before their dendritic cells could activate the T cells. With Robbins and Hoffman, she then injected groups of neonatal mice with ever smaller doses of the leukemia virus, and eventually, the mice were primed to fight off subsequent injections of virus. "The dogma is that newborns don't respond," says Sarzotti. "But [their] response was just like the response we see in adults." The finding means that the proper amount of antigen is key to successful vaccination and that vaccination failure in infants may be due to too high a dose of antigen.

And the third paper in this trio points to still another route for inducing immunity. In many immunological experiments, the antigen is injected with a nonspecific immune-system booster known as an adjuvant. Lehmann and Case Western Reserve colleagues Thomas Forsthuber and Hualin Yip suspected that the choice of adjuvant used by many researchers since Medawar might account for the differences they observed between adult and newborn mice in responses to antigens. If that were true, they reasoned, then there probably was no such thing as a window for neonatal tolerance. Their hunch was right.

They found that newborn mice, as well as adults, could mount an easily detectable immune response to either a mouse protein called myelin basic protein or a protein from chickens (the enzyme lysozyme) if the proteins were given along with Freund's complete adjuvant, a preparation containing killed mycobacteria. But if the proteins were administered with an adjuvant lacking the mycobacteria, both the newborns and the adults did not show that response to the two proteins.

"These three papers each have a different statement, but they [convey] one very strong message: There's nothing special about the neonate," Lehmann says. "[That message] challenges the classical knowledge upon which Nobel Prizes have been based."

While many immunologists had already sensed this challenge in the backs of their minds, says Lafferty, they had not really paid heed to it. Now, "these new ideas have the potential to radically change our approach to immunobiology," he adds.

If researchers can control the type of immune response they induce by adjusting how much antigen they give relative to the amount of T cells and antigen-presenting cells in the recipients, they might, for example, be able to make people undergoing organ transplants tolerant to the donor tissue or arrest autoimmune diseases. The work "leads to a way that you can directly control the outcome [of giving an antigen]," Fuchs says.

Dangerous liaisons

But Matzinger, Fuchs, and Ridge think the papers emphasize the need for a more drastic revision of ideas about how the body decides whether or not to respond to an antigen. As they see it, three signals—not just two—are needed to activate the immune system. Two of these are the same ones other immunologists think are needed to trigger T cells: one triggered when a specific antigen is recognized by the T cell receptor, plus a nonspecific "costimulatory" signal, provided by dendritic cells. But then Matzinger's group de-

parts from the conventional view, suggesting that the second signal is delivered only after the dendritic cell has been activated by an alarm signal, a primary message received as a "May Day" from stressed, damaged, or lysed or necrotic cells.

That picture is consistent with all three experiments, she says. In her own group's work on inducing immunity to the H-Y antigen in newborn mice, the surgery required to obtain the dendritic cells provided the activating danger signal. In the other experiments, the mycobacteria in adjuvant or damage done by the murine leukemia virus could have done the trick by stressing or damaging cells.

But in the absence of danger, or in Medawar's experiments where there were relatively too few dendritic cells conveying the costimulatory signal, T cells lose their ability to respond, and tolerance results. Similarly, a T cell encountering an antigen normally found in the body, such as the proteins located on the body's own healthy tissues, dies or becomes unresponsive. In this way, "each tissue is constantly inducing tolerance to itself over its lifetime," Matzinger says.

Some immunologists find the danger theory an intriguing alternative to the self-nonself theory. "We've come to a theoretical dead end with self-nonself," Lafferty says. Others argue, however, that even if the danger theory is valid in some circumstances, "it's really an extension of the self-nonself model," as Singer puts it. In his view, it may explain how T cells become tolerant of self antigens not encountered in the thymus gland, where T cells are supposed to learn to distinguish self and nonself, but would not preclude that education from occurring in the first place.

And still others argue that Matzinger's ideas are not all that new. "It's just putting new words on what's been known before," says J.F.A.P. Miller, an immunologist at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia. Matzinger is the first to concede that the danger model builds on the ideas of others, such as Melvin Cohn of the Salk Institute for Biological Studies in San Diego and Lafferty's group. Both pioneered two-signal models for T-cell activation.

Still, Bendelac gives her credit for tying all these ideas together in a novel way, and particularly for introducing the idea of danger as critical for inducing immune responses. "[The model] is one coherent way of putting things together and one that makes evolutionary sense," he says. "It focuses the whole issue of tolerance on danger or stress inducing a particular signal that gives a green light for the response."

And if she's right, the shift in thinking could make an enormous difference in understanding everything from cancer development to graft rejection. Matzinger points out that many cancer researchers think the immune system usually keeps watch for tumors, successfully wiping out most that arise. But she proposes that it will attack tumor cells only after they are assaulted by some pathogen. "What you need is danger, and the tumor isn't giving you danger," Matzinger says.

The danger theory also implies that giving immunosuppressive drugs, such as cyclosporin, to organ transplant recipients may ultimately be counterproductive. First, the trauma of surgery creates the danger that normally would trigger a costimulatory signal, causing the immune system to activate and make lots of the T cells that could destroy the graft. At first, the drug forestalls this rejection by blocking the other signal, that which the T cell receives when it recognizes the graft. But after the graft is healed—and the danger has passed—the drug's blocking action keeps that same signal from making those T cells tolerant. Thus, if the recipient stops taking the drug, the organ will be rejected, having been unable to disarm the T cells that recognize its antigens.

Identifying the alarm signal would be one way of convincing the many skeptics that the danger theory is correct. And Matzinger has a candidate in mind, a group of molecules called heat shock proteins, which are gene-regulating molecules that are produced when cells are stressed by heat or other environmental conditions. But she expects that proving this hunch may be difficult. The chemical milieu in and around cells is very complex and constantly changing, so teasing out any one protein as a danger signal will be difficult. "I'm in the position of a physicist who proposes a new particle because she sees tracks in a cloud chamber. She just hopes she lives long enough to find it," she says.

But even if Matzinger never finds her "particle," and the self-nonself paradigm pillar never falls, her supporters still think her efforts will have been worthwhile. "She's put her fingers exactly where the paradoxes are," says Bendelac. That alone should get her colleagues thinking.

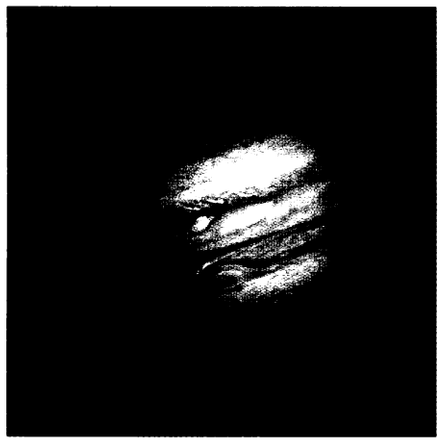
–Elizabeth Pennisi

PLANETARY SCIENCE

Shock Forges Piece of Jovian Interior

The most abundant material in the nine planets isn't rock or ice or iron. Instead, it is hydrogen crushed under such great pressures that it turns into a silvery, electrically conducting metal. This exotic substance makes up most of the interiors of the giant planets Jupiter and Saturn. Earth, however, is devoid of it—or was, until a group of high-pressure researchers at Lawrence Livermore National Laboratory briefly forged it in the impact of a high-speed projectile.

The achievement, reported this week at the American Physical Society meeting in St. Louis by Livermore researchers William Nellis, Samuel Weir, and Arthur Mitchell, ends a long quest. For more than 2 decades, researchers have been trying to create metallic hydrogen in the hopes that it would answer some crucial questions. While theory firmly predicts the existence of the metallic state, it is vague on the details, such as exactly how much pressure is required to make it. And that has been a major uncertainty for scientists studying Jupiter and Saturn, casting doubt on calculations of everything from how Jupiter manages to generate its outsized magnetic field to how it heats itself.



JPL

Stealing a bit of Jupiter. Giant planets no longer have a monopoly on metallic hydrogen.

Now the Livermore result may dispel some of those uncertainties. "It really has been a long haul on hydrogen," notes theoretician Neil Ashcroft of Cornell University. "This is quite an exciting development." One cause of the excitement is the Livermore group's report that it took less pressure than expected to create metallic hydrogen, implying that it fills even more of the two planets than had been thought. "The perceived constitution of [Jupiter], I think, could undergo quite a change if these experiments prove to be correct," says Ashcroft. Coming just a few weeks after the arrival of data on the composition of Jupiter from the Galileo spacecraft's probe (*Science*, 2 February, p. 593), this first look at that planet's major constituent "will make for very interesting times" for Jupiter specialists, says Edwin Salpeter of Cornell.

The key to those interesting times turned out to be a hotter recipe for metallic hydrogen—a recipe the researchers themselves didn't expect to succeed. In recent years, most experimentalists trying to metalize hy-