# Taming Rogue Immune Reactions

*Immunologists are trying to apply their hard-earned knowledge about how the immune system works to improve therapies for autoimmune diseases and organ rejection*

"FRIEND OR FOE?" goes the ancient question. But medicine doesn't always have a simple answer. The immune system, for example, can be both. Friend when it protects the body against disease by recognizing and destroying foreign invaders such as pathogenic bacteria and viruses. Foe, if it attacks a life-saving heart or kidney transplant or if it mistakenly turns on the body's own tissues, a root cause of such autoimmune diseases as multiple sclerosis, rheumatoid arthritis, and juvenile diabetes.

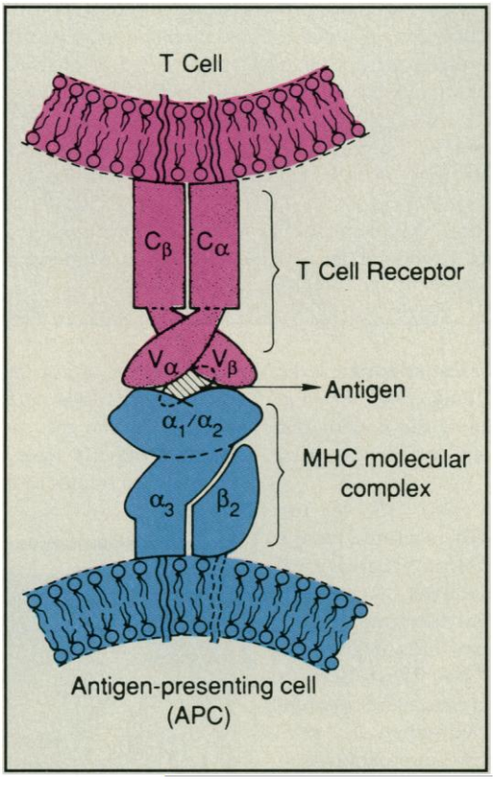
Only a decade ago immunologists didn't even understand normal immune responses. But with the cloning of genes for several key immune system components in the early 1980s, both normal and abnormal responses began to come clear. At last immunologists could characterize—on the molecular level—the types of immune cells and work out the myriad ways in which those cells communicate with one another.

So with science having largely demystified the "witchcraft" of immune responses, immunologists are turning to the next challenge: putting their new knowledge to clinical use in taming pathological immune responses. Successes are still mostly on the horizon. "The progress in basic immunology hasn't reached the point where it has started spilling over into the clinic," says immunologist Hugh McDevitt of Stanford University School of Medicine. "But," he adds optimistically, "that will happen in the next 5 to 10 years."

A few small trials of new immunosuppressive therapies support his optimism. Aimed at treating organ graft rejection and autoimmune diseases, including multiple sclerosis and rheumatoid arthritis, these are at the vanguard, with yet more trials now in the planning stages. And new immunosuppressive therapies are badly needed because the current ones are blunt instruments, sledge-hammers that unselectively depress the entire immune system, leaving patients vulnerable to infections. These days immunologists want to develop scalpels that will excise only the particular immune reaction that is causing the trouble.

Many of these scalpels are aimed at the immune system's T cells—a logical place to start, because those cells cause much of the tissue damage in graft rejection and autoimmune disease. "We feel that the auto-reactive T cell can be treated as a pathogen," says Steven Brostoff of the Immune Response Corporation in San Diego, the leader of one of the groups doing the work. "You can make a vaccine for it as you do for any pathogen."

How? Some researchers are turning foes into friends. The idea is to use auto-reactive T cells, the cells that attack the body's own tissues, as the active ingredient of the vaccine. This approach is based on findings made a few years ago by Irun Cohen of the Weizmann Institute in Rehovot, Israel.




**Activating T cells.** *An antigen-presenting cell gets things under way by displaying an antigen fragment on its surface as part of a complex with an MHC protein. A T cell is triggered when the variable region of its receptor (designated Vα and Vβ) on the presenting cell surface.* [*Reproduced with permission from the* Annual Review of Immunology, *vol. 7, © 1989 by Annual Reviews, Inc.*]

Cohen was studying rats with experimental allergic encephalomyelitis (EAE), a model for multiple sclerosis. Researchers produce EAE by injecting animals with myelin basic protein, a component of the membranous sheath surrounding some nerve fibers. The protein triggers an immune attack on the fibers, causing nerve damage and symptoms, such as paralysis, similar to those seen in multiple sclerosis.

Cohen's group showed that T cells can cause the nerve fiber damage in EAE. The researchers found that T cells prepared from rats with the allergic condition could trigger it in other animals who had never even been exposed to myelin basic protein. But the Weizmann workers also found that if they first inactivated the T cells so that they could no longer mount an immune attack, the cells would actually protect rats against a subsequent challenge with either the active T cells or myelin basic protein. The animals apparently mounted an immune response capable of suppressing or destroying myelin-reactive T cells.

In recent months, at least two groups have begun small clinical trials to see if a similar approach will work in human beings. Rene de Vries of University Hospital in Leiden in the Netherlands has given T cell vaccines to four patients with rheumatoid arthriti,s and Howard Weiner and David Hafler of Harvard's Brigham and Women's Hospital in Boston have administered the vaccines to four multiple sclerosis patients. The researchers prepare the vaccines by recovering T cells from the joint fluid of the arthritis patients or the spinal fluid of the multiple sclerosis patients, growing the cells in culture, then inactivating them and injecting them into the patients from whom they were taken.

Both de Vries and Weiner say it is much too early to tell whether these treatments will improve the patients' symptoms. Both report at least one encouraging finding, however: The T cell vaccines do not appear to be toxic. "We can tell that we don't see unwanted side effects," de Vries says.

But even if the vaccinations eventually

prove effective, they have a potential logistical liability—an individual vaccine must be prepared for each patient. Someone else's auto-reactive T cells might have a different specificity, and immunity against them would not help. And of course it would protect against the chance of infecting the recipient with a virus or other pathogen.

But a mass-produced vaccine would probably be cheaper and logistically easier to handle, and that's where Brostoff and his colleagues at the Immune Response Corporation come in. They and Arthur Vandenbark and his colleagues at the Veterans Affairs Medical Center and Oregon Health Sciences University in Portland have shown that small peptides—containing 10 to 20 amino acids and easily made synthetically—can be used to vaccinate rats against EAE. If such an approach proved applicable to humans, it would be a great advance toward practical treatments.

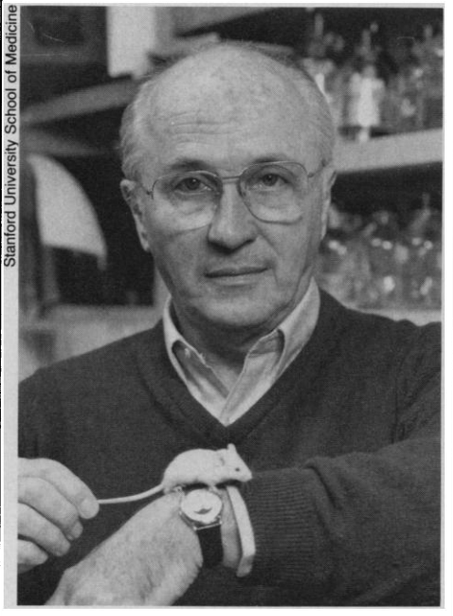
The work of the Brostoff and Vandenbark groups would not have been possible without the detailed molecular picture of T cell activation that immunologists have been sketching for nearly a decade. Each T cell is triggered by a specific antigen, which it recognizes by means of a receptor protein on the T cell surface. Before the receptor can recognize the antigen, however, the antigen must be first taken up by another immune cell, the macrophage. There, it is broken into pieces, forming peptides some 10 to 20 amino acids long. The macrophage displays one of those peptides on its surface in conjunction with another key immune molecule, one of the MHC proteins. (The MHC proteins are so called because their genes are contained within the major histocompatibility complex.) It is the combination of antigen peptide with the MHC protein that the T cell receptor recognizes.

The antigen specificity of the T cell receptor makes that receptor an inviting target for a vaccine or other therapy for autoimmune diseases. By homing in on the receptor it might be possible to knock out only those T cells that cause an autoimmune attack. Potentially, however, the total repertoire of an individual's T cell receptors numbers in the millions. So if a given autoimmune disease was caused by large numbers of T cells, all bearing different receptors, then vaccine development would be daunting indeed.

Fortunately, work done in the past year or two suggests that is not the case. Various researchers, including McDevitt and Lawrence Steinman of Stanford University School of Medicine and Lee Hood of the California Institute of Technology, have found that the array of T cell receptors participating in the development of EAE seems to be limited: as few as four may account for the autoimmunity.

It is that limited array that provides the basis for the T cell vaccines the Brostoff and Vandenbark groups have been using experimentally against EAE in rats. The researchers synthesized peptides corresponding to the antigen recognition sites on those few T cell receptors and used them to vaccinate the rats before injecting them with myelin basic protein; the vaccinations prevented the animals from getting EAE.

That's all well and good for rats. But will the same strategy work in humans? Recent developments lead immunologists to hope it might. In May, Hafler, Weiner, and their colleagues reported results suggesting that the neuronal damage seen in multiple sclerosis might be caused by a limited repertoire of T cells. Their work was done with cloned T cells from the blood of multiple sclerosis patients, but the finding is supported by results obtained with T cells from the disease focus itself.




**Optimistic.** *Hugh McDevitt hopes to see new immune therapies in 5 to 10 years.*

Steinman and his colleagues examined T cells from the nerve-damaged regions of the brains of multiple sclerosis patients—and came to a similarly hopeful conclusion. "In a few individuals [with multiple sclerosis] the results were very encouraging," Steinman says. "Only three or four receptors may be involved. Now we have to extend the finding to see whether other patients have the same T cell receptors." He points out, however, that even if the patients' receptors turn out to show great variability, it might still be possible to use peptides for vaccination, although they, too, would have to be individualized.

The vaccine approach is promising, but it isn't the only strategy that's being tried out on the autoimmune disorders. Other groups are exploring alternative ways of knocking out the T cells that cause autoimmunity. One possibility is to use monoclonal antibodies directed against the pertinent T cell receptors. Such antibodies probably would not have the long-lasting effect of vaccination—but they might have another advantage. Both the Steinman and McDevitt groups and the Hood group have shown that monoclonal antibodies cannot only prevent EAE, but can reverse already established disease. Apparently once the myelin-damaging T cells are out of the way, the animals' nerves can recover.

Inspired by those findings, Weiner and Hafler undertook a pilot study of T cell–reactive monoclonal antibodies in 20 patients with multiple sclerosis. It was not possible, Weiner says, to draw any conclusions about whether the patients experienced any clinical improvement. Multiple sclerosis is a notoriously variable disease to start with, and the patients knew they were getting the antibodies. The power of suggestion might have led them to think they were better when they really weren't.

But, as expected, the antibody treatments suppressed the activity of the patients' T cells, Weiner says, and they did not produce toxic effects. There was a problem, however. Because the monoclonal antibodies were of mouse origin, the patients mounted a treatment-nullifying immune reaction against them.

If researchers could identify the antigens that set off the auto-reactive T-cells, still another approach to blocking the T cell activity might be possible. In most human autoimmune disorders, the triggering antigens have not been pinned down, but they are precisely known in the experimental disease EAE. Immunologists have pinpointed one or two segments of myelin basic protein as being at fault.

Once the identity of the offending peptide is known, McDevitt says, it is possible to modify its structure, producing a peptide that can still bind to the MHC protein on the antigen-presenting cell, but forming a complex that can no longer be recognized by T cells. Such "blocking peptides" can prevent the development of EAE when given with myelin basic protein, or shortly after, although McDevitt says, "What hasn't been shown is that if you once get the autoimmune disease established that you can reverse it with blocking peptide."

Blocking peptides do have a significant drawback in comparison with vaccination. It is likely, Steinman notes, that any therapy using blocking peptides would have to be

administered on a continuing basis, unlike vaccinations, which have the potential of providing permanent protection. Nevertheless, he sees the two approaches as "quite complementary." Blocking peptides might be used to treat a patient with acute disease—one who is developing diabetes, for example—while vaccinations could be given for long-term protection.

Vaccines, blocking peptides, or other immunosuppressive therapies that can be directed specifically at the T cells at fault in autoimmunity or graft rejection are the ideal. But immunologists are also hard at work developing therapies that may not be so selective but could still be an improvement over current methods of immunosuppression. Cyclosporine, for example, has been a major contributor to current successes with organ transplants, but the drug leaves much to be desired. Not only does it depress the immune system generally, but also it can cause kidney damage. As a result, clinicians hesitate to use cyclosporine in children who are developing diabetes, even though clinical trials have shown that it will delay the destruction of the insulin-producing cells of the pancreas.

Cyclosporine has such wide-range immunosuppressive effects because it apparently works by suppressing secretion of the lymphokines, a group of perhaps 20 proteins that play a variety of roles in bolstering immune reactions. But there may be more specific—and gentler—ways of interfering with lymphokine action.

Take, for instance, the lymphokine called interleukin-2, or IL-2, which stimulates the growth of T cells once they are activated, thereby greatly amplifying the T cell response. According to Thomas Waldmann of the National Cancer Institute, the receptor through which interleukin-2 acts is a "marvelous target" for immunosuppression. "The receptor is not expressed on resting cells," he explains. "It's a marker for T cell activation. It's present on cells you might wish to eliminate in disease." By selectively destroying just those T cells that become activated by an organ transplant, for example, it might be possible to induce tolerance to the graft, thereby reducing or eliminating the need for further immunosuppression.

There was a hitch, however, when Terry Strom, Vicki Kelly, and their colleagues at Harvard Medical School tested a monoclonal antibody to the IL-2 receptor in patients who had kidney transplants. Although the antibody, which it was originally made by Takashi Uchiyama in Waldmann's lab, was well tolerated by the patients, it failed to kill human T cells. That may have happened, Strom says, because the antibody was originally made in mice and incapable of activating the human cell-lysing machinery.

Researchers are tinkering with the design of monoclonal antibodies as away of getting around this problem. Cary Queen of Protein Design, Inc., in Palo Alto has exploited genetic engineering methods to "humanize" the mouse monoclonal antibody to the IL-2 receptor by replacing the entire molecule—except the regions that bind the receptor—with human antibody sequences. Moreover, the human immune system generally attacks mouse monoclonal antibodies as foreign and such humanization might solve that problem, too.


**Combatting autoimmunity.** *Lawrence Steinman is working on new therapies for diseases such as multiple sclerosis.*

An alternative method of getting T cell killing, which also relies on the tricks of genetic engineering, is to tie a toxin either to an antibody to the IL-2 receptor or to IL-2 itself. Strom, Kelly, and their colleagues have combined IL-2 with the diphtheria toxin, for example. The advantage of using the lymphokine itself, rather than the antibody is, Strom says, that once the lymphokine portion binds to its receptor, the fusion protein is taken into the cell, where the toxin can exert its deadly effects. The fusion protein proved to be a potent immunosuppressant in animal experiments and the Strom group has now begun a clinical trial with it in patients with adult T cell leukemia.

The patients' leukemia cells carry high concentrations of the IL-2 receptor, and Waldmann and his colleagues have already found that therapies directed at the receptor show promise. They treated 16 patients with Uchiyama's antibody. Although adult T cell leukemia is highly virulent—it normally kills in 20 weeks—and hasn't responded to any therapies, six of the patients experienced remissions, Waldmann says. Four of them were complete remissions, lasting several months.

There may also be other ways of interfering with the action of lymphokines, as work by Steven Gillis's group at Immunex Corporation in Seattle has shown. Over the past 2 years, Gillis and his colleagues have cloned the genes for the receptors for several lymphokines and found that the receptor proteins may be made in soluble forms, which can be secreted by cells, as well as in the expected membrane-bound form.

Along with some interesting therapeutic possibilities, this discovery raises basic questions. For instance, why should there be soluble receptors at all? After all, receptors generally do their signal-receiving work at the outer membrane of the cell. Why should they float around in the bodily fluids, where they can't pass the message directly to a cell? Gillis hypothesizes that the immune system may use these soluble receptors to shut off immune responses by modulating the action of the potent lymphokines. A soluble receptor could bind a lymphokine, taking it out of action as soon as its job is done—and before the response gets out of control.

The capacity of soluble receptors to bind lymphokines might also make them good immunosuppressants, according to Gillis. The receptors cloned by the Immunex group include the one for interleukin-1, or IL-1, which promotes inflammatory reactions; IL-1 has been detected in joints of rheumatoid arthritis patients and in pancreatic cells that are coming under immune attack.

Gillis and his colleagues have made a soluble IL-1 receptor and tested it in animal models of both autoimmunity and graft rejection. They found that it is a potent immunosuppressant. "What's interesting," Gillis says, "is that [the soluble receptors] have been incredibly potent molecules in the animals as well as in the test tube. We've been continuously surprised at the low concentration of product that's able to shut off a cytokine response."

The next step for the Immunex group is to make enough of the soluble receptors to begin clinical trials in humans. If all goes well, Gillis says, trials with the soluble IL-1 receptor might begin sometime next year.

For decades immunologists have had to rely on broad-spectrum immunosuppressants to fight graft rejection and autoimmunity in their patients. But the new developments in T cell vaccines, blocking peptides, monoclonal antibodies, and soluble receptors may soon give them a whole new arsenal of weapons. ■ JEAN MARX