Suppressing Autoimmunity in Mice

Monoclonal antibodies against certain antigens that help to regulate immune responses can prevent or suppress autoimmunity in mice

Current therapies for treating autoimmune diseases, conditions caused by inappropriate attack of the immune system on the body's own tissues, require broad-scale suppression of immune responses. Although often effective in controlling the diseases, which include serious conditions such as multiple sclerosis and systemic lupus erythematosus (SLE), the severe immunosuppression caused by the treatments may leave patients highly vulnerable to complicating infections.

What is needed is a more specific therapy that would knock out only the portion of the immune system causing the inappropriate attack while leaving intact most other immune responses. Recently, Hugh McDevitt and his colleagues at Stanford University School of Medicine have been exploring an approach that appears to do just that in mouse models of human multiple sclerosis, SLE, and myasthenia gravis.

Susceptibility to these and other autoimmune conditions has been linked to certain histocompatibility antigens that regulate immune responses. These antigens, which map to the HLA-D or -DR regions of the human major histocompatibility complex (MHC) and to the I region of the mouse MHC, are needed to produce antibodies in response to many antigens. Their presence may contribute to the development of autoimmunity by permitting the immune system to mount a response against normal cellular constituents.

The Stanford workers have found that they can prevent or suppress the autoimmune conditions in mice by treating the animals with monoclonal antibodies against the products of genes in the I-A subdivision of the I region. The antibodies may work by specifically depressing the immune responses mediated by these I-A antigens.

A serious obstacle must be overcome before a similar therapy can be attempted in human patients, however. Although the monoclonal antibodies did not cause any ill effects in the mice, there is evidence that relatively low doses may be lethal to monkeys.

McDevitt, who has been studying the control of immune responses for many years, was prompted to begin the current experiments about 3 years ago by reports of the production of human monoclonal antibodies, which might be more suitable for treating human diseases than mouse monoclonal antibodies, the only type available until then. Other investigators, including Mark Greene and Baruj Benacerraf of Harvard Medical School, had already shown that antibodies against I-A region products could suppress immune responses in experimental animals. The idea, McDevitt says, was to attempt to treat an autoimmune disease with a monoclonal antibody directed against the particular I-A antigen linked with the disease in the hope that this would block only the causative immune response. Most human autoimmunity patients are heterozygous for the I-A antigen in question; that is, they have two different gene variants at the appropriate genetic locus—the susceptibility gene plus another. Presumably the responses mediated by the second gene would remain intact.

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In an early experiment, McDevitt with Nancy Adelman of Stanford and James Rosenbaum, who is now at the University of California School of Medicine at San Francisco, showed that it was possible to obtain specific suppression of the production of an antibody that is under the control of an immune response gene. They used mice that are genetically capable of making antibodies against two synthetic peptide antigens. The ability to make antibodies to the peptides is controlled by two different immune response genes and the heterozygous mice used in these experiments carried both the necessary I-A–encoded genes.

The Stanford workers found that a monoclonal antibody against the product of one of the I-A genes prevented production of antibody to the corresponding peptide, but not of antibody to the other peptide. Moreover, McDevitt says, "The response was persistent. It didn't require the continued injection of the antibody." It lasted at least 3 to 6 months after the treatment was stopped.

Encouraged by these findings, McDevitt and his colleagues have gone on to test the use of the monoclonal antibodies for treating mouse models of human autoimmune disease. The first of these was experimental allergic encephalitis, in which the brains and spinal cords of mice that have been immunized with myelin protein develop pathological changes similar to those that occur in multiple sclerosis.

The McDevitt group, in collaboration with that of Stanford neurologist Laurence Steinman, found that the encephalitis could be prevented if the animals were given appropriate antibodies just before or after they were immunized. The most effective antibodies were directed against the product of the $I\text{-}A^s$ gene, an I-A variant that has been linked by some investigators to susceptibility to the encephalitis. Antibody against the $I\text{-}A^k$ variant was less effective.

The Stanford workers also found that monoclonal antibodies against I-A antigens could ameliorate experimental myasthenia gravis in mice. This autoimmune disease is triggered by antibodies against the receptor for the neurotransmitter acetylcholine, leading to receptor destruction and decreased strength of contraction of the voluntary muscles, which are stimulated by acetylcholine-releasing neurons. The ability to make antibodies in response to the acetylcholine receptor is under the control of immune response genes that have been mapped to the I-A subregion.

Myasthenia gravis can be induced in mice by immunizing them with acetylcholine receptor protein. The Stanford workers used two susceptible strains, bearing different I-A gene variants. Monoclonal antibody against each variant suppressed production of antibody against the receptor in the corresponding strain, but not in the other strain. The antibodies also depressed cellular immunity to the acetylcholine receptor, as measured by a decrease in the lymphocyte proliferation elicited by the receptor, and appeared to alleviate the clinical symptoms of the animals.

The antibody treatment showed a degree of specificity for the response suppressed. It did not decrease lymphocyte proliferation induced by purified tuber-

culin protein, which is not under the control of immune response genes.

Monoclonal antibodies against I-A gene products not only suppress the development of autoimmunity, but may also induce remission of an already established disease, in this case an SLE-like condition in mice. Both the human and murine diseases are characterized by severe inflammation of the kidney, which may lead to kidney failure and death. The inflammation is caused by deposition of complexes of the abnormal antibodies associated with the disease and their antigens in the filtering apparatus of the kidney.

McDevitt, Adelman, and David Watling of Stanford treated mice that had already begun to show symptoms of the nephritis with a monoclonal antibody against either of two I-A antigens. The mice, which develop the kidney inflammation spontaneously, carried both antigens. The antibodies were administered weekly for a 4-month period. They reduced the animals' kidney disease, as indicated by a decrease in the protein lost in the urine, and improved their survival.

One antibody was more effective in both regards than the other. For example, 90 percent of the animals receiving it survived for 1 year, compared to 10 percent of the untreated controls and about 60 percent of the animals receiving the other antibody. The greater efficacy of this antibody may be attributable to a more direct role of the I-A antigen against which it is directed in the development of the nephritis.

In all the studies, the mice tolerated the treatment with monoclonal antibodies without apparent ill effects. "The animals didn't show any signs of toxicity," McDevitt says. "They hopped around the cage happily."

Despite the encouraging results with mice, recent reports of toxicity in nonhuman primates make McDevitt extremely cautious about the potential for treating human autoimmune diseases with monoclonal antibodies against I-A antigens. For example, R. Billing of the University of California School of Medicine in Los Angeles and S. Chatterjee of UC's Davis campus reported that four of nine rhesus monkeys treated with relatively low doses died. "At the moment," McDevitt says, "this therapy isn't possible in humans." However, he plans to continue animal experimentation to see if it is possible to administer the antibodies safely to primates.

Monoclonal antibodies against other types of antigens have been used to treat human patients—in experimental cancer therapy, for example—without causing serious side effects. The problems seen in the monkeys might be peculiar to antibodies against I-A antigens.

Billing and Chatterjee found that the monkeys, which died of a severe type of allergic response resembling anaphylaxis, had disseminated intravascular coagulation, a condition in which blood clotting is activated throughout the entire circulatory system. "It is possible that because I-A antigens are on the endothelial cells of blood vessels, the antibodies may induce disseminated intravascular coagulation the way other antibody does not," McDevitt remarks.

According to recent reports, the I-A antigens are present all the time on the endothelial cells lining some human blood vessels. Binding of the monoclonal antibodies to the antigens on primate vessel walls may activate the complement system, leading to damage to the endothelial cells and consequent activation of the clotting system. Mice may be less susceptible to this problem because the I-A antigens are not always present on the endothelial cells, although their expression there can be induced.

The antibody treatment may lead to the production of suppressor T cells.

It may be possible to avoid activating the complement system, McDevitt says, by removing the complement-binding portion of the monoclonal antibody molecules and using only the antigen-binding fragments. This might increase the likelihood of suppressing an immune response without causing endothelial cell damage.

The monoclonal antibodies used by the Stanford workers and also by Billing and Chatterjee bind to framework regions of the I-A molecules. These regions, the less variable portions of the molecules, are located next to the cell membrane. Another approach that may be tried to avoid damaging endothelial cells is to make monoclonal antibodies that react with the outer, more variable regions of the I-A antigens.

Even if it does not prove possible to use monoclonal antibodies against I-A antigens to treat autoimmune disease, McDevitt says, "The work is still interesting. You can ask how I-A antigens contribute to these diseases." The linkages have been known for some time. Less clear is the manner in which a particular antigen contributes to the susceptibility to a given autoimmune condition.

The current work suggests that products of the I-A region genes participate actively in the generation of the aberrant immune responses, perhaps working as they normally do. The I-A antigens are needed for the interactions that must occur between immune cells in order to respond to certain antigens. These interactions include that between the antibody-producing B cell and the helper T cell that activates it. The helper cell itself must be activated by an antigen-presenting cell such as a macrophage. None of these interactions can occur unless the partners bear the same I-A gene products on their surfaces.

The monoclonal antibodies used by the Stanford workers probably do not suppress autoimmunity solely by blocking I-A antigens on immune cell surfaces and thus preventing the antigen presentation required for the initial triggering event, although this may contribute. The effects are too long-lived, lasting beyond the time the antibody would be expected to persist, for interference with antigen presentation to be the only mechanism. Moreover, this could not explain suppression of already established autoimmune disease, as in the mice with the SLE-like nephritis.

McDevitt and Adelman have preliminary evidence that the antibody treatment leads to the production of suppressor T cells that might persistently block the activity of helper or B cells. Lymphocytes from treated animals also suppress antibody production in untreated mice. This suppression is not seen if the transferred cells are first exposed to an antibody that attacks all T cells. The Stanford workers do not have direct evidence that a suppressor T cell produces the effect, although other investigators have found that antibodies against I-A antigens induce such cells.

McDevitt also cites the work of Samuel Strober of Stanford University School of Medicine in support of the hypothesis that a suppressor cell is involved. Patients who had been given total lymphoid irradiation for Hodgkin's disease, a therapy pioneered by Henry Kaplan of Stanford, had lower than normal T-cell functions as much as 10 years later. Strober found that the decreased activity was caused by the presence of a suppressor T cell. He is now beginning to use total lymphoid irradiation to treat patients with autoimmune disease. "I think that by using antibody against I-A at the time antigen is administered we are inducing this same type of cell," McDevitt says.

The use of monoclonal antibodies in

disease therapy is barely in its infancy. Many problems will have to be solved before their application becomes widespread. The discovery that monoclonal antibodies against I-A antigens could kill monkeys was unexpected in view of the apparent safety of other types in limited trials in humans. Nevertheless, McDevitt is hopeful that a safe way of treating autoimmune disease with the antibodies can be found. He points out, "I wouldn't have done the experiments if I didn't think that I would ultimately get to the point where I could treat someone."

—JEAN L. MARX

Science Underground

An underground laboratory could house ultrasensitive experiments; Los Alamos wants to build one at the Nevada nuclear test site

Spurred by the predictions of the grand unified field theories, and by the astrophysical conundrum of the missing solar neutrinos, a number of physicists are now arguing that the United States should build a permanent, dedicated laboratory at least 1 kilometer underground, where a new generation of ultrasensitive experiments could be shielded from cosmic rays and mechanical disturbances. The Los Alamos National Laboratory is in fact pushing hard for such a laboratory, a $45-million "National Underground Science Facility" that it would build and operate in an unclassified portion of the nuclear weapons test range in Nevada.

The possible uses of an underground facility were laid out last fall at an international conference in Los Alamos;* then again last spring in the report† of an ad hoc advisory committee to Los Alamos director Donald M. Kerr; and yet again last month in a discussion before HEPAP, the Department of Energy's High Energy Physics Advisory Panel (*Science*, 22 July, p. 344). Some highlights:

• *Nucleon decay*. This most famous prediction of the grand unified theories is currently being tested by some ten dedicated experiments in deep tunnels and mines around the world. No definitive events have yet been forthcoming. However, even if nothing is found in the existing detectors, physicists will still want to extend the limits with more sensitive, second-generation detectors. And if nucleon decay *is* found, they will want new detectors to test the theories all the harder with detailed measurements of the decay modes.

• *Solar neutrinos*. Raymond Davis's pioneering experiment in neutrino astronomy is as baffling as ever. Astrophysicists say that nuclear reactions in the sun should produce neutrinos at a certain calculable rate. But Davis and his colleagues from the Brookhaven National Laboratory have spent more than a decade running their detector in South Dakota's Homestake Gold Mine, and still find neutrinos at only a fraction of that rate. On the other hand, Davis's detector is sensitive only to the relatively energetic neutrinos produced in one of the sun's minor side reactions. So perhaps the astrophysicists are wrong, their models of the sun too coarse to give the right answer for such a small detail. Perhaps Davis is wrong, his experiment concealing some subtle flaw that no one has yet noticed. (His apparatus is essentially a huge tank of perchloroethylene cleaning fluid, wherein solar neutrinos convert chlorine-37 into argon-37; elaborate radiochemical techniques are needed to find and measure the argon.) Or perhaps the solar neutrinos are doing something exotic, oscillating from one form to another on their journey to Earth. Whatever is going on, everyone agrees that the only way to resolve the question is to measure the low-energy neutrino flux from the sun's main powerhouse, the proton-to-helium reaction. The most promising technique involves some 50 tons of gallium and the neutrino-induced transformation of gallium-71 to germanium-71. The scale of the experiment is certainly worthy of a national facility: the germanium alone would cost some $25 million. Fortunately, it could be resold at the completion of the experiment.

• *Cosmic-ray neutrinos*. Energetic neutrinos produced by cosmic rays high in the atmosphere *will* penetrate the underground laboratory. In fact, a directional detector could measure the number of neutrinos coming down through the 1 or 2 kilometers of rock over the laboratory, then compare it with the number coming up through the 12,900-kilometer diameter of the earth, and thereby derive a very sensitive test of the neutrino oscillation idea. A large detector might also be able to localize astronomical sources of energetic neutrinos to within 5°—in effect, serving as a neutrino telescope. (This would be far more difficult to do with the much lower energy solar neutrinos, unfortunately.)

• *Gravitational physics*. A number of attempts are under way in surface laboratories to detect gravity waves. For very low frequency waves (less than 100 hertz), however, sensitivity is limited by changing gravitational gradients from nearby moving objects. What is needed is an exceptionally quiet location underground. Such an environment would also be conducive to improved measurements of the gravitational constant and its hypothetical variation over time, as well as to precise tests of the inverse-square law. (Some theories predict deviations from this law at laboratory distances.)

The underground science facility idea has been persuasive overseas: the Soviet Union has constructed such a laboratory near Baksan in the Caucasus, Italy is constructing one in the Gran Sasso tunnel in the Apennines, and the French are completing a third in the Frejus tunnel in the Alps. In the United States, the underground facility has been championed since 1981 by neutrino physicist Alfred K. Mann of the University of Pennsylvania, who conceived of the notion as he was developing ideas for second-generation nucleon decay detectors. "These things are complex and huge," he says, "and when I thought about trying to do it all in some mine, with water dripping over everything and a half-day wait at the lift, and without the resources and technical support I was used to at the national labs, I got discouraged—until I thought, 'Just build a national lab underground.' "

Ultimately he was led to the Nevada Test Site, where there is plenty of land, where the geology and hydrology is thoroughly understood, where support facilities are already available, and where

**Science Underground (Los Alamos, 1982)* (American Institute of Physics, New York, 1983).
†"Report of the Advisory Committee for the Proposed National Underground Science Facility," Norman F. Ramsey, chairman, 15 April 1983 (Los Alamos National Laboratory, 1983).