Making Antibodies Without the Antigens

Anti-idiotype antibodies may provide a new way of making vaccines and are also proving useful for the isolation of receptors

Current methods of making vaccines, despite their many successes, are not without problems. A vaccine is typically composed of all or part of the pathogenic organism against which immune protection is desired. But this approach may be limited, either because injection of the pathogenic material may occasionally be associated with harmful side effects—the whooping cough vaccine is a recent case in point—or because the organism can not be grown in sufficient quantities. A new approach to vaccine production, one which has the singular advantage of not exposing the vaccinated individual to pathogenic materials, may help to circumvent these problems.

The new technique takes advantage of the existence of "immune regulatory networks," in which production of an antibody in response to a foreign antigen elicits the formation of other antibodies directed against the first. Some of this second group of antibodies, called "anti-idiotype antibodies," may carry the image of the foreign antigen as part of their structures. If that is the case, then injection of the anti-idiotype antibody may be equivalent to injecting the antigen itself and elicit the same type of protective antibodies. In other words, it may be possible to vaccinate without actually using pathogenic material.

The idea that antibody production is controlled by a complex interacting antibody network was first proposed a little over 10 years ago by Niels Jerne of the Basel Institute of Immunology, who was awarded a share of the 1984 Nobel Prize for Physiology or Medicine for this and his other contributions to immunology. The current research on anti-idiotype antibodies provides further evidence for the network theory.

In addition, the antibodies made by the new approach are proving to be valuable tools for identifying and studying hard-to-obtain receptors for hormones, neurotransmitters, and other physiologically active agents. Moreover, these studies suggest that autoimmune diseases, especially those such as Graves' disease and myasthenia gravis that are caused by antibodies directed against receptors, may result from disturbances in the antibody regulatory network.

Jerne based his network theory on the previous discovery by others that antibodies carry "idiotypes," regions in or near their antigen-recognition sites that are themselves capable of acting as antigens and stimulating antibody production. Recognizing that the anti-idiotype antibodies might participate in the regulation of antibody production, he proposed that the normal immune system features an interlocking network of antibodies directed at one another's idiotypes.

Production of any particular antibody would then be kept in check by the corresponding anti-idiotype antibody unless the system was perturbed by the introduction of a foreign antigen, leading to an increased synthesis of whatever antibodies recognize it. The reaction would eventually be terminated because these antibodies would also trigger increased synthesis of their anti-idiotype antibodies, and so on. In effect, the perturbation caused by the foreign antigen spreads through the network much as ripples spread over the surface of a pond.

> "You can make an antibody that will interact with a receptor without having to isolate the receptor."

Inherent in the network theory is the idea that at least some of the anti-idiotype antibodies are internal images of antigens. For an antigen and an anti-idiotype antibody to bind to the same region of an antibody they must have similar three-dimensional shapes, even though they may otherwise be very different biochemically.

In 1981, Alfred Nisonoff and Edmundo Lamoyi of Brandeis University and independently I. M. Roitt and his colleagues at Middlesex Hospital Medical School in London suggested that internal image antibodies might be exploitable as a new type of vaccine. The idea was to make first an antibody to antigenic material from the pathogen in question and then make a second antibody to the idiotype of the first. If all goes well, the second antibody should be the image of the original antigen and, if given as a vaccine, should elicit immunity to the pathogen. Moreover, monoclonal antibody technology now makes possible the virtually unlimited production of antibodies in a readily purifiable form.

Although research aimed at developing anti-idiotype vaccines is still in the early stages of development, several groups have shown that it is possible to produce anti-idiotype antibodies that evoke the formation of the correct type of protective antibodies in animals. For example, Ronald Kennedy and Gordon Dreesman of the Southwest Foundation for Biomedical Research in San Antonio have produced an anti-idiotype antibody by immunizing rabbits with antibody to the surface antigen of hepatitis B virus. "The rabbit anti-idiotype antibody mimics hepatitis B surface antigen," Dreesman says. "When you inject it [the antibody] into other animals, you produce antibody to the antigen."

Although the anti-idiotype antibody is of rabbit origin, it elicits antibody to the hepatitis B antigen in mice and chimpanzees, as well as in rabbits. This is important, Dreesman points out, because it shows that the ability to respond to the anti-idiotype antibody is not restricted to animals of a specific genetic composition, a requirement that would have to be met if the approach is ever to be used to prepare vaccines for genetically diverse human populations. There is currently a safe and effective conventional hepatitis B vaccine, but its availability is somewhat limited because the hepatitis antigen from which it is made must be obtained from human serum.

A critical question remains to be answered by the San Antonio workers. Although they have demonstrated the formation of protective antibodies in chimpanzees, the only species other than man to get hepatitis B, they have not shown that the antibodies will protect the animals against the infection. Those experiments are currently under way. Meanwhile Kennedy and Dreesman are among the reseachers who are beginning

studies to see whether an anti-idiotype vaccine might be developed to protect against the virus that causes AIDS (acquired immune deficiency syndrome).

The immune responses produced by immunizing mice with anti-idiotype vaccines have proved capable of improving the animals' resistance to infection by viruses, bacteria, and a unicellular parasite, a trypanosome that causes a form of sleeping sickness. However, in the case of the parasite, David Sacks and Alan Sher of the National Institue of Allergy and Infectious Diseases (NIAID) found that only certain strains of mice were genetically capable of developing the immunity.

Even if the anti-idiotype antibody does not induce protective immunity by itself, it may do so when attached to an appropriate carrier that boosts an otherwise weak immune response, according to Heinz Kohler and his colleagues at Roswell Park Memorial Institute. They found that they could protect mice against a lethal bacterial pneumonia by immunizing them with an anti-idiotype antibody coupled to keyhole limpet hemocyanin, a protein used by immunologists to increase the ability of antigens to stimulate antibody production.

Kohler suggests that an analogous approach might be used to develop cancer vaccines. The tumor-associated antigens that might serve as such vaccines have been difficult to isolate and there is also

Nuclear Winter Won't Blow Away

Nuclear winter is still far from confirmed, but the results of a recent study add new support for the theory. Presented at last month's National Academy of Sciences Nuclear Winter Symposium, the study shows that at least one step leading from fiery nuclear holocaust to climatic chilling is not likely to be short-circuited by natural atmospheric processes. Critics had conceded that the detonation of 25,000 nuclear weapons with 6500 megatons of explosive force could possibly ignite forests and cities, create huge amounts of smoke, blot out the sun, and chill the land below. The catch, they said, was that even if the fires created enough smoke, rain and other natural removal processes would likely cleanse the atmosphere too rapidly to allow any significant climate change. The new study and others show that the smoke would create its own defenses against atmospheric cleansing, keeping much of its original mass in the atmosphere for many months.

The new study, conducted by Robert Malone, Lawrence Auer, Gary Glatzmaier, and Michael Wood of Los Alamos National Laboratory and Brian Toon of NASA's Ames Research Center, uses a third-generation computer model to predict the behavior of the smoke-laden atmosphere. The simplistic first-generation model on which the original nuclear winter theory was based was an easy target for critics. It was one-dimensional—a vertical line up through an atmosphere that had no winds to disperse the smoke, no ocean to warm the land, no seasons, and no way to calculate how fast rain would remove smoke particles.

Subsequent second-generation, three-dimensional models containing far more realistic atmospheres, continents, and oceans also indicated that, given a large enough initial injection of smoke, continents would cool by tens of Celsius degrees and take months to recover. The cooling would on the whole be a bit lower than first calculated, coastal regions would be much less affected, and wintertime temperature effects would be much reduced, but on the other hand regions of dense smoke might quick-freeze the land beneath them.

The Los Alamos model is the most sophisticated of four third-generation models now simulating nuclear winter. These models allow the smoke to move about and respond to the atmospheric changes that it induces. Smoke is free to move in any direction; it is warmed by the absorption of sunlight; and it is washed out by rain at a rate determined by the precipitation rate that the model actually predicts. The smoke is released over North America and Eurasia below the tropopause, the boundary between the troposphere where weather and rain removal occur and the far more stable stratosphere. As critics warned, rain in the model begins to remove tropospheric smoke rapidly. But as proposed earlier by Carl Sagan of Cornell University and suggested by second-generation models, much of the smoke did not stay in the troposphere.

Warmed by the sun, the upper parts of the model's smoke clouds become buoyant and rise away from the cleansing rain. In addition, the warming of high smoke clouds pushes the tropopause downward from its usual altitude of 10 to 13 kilometers to 4 to 8 kilometers, confining the weather below the remaining smoke. During the winter, when less sunlight reaches the Northern Hemisphere to induce this lofting of smoke, rain would still remove 95 percent of the smoke within the first 40 days. During summer, scavenging by rain would get a jump on lofting of the smoke, removing 50 percent of it in the first week. But only two-thirds would have been lost by 40 days; and the remaining third of the smoke would by then be largely out of the reach of efficient tropospheric removal. Once it escapes, the smoke in the atmosphere decreases by a factor of 3 every 180 days, according to this model. The maximum summertime cooling of more than 15°C would occur over North America and Eurasia during the first 2 weeks, assuming an initial release of 170 million metric tons of smoke. Even 40 days later, continental cooling of 5° to 15°C would persist. That is still a significant nuclear winter effect.

Thanks in part to the lofting of smoke due to solar heating, the atmospheric component of the nuclear winter theory is proving rather robust. Not that even this latest model is sufficient to predict accurately the behavior and effects of smoke. Researchers will be further refining their atmospheric models, but they will never be able to include some processes that operate on too fine a scale for their global models to portray. One such process is the condensation and resulting scavenging that might occur in smoke plumes rising over a burning city.

The greatest uncertainty now, atmospheric scientists say, is the initial amount of smoke. The National Research Council committee on nuclear winter settled on a baseline amount of 180 million tons of smoke, but found plausible amounts to range from 20 million tons—which would have negligible climatic effects—to 650 million tons—which might plunge the Northern Hemisphere and perhaps the globe into a deep freeze. No one sees a quick way to reduce those uncertainties.—**RICHARD A. KERR**

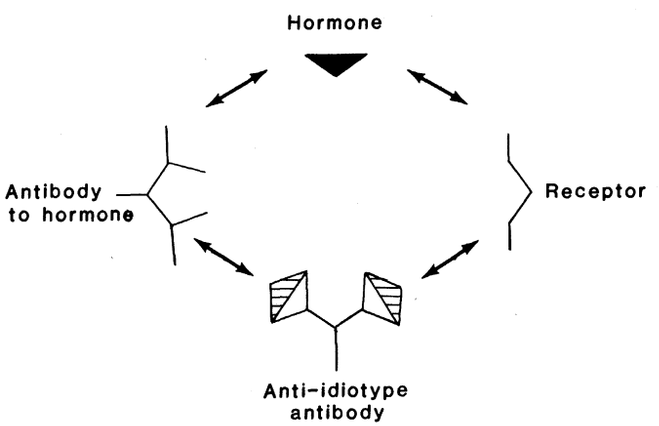
the possibility that vaccination with an incompletely purified tumor material might result in the transfer of a cancer-causing virus or gene. Moreover, there are indications that cancer patients become tolerant to the antigens of their own tumors and fail to mount an effective immune response. By joining the antibody image of a tumor antigen to keyhole limpet hemocyanin or another carrier that boosts immune responses, it might be possible to break the tolerance.

Although most investigators have used anti-idiotype antibodies to stimulate humoral immunity, which is a B-cell response, they also work in boosting the responses of T cells, which bear idiotypes of their own. For example, Robert Finberg and Hildegund Ertl of Harvard's Dana-Farber Cancer Institute prepared an antibody to the idiotype of a T-cell line that had been primed to recongize Sendai virus. When mice were immunized with this antibody, their T cells became activated and able to attack specifically Sendai virus–infected target cells in culture. Moreover, the immunization protected the animals against what would otherwise have been a lethal Sendai virus infection.

Killer T cells usually recognize foreign antigens, such as viruses, only when they are presented on target cells of the same histocompatibility type. However, the responses of the T cells that were activated by the anti-idiotype antibody were not restricted to Sendai virus–infected cells of the same histocompatility type. This T cell result parallels the results obtained with antibody production by B cells, which often requires the participation of the helper class of T cells. The reasons for this apparent lack of restriction are unclear, although it is an encouraging finding in regard to eventual vaccine development.

A number of potential problems will have to be overcome if anti-idiotype vaccines are ever to be developed for human use. In particular, the current inability to produce monoclonal antibodies of human origin may be a handicap. Most monoclonals are now produced by mouse cells and, as foreign proteins, could themselves be targets of an immune attack that might destroy them before they could induce long-lasting immunity to the corresponding pathogen. They might even trigger the severe, sometimes fatal, immune reaction known as anaphylaxis, especially if more than one injection is required to elicit protective immunity.

But, says Bernard F. Erlanger of Columbia University, "If that's a problem, the problem can be solved." For example, Sherie Morrison of Columbia University's College of Physicians and Surgeons and Vernon Oi of the Becton-Dickinson Monoclonal Center in Mountain View, California, and their colleagues have recently described a method for making chimeric antibody molecules in which the variable, antigen-binding portion is of mouse origin and the constant region is of human origin. Such antibodies might be less likely to produce an untoward immune response than all-mouse antibodies.



Simplified network

The antibody to the hormone and the receptor are equivalent. The anti-idiotype antibody carries the image of the hormone and can also recognize the receptor.

There is also the possibility of combining the anti-idiotype approach with another vaccine strategy now receiving much attention, namely, the use of short antigenic peptide fragments to evoke antibody production. This would depend on being able to identify a small region of the anti-idiotype antibody as the actual target of the antibodies whose formation it stimulates.

The peptide vaccine approach shares many of the potential advantages of anti-idiotype vaccines, but there is one aspect in which the latter have the edge. At least a portion of the amino acid sequence of the antigen must be known if a peptide is to be synthesized and used for immunization. In contrast, it is possible to obtain an anti-idiotype antibody in the complete absence of structural information about the antigen. This is one reason why anti-idiotype antibodies are proving valuable for studying receptors. "You can make an antibody that will interact with a receptor without having to isolate the receptor," explains A. Donny Strosberg of the Institut Jacques Monod and the University of Paris VII.

Antibodies that specifically recognize receptors can be made by employing a strategy quite similar to that used for the vaccine work, except that the first antibody is made against an agent that specifically binds to the target receptor. The second antibody, which is then made to the idiotype of the first, may not only bind to the receptor but also activate it just as the normal binding agent does. "With receptors you can really test the activity of the antibody," Strosberg explains. "Binding is not enough. It may not be specific."

Investigators have made anti-idiotype antibodies that mimic the receptor action of small binding agents, including the neurotransmitters acetylcholine and the catecholamines, and of larger protein hormones, such as insulin. In addition, Mark Greene and his colleagues, first at Harvard Medical School and more recently at Tufts University Medical School have used an anti-idiotype antibody to identify and isolate the receptor by which a reovirus attaches to and infects cells. Viruses apparently recognize the same receptors used by normal endogenous agents. The indications are, Greene says, that the reovirus attaches to the β-adrenergic receptor.

Anti-idiotype antibodies are usually prepared in two immunization steps, but can be obtained in just one, according to Erlanger, W. Louis Cleveland, who is also from Columbia, and their colleagues. They immunized mice with BisQ, a compound which was synthesized by Norbert Wasserman of the Columbia group and is a potent synthetic mimic of acetylcholine, and then used spleen cells from the animals to produce monoclonal antibodies. "We were essentially taking a snapshot of the state of the idiotype network in the animal at the particular time the spleen was removed," Erlanger says.

As expected, they were able to identify monoclonal antibodies that react with the immunizing agent. But they also found a monoclonal that recognizes both the BisQ-binding antibody and the acetylcholine receptor—in other words an anti-idiotype antibody. The result shows that the idiotype network was functioning during a normal immunization.

The autoimmune disease myasthenia

gravis is caused by an antibody-mediated attack on the acetylcholine receptor. During their studies, the Erlanger group noted that rabbits that had been immunized with antibody to BisQ and were producing anti-idiotype antibody to it developed symptoms characteristic of the disease. "This raises the possibility that autoimmune diseases could be caused by aberrant network regulation," Erlanger suggests.

Although the standard view holds that the acetylcholine receptor somehow elicits its production of the harmful antibodies, he proposes that the trigger might be the production of antibody against a substance with a shape similar to that of acetylcholine. If the antibody, which is the receptor image, elicits the production of anti-idiotype antibodies the result might be destruction of the receptor.

The trigger might even be a virus that binds to the acetylcholine receptor, Erlanger suggests, although presumably not the rabies virus which uses that receptor to enter nerve cells. There is a long history of anecdotal evidence suggesting that viruses might initiate autoimmune diseases, and Greene's work shows that it is possible to make an anti-idiotype antibody to an antibody to a virus that will recognize a cellular receptor.

Graves's disease is also caused by antibodies to a receptor, in this case the one for thyroid-stimulating hormone (TSH). Instead of destroying the receptor, the antibodies activate it, much as the hormone does, and the thyroid gland becomes overactive. Leonard Kohn of the National Institue of Allergy and Infectious Disease thinks that anti-idiotype antibodies may play a role in Graves' disease, but opposite to that suggested by Erlanger for myasthenia gravis. According to Kohn, they are more likely to ameliorate the symptoms of the autoimmune disease by blocking the antibodies that react with the TSH receptor.

He points out, however, that it is very difficult to determine which antibody in an interlocking idiotype network might have initiated an abnormal immune response. Nevertheless, regardless of whether antibody to the receptor or its ligand came first, a defect in the regulatory network might have contributed to the autoimmunity. And in either case, an anti-idiotype antibody to the receptor-binding antibody might aid in controlling the disease, which is another path of inquiry currently being explored by immunologists.—**JEAN L. MARX**

Plant Communities Resist Climatic Change

A combination of self-generated microclimates and the dynamics of seedling competition gives plant communities an unexpected stability

The change in vegetation coverage through time represents a clear record of climatic change. For instance, the replacement of deciduous forest by conifers betokens cooler times. However, the response of plant communities to shifting climes, when viewed in relatively close focus, is more complex than is often imagined. In a detailed study of vegetation patterns of the Grand Canyon through the past 24,000 years Kenneth Cole of the Indiana Dunes National Lakeshore records a dynamic set of interactions—plants with climate and plants with plants—that clearly reveals this sometimes hidden complexity (*1*).

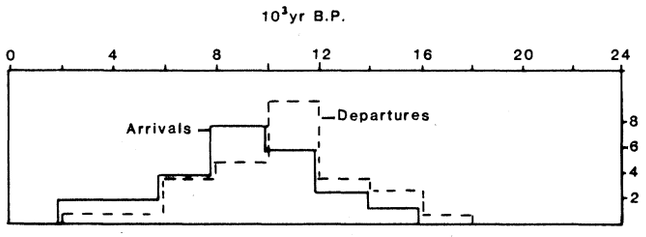
The past 24,000 years includes the Pleistocene/Holocene boundary—the transition between the last ice age and the current interglacial. The retreat of the Northern Hemisphere ice sheets, which began between 16,000 and 13,000 years ago, triggered dramatic biotic change, as temperature and rainfall patterns ameliorated. Specifically, sub-Arctic vegetation was replaced by temperate plant assemblages. In the eastern United States, there was a general northward migration of vegetation. In the west, where the great mountain ranges presented both geographical barriers and opportunities, this latitudinal migration was accompanied in places by altitudinal shifts.

Where geological and paleoecological records have coincided in locality and quality, it has become apparent that a significant lag time—some 2000 years or so—separates the climatic and consequent vegetational change at this boundary. The constraints of migration in essentially immobile organisms have, very reasonably, been adduced to explain this. From his analysis of the Grand Canyon record, Cole deduces an additional constraint: to wit, vegetational inertia, an idea that has a mature pedigree in plant ecology but one that until now has been given little prominence.

Put simply, the model suggests that a combination of factors, including competition and physical microenvironment, allows a plant community to persist long after the disappearance of conditions that were necessary for its initial establishment. Eventually, such a community will be replaced but rather more slowly than might have been predicted. This evanescent uncoupling of flora from prevailing environment means that if climatic change has been rapid, such as at the Pleistocene/Holocene boundary, plant fossil records might be less reliable as climatic indicators than is usually the case.

The precipitous slopes of the Grand Canyon offer excellent conditions from which to determine patterns of vegetational change through time. Not only do the high cliff sides encompass several different vegetational zones, ranging from ponderosa pine to sagebrush and desert environments, but their inaccessibility has protected them from disturbance by all but the most nimble bighorn sheep and deer.

The paleoecologist is also blessed by the existence there of so-called pack rats (of the genus *Neotoma*). These little rodents, of the size of Norwegian rats, live



Species flux

A compilation of the appearances and disappearances of species over the Pleistocene/Holocene boundary shows a clear separation of peaks of activity, which implies a period of species paucity.