# The EAE Model: A Tentative Connection to Multiple Sclerosis

Studies on multiple sclerosis (MS) have been hindered by the lack of a suitable animal model that mimics the pathology of MS in humans. The closest approximation to such a model is a disease known as experimental allergic encephalitis (EAE), an autoimmune disease in which the immune system attacks and destroys myelin, a lipoprotein that surrounds and protects components of the central nervous system. There is no firm evidence that MS is an autoimmune disease, and therefore it is not clear how closely EAE is related to it. But EAE reproduces at least some aspects of MS, and may thus offer some new insights into its nature and, possibly, its therapy.

The strongest evidence that MS may involve the immune system is the presence of much higher concentrations of antibodies in the cerebrospinal fluid of MS patients than in healthy individuals. As was noted in an earlier article, some of these antibodies are directed against viruses. But the nature of most of the antibodies is still unknown. One portion that has remained particularly mysterious is known as oligoclonal immunoglobulin because it contains at least seven proteins that remain very close together during electrophoresis. Many investigators have attempted to determine the specificity of these presumed antibodies, but they have had little success. These antibodies are apparently not specific for any known virus or any recognized component of the central nervous system.

The best clue to the nature of this antibody material was provided last year by Nelson L. Levy and Thad M. Schoen of the Duke University Medical Center. They theorized that at least some of the oligoclonal protein might actually be complexes of antigen and antibody, and they accordingly tried to isolate such complexes.

Levy and Schoen found that a substantial portion of the material apparently consists of protein associated with nucleotides, which may be either DNA, RNA, or oligonucleotides. This material is not found in healthy individuals or in patients with other neurological diseases. Its absence in patients with other neurological diseases suggests that the nucleotides are not a product of general tissue destruction in the central nervous system. They must thus come either from elsewhere in the body or from an external source, such as a virus. The two investigators are attempting to collect enough of this material to make a firm identification of the nucleotide and to try to get a better idea of its origin.

In order to learn the role of the immune system in MS, most investigators have directed their research toward EAE, a disease first recognized about 40 years ago in humans given rabies vaccine which contained rabbit brain cells. This type of vaccine is no longer used. EAE has been commonly produced in laboratory animals by homogenizing central nervous system tissues and injecting the homogenate into the animals in combination with Freund's complete adjuvant (an emulsion of heat-killed mycobacteria in mineral oil that stimulates activity of the immune system). Within about 2 weeks after the injection, most animals begin to lose weight and develop muscular atrophy, lack of muscle coordination, and incontinence. These symptoms are usually followed by paralysis and death.

Initiation and progression of EAE is mediated by T (thymus-dependent) lymphocytes, cells that become sensitized to an antigen in the injected tissue and then attack central nervous system tissues that contain the same antigen. Sanford H. Stone of the National Institute of Allergy and Infectious Diseases has shown that the disease can be transferred from animal to animal by transplantation of lymph node cells that contain the sensitized T lymphocytes.

In the early 1960's, Elizabeth Roboz Einstein, now at the University of California at Berkeley, and Marian W. Kies of the National Institute of Mental Health (NIMH) discovered that the crucial agent in the ground tissue preparations is a protein known as myelin basic protein or BP. This protein accounts for as much as 50 percent of the protein found in myelin and may be its major structural protein. The BP's from various species have subsequently been purified, and their amino acid sequences have been determined independently by Edwin H. Eylar, George A. Hashim, and their associates at the Salk Institute and by Patrick R. Carnegie and his associates at the University of Melbourne.

BP consists of a single chain of 170 amino acids whose sequence is very similar in different species. It is an unusual protein in that it has no disulfide bridges, has no $\alpha$-helical structure, and does not assume a globular structure in solution. Einstein, Kies, and Ellsworth C. Alvord, Jr., of the University of Washington Medical School demonstrated that purified BP, in conjunction with Freund's complete adjuvant, is sufficient to establish EAE in animals.

It has now been shown that each species seems to develop EAE in response to different short sequences of the molecule. Eylar, now at the University of Toronto, and Alvord have found that the guinea pig is especially sensitive to a segment of nine amino acids at one site on the molecule. Robert F. Kibler and Raymond F. Shapira of the Emory University School of Medicine have found that rabbits are most sensitive to a peptide of similar size at a different site within the molecule. And several investigators have shown that rats are sensitive to at least two sites and that monkeys are sensitive to several sites.

In each case, the sensitive site is the primary stimulus for the production of sensitized T lymphocytes. According to Kies, Håkan Bergstrand of the University of Lund in Sweden, and John Whitaker of the Memphis Veterans Administration Hospital, there are at least eight other sites throughout the molecule that stimulate the production of antibodies against BP. These antibodies, however, do not seem to play a role in the development of EAE.

One of the most paradoxical aspects of BP is that it not only can initiate EAE, but that it also can prevent the initiation of the disease and alleviate or even stop the symptoms after the disease has started. For instance, Alvord and Kies initially found that injection of an encephalitogenic (EAE-producing) fraction of homogenized nervous tissue without adjuvant alleviates the symptoms of EAE in guinea pigs. They later found that inoculation of the animals with BP in Freund's incomplete adjuvant (which contains no mycobacteria) over a period of 3 weeks prevents the development of EAE when the animals are subsequently injected with a normally fatal dose of BP or whole nervous tissue in Freund's complete adjuvant.

The development of the EAE-resistant state is accompanied by the buildup of antibody to BP, but this antibody appar-

ently does not play a role in the resistance. In fact, Robert Lisak of the University of Pennsylvania has shown that resistance to EAE develops even if antibody production is blocked by immunosuppressant drugs during the immunization period. Bernard Driscoll of NIMH also demonstrated that, in at least one strain of guinea pigs, the symptoms of EAE can be abolished if the animals are inoculated with BP within a day after their appearance. Driscoll has shown that this process involves the short-term removal of sensitized lymphocytes from the bloodstream.

Many other investigators, including Einstein, Eylar, and Cedric S. Raine and his associates at the Albert Einstein College of Medicine, have demonstrated that it is possible to delay or halt the development of EAE in many species by treatment with BP in Freund's incomplete adjuvant. In some cases, they have also shown remission of the disease in these animals if the treatment is begun after initiation of the disease. Some, including Driscoll, Kibler, and Robert H. Swanborg of the Wayne State University Medical School, have also shown that protection and therapy can be accomplished with smaller fragments of BP.

The application of these results to MS in humans is a matter of great controversy now. Much of the controversy surrounds Eylar, an aggressive, occasionally abrasive investigator who seems to have greatly antagonized many of the investigators who have been in the field longer. One investigator even refused to talk to *Science* because he did not want his work discussed in the same article as Eylar's. But Eylar's recent work, itself, is also controversial, for he believes that it might be possible to treat MS with BP.

Eylar and William Sheremata of the Montreal Neurological Institute have reported that the blood of MS patients contains immune leukocytes sensitized to BP. Eylar and Sheremata say that these leukocytes are very similar to those known to participate in the destruction of myelin in monkeys with EAE, which suggests that EAE and MS share a pathologic mechanism. Eylar has also reported that injections of BP of about 2 mg daily for 12 to 16 days brings about a complete and permanent regression of EAE in rhesus monkeys.

As a result of these observations, Eylar, William McIlroy, and John Wherrett of the University of Toronto are beginning a clinical trial of BP in a few carefully selected MS patients. The patients will be treated with 20 to 30 mg of BP per day for 20 to 30 days. During this time, in collaboration with investigators at several other laboratories, Eylar and his associates will be monitoring as many immune functions in the MS patients as possible. Eylar concedes that the chances of success in the trial are small, but he argues that this monitoring will provide a great deal of information about the immune response to BP.

Some investigators think that Eylar's clinical trial may be based on faulty data. Alvord, for example, has been unable to reproduce Eylar's cure rate in rhesus monkeys. He finds that BP alone provides little therapeutic benefit. Suppression of EAE was obtained only when BP was given in conjunction with penicillin (which Eylar used routinely in his monkeys to prevent infections), and even then only in about 60 percent of the monkeys. Alvord observes no therapeutic benefit at all when BP was given to

## New Tests for Diagnosis of MS

A definitive diagnosis of MS is exceptionally difficult when the disease process is first beginning. It has been estimated that in Britain, for example, a period of 4 years elapses between the appearance of the first somewhat vague signs and symptoms which herald the disease and a definitive diagnosis. Many scientists have thus sought a biochemical, immunological, or other test that would make it possible to recognize MS when the symptoms first appear. A procedure developed by Nelson L. Levy of the Duke University Medical Center and described in the second article in this series is one candidate for such a test. Two other candidates have been developed by Ephraim J. Field, B. K. Shenton, and Greta Joyce of the Royal Victoria Infirmary in Newcastle-upon-Tyne, England.

Field's tests are based on observations by the English investigators J. H. D. Millar and R. H. S. Thompson that MS may involve a defect in the functioning of unsaturated fatty acids in cellular membranes. The first test involves the migration of macrophages (a kind of leukocyte) in an electric field. When human leukocytes are exposed to an antigen to which they are sensitized (Field uses a thyroid protein), they release a substance that slows the migration of macrophages from healthy guinea pigs. Field has found that leukocytes from MS patients release more of this substance and that the effect is potentiated in the presence of a small concentration of linoleic acid. When leukocytes from MS patients are exposed to the antigen and to linoleic acid, the migration of macrophages is slowed by 90 percent. Under the same conditions, leukocytes from healthy individuals and from patients with other neurological diseases slow migration by 57 percent and 46 percent, respectively.

The second test involves the migration of erythrocytes (red blood cells) in an electric field. Field finds that, in the presence of a small, defined quantity of linoleic acid, erythrocytes from MS patients migrate significantly more slowly than do those from healthy individuals, while erythrocytes from patients with other neurological diseases migrate significantly faster.

The first test is difficult to perform accurately and requires at least 8 to 10 days to obtain a result. Apparently because the test is so sensitive to slight alterations in a complicated procedure, some investigators have not been able to reproduce Field's results. It is thus unlikely that the test will see widespread use. The second test, developed last year, is quite a bit simpler, Field says, and other investigators should be able to reproduce it easily.

The tests also seem to be able to identify a genetic defect that predisposes toward the development of MS. When close relatives of MS patients are tested with either test, Field says, they fall into two categories. About 60 percent produce the same results as healthy individuals, but about 40 percent (mostly mothers and sisters of MS patients) show a response that is about midway between those of MS patients and healthy individuals. These results imply, Fields suggests, that some genetic error causes certain individuals to produce myelin in which the membrane is defective in some way particularly suited to the development, from whatever cause, of MS.

There are thus at least three candidates for MS diagnostic tests. Each is being evaluated in coded trials that will show whether the tests really can identify MS, and these trials will require time and effort. But if any of the tests is shown to be accurate, it should become possible to identify MS patients at an early stage in the disease.—T.H.M.

another strain of monkeys called vesicularis. He is thus concerned that the results with the rhesus monkey may be species-related, and thus not applicable to humans. He is also concerned that Eylar's regimen for humans might not incorporate penicillin and, perhaps, other minor agents whose therapeutic contribution has not been detected.

Some other investigators have not been able to identify leukocytes sensitized to BP in MS patients. Lawrence Myers and George Ellison of the University of California Medical School at Los Angeles, for example, conducted a carefully controlled study and were unable to find good evidence for the existence of such leukocytes. Negative results have also been obtained by Donald Silberberg of the University of Pennsylvania School of Medicine. Eylar and Sheremata's results have been confirmed, however, by Harry Bartfeld of St. Vincent's Hospital and Medical Center in New York City. Eylar suggests that the apparently contradictory results arise because the sensitized cells appear in MS patients only during exacerbations of the disease.

Other investigators offer a more fundamental objection—that humans should not be given any agent, such as BP, that might produce a neurological disease or exacerbate an existing one. These investigators, Kies among them, argue that therapy of MS patients with BP has already been attempted unsuccessfully by Barry Campbell and his associates at the University of California at Irvine. Since there is no reason to believe Eylar's trial will be any more successful, Kies argues, it seems unwise to run the risk of exacerbating MS in those patients. Eylar replies that a great deal more about how to use BP has been learned since the time of Campbell's trials and this will increase the chance of success.

Some investigators think it may be possible to separate the therapeutic or protective effects of BP from the encephalitogenic effects. So far, though, there have been conflicting reports about success in this endeavor. Driscoll, for instance, has found that the encephalitogenic site of BP must be present in BP fragments if they are to provide protection in guinea pigs. Einstein, in contrast, has shown that it is possible chemically to modify a crucial tryptophan moiety in BP so that the modified protein is no longer encephalitogenic in guinea pigs, but still provides protection against EAE. Swanborg has reported that some synthetic and modified peptides also provide protection without being encephalitogenic, and Kibler also has some promising preliminary results. Many investigators argue that it would be safer to attempt to treat MS in humans with these materials because there would be less risk of exacerbating the disease.

Even if such therapeutic oligopeptides should become available, Kies and others say, it is questionable if the oligopeptides would be of great value in treating MS. For one thing, the encephalitogenic site for humans is not and may never be known, and hence there will be no assurance that a fragment is not potentially harmful. Furthermore, despite the similarities, there are profound differences between EAE and MS and it is still not even clear that the one is a good model for the other. Some of the differences are striking. Whereas MS is a persistent disease characterized by alternating periods of disease activity and remission, EAE is usually an acute disease that quickly leads to death. MS produces large, well-defined lesions in the central nervous system, while EAE in most laboratory animals produces inflammation around blood vessels and much less destruction of myelin. And antibodies against BP are common in EAE, but have never been demonstrated in MS.

### Looking for New Models

Because of these differences, some investigators are trying to develop models that may provide a more accurate reflection of the disease process in MS. Stone, for instance, has observed that at least one strain of guinea pigs develop a different form of EAE when BP is given to the newborn rather than to adults. The adult animals develop an acute, fatal form of EAE within 2 weeks after inoculation with BP in Freund's complete adjuvant. But Stone and Raine have shown that symptoms do not appear in the immature animals for 8 to 12 weeks after the inoculation.

EAE in the immature guinea pigs is rarely fatal and is characterized by a progressive deterioration of the central nervous system with occasional periods of remission. The central nervous system lesions in these animals are also more characteristic of those observed in MS patients. This development of a neurological disease in adults after exposure during immaturity is similar to the epidemiology of MS in humans, Raine says, and may provide a better understanding of the MS disease process.

Other investigators have been examining organized tissue culture systems that mimic some structural aspects of the central nervous system. Interest in this area was stimulated in 1961 when Murray B. Bornstein and his associates at the Albert Einstein College of Medicine reported that blood serum from both MS patients and animals immunized with homogenized nervous tissue could destroy myelin or prevent its formation in the tissue culture systems.

It has recently been shown by Sarka Hruby of the University of Wasington Medical School and Frederick Seil of the Portland Veterans Administration Hospital that this effect is also obtained with serum from animals immunized with cerebrosides, a component of the membranes of myelin cells. Frederick Wolfgram of the University of California School of Medicine at Los Angeles has also shown recently that the factor in MS serum is different from that in EAE serum, so that the original association between MS and EAE no longer seems valid. Nonetheless, several investigators responded to the initial report by developing good tissue culture systems for studying demyelination.

It is thus fair to say that the study of EAE has so far made only a few significant contributions to the understanding of MS. But the number of contributions should increase, and there has already been at least one side benefit. Guy M. McKhann, Steven R. Cohen, and Robert M. Herndon of the Johns Hopkins University School of Medicine have used the purified BP to develop a radioimmunoassay to measure the concentration of myelin fragments in the cerebrospinal fluid of MS patients. They have since demonstrated that there is a high concentration of BP in the fluid during acute episodes of MS and a much lower concentration during remission.

The radioimmunoassay is not specific for MS since myelin fragments are also present in the cerebrospinal fluid in other diseases in which there is tissue destruction in the central nervous system. The assay does, however, provide the best way available to determine when active destruction of myelin is occurring. It thus provides an objective technique to follow the course of the disease. McKhann hopes to improve the test so that it can be used on blood and urine samples.

It now seems clear that MS is an exceptionally complicated disease that results from a complex interaction of genetics, environment, geography, viruses, and the patient's own immune system. Some progress is being made in understanding each of these individual components, but there is still no clear picture of the entire disease process and no therapy for it. The best hope now seems to be that a breakthrough in one of these smaller areas will at least provide a way to halt the spread of the disease and, perhaps, alleviate some of its symptoms.

—THOMAS H. MAUGH II

*This is the last of three articles.*