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Medical Progress

Multiple Sclerosis

Current Etiological Concepts

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■ *An animal model for acute multiple sclerosis* (MS) *is experimental allergic encephalomyelitis* (EAE). EAE *is produced by intradermal injection of a protein component of central nervous system* (CNS) *myelin. Ultrastructural studies of* EAE *and of a peripheral nerve analog, experimental allergic neuritis* (EAN), *have revealed an orderly sequence of cellular events leading to the destruction and removal of myelin with sparing of axons (primary demyelination). Acute* MS *has not been studied electron microscopically, but the ultrastructural similarities between* EAN *and a case of acute Landry-Guillain-Barré syndrome, a primary demyelinating disease of the peripheral nervous system, suggest that a similar sequence of events might be found in acute* MS. *While the pathological findings support a cell-mediated or delayed hypersensitivity response, there is also evidence for the pathogenetic role of circulating antibodies. Among such evidence is included the finding that sera from animals with* EAE *and humans with acute* MS *rapidly produce a reversible block of complex (polysynaptic) electrical activity when applied to* CNS *tissue cultures, which suggests a possible mechanism for transient symptoms in* MS. *Epidemiological and other studies link* MS *with a viral cause, although no direct evidence that* MS *is caused by a virus exists. Viral and immunological mechanisms are not mutually exclusive in considering pathogenetic possibilities for* MS, *for it can be postulated that a viral infection of the central nervous system acts as a triggering agent for a series of immune responses, including production of a bioelectric blocking antibody and demyelination mediated by sensitized cells, the combination of which ultimately produces the total clinical picture of* MS.

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As two comprehensive reviews of experimental work related to multiple sclerosis (MS) have recently been published,[1,2] the purpose of this report will be to highlight some aspects of research directed at the etiology of MS. In particular, studies dealing with the animal model, experimental allergic encephalomyelitis (EAE), and those employing the nerve tissue culture technique will be emphasized. In view of the attention attracted by the possibility of a viral cause for MS,[1-3] some consideration will be given to the conceivable role of a virus.

Experimental Allergic Encephalomyelitis

Experimental allergic encephalomyelitis is an inflammatory disease of the central nervous system (CNS) induced in animals by injection of normal CNS tissue of either the same or of a different species, usually in the company of Freund's complete adjuvant.[4,5] An animal's own brain tissue could as well serve as the antigen.[1] The disease is characterized clinically by weight loss, ataxia and paralysis of the limbs, and pathologically by perivenular infiltration of the CNS by mononuclear cells and demyelination.[5,6] The pathologic changes of the acute phase of the disease resembles, at the light microscopic level, the changes of human postvaccinial encephalomyelitis, postinfectious encephalomyelitis and acute MS; hence EAE has been considered to be an animal model for these human diseases.[7] If B. pertussis is substituted for M. tuberculosis in the preparation of Freund's adjuvant, a "hyperacute" form of EAE is produced, characterized by rapid clinical onset, necrosis of vessel walls and perivascular hemorrhages, infiltrates of mononuclear and polymorphonuclear leukocytes and demyelination.[8] The hyperacute form of EAE has been regarded as an animal model of human acute necrotizing hemorrhagic encephalopathy.[1] If peripheral nervous system (PNS) tissue is substituted for CNS in the preparation of the antigenic material, pathologic changes similar to EAE are confined to peripheral nerves and nerve roots.[9] This experimental animal disease, experimental allergic neuritis (EAN), has been regarded as a model system for the Landry-Guillain-Barré syndrome.[10,11]

While the specific antigenic factors in PNS remain to be elucidated, it has been found that a protein fraction of CNS myelin which migrates toward the cathode on electrophoresis is encephalitogenic.[12] Small doses of this myelin basic protein (BP), given with Freund's complete adjuvant, produce a clinical and pathological picture of EAE identical with that produced by larger doses of whole CNS.[13] The amino acid sequence of myelin basic protein, which constitutes at least 30 percent of the total myelin protein,[14] has been determined for human and bovine basic protein.[2] The whole molecule of 170 amino acids has been further fractionated, with encephalitogenic activity having been found with a peptide consisting of a linear sequence of nine amino acids, and an encephalitogenic peptide containing 11 amino acids has been synthesized.[15]

Pathological Aspects of EAE and MS

The essential pathological lesion in both EAE and EAN is primary demyelination, by which is meant the destruction of myelin with sparing of the axon.[7] Electron microscopic studies have revealed an orderly sequence of events leading to the destruction and removal of myelin sheaths, common to both EAE and EAN.[13,16-19] The pathological changes in EAE and EAN are so similar that these two disease models may be considered together.

Before further discussion of the pathological events, it might be well to briefly review some of the pertinent ultrastructural features of myelin. PNS myelin is formed by the concentric wrapping of peripheral axons by Schwann cell cytoplasmic membranes.[20] Each myelinated segment, or internode, is formed by a single Schwann cell. The central myelin forming cells are the oligodendrocytes.[20] Each oligodendrocyte contributes to the myelin of several central axons, and therefore is involved in the formation of at least several internodal segments. As the processes of myelin-forming cells become wrapped around axons, cytoplasm is extruded, resulting in apposition of the inner surfaces of the cytoplasmic membranes with consequent formation of the major dense lines of the myelin sheaths. Apposition of the outer surfaces of the encircling cytoplasmic membranes results in formation of the minor dense or intraperiod lines. The electron microscopic picture of a myelin sheath thus consists of a repeated pattern of alternating dark osmiophilic major dense lines and less heavy minor dense lines, separated from each other by non-osmiophilic light intervals. It is believed

that the major and minor dense lines represent the protein elements of the myelin sheath, while the light zones represent the lipid elements.[20]

The process of demyelination in both EAE and EAN begins with the traversing of the walls of venules in the nervous system by mononuclear cells, which are believed to be transformed lymphocytes.[18,21,22] The mononuclear cells pass from the vascular lumen either through or between endothelial cells and subsequently penetrate the vascular basement membrane.[18,22] In the CNS the next step is the surrounding of myelin sheaths by invading mononuclear cells or their processes (Figure 1,A), while in the PNS this is accomplished after penetration of the Schwann cell basement membrane and separation of the Schwann cell from the myelin sheath.[16-19] The next and possibly key step according to Lampert[18,19] is a vesicular myelinolysis, with splitting of the myelin lamellae along major dense lines (Figure 1,B). Subsequently phagocyte cell processes, which may originate from the mononuclear cells causing the vesicular myelinolysis or from other "nonsensitized" mononuclear cells, invade the myelin sheaths at points of lysis (Figure 1,C), progressively peel myelin lamellae along minor dense (intraperiod) lines, and remove and digest the resultant myelin debris[13,16-19] (Figure 1,D). Vesicular myelinolysis is not always observed however, and processes of phagocytic mononuclear cells invade myelin lamellae at nodes of Ranvier or via the outer glial loops without preceding lysis.[13,16-19] Axons are usually spared in this process, being only occasionally secondarily involved, so that the end result is a normal-appearing axon completely stripped of its myelin (Figure 1,E). In EAN, Schwann cells are neither destroyed nor do they participate in myelin removal, contrary to their behavior in Wallerian degeneration or experimental diphtheritic neuropathy, in which damaged myelin is initially taken up by Schwann cells.[19,23] In EAE, oligodendrocytes may be destroyed, but the fate of these cells has not been definitely established.[18]

That a similar sequence of events may occur in human demyelinating disease was demonstrated by an ultrastructural study of a patient with the Landry-Guillain-Barré syndrome who died during the acute phase of the illness.[11] The findings on electron microscopic examination of peripheral nerves and nerve roots included myelin destruction with axonal sparing, a vesicular myelinolysis associated with macrophages or mononuclear cells and a failure of Schwann cells to participate in myelin phagocytosis, which was accomplished by macrophages. The pathological picture was very similar to that seen in EAN. To date, no case of MS has been studied electron microscopically in the acute phase. Those MS lesions which have been studied at the ultrastructural level have all been chronic,[24-26] and the pathological features are comparable to those seen in chronic EAE.[27] The acquisition of tissue in an appropriately acute stage of MS and in a state of preservation adequate for the type of detailed ultrastructural examination that has been performed in animals with EAE will require an extraordinary set of circumstances. Thus there may be some interval of time before comparisons can be made between the acute lesions of MS and EAE.

Immunological Aspects of EAE and MS

One reason for pursuing a comparison between EAE and MS relates to the consideration that MS may be an "immunological" disease. EAE clearly follows injection of an antigenic protein component of myelin, and the sequence of pathological events suggests a cell-mediated or delayed hypersensitivity type of response, with the essential steps appearing to be invasion of the nervous system by sensitized cells and the encirclement and lysis of myelin sheaths, which occurs in the presence of mononuclear cells.[18] The phagocytic stripping of myelin lamellae which follows is a non-specific reaction which can be seen in conditions other than EAE.[28] The finding of a similar sequence of cellular events in acute MS could suggest that similar immune mechanisms play some role in its pathogenesis.

While the described pathological events in EAE suggest a delayed hypersensitivity response, there is considerable controversy as to whether EAE induction is cell-mediated or whether circulating antibodies participate in the development of the CNS lesions. Additional evidence in favor of the delayed hypersensitivity concept includes the fact that EAE can be passively transferred from diseased to healthy animals by injection of lymph node cells[29,30] or whole blood,[31] while such passive transfer is not possible by either intraperitoneal or intravenous injection of se-

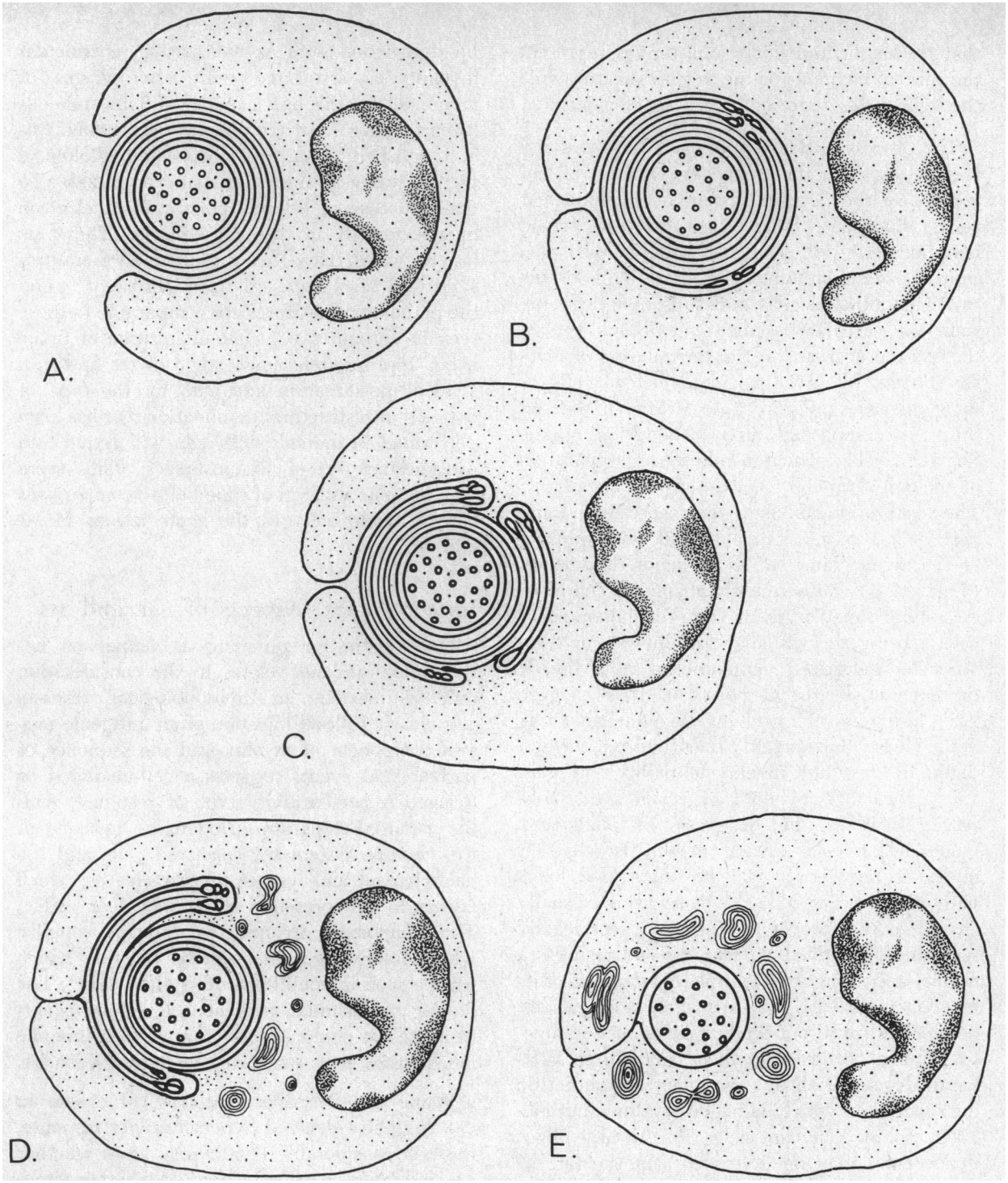

Figure 1.—Semi-schematic illustration of cell-induced myelinolysis and subsequent myelin lamellar stripping as occurs in EAE and EAN, based on electron micrographs by Lampert (see text for references). A, a myelinated axon is surrounded by an invading mononuclear cell of hematogenous origin. B, vesicular myelinolysis occurs in the presence of the "sensitized" mononuclear cell, with splitting of the myelin lamellae along major dense lines. C, mononuclear cell processes invade the myelin sheath at points of lysis and begin to separate myelin lamellae along minor dense (intraperiod) lines. D, at a more advanced stage myelin lamellae are progressively peeled, with phagocytosis of the resultant myelin debris. E, the end result of this process is a normal-appearing axon completely stripped of its myelin, with myelin debris continuing to undergo phagocytosis.

rum.[32] Also consistent with cellular hypersensitivity is the correlation of delayed skin reactions to basic protein (BP) with the induction of disease.[33] Skin reactions after injection with BP reach a peak a few days before onset of EAE, and subsequently decrease as signs of EAE develop. This has been interpreted to mean that the lymphoid cells which have been involved in the delayed skin reaction are withdrawn from the periphery by subsequent attraction to the antigen in the CNS, where they then produce delayed hypersensitivity reaction of the same type.[2]

Other supportive data favoring delayed hypersensitivity as the pathogenetic mechanism of EAE include the presence of low titers or absence of circulating anti-BP antibodies in guinea pigs with EAE produced by a single injection of BP in Freund's adjuvant,[34] and the presence of cell-migration inhibition when macrophages from EAE animals are exposed to either whole CNS or BP.[35,36] The latter is tested by packing peritoneal exudate cells (lymphocytes plus macrophages) collected from sensitized animals into capillary tubes and maintaining them in a suitable medium. The addition to the medium of the antigen to which the animal has been sensitized results in an inhibition of the migration of macrophages from the open end of the capillary tube. The inhibitory factor is believed to be produced by sensitized lymphocytes, and the purpose of such a factor may be to keep macrophages at the antigenic site in order to perform their function of phagocytosis.[2,37] The test is currently under evaluation as a possible investigative and diagnostic tool in MS and the Landry-Guillain-Barré syndrome.[37,38]

Evidence which favors the role of circulating antibodies in EAE induction includes the ability of serum from animals with EAE to produce periventricular demyelinating lesions when injected directly into the ventricles of normal animals,[39] the finding of deposition of γ-globulin in the CNS before the appearance of cells in EAE,[40] and the ability of serum from animals with EAE produced by sensitization with whole CNS to demyelinate CNS tissue cultures.[41] The tissue culture demyelinating factor was shown to be complement dependent and was localized in the $7S\gamma_2$-globulin fraction.[42] It could be absorbed out by previous exposure to CNS tissue, and immunofluorescent studies demonstrated attachment of the globulin to myelin sheaths and glial cell membranes during demyelination.[42] The presence of this demyelinating antibody in the sera of EAE animals provides a link with MS, for a high proportion of sera from acute cases of MS also demyelinated CNS tissue cultures.[43,44] In both instances, removal of the demyelinating serum and return of the cultures to their normal nutrient medium was followed by remyelination.[43] On the other hand, chronic exposure of cultures to high doses of EAE serum resulted in an irreversible state of "sclerosis."[45]

Electron microscopic examination of cultures exposed to EAE sera revealed a selective degeneration of oligodendrocytes.[46] It was further demonstrated that if CNS cultures, which are usually derived from fetal or newborn animals before myelination, were exposed to low concentrations of EAE serum from the day of explantation on, the formation of myelin *in vitro* was inhibited and oligodendrocytes failed to differentiate.[47] Upon withdrawal of the EAE serum and replacement with normal nutrient medium, differentiated oligodendrocytes appeared and myelin was formed.

The relevance of these studies to the pathogenesis of EAE is opened to question by the demonstration that substitution of either a diffusible encephalitogenic peptide[48] or myelin basic protein[49] for whole CNS as the disease-producing antigen resulted in failure to produce tissue culture demyelinating antibody. In the latter study,[49] steps were taken to insure high levels of circulating anti-BP antibody, including sensitization with large doses of a high molecular weight BP and hyperimmunization. Hyperimmunized or "protected" animals are produced by sensitization at intervals with BP in Freund's incomplete adjuvant (without M. tuberculosis), followed by a challenge dose of BP in complete adjuvant. These animals do not develop EAE, but do exhibit high titers of anti-BP antibodies. None of the sera with high levels of anti-BP antibodies demyelinated CNS tissue cultures, indicating that antibodies to the encephalitogenic BP were not responsible for demyelination *in vitro*. The conclusion drawn from this study was that the demyelinating antibody present in the sera of whole CNS-sensitized animals was formed in response to an antigen not involved in the pathogenesis of EAE, as whole CNS contains a multiplicity of antigens which are not encephalitogenic.[2] A dissenting note is a study which claimed that demyelinating antibody

was found in the sera of BP-sensitized animals, though in a smaller percentage than with whole CNS sensitization.[50]

Similar doubts have been raised about the significance of demyelinating antibody in MS.[1,2] Although the highest proportion of demyelinating sera have been reported in cases of active MS, tissue culture demyelinating activity has also been found in some normal sera, in sera from two cases of cobalt-irradiated brain tumors and in a high percentage of cases of amyotrophic lateral sclerosis,[51-52] in which demyelination is secondary to axonal degeneration rather than primary. The presence of demyelinating antibody in conditions involving degeneration of myelin rather than primary demyelination raises the possibility that such antibody represents a nonspecific response, perhaps to the breakdown of myelin, and is not a causative factor in disease production.

Of perhaps greater relevance than demyelinating antibody to the pathogenesis of both EAE and MS is the observation that sera from animals with EAE and humans with MS abolished evoked complex electrical activity in tissue cultures soon after application.[53] The effect was rapidly reversible after replacement of the test sera with normal nutrient medium. A similar phenomenon was demonstrated upon application of MS sera to isolated frog spinal cord.[54] In both instances complex activity indicative of transmission over polysynaptic pathways was affected, but the assumption that the block occurs at the synaptic level remains to be proven by intracellular recording. A dissociation between demyelinating antibody and the factor blocking complex bioelectric activity is evident from a study which confirmed the absence of demyelinating activity in the sera of a majority of animals sensitized with encephalitogenic protein and demonstrated that these negative sera still blocked polysynaptic evoked responses.[55]

Electron microscopic examination of tissue cultures exposed to EAE sera was reported to reveal a 30 percent reduction in the total number of synapses present after 24 hours, and a 50 percent reduction after six days of exposure.[56] These effects were reversible after return of the cultures to normal nutrient medium. The statistical difficulty with such a study, especially when there is variation from culture to culture normally, is monumental. Furthermore, the functional blocking effects were present minutes after application of the test sera, long before any morphological changes, either of myelin or synapses, were evident.[51] The significance of the report of morphologically altered synapses is, therefore, not entirely clear.

The significance of the functional blocking activity exhibited by EAE and MS sera may, however, be profound. It has always been difficult to explain the commonly observed transient visual, sensory and motor deficit phenomena of MS on the basis of such pathological mechanisms as demyelination and remyelination. Furthermore, the degree of clinical involvement and the extent of histological lesions in animals with EAE do not always correlate.[57,58] A factor such as a functional blocking antibody, operating in addition to and independently of the factors causing demyelination, could provide a plausible explanation for these clinical and laboratory phenomena. Whether the functional blocking agent is an antibody or not is unclear, although the fact that it is complement-dependent[53] suggests that it is.

The consideration that more than one pathogenetic mechanism may underlie the clinical and histological events of EAE could bring together the opposing schools of delayed hypersensitivity versus circulating antibody. It is possible that both have a role in evoking the full picture of EAE. Such a concept is supported by the finding that maximum immunofluorescent staining of lymph node cells from EAE animals for γ-globulin occurred at a time interval after sensitization different from that when the cells exhibited maximum ability to passively transfer the disease.[59] It is conceivable that the acute clinical manifestations of EAE are produced by a functional blocking antibody, while the demyelination is a manifestation of a delayed hypersensitivity reaction. A similar line of reasoning might be applied to MS.

Viruses as Etiological Agents

Epidemiological studies of MS have indicated increased incidence of the disease in temperate climates and among the upper socio-economic classes of the more developed countries, a notable exception being Japan, where the incidence is low.[60] Studies of immigrant populations from areas of high incidence to areas of low incidence have demonstrated a prevalence rate of MS con-

sistent with that of the country of origin. Thus, while the disease is rare in native-born white South Africans, the occurrence rate is significantly higher among European-born white South Africans, and the prevalence of MS in various groups migrating to Israel correlates with the prevalence in their countries of origin with regard to both higher and lower incidence than among native-born Israelis.[60,61] Such data, plus the long delay in onset of MS after immigration, have been interpreted as being consistent with a viral cause, the virus in question being one with an incubation period measured in years.[60,62]

That viruses which have long incubation periods and are infective for extended periods (so-called "slow" viruses) may be involved as causative agents in human disease is suggested by the finding of transmissible agents in two chronic, progressive neurological disorders, kuru and Jakob-Creutzfeldt disease.[3,63,64] Kuru, which means the "shakes," is an unremitting invariably fatal disease with predominantly cerebellar symptoms that is found among the Fore tribe in New Guinea. Jakob-Creutzfeldt disease, one of the pre-senile dementias, is characterized by a rapidly progressing dementia and myoclonic jerks. Both diseases have been transmitted to chimpanzees by intracerebral injection of brain homogenates from human patients, and subsequently from chimpanzee to chimpanzee by similar inoculations.[63,64] In each instance, the clinical disease transmitted to the chimpanzees resembled the human disease, and the two conditions were easily distinguishable in the involved animals.[64]

In the first instance of transmission of kuru to chimpanzees, there was a latent period of from 18 months to four years between inoculation with human material and initial appearance of signs.[63] This was subsequently lowered to 12 months in chimpanzee to chimpanzee passages. In the case of Jakob-Creutzfeldt disease, onset of disease was noted in chimpanzees 12 to 14 months after inoculation with suspensions of brains from afflicted humans.[64] This incubation period was not altered by subsequent passage to chimpanzees. Virus particles have not been seen in kuru on electron microscopic examination, nor have antibodies been detected.[3] Virus-like particles were seen in one of five chimpanzees with experimental spongiform encephalopathy, but the difficulty in interpreting such material in light of the isolation of a great number of viral strains from experimental chimpanzees is discussed by the investigators.[65]

Although causative agents in these conditions have not been isolated and identified, the successful transmission of these diseases from man to higher primates, even with material passed through a 220 mμ filter, is nevertheless suggestive that a viral or virus-like agent is involved.

Similar attempts have been made to transmit other chronic progressive neurological diseases of man to animal hosts, including multiple sclerosis.[66] To date, no successful transfer of MS from man to higher primates by intracerebral inoculation of CNS tissue has been accomplished. There is a report, however, that inoculation of Icelandic sheep with brain homogenates from a patient who died of acute MS resulted in the development of scrapie in the sheep.[67] Scrapie is a naturally occurring disease of sheep which can be transmitted by intracerebral inoculation of filtered homogenates of CNS from affected animals to healthy sheep and to other species as well, including goats, hamsters, rats and mice.[3] The incubation period of scrapie in sheep ranges from nine months to four years. Clinically affected animals develop weakness, ataxia, tremors and hyperexcitability or lethargy, while pathological changes in the CNS are similar to those found in kuru and Jakob-Creutzfeldt disease.[3,65,68] The report of production of this disease by inoculation of tissue from a patient with MS is therefore of considerable interest. However, this study remains to be confirmed and it needs to be more clearly established that a latent natural infection was not incited by the experimental procedures.

Other findings suggestive of the possible etiologic role of viral agents in MS include the presence of higher viral antibody titers in the sera of MS patients than in the sera of matched controls. This was true for measles, type C influenza, herpes simplex, parainfluenza 3, mumps and varicella-zoster.[69-70] Such studies, however, by no means provide a direct relationship between viruses and MS, as the authors reporting these data readily admit.[70] Thus, while there are a number of suggestive links between viruses and MS, the relationship of these agents to the disease can be summarized by the statement that there is at present no direct evidence that MS is of viral origin.

Virus and Immune Mechanisms Combined

The possibility of a viral factor and the possibility that immunological mechanisms play a prominent role in the pathogenesis of MS are not mutually exclusive. Unlike the animal model, EAE, and unlike human postvaccinial encephalomyelitis, both of which are produced by intradermal injection of CNS tissue, no such direct exposure to an antigenic agent is known to exist in MS. If MS is an immunological disease, some triggering event is required to set the immunopathogenetic mechanisms in motion. A viral infection of the CNS could conceivably play such a triggering role, as has been suggested previously,[1] by causing a breakdown of some myelin with resultant release of encephalitogenic proteins or peptides. The response to the presence of the released encephalitogens might be the production of a functional blocking antibody and the sensitization of lymphocytes. The former might be responsible for acute, transient deficit phenomena while the consequence of the latter would be a cell-mediated demyelinative reaction, with the combination of these factors ultimately producing the total clinical picture of MS.

What causes MS remains unknown. Some progress has been made toward understanding possible pathogenetic mechanisms underlying MS and other demyelinating diseases, but much remains to be learned. The point of continuing to pursue studies such as those described in this review is eventually to provide a rational basis for therapy, a need evidenced by the almost annual appearance of a new "cure" for this still puzzling disease.

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