The Path to Damage in Multiple Sclerosis

For several decades students of multiple sclerosis (MS) have generally accepted the cause of the MS lesion as being entirely T cell mediated. More recently, however, numerous reports of studies of the animal model, experimental allergic encephalomyelitis (EAE), and detailed analysis of the pathology of MS have indicated that the events involved in the MS lesion may be more complicated. In this issue of the *Annals*, Raine and his colleagues[1] provide additional evidence that damage to the myelin sheath may, at least in some patients and in some animal models, result from an antibody-mediated step. The current article, which extends the recent report of these authors in *Nature Medicine*,[2] provides important details of the pathological changes found in three cases of MS and in marmosets immunized with either myelin oligodendrocyte glycoprotein (MOG) or whole white matter. In both cases, the authors present evidence that an early event in the steps leading to destruction of the myelin sheath appears to be associated with antibody binding. Based on results obtained from eloquent studies using immunogold and silver staining, the authors report immunoglobulin (Ig) bound to the vesiculated myelin networks demonstrated by electron microscopy. Using gold-labeled MOG and myelin basic protein (MBP) peptides, the authors report that antibody to both MOG and MBP can be found in the MS cases.

That antibody may have a role in demyelination seems increasingly likely. In 1988, Linington and colleagues[3] demonstrated that when antibody to MOG, which is found on the outer lamella of the myelin sheath and which has extracellular domains, was administered to rats with EAE; it converted the disease to a more severe clinical form and produced more extensive demyelination. Generally, rats with EAE show only minimal myelin damage. These initial studies have been confirmed and extended by many investigators, and Genain and colleagues have shown that EAE induced in marmosets using MOG shows more extensive demyelination than that found in animals immunized with MBP.[4]

Further, a detailed study of the pathology of MS that examined a large number of samples obtained by biopsy or at autopsy showed that, although the pathology is heterogeneous,[5] the most common finding was inflammation associated with Ig binding to myelin, suggesting an antibody-mediated process producing damage to myelin. In addition to Ig, deposits of complement components have also been demonstrated in regions of myelin destruction.[6] The study reported in this issue[1] and the previous report in *Nature Medicine*[2] have extended these findings further, supporting a role for antibody in myelin damage.

As Raine and colleagues note, an important question is whether antibody binding actually represents the initial step in myelin damage. They describe myelin vacuolization at the leading edge of active lesions in both marmosets and the MS cases. Bound antibody was not demonstrated at this step, so it is possible that other processes such as those mediated by cytokines could contribute to the initial step in myelin damage. Efforts to demonstrate antibody to MOG or other myelin components have been thus far unsuccessful in the model of relapsing EAE in the SJL mouse. Despite the lack of antibody, substantial demyelination is observed.

One note of caution is also needed. Several investigators have shown that antibody to MOG or B cells specific for MOG can occur in controls as well as in patients with MS.[7,8] The presence of the antibody in healthy controls indicates that caution must be used in interpreting the presence of the antibody in a lesion with an open blood-brain barrier. Although the immunohistochemical evidence presented in the current work, as well as that of others, strongly supports a role of antibody and specifically antibody to MOG in the myelin destructive process, it does not provide definitive proof. The ability of antibody to MOG to augment demyelination in various EAE models, however, provides additional circumstantial evidence in support of an antibody-mediated step.

If antibodies contribute to the disease process, how does this fit with the notion that MS, and certainly EAE, is a T-cell–mediated disease? The evidence that T cells specific for an antigen found within the central nervous system are required to initiate the EAE lesion is without question.[9] The ability to augment disease with antibody to MOG in some EAE models indicates that in these instances various steps are involved in the EAE lesion and, by extrapolation, in the MS lesion. It is not unreasonable to assume that a T-cell–mediated process initiates the lesion but that the effector mechanisms, which actually result in damage to the myelin sheath, are related to mechanisms that are immunologically distinct from those that initiate the lesion. The increasing evidence that some degree of axonal damage can occur even in relatively early lesions further complicates the development of the MS lesion. Finally, progression of damage may continue to occur even after the acute lesion has resolved and, as Raine and colleagues[1] suggest, this may well be due to prolonged effects of cytokines or other mediators that result in prolonged tissue damage and destruction and contribute to progression of the lesion and of the disease. Understanding the events involved in the various stages

of the MS lesion is central for designing treatment strategies.

Finally, while important insights can be gained from studies of EAE, it will be necessary to do careful pathological studies of patients with MS, such as the one reported in this issue or the recent detailed pathological analysis of MS lesions presented by Lucchinetti and colleagues,[5] to define the events involved in the lesion. Neurologists and especially those caring for patients with MS can facilitate this research effort by placing a higher emphasis on tissue donation.

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Solving the COX Puzzle

Denis Leigh carefully examined the nervous system of an infant who died after a devastating 6-week neurological illness.[1] He was impressed with the distinctive histopathology and compared his findings with the neuropathology of Wernicke encephalopathy associated with chronic alcoholism and thiamine deficiency. What has emerged to some extent from this seminal observation is the modern discipline of mitochondrial genetics—undoubtedly far beyond Prof Leigh's wildest expectations or speculations.

The report by Willems and colleagues in 1977[2] may have a similar impact historically as we learn more about the biochemical basis for Leigh syndrome. These investigators suggested that cytochrome *c* oxidase (COX) deficiency could cause Leigh syndrome. Their patient had died of respiratory insufficiency at age 6 years after suffering a progressive clinical disorder characterized by ataxia, dementia, and optic atrophy. COX activity was quite deficient in muscle, decreased in heart, and normal in liver. Several researchers, myself included, speculated that the finding was an "epiphenomenon"—absent complex IV activity in some tissues but not all, and consistent with postnatal survival for several years. Hard to believe but clearly true, as others would show later.[3,4] My colleagues and I in 1987 reported on 5 children with COX deficiency in Leigh syndrome in this journal.[5] Partial COX deficiency was noted in brain, muscle, kidney, heart, liver, and cultured skin fibroblasts in some but not all of the 5 patients. For example, COX deficiency was normal in both liver and cultured skin fibroblasts from 1 patient. All patients had COX deficiency in brain (30% residual activity) and muscle (15% residual activity). Residual cross-reacting material was present in all tissues, and later studies would show that all 13 COX subunits were present, and the 3 mitochondrial and the 10 nuclear genes encoding these subunits harbored no pathogenic mutations.[6,7] Also, we and others had noted that the COX activity in cultured fibroblasts from the parents of COX-deficient patients was consistently normal—an unusual finding if the parents were carriers of an autosomal recessive trait. The aggregate findings were indeed puzzling but implicated a mutation in another gene that was important in the assembly and regulation of the COX holoenzyme. In addition to the 10 smaller COX subunits encoded by nuclear DNA and the 3 larger subunits encoded by mitochondrial DNA, there are other nuclear genes that are responsible for the synthesis of hemes a and a_3, transport and insertion of metal cofactors, and the coassembly of the 13 subunits. Many COX assembly genes have been identified

in yeast, and several of the human homologs have been characterized. SURF-1 is one of these COX assembly genes. SURF-1 has been mapped to chromosome 9q34, and this gene product is involved in the maintenance of COX activity and mitochondrial respiration.[8] In 1998, two groups identified SURF-1 mutations in patients with COX-associated Leigh syndrome.[9,10] These observations helped unravel the earlier mystery regarding partial COX deficiency in a subgroup of patients with Leigh syndrome. The reduced COX activity was not the result of a primary mutation in one of the genes encoding a COX subunit. Rather, the primary molecular defect was located elsewhere in the nuclear DNA, affecting a gene that was responsible for the assembly and maintenance of COX activity. Now the earlier observations made more sense, but new questions emerged.

Tiranti and colleagues in this issue report loss-of-function SURF-1 mutations in 18 of 24 patients with classical COX-associated Leigh syndrome.[11] The genetic errors are of several types, including frame-shift, stop, and splice mutations. No patients had missense mutations, and the authors speculate that the anticipated phenotype resulting from a putative missense mutation might be milder. Another series of patients with decreased COX activity in affected tissues had no mutations in the SURF-1 gene. Six of these patients were "Leigh-like" because of atypical clinical and radiological features, and 16 patients were "non–Leigh syndrome" COX-deficient cases lacking the typical magnetic resonance imaging or neuropathological lesions. Furthermore, 25% of the classic COX-associated Leigh syndrome patients had no SURF-1 mutations, suggesting that other assembly genes might be responsible for this subset of patients. Undoubtedly, we can anticipate more mutations in this array of assembly genes as a new class of gene defects causing human disease is defined.

These new findings also emphasize the complicated interplay between the two cellular genomes and their respective gene products. Most of the mitochondrial proteins are encoded by nuclear genes and synthesized in the cytoplasm. These proteins are imported into the mitochondrial matrix by an adenosine triphosphate–dependent process. This process also is regulated by numerous nuclear genes and adversely affected by deleterious mutations.[12,13] Once imported into the mitochondria, these proteins need to be directed to the proper region of the subcellular organelle and assembled with both mitochondrial and nuclear-encoded subunits and prosthetic groups to perform the designated biological function. Given the complexity of the intergenomic "cross-talk," we can expect to uncover more defects in this nuclear DNA-driven process that will explain many human neurodegenerative diseases. Phenotypic diversity appears to be the rule as witnessed by the clinical features of the classical COX-associated Leigh syndrome cases in the report by Tiranti and colleagues.[11] One patient (Case 20) had symptoms at birth and died at age 14 months, whereas others had symptoms in infancy but remain alive 3 to 10 years later. This phenotypic diversity will continue to challenge clinicians as more molecular defects are identified at the bench. Scientists also will be challenged by the need to unravel the mysteries surrounding phenotype-genotype correlations, and patients and their families will remain hopeful that these extraordinary scientific insights will eventually lead to much-needed advances in treatment. The most immediate clinical benefit derived from these laboratory findings is the possibility for prenatal diagnosis.

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