ABSTRACT

Multiple sclerosis (MS) is a complex condition, characterized by considerable variability in its presentation and in the disease course within each individual, in addition to across patients. The future of MS research will strive to better understand both its autoimmune as well as neurobiological underpinnings. This complexity of pathophysiology presents challenges in terms of developing diagnostic techniques and therapeutic strategies. Research to date indicates that the MS disease process involves several mechanisms of tissue injury, and that damage to the central nervous system occurs earlier and probably more diffusely than was previously suspected. Emerging concepts and future areas of basic investigation include an integrated understanding of the pathology of MS, including the concepts of autoimmune and neurobiological etiologies, acute inflammatory injury, demyelination, axonal injury, and processes involved in tissue protection and recovery. In terms of therapies, the future of MS relies on the development of novel imaging techniques and biomarkers, in addition to strategies that allow for the optimal balance of target-specific treatments, immune modulation, and strategies that promote neural protection and repair.


PARADIGM SHIFTS AND EMERGING CONCEPTS

Scientific progress within the field of multiple sclerosis (MS) research will be predicated on our ability to understand more about the biology of the disease, and specifically to advance in the areas of pathology, immunology, neurobiology, biomarker development, and novel imaging modalities. One of the fundamental questions that must be addressed is whether MS represents many different diseases, or whether the variations we see are an expression of a similar disease in patients with different host backgrounds and/or environmental exposures. Certainly, MS is a complex disease, and there are multiple and varied mechanisms contributing to tissue injury. All of these factors increase the challenges confronted by researchers and clinicians, especially when we realize that pathologic mechanisms are likely occurring much earlier in the course of the disease than was previously believed, and that they extend beyond the classic, multifocal lesions visible on routine magnetic resonance imaging (MRI) scans. As scientists look ahead to the future of MS therapies, we also need to understand how peripherally administered treatments benefit the central nervous system, and to identify biomarkers that will help us predict drug efficacy and safety.

EVOLVING THEORIES OF THE UNDERLYING PATHOPHYSIOLOGY OF MS

AUTOIMMUNE VERSUS NEUROBIOLOGICAL DISEASE?

The consensus for most scientists is that MS, like type 1 diabetes, is an autoimmune disease that emerges in individuals who have a certain genetic susceptibility, are exposed to 1 or more particular environmental factors, and as a result undergo a dysregulation of the immune system and develop clinical disease. Such interactions of genes, environment, and immune regulation may be relevant as initiators and as propagators of the
disease process. Figure 1 provides a simplistic view of the clinical course of MS and some of the MRI metrics that have been used to assess disease activity. It illustrates that, over time, there is commonly a change in the course of clinical disease, as patients are seen to transition from relapsing-remitting MS (RRMS) to secondary progressive MS (SPMS). Much of the biological disease process is hidden such that ongoing activity and damage may not be clinically apparent. The burden of inflammatory disease as measured by the extent of T2 lesion damage on MRI appears to increase as the disease progresses in the earlier phases, followed by a relative stabilization in terms of fewer new T2 lesions in the face of progressive disability. Likewise, the presence of gadolinium (Gd)-enhancing lesions appears to be concentrated early on in the disease. Gd enhancement is a marker of a local breach of the blood-brain barrier, and is thought to represent focal areas of active inflammation. With time, fewer Gd-positive scans are observed in the face of progressive worsening of neurological disability. These data illustrate the types of mismatch between what we see on serial imaging and what happens to patients clinically particularly with respect to the progressive component of the disease.

These and other data have raised the question as to whether the biological substrate of what has been termed progressive MS should be conceptually revisited. How might the mechanism underlying progression differ from the biology of acute MS relapses? How early does this process begin, and might it even contribute to the initiating process in MS? Could it be that a primary abnormality in MS is neurobiological, not just autoimmune? Conceivably, the RRMS and subsequent SPMS clinical pattern may involve an ongoing neurobiological process of ‘progression,’ which could be subclinical early on, and superimposed by waves of immune response manifesting as attacks and remissions. The latter may abate or acquiesce (as many human autoimmune diseases do) later in life, such that the biology of progression finally emerges as SPMS. To extend the theory even further is the notion that primary progressive MS (PPMS) occurs in those few individuals in whom the superimposed phase of relapses and remissions is absent. The above scenarios may well not be correct, but point to an important and, as of yet, unresolved theme—namely the mechanism underlying disease progression.

In the early phases of typical RRMS, newly evolving lesions seem to involve an inflammatory cellular infiltrate that leads to the dual pathology of demyelination and axonal compromise mediated by an invasion of immune cells from the circulation across the blood-brain barrier and into the surrounding parenchyma. Thus, with respect to these waves of immune-mediated inflammation, the active lesions of MS are no different than the conceptual model for other autoimmune diseases, with the central nervous system (CNS) as the target. Some as yet unidentified factor is thought to initiate the activation of immune cells in the periphery, with the upregulation of molecules that then enable the cells to more efficiently traffic across the normally intact blood-brain barrier. This includes interactions between adhesion molecules, and between chemokines and their receptors, as well as active breakdown of the blood-brain barrier through the elaboration of tissue damaging enzymes (eg, matrix proteases). Subsequent reactivation of the immune cells is then thought to occur within the CNS by resident antigen-presenting cells (microglia) or by invading antigen presenting cells (macrophages), which are known to be present in large numbers in MS lesions. All of these conceptual steps (Figure 2) are potential targets for research with the ultimate goal of therapeutic intervention.¹

**DISTINCT PATTERNS OF WHITE MATTER DEMYELINATION**

Focal demyelinated plaques in the white matter are the hallmark of MS pathology. To investigate the con-
cept that “MS may be a disease with heterogeneous pathogenetic mechanisms,” Lucchinetti et al examined specimens obtained from biopsies and autopsies that contained actively demyelinating lesions using several immunological and neurobiological markers. The investigators described 4 distinct patterns of demyelination, defined according to features including myelin protein loss, the pattern of oligodendrocyte destruction, and specific immune markers. Patterns designated I and II demonstrated similarities to T-cell–mediated or T-cell plus antibody-mediated immune-mediated injury, respectively. The other patterns (III and IV) were suggestive of a primary oligodendrocyte dystrophy that may be seen in the presence of virus- or toxin-induced demyelination rather than primary immune-mediated injury. Although patterns of demyelination were heterogeneous between patients, they tended to be homogenous in multiple lesions from the same patient. These findings have driven some of the research to evaluate whether one can subtype patients in vivo to understand what type of therapy may be most effective in a given patient. However, although this evidence is intriguing, it is important to remember that the authors only evaluated demyelinating lesions, not nondemyelinating or normal-appearing white matter. Cortical demyelination and diffuse white matter abnormalities are thought to be important elements of secondary and primary progressive MS.

**Distinct Patterns of Cortical Demyelination**

Similarly to the investigation conducted by Lucchinetti et al, Peterson et al characterized demyelinated lesions, but in the cerebral cortex rather than in the white matter, of patients with MS. The authors identified 3 patterns of cortical demyelination: type I lesions were contiguous with subcortical white matter lesions; type II lesions were small, confined to the cortex, and typically perivascular; type III lesions extended from the pial surface to cortical layer and, interestingly, did not appear to follow a perivascular distribution. Inflammation and neuronal pathology were also studied in a subset of patients. Compared to white matter lesions, cortical lesions contained 13 times fewer CD3-positive lymphocytes and 6 times fewer CD68-positive microglia/macrophages. Transected neurites (axons and dendrites) were most common in active cortical lesions, followed by chronic active cortical lesions, chronic inactive cortical lesions, and myelinated MS cortex, and were nearly nonexistent in the control cortex. The authors suggested based on their data that demyelination, axonal transection, dendritic transection, and apoptotic loss of neurons in the cerebral cortex contribute to neurological dysfunction in patients with MS. It is interesting to note from the Peterson et al data that the different patterns of demyelination in the cortex again suggest that there are different underlying biological mechanisms to the MS injury process.

**Cortical Demyelination and Diffuse White Matter Injury in Different MS Presentations**

Taking it a step further, Kurtzelnigg et al analyzed so-called “global brain pathology” in MS, focusing on the normal-appearing white matter (NAWM) and the cortex. The investigators examined autopsy tissue from patients with MS with RRMS, in addition to those with PPMS and SPMS, comparing the specimens to those of control patients. New and active focal inflammatory demyelinating lesions in the white matter were mainly present in patients with acute and relapsing MS, whereas diffuse injury of the NAWM and cortical demyelination were characteristic of PPMS and SPMS. Cortical demyelination and damage to NAWM was marked by diffuse axonal injury with microglial activation, and was suggestive of a rather diffuse inflammatory response in the whole brain and meninges. The authors found little relationship between the number of
focal lesions in the white matter and the amount of diffuse white matter injury or cortical pathology. They concluded that MS may begin as a focal inflammatory disease of the CNS, which initially causes distinct plaques of demyelination in the white matter. However, as the disease becomes more chronic, a more diffuse inflammatory pattern occurs throughout the whole brain, and results in slowly progressive axonal injury in the NAWM and in demyelination of the cortex. To summarize their data, in RRMS, it is the focal inflammatory demyelinated lesions that predominate, whereas in SPMS and PPMS, cortical demyelination and diffuse white matter infiltration are thought to be hallmarks of the disease.

NEW NEUROIMAGING TECHNIQUES FOR EVALUATING MS PATHOLOGY

Although quite helpful in initial diagnosis of MS, the commonly used T2 MRI sequences are very non-specific. This is because any increase in free water, whether due to edema, cellular infiltrates, demyelination, or axonal loss and gliosis, will appear as a T2 hyperintense lesion, with no pathologic specificity to help clinicians understand what is happening within that tissue. Two techniques that are thought to provide more specific insights to pathology are magnetization transfer ratio (MTR) imaging and magnetic resonance spectroscopy (MRS). Currently, these approaches are largely used in research protocols. MTR imaging is able to detect water that is associated with the macromolecules of the brain. Because much of this water is trapped by myelin, MTR serves as a surrogate marker of myelin—a higher MTR signal implies a better status of myelin. By performing serial MTR studies beginning from a time period before patients develop a new T2 or Gd-enhancing lesion, scientists have been able to demonstrate that brain abnormality in that location is progressive, and that it precedes the development of a new MRI lesion by a period of years. This MTR data indicate that a disruption in myelin integrity occurs considerably before there is evidence of a typical lesion detected by conventional MRI.

Magnetic resonance spectroscopy evaluates the biochemical content of tissue. For example, N-acetylaspartate (NAA) is a metabolite produced by neurons and present in neuronal bodies, axons, and dendrites. In the white matter, NAA content thus provides a useful marker of axonal integrity. Control subjects without MS will have a tall NAA peak on MRS in their white matter, indicative of healthy axons. However, in the NAWM of an individual with MS, even when there is no evidence of abnormality on conventional (T2) MRI, there is typically an abnormally low level of NAA—a surrogate marker for impaired axonal integrity. MRS evidence indicates that NAWM/axonal integrity becomes progressively disrupted from the relapsing-remitting stage to the secondary-progressive stage of MS.

PUTTING IT ALL TOGETHER: THE TWO FACES OF MS

Axonal injury (and not demyelination) is almost certainly the major contributor to the progressive, sustained, and percolating disability of patients with MS. There are 2 contexts of axonal injury that bear analysis, pathophysiological and therapeutical. In the context of the active inflammatory lesion, it is important to try to understand what triggers these responses at the level of the immune cells, the genes, and the environment, and how one might modulate these responses. However, there are also the extraleSIONAL regions of the brain that are now being recognized as potentially important sites of injury. A complex neurobiology is likely to impact this aspect of MS pathology. Genetics can play a role here (eg, by modulating the susceptibility of the target for injury and/or repair). For example, patients with MS who lack the gene for ciliary neurotrophic factor (CNTF) tend to have a more severe course of MS, with a more rapid progression of disability, than patients who have a normal CNTF gene. Environmental-gene interactions may also be relevant. For example, viruses such as the human endogenous retroviruses can be incorporated into the human genome, and may become reactivated in the context of local inflammation. Also, toll-like receptors, expressed on immune cells and on CNS microglial cells, can sense the environment and respond to various substances, including pathogens. These interactions represent another element of the complex neurobiological and immune processes that may contribute to lesional and extraleSIONAL pathology in MS.

Thus, evidence from the clinical evolution of MS, and data from pathologic and imaging studies, suggest that there may be 2 distinct yet concurrent biological processes—both contributing to axonal injury. In addition to pathology occurring within inflammatory lesions, there is likely to be another context of axonal injury that may relate more to the neurobiology of the
disease. These processes may be relatively independent of one another, although both probably start very early in the disease course.

THE FUTURE OF MS THERAPY

The following brief discussions represent just a few of the many ongoing trials and targets of research for this multifaceted disease.

ABLATION OF THE PERIPHERAL IMMUNE SYSTEM

Global immune suppression represents a nonselective therapeutic approach for a variety of conditions that are thought to be immune mediated. In MS, phase I and II studies have been conducted in which patients with very severe disease underwent aggressive ablation of the immune system via chemotherapy and then received bone marrow transplantation or autologous stem cell rescue. The goal was to allow patients with aggressive disease who had failed conventional therapy to develop a new and functional immune system that may no longer contribute to progression of MS. With this intervention, researchers essentially eliminate all of the peripheral immune response, isolating whatever is occurring in the CNS. Early cohorts for these studies included patients with advanced disability and relatively little ongoing inflammatory activity, who were nonetheless at considerable risk for adverse events from the procedure. More recent cohorts included younger, more recently diagnosed patients with highly active disease. These individuals had multiple Gd-enhancing lesions and multiple clinical attacks, and had failed a range of other therapies. Results have suggested that such intervention may be quite effective at preventing new Gd-enhancing MRI lesions or clinical attacks. However, it is not clear that the disease is completely arrested. It is too early to know whether this finding reflects an under-recognized CNS toxicity of this ablative chemotherapy regimen, or whether it underscores an inflammation-independent component of the disease that may continue relatively unchecked despite aggressive immune-targeted treatment.

B-CELL MODULATION

When the immune-mediated aspects of MS are considered, much of the attention has typically focused on the roles that T cells play. Recently, pathologic studies have identified regions where immune cell clusters are seen within the meningeal membranes that wrap around the brain. It is thought that as part of the chronic inflammation that occurs in the CNS of patients, there may be the creation of these chronically activated cell clusters as a form of ectopic lymphoid tissue formation within the target organ. In these clusters, there are reported to be chronically activated B cells. In parallel to these findings in patients, there has been progress in our understanding of the roles that B cells may play in the normal immune response, beyond their potential to become antibody-producing cells. For example, B cells are now recognized as being able to release distinct effector cytokines that could contribute to the regulation of local immune responses. As a corollary, abnormal B cells may fail to properly regulate the immune response, thereby contributing to autoimmune disease processes, including MS.

The brain may be a permissive environment to B cells, as Krumholz et al have shown that B-cell-activating factor (BAFF) is expressed in the normal human brain, and that it is produced by astrocytes, which are not traditionally thought of as being part of the immune system. Of note, the authors also found that in MS plaques, BAFF expression is strongly upregulated to levels observed in lymphatic tissues. BAFF is a critical survival factor for B cells, helping them to maintain a chronically activated state and survive long term. Collectively, these data implicate B-cell responses as potential contributors to the disease and suggest that these cells may be a potential therapeutic target in patients with MS.

THE MULTIHIIT MODEL OF CNS INJURY

As noted earlier in this article, damage in MS reflects not only the contribution of immune responses but also the state of the target tissue, including oligodendrocytes (the myelin-producing cells of the CNS). In a chronic disease like MS, a multihit model of injury is appealing. For example, when oligodendrocytes are exposed to inflammatory mediators, such as interferon-α or tumor necrosis factor α, they may become more vulnerable to subsequent cell death signals. Exposure to inflammatory mediators may lead the oligodendrocytes to alter the expression of certain molecules, such as p53, which may in turn predispose them to receiving additional immune or neurobiological signals, eventually leading to apoptosis. This “multihit” model may provide a model for the chronic, neurodegenerative process seen in MS, and may in the future present another opportunity for therapeutic intervention.
The examples earlier in this article list just some of the many potential strategies for treating MS, and there are currently a growing number of drugs in different phases of development and clinical trials. There are also many challenges in assessing these potential therapies. The drugs currently under investigation almost exclusively target the immune aspects of the disease. At one end of the spectrum are therapies that go to the extreme of global immune ablation and reconstitution, whereas at the other end of the spectrum investigators are attempting to modulate the immune system by targeting specific antigens in specific individuals that may play a role in their unique disease presentation. One of the challenges in MS is that although putative antigenic targets exist, these targets are likely to differ across patients, and almost certainly change in an individual patient over time. If one target is initially attacked, this injury exposes additional targets, posing a conceptual challenge to antigen-specific therapies. Thus, the concept of individualized therapy may still be far off in the future.

In the meantime, what lies in the middle of the spectrum of immune-modulating therapies is to target a family of non–antigen-specific molecules that are involved in the complex interaction between T cells and antigen-presenting cells, such as myelin basic protein, costimulatory molecules, and cytokines. However, further complicating this approach is the reality that there are 2 sides to inflammation: the destructive and protective sides. Some of the molecules under study in trials are being considered based on their potential to have neuroprotective mechanisms of action and perhaps also play a role in neural repair, but much work remains in terms of developing modalities to target the neurobiology of MS. Ultimately, in designing therapeutic interventions for MS, one must be able to strike a balance between proinflammatory and potentially neurotoxic substances and other anti-inflammatory and potentially neuroprotective ones.

To assist in the process, there is a need for more effective biological markers of this complex disease that would be able to capture features relevant to the inflammatory and the degenerative components of MS. Ideally, these biomarkers would be in vivo, non-invasive measures, perhaps building upon current imaging modalities to allow us to reach the level of molecular imaging.

Conclusions

Looking ahead, as clinicians and researchers, our therapeutic strategies must be able to impact the complex immunology and the neurobiology of MS. We need to develop treatments that will be efficacious in the long term, safe, and truly beneficial to patients. The emergence of many potential novel therapies is welcome, although it poses challenges to the community's ability to properly evaluate all candidates. We must continue to try to understand how our current and future treatments work, as this will provide important windows into the disease. In addition, scientists must begin to identify biological phenotypes for responders and nonresponders to various therapies, and attempt to develop biomarkers that are able to identify these patients and tailor therapies to them.

References