ABSTRACT

Multiple sclerosis (MS) is often thought of as an autoimmune condition in which the immune system attacks the central nervous system (CNS) through perivascular lesions and leads to demyelination. It is becoming appreciated that this model is simplistic. For one, CNS tissue injury involves both demyelination and axonal injury and furthermore, there appear to exist multiple mechanisms that may contribute to damage. There is evidence for more diffuse defects beyond the typical perivascular lesions visualized on standard magnetic resonance imaging, as well as sites of tissue disruption with relative paucity of classical immune cell infiltration. Additionally, careful histologic analysis of perivascular lesions has suggested that there exist several types of lesion pathology, implicating multiple mechanisms of injury. Recent work has implicated cellular mechanisms by which distinct T cells and B cells can promote demyelination. Interestingly, another theme is beginning to emerge implicating certain subsets of the immune system in a potentially protective role. Recent follow-up studies on patients with MS who had received aggressive immune ablative therapy (bone marrow transplantation) supply further evidence for immune and nonimmune processes involved in the disease process.

This review will cover emerging themes in the field of multiple sclerosis (MS) as they pertain to our insight into the biology of the disease. One is that there are multiple mechanisms of injury. This is partly why we have had difficulty identifying a single mechanism by which the nervous system is damaged in MS. Another important theme is that of disease heterogeneity across individuals. MS could be, in fact, a syndrome of several diseases, or the predominant pathophysiologic process may actually change in a given individual over time. It is also important to understand the different contributions of the immune system, both from outside and within the central nervous system (CNS), which leads to the notion of compartmentalized processes in the disease—the periphery and the CNS. Finally, there is the theme that we are dealing with a neuroimmune condition, one that involves very important neurobiologic principles in addition to principles of immune response and immune-neural interaction.

EVIDENCE FOR MULTIPLE DISEASE MECHANISMS

Disease course at early stages is most commonly characterized as relapsing-remitting multiple sclerosis (RRMS) and is thought in many cases to then transition into a secondary progressive phase (SPMS; Figure, Panel A). One of the typical ways we consider the burden of disease from an inflammatory standpoint is represented by the total volume of T2 hyperintense lesions (Figure, Panel C). This tends to increase overall, particularly in the early phase of MS, and then levels off without much further increase, yet the patients often continue to progress clinically. This represents one of several “disconnects” between imaging parameters that can be followed over time versus what happens to patients with respect to disability.
Each of the arrows at the bottom of the Figure can be taken to represent a time point in which a patient had gadolinium-enhancing lesions on a scan. Such lesions are thought to represent local areas of breach of integrity of the blood-brain barrier, suggestive of local inflammation at the level of the barrier. Early in the disease there is a higher concentration of gadolinium-positive lesions, whereas later, fewer and fewer gadolinium-enhancing lesions are seen—once again, in the face of worsening neurologic function. This has led to the notion that there may be, on one hand, a peripherally mediated immune response that is likely contributing to periodic breach of the integrity of the blood-brain barrier (and presumably clinical relapses), and on the other hand, an independent process that may be compartmentalized to the CNS. The latter may be due to local CNS inflammation and/or degeneration (Figure, Panel B).

It is becoming apparent that typical T2 hyperintense magnetic resonance imaging (MRI) lesions in MS only capture part of the picture. Using magnetic resonance spectroscopy, one is able to look at a metabolic profile of the tissue. The ratio of N-acetyl aspartate (NAA) to creatine provides an indication of the integrity of axons because this metabolite is made only in neuronal cell bodies and present in their extensions (in the white matter NAA is present essentially in the axons). In a typical MS lesion, one can see an impressive decrement of NAA as compared to a normal brain—representing compromise of the axons. If one looks at a voxel within the MS brain that appears normal (called “normal appearing white matter” or NAWM) and compares it with the corresponding voxel from a normal brain, an intermediate-level abnormality in NAA is seen, suggesting that in the MS brain there may be an abnormal process that is much more diffuse and widespread than the multifocal T2 hyperintense lesions seen on traditional MRI. The biology underlying these more diffuse abnormalities requires further study.

One also should consider the context in which myelin and axons may be degenerating or injured over extended periods of time. As shown by the work of Trapp et al in the relative absence of immune mediators (such as classical T-cell infiltrates), one can still see a loss of myelin integrity and injury to axons. Although there are reportedly many thousands of transected axons in the MS lesion, there also appear to be abnormalities of this type in NAWM. His work elegantly reminds us there are at least 2 aspects of pathology we must be attentive to.

Much of our understanding of MS comes from studies of experimental autoimmune encephalomyelitis (EAE). The model is created by injecting a healthy mouse with a component of the CNS. The animal will predictably come down with an ascending paralytic illness with pathologic features that one might see in MS. If T cells from a sick animal that react to myelin are injected into a healthy animal, the recipient animal will come down with the same disease. That is called “adoptive transfer”, and in the animal model, represents a very compelling case that T cells, in particular CNS-directed T cells in an activated form, can themselves mediate the disease.

When comparing human MS with EAE, one must realize that the model has significant limitations. In humans, a multiplicity of genes are interacting with several different environmental factors (very possibly

---

**Figure. Compartmentalization of MS**

A. Clinical course of relapsing-remitting MS, which evolves into progressive disease. Y axis represents disease progression. Vertical bars represent acute relapses. Horizontal regions represent remission periods. Sloped line represents progressive disease phase.

B. Conceptual division of MS into a phase in which disease is driven primarily by inflammatory injury (left) and by degenerative CNS compartmentalized injury (right).

C. Accumulation of T2 hyperintense involvement. Y axis measures T2 volume. Arrows represent times of appearance of gadolinium-enhanced lesions.

CNS = central nervous system; MS = multiple sclerosis.
different combinations in different individuals) that ultimately leads to a state in which the immune system is not properly regulated. All humans have the capacity to react against self; this is normal. Only when this self-reactivity is improperly regulated and causes tissue injury do we have a state of autoimmune disease. Normally these responses are kept in check by an array of immune regulatory mechanisms.

One can conceive of autoimmune disease as a 3-compartment system in which there is the following: (1) a “target”; (2) the peripheral immune system; and (3) a barrier that separates them. In MS the target is the CNS, and the barrier is the famous blood-brain barrier, which has unique biologic properties that are very relevant to the processes of disease in the CNS. From work performed in the EAE model, T helper type 1 (Th1) cells, which play a role in normal antiviral responses, have been the focus of attention for many years. These cells normally secrete interferon \( \gamma \); however, too much IFN\( \gamma \) secretion is thought to be pathologic. Additionally, there are emerging data concerning a new subset of Th cells termed ThIL-17, because of their secretion of interleukin (IL)-17, and these are now thought to be equally if not more important than Th1 cells in EAE and MS. These immune cells represent the potentially pathogenic mediators in MS. If they are inappropriately activated or inadequately regulated, they can adhere to and ultimately break down the blood-brain barrier through the release of matrix proteases (enzymes normally used to allow the immune system to get cells to the right place when necessary), and then participate in damage of the CNS.

**DISEASE HETEROGENEITY**

Lucchinetti et al looked at a series of biopsy and pathology specimens across MS brains and generated a classification scheme involving 4 different subtypes existing within the context of different demyelinating lesions. Patterns 1 and 2 showed clear evidence of T-cell infiltration, with antibody and complement deposition particularly marked in pattern 2. In contrast, patterns 3 and 4 were more reminiscent of injury that may involve ischemia-like injury or oligodendrocyte dystrophy. Thus, within demyelinating lesions one can see very different patterns of pathology invoking different mechanisms of injury. The authors noted that lesion types tended to be different across patients; however, within any one MS brain, the lesions were quite homogeneous. This in turn leads us to consider 2 possibilities. We may be dealing with a disease syndrome that has different subtypes in different patients that pathologically have different diseases, even though clinically they may appear similar. Another possibility is that the predominant pathologic process may be changing in a given individual over time (biopsy and autopsy specimens are really just snapshots in time).

Another study that illustrates heterogeneity comes from work looking at cortical demyelination. Interestingly, some lesions were clearly associated with a vessel (invoking a perivascular process, similar to what is seen in a typical white matter lesion), whereas other lesions were clearly not associated with any vessel. These data suggest that a mechanism other than the typical perivascular biology must explain the pattern of these cortical lesions in the MS brain.

**IMMUNE SYSTEM CONTRIBUTION TO MS**

T cells have traditionally been thought to be orchestrators of immune responses by activating B cells and microglia/macrophages. However, the role of the T cell has been revisited and evidence is emerging, identifying different types of T cells as having the potential to directly mediate injury to the tissue. CD8 T cells can secrete cytotoxic agents and thus have machinery that can cause injury. Indeed CD8-mediated neuronal transection has been demonstrated in vitro. Activated CD4 T cells also are able to kill neurons in vitro, although here the mechanisms are less clear. How relevant this is to what is happening in vivo remains uncertain. There are data to suggest that microglia/macrophages might be involved with the release of a variety of toxic substances.

Interestingly, some CD4 T cells within MS lesions can be stained for brain-derived neurotrophic factor (BDNF). Hence immune cells can make what were typically considered to be CNS factors and carry them into the MS lesion. BDNF serves several roles; not only does it function as a neurotrophic factor, supporting the growth and survival of transected neurons and oligodendrocytes as well as the process of remyelination, but BDNF also serves an immunomodulatory function quite separate from its CNS function, including the inhibition of co-stimulatory molecule expression and the inhibition of chemotaxis. This could lead to decreased T-cell activation and decreased infiltration. Thus, immune
cells, injurious on one hand, may be anti-inflammatory on the other, and also provide molecules that may have both neural and immunologic effects.

There exists clear evidence for B-cell involvement in MS. When we think of B cells in normal or abnormal immune responses, we tend to think of them as precursors to plasma cells that make antibodies. Thus, if B cells are relevant (whether in a normal or abnormal context), it is assumed that they function through their capacity to become antibody-producing cells. Several studies provide evidence that antibody directed to myelin or axonal elements is relevant to the MS disease process. Others have suggested that certain antibodies might actually be beneficial, such as antibodies that may bind to oligodendrocytes, stimulate them, and promote remyelination. Additionally, antibodies also may be involved because of their backbone (the Fc fragment). A variety of immune cells and other cells express Fc receptors, and when the antibody—regardless of its specificity—binds to this receptor it changes the biology of those cells. Some myeloid cells, when they pick up antibody, become activated and more aggressive, and others become relatively acquiescent and may contribute in an anti-inflammatory fashion.

Importantly, B cells may not merely be passive recipients of instruction from T cells but might actually “talk back.” This has become particularly interesting in the context of MS. In addition to the typical perivascular lesions seen in MS, B cells also are present within the meninges. These cellular complexes at times seem to replicate the architecture of a lymph node. Lymphatic architecture should not be present in the normal brain. Interestingly, in the salivary glands of Sjogren’s syndrome and in the knee joints of patients with rheumatoid arthritis, one can find similar ectopic lymphatic tissue. One of the hallmarks of this type of a germinal center architecture is the presence of high numbers of B cells and plasma cells that are seemingly in a chronic state of activation.

The CNS, which many of us learned is “immune privileged,” is actually less immune privileged than we had initially thought. In fact, there is a normal process of immune surveillance, wherein immune cells (such as T cells and B cells) migrate through the CNS, presumably looking for foreign pathogens. These cells generally find nothing of interest and eventually leave. Still, there are several interesting mechanisms within the CNS that involve active downregulation of immune responses, designed to minimize immune-mediated damage to the CNS tissue itself. For example, production of the pro-inflammatory Th1 cytokine IFNγ by T cells in the inflamed brain results in rapid upregulation of human leukocyte antigen-G (HLA-G) molecules on local antigen presenting cells (resident microglia or invading macrophages). HLA-G is a downregulatory molecule that binds to its receptor on the invading T cells and inactivates them. In contrast to this and other mechanisms that act to downregulate T-cell responses during inflammation in the CNS, the same environment may upregulate B-cell responses. For example, in the inflamed MS brain, astrocytes are known to make high levels of BAFF, which is a very important B-cell activating and survival factor. Thus, the CNS may be a rather supportive environment for B cells in particular.

It is now appreciated that B cells also have the capacity to regulate immune responses of other cells. A study that we performed was based on the observation that normal human B cells can produce very different effector cytokines, depending on their context of activation. More recently we found that in patients with MS there is a specific defect in this pattern of cytokine secretion. IL-10, a cytokine important for the suppression of the immune response, is greatly diminished in activated B cells derived from patients with MS, whereas other cytokines, such as tumor necrosis factor α and lymphotoxin, are unaffected. This becomes particularly salient when one considers animal models of several autoimmune diseases, in which selective depletion of IL-10 just from B cells results in worsening of the disease. This is true for models of inflammatory colitis, inflammatory arthritis, and EAE. These data do not point to IL-10 deficiency in B cells as the cause of MS, but in keeping with the animal model data, suggest that abnormal regulation by B cells may be associated with a worse phenotype of autoimmune disease. It is well established that multiple mechanisms regulate the immune response, and each one of these could potentially be dysregulated. There may be different mechanisms that are dysregulated in different patients. The target may be defined by the gene-environment interaction, and the mechanisms that are dysregulated may indeed be different across individuals. Thus, perhaps we should be measuring several things at the same time to capture a more complete picture of the state of immune dysregulation in a given patient at a given time point.
**MS as a Neurodegenerative Disease**

Given that elements of the immune system are clearly implicated in MS, several groups have been exploring the effect of immune ablation followed by bone marrow transplant (BMT) on the course of MS. The question is whether the disease will re-emerge or will it be fully eliminated? In the Canadian BMT cohort, recruited patients suffered from very aggressive MS, experiencing multiple relapses and developing multiple new lesions on MRI, despite standard therapies. After an average of 3.5 years of follow-up in 8 treated patients, none experienced relapses or new MRI lesions. However, an important observation is that despite the profound effect of BMT on new relapses and typical T2 or gadolinium-enhancing MRI lesions, the brains of these patients continue to atrophy. This may be an effect of chemotherapy (a treatment discussed in subsequent articles in this monograph). Alternatively, this may represent an example of the dissociation of the effects of the peripheral immune system from the intrinsic properties of the CNS contributing to the disease state.

An important point that must be stressed is that the brains of these patients continue to atrophy. This ongoing atrophy is seen in the second to third year and beyond. A possible explanation is that the toxicity of the aggressive chemotherapy associated with the transplant process triggers a chronic injury process in the CNS. It also may be that this is one of the most direct windows showing a dissociation of the different biologies that contribute to the MS process, namely, peripherally mediated inflammation and a CNS compartmentalized disease process, which again, may be inflammatory and/or degenerative.

There may be several mechanisms of neurodegeneration in MS. Glutamate toxicity is one such mechanism that acts through activation of N-methyl-D-aspartic acid receptors. Indeed, activated immune cells can produce large amounts of glutamate. Perhaps one reason it has been difficult to identify a particular mechanism for MS is the multiplicity of processes that can contribute to injury. Another is the notion that any given mechanism may not kill, but may make a cell more susceptible to another mechanism that may subsequently kill. One way in which multiple mechanisms might induce demyelination is through multiple sublethal "hits." Each insult, by itself, is unable to cause the death of the oligodendrocyte but a combination of insults will induce death and the pattern observed will be dependent, in part, on the specific insults causing the damage.

**Conclusions**

Our concept of MS is evolving. We now realize that MS can involve several different mechanisms, and we are just beginning to establish the cellular processes associated with these mechanisms. Molecular targets associated with different aspects of immune-mediated damage are being elucidated. Additionally, components of the immune system may also serve protective/reparative roles. These new discoveries will supply us with new targets for the development of the next generation of MS therapies.

**Discussion**

Dr Greenberg: In the BMT, how are the T cells depleted?

Dr Bar-Or: Before the actual chemotherapy, you give cyclophosphamide and mobilize CD34+ stem cells from the bone marrow. These cells are multipotent and will turn into a new immune system. The stem cell preparation is highly depleted of T cells, and then frozen for later use. You then ablate the patient's immune system with aggressive chemotherapy, and then give back their stem cells to reconstitute a new immune system.

Q: It seems that if you deplete the autologous T cells you can get rid of the disease. Does this basically prove that the autologous T cells are a key player for these patients with MS, in this group at least?

Dr Bar-Or: I want to be sure that people understand 2 things. Firstly, this BMT approach depletes many more cells than just autologous T cells. Secondly, that the results I showed should not be taken to advocate BMT as a cure for MS. The ongoing brain atrophy that we see may be because not all aspects of the disease process have been halted. I think that with this peripheral intervention we are not fully targeting the compartmentalized process within the CNS, whether it is inflammatory, or degenerative, or both. I think BMT is an example of how we can learn a lot about the biology of disease if we pay attention to the biology of the treatments that we expose patients to.

Dr Greenberg: Could you comment on the selection of patients? Are we self-selecting a subtype of MS to enroll in these trials, and thereby getting these dramatic results?
**Dr Bar-Or:** That is very possible. This is not a straightforward recruiting process, and it is certainly not the kind of trial that you can randomize populations into placebo or control groups. So, these are patients who have severe disease. Patients in our cohort in Canada have to be within 5 years of their diagnosis, and in fact, within 5 years of their first symptoms. Someone who had an attack 20 years ago would not qualify. The patient must be within 5 years of the first symptom of what turns out to be MS and have multiple attacks per year with multiple new gadolinium-enhancing lesions, despite having tried at least 1 year of the standard therapy. The patient receives a very detailed disclosure, because this protocol can be fatal. We already have 1 patient who succumbed to a busulfan-related liver toxicity and multisystem failure.

Thus, we think that while autologous BMT is certainly safer than allogeneic transplantation, it is still a potentially very risky approach. The issue is, is there any role for this intervention? In fact, one of the endpoints for us is that as soon as it is felt that this is not helping enough, the trial ends. Indeed the Canadian group stopped recruiting new patients for a year after the first death to see what would happen to the others, and we have now just started again to continue with the initial cohort of patients. Data from this trial will have a big impact on aspects of the disease biology but I do not think it cures MS completely.

**Q:** I am sure somebody has looked at brain atrophy in other patients with BMTs, which would give you an idea of what the toxicity of the chemotherapy and the preparation for BMT is in other autoimmune diseases.

**Dr Bar-Or:** Dr Richert may want to comment on this, but first of all, the regimen, the particular autologous regimen that we use, is one that is not very commonly used. It is rarely used because it is a highly ablative. Many of the other regimens called “immune ablative” are actually “subimmune ablative.” And thus, we actually do not have a good control, and this is one of the issues that is being actively pursued, whether it is other autoimmune disease—myasthenic, for instance—or cancer populations with autologous BMT.

**Dr Richert:** Most of your patients for BMT had SPMS. Is that correct?

**Dr Bar-Or:** No. In fact, in our cohort there were 2 patients with SPMS and the others had very aggressive RRMS.

**Dr Richert:** I ask because atrophy and clinical disability continue in the SPMS population even in the absence of further inflammation. However, you have shown a disconnection between disability and atrophy. The rate of atrophy is extraordinarily high, but clinically these patients are relatively stable.

**Dr Bar-Or:** Dr Richert is implying that what I was showing is a disconnection between ongoing atrophy and stable Expanded Disability Status Scale (EDSS). However, I think the measure of change in EDSS at that level is very insensitive (6–6.5 for example). Also, atrophy may be affecting cognition, which is not measured. Thus, I would be very cautious not to overinterpret the EDSS data. What we can say is the patients are not continuing to spiral down. That is all I think we can say. But, I remain concerned about this atrophy, and you are right, whether we want to call it inflammation of the compartmentalized type, if there is activated microglial response and toll-like receptor stimulation, some would consider that very much inflammation. Others would say if you do not have a T cell it cannot be called inflammation. Of course, the additional discussion, which will be elaborated on, relates to actual neurobiologic degeneration.

**REFERENCES**