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The immuno-pathophysiology of multiple sclerosis

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Overview

The etiology of multiple sclerosis (MS) remains elusive. However, clues to its pathogenesis have traditionally been derived from the basic pathologic characterization of central nervous system (CNS) tissues of MS patients. While ongoing debate lingers over the autoimmune nature of MS, it is well established that the immune system directly participates in the destruction of myelin and nervous cells. Understanding the mechanisms of immune-mediated destruction of CNS components in MS promises to not only promote effective design of MS therapeutics, but also provides a broader understanding of immune-mediated diseases affecting the CNS.

This review will explore the principle features of the immuno-pathology of MS, in particular of relapsing-remitting MS (RR-MS). Herein, emerging concepts in the pathogenesis of MS in the context of known features of pathology have been highlighted. These include the characterization of cytokine networks promoting inflammatory damage of the CNS, B cell involvement, and inflammatory damage of axons and neurons. An effort has been made to preferentially focus on MS rather than animal models of the disease, such as experimental autoimmune encephalomyelitis (EAE). For a more comprehensive examination of data derived from studies on animal models, readers are referred to other outstanding reviews [52,53]. From human studies alone, it is clear that the past 5–10 years have been highly productive in advancing the understanding of the pathogenesis of MS. For both the general neurologist and MS specialist, fundamental appreciation of the immunopathology of MS should lead to a broader understanding of the disease course and the emerging MS therapeutics that are now more than ever tailored to the intricacies of the immune system.

Pathologic characterization of MS lesions

Plaques of inflammatory demyelination within the CNS are the pathologic hallmark of MS [29,32,47]. Myelin destruction is an essential element of the plaque. Yet the MS plaque is not simply a static entity of myelin loss in isolation; rather, lesions are comprised of a wide variation of immunologic and pathologic features. These features have been categorized in an effort to understand the neural-immune mechanisms underlying MS. Constructing a

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framework around which myelin loss and neuronal/axonal injury occur entails a close examination of the cellular and molecular constituents, timing of damage, and repair processes. Thus, the various features of plaques provide a platform on which hypotheses regarding pathogenic mechanisms underlying MS have been formulated for over a century.

Traditionally, MS plaque classification has been based on temporal progression, or stages, of inflammatory destruction. Accordingly, acute, chronic active, and chronic silent lesions are thought to occur along a continuous timeline, eventually producing the scarred and hardened areas within the CNS that can be appreciated grossly (FIGURE 1). The acute MS plaque represents the earliest stage of lesion formation. It is typified by robust inflammatory infiltration combined with demyelination distributed throughout the lesion [47,54,98] (FIGURE 1). Typical features of the acute plaque include ill-defined margins of myelin loss, infiltration of immune cells and parenchymal edema [47]. The constituents of immune cell influx centered around vessels (termed perivascular cuffing) include lymphocytes (predominantly T cells), monocytes and macrophages. A portion of major histocompatibility class II (MHCII)-expressing cells, distributed evenly throughout the lesion, are loaded with lipids (foamy macrophages; FIGURE 1) and participate in active stripping of myelin from axons. While oligodendrocyte apoptosis has been observed [61], the degree of oligodendrocyte loss within active lesions can be variable [54]. In spite of the relative degree of axonal sparing, axonal injury can be extensive in acute lesions (see section below; [129]). Glial reactivity throughout the lesion is noted, particularly hypertrophic astrocytes. However, dense glial scarring is not typical of the acute plaque.

The chronic plaque is characterized by a region of hypocellularity with loss of myelin and glial scarring. On gross examination of post-mortem tissue, the hardened and discolored appearance of chronic plaques is often appreciable in frequently targeted areas of the CNS (e.g. the corpus callosum; FIGURE 1). Histologically, the lesion borders of chronic plaques are more distinct than those of acute plaques (FIGURE 1). A division into two forms of chronic plaque is made to signify temporal evolution from active destruction at the edge of the lesion (chronic active plaque) to an entirely “burned out” lesion devoid of active inflammatory destruction (chronic silent plaque). In chronic active lesions, inflammation continues along the outer border with the histologic appearance comparable to acute lesions [54]. Thus, borders of the chronic active plaque are populated with activated microglia and macrophages, vessels demonstrating perivascular cuffing, and reactive astrocytes. The presence of antibody and complement are more prominent in chronic active lesions. Areas of re-myelination are often observed on the edge of lesions, but can encompass the entire of lesion [77]. The core of the chronic plaque is typically hypocellular, though, and often contains thickened vessels with enlarged perivascular spaces. Chronic silent lesions are characterized by loss of the inflammatory traits along the border of chronic active lesions. Remyelination and the presence of oligodendrocyte progenitors are uncommon [136]. Essentially, the chronic silent plaque is “burned out”, containing minimal inflammation and devoid of the active inflammatory border. This is accompanied by a complete loss of oligodendrocytes and a variable but demonstrable reduction in axonal density (FIGURE 1). Overall, the gross and histologic features of the MS plaque imply a complex progression of inflammatory damage culminating in a scarred region of demyelination.

Extensive variability across MS plaques exists, including different degrees of inflammation, demyelination, re-myelination and axonal injury. Controversy has emerged in the “taxonomy” by which lesions are grouped, possibly in part as a result of studying a combination of autopsy and biopsy tissues at different stages of the disease. A relatively recent attempt was made to re-define the process of categorizing MS lesions by proposing a system for classification based on suspected pathologic mechanisms. Alongside a temporally-based system, Lucchinetti et al described various types of lesions based on the

pattern of leukocyte markers, myelin proteins, immunoglobulin and complement within lesions [79]. From 83 pathologic specimens obtained at autopsy or from biopsy, four distinct patterns of immunologic and pathologic features of actively demyelinating lesions were discerned, patterns II and III being the most common. Pattern I is characterized by the predominance of T cell and macrophage inflammatory content; pattern II is characterized by T cell and macrophage infiltration, along with accentuated immunoglobulin deposition and myelin degradation products within macrophages; pattern III is distinguished by pronounced oligodendrocyte loss at the active edge of the lesion and preferential loss of myelin-associated glycoprotein (MAG); and pattern IV is characterized by oligodendrocyte dystrophy and the absence of remyelination or shadow plaques. Importantly, these distinct patterns were consistently observed within samples from the same patient, but varied between patients. The authors, based on the results from one open-label clinical trial in patients who had brain biopsies, hypothesized that deficit recovery after plasma exchange was indicative of the individual pattern of MS pathology. Plasma exchange was effective in patients with biopsy-proven pattern II (with prominent antibody and complement involvement) but not for patients with pattern I or III lesions [66]. Overall, categorization of MS lesions based on immuno-pathologic mechanisms has added to the complexity of paradigms for lesion classification.

While the thought that distinct pathologic processes underlie a singular clinical entity referred to as MS is attractive, this concept has recently been disputed. In particular, challenges have emerged to the idea that there is a clear delineation between the different patterns of pathology [106]. Prineas and colleagues have reported acute lesions from the same patient consisting of features from several of the four pathologic patterns [12]. Furthermore, in a recent study, 131 tissue specimens from 39 MS patients all had lesions with little inter-individual variation in pathologic features. Indeed, lesions from this study exhibited traits consistent with inclusion into pattern II (i.e. deposition of complement and antibodies in proximity to macrophages and the absence of oligodendrocyte apoptosis) [22]. Therefore, various pathologic patterns of disease may more precisely refer to the stage of lesion; the relevance of the classification system based on four individual subtypes of plaques remains to be confirmed.

The timing of lesion evaluation may be one critical factor in reconciling the conflicting data described above. One hypothesis proposed regarding initial events during plaque genesis is that early intrinsic oligodendrocyte injury leads to the inflammatory damage historically associated with the pathologic features of MS plaques. Thus, toxic insults directly affecting the oligodendrocyte could serve as a trigger for a common immuno-pathologic pathway associated with evolution into later stages of the plaque. That 30 out of 39 cases examined by Breij et al [22] were from patients who were in the progressive phase of disease would suggest that early heterogeneity of lesions could have been missed. Further support of this hypothesis comes from a study in which 26 active lesions were examined from 15 patients at autopsy, 11 of whom were early in the course of their disease [61]. In this study, tissue immediately adjacent to lesion borders showed microscopic evidence of cellular injury without the presence of immune infiltration. Prineas and colleagues speculate that there are possibly toxic factors diffusible to the edge of the lesion resulting in oligodendrocyte fragility, and refer to these areas of initial injury as “pre-phagocytic” [61]. Therefore, a critical question remaining in the field of neuropathology is whether differences exist between borders of acute versus chronic active lesions. Further work is required in order to more clearly identify white matter within the CNS that is undergoing early changes or at risk for doing so and how this relates to features described in more established MS plaques.

Normal appearing white matter

There has been a long-standing interest in early white matter changes in MS, with some of the earliest studies on myelin examining tissue outside of plaque regions that appears grossly unaffected [7,123]. More recently, investigation into normal appearing white matter (NAWM) defined on conventional MRI sequences has been explored extensively using a variety of novel neuro-imaging techniques that show abnormalities in these areas suggestive of decreased myelin integrity and diminished axonal density within non-lesional regions [44,141 27].

Histo-pathologic examination of NAWM in patients with MS also support the concept that areas outside of plaques have immuno-pathologic changes. Microglial activation, T cell infiltration and perivascular cuffing have been reported in NAWM [1]. [72]. Not surprisingly, these features were found more diffusely in cases of progressive MS. As a transition zone, dirty appearing white matter (DAWM) - defined by MRI as having intermediate signal intensity between NAWM and T2 lesions - showed diffuse loss of myelin histologically [88]. Thus, greater involvement of NAWM in MS, associated with the progression of disease, may be related to the extent to which inflammation extends beyond focal lesions [72]. Overall, the extent of white matter abnormalities and the immuno-pathologic features of NAWM have yet to be precisely defined.

Gray matter plaques

MS plaques are not confined to the white matter. Gray matter lesions are detected by MRI and by examination of pathologic specimens [50]. Almost all of the gray matter nuclei within the CNS can be affected, as observed in a cohort of mostly progressive MS patients [51], but out of several regions of the CNS, including motor cortex, the spinal cord and cerebellum are particularly vulnerable, resulting in demyelination in up to 28.8% of the gray matter on average. As might be expected, inflammatory lesions within the gray matter are associated with neuronal loss and transected axons, which are more common in active lesions [99]. While gray matter lesions seem to be more common in patients with progressive forms of MS [72], they can develop early in the disease process and possibly account for some of the disability seen in MS patients that cannot be explained by white matter lesions. For example, lesions located within the gray matter correlate better with cognitive disability than white matter lesions [3].

There are several unique features of pathology associated with gray matter lesions. Histologically, gray matter lesions are less inflammatory with fewer infiltrating T-lymphocytes and microglia/macrophages [99]. Purely cortical gray matter lesions have also been described as lacking complement deposition [23] and blood-brain barrier breakdown [132]. Whether gray matter plaques arise from distinct immunologic mechanisms is unclear at this time. In an attempt to more carefully evaluate the processes involved, cortical lesions have been separated into several categories based on the depth of penetration from the surface into the brain (FIGURE 2). Type I lesions include discernable injury to both white and gray matter; Type II lesions have perivascular areas of demyelination isolated to the cortex; and Type III lesions demonstrate cortical demyelination below the pial surface that often cover several gyri and stop at cortical layers three or four [99]. Others have proposed a fourth category, Type IV, to describe lesions that affect all cortical layers without extending into the white matter [18 19] (FIGURE 2). Type III and IV lesions are the most extensive and difficult to visualize. Although scarce inflammatory cells are found within these lesions, the meninges overlying them contain inflammatory cells that collect in structures resembling ectopic B-cell follicles [72,80]. In support of a role for lymphoid neo-genesis in the pathogenesis of these lesions is that patients with ectopic B-cell follicles (41% of secondary

progressive MS patients) had a more rapid disease progression [80]. More solid determination of the role for ectopic B cell follicle formation in MS will depend upon the reliable detection of cortical lesions by MRI and identification of the immuno-pathologic mechanisms leading to lymphoid neogenesis within the CNS.

The role of T lymphocytes in multiple sclerosis

The lymphocytic presence within plaques and bordering areas suggests that inflammatory destruction in MS is driven by antigen-specific targeting of myelin and other CNS components. In particular, adaptive immune responses by T lymphocytes are thought to mediate injury to myelin and nerves within the CNS during MS. The determination that EAE can be mediated by CD4 T cells has promoted intense investigation into the potential CD4 T cell targets in MS. The relevance of antigenspecific CD4 T cell responses in MS was highlighted by the results of trials using an altered peptide ligand of MBP designed for therapeutic suppression of CD4 T cell responses, which resulted in disease exacerbations in multiple patients[16]. T cells from MS patients can recognize a variety of myelin protein targets, including MBP [100,131], PLP [55], MOG [139] and MOBP [36], among others. Non-myelin T cell antigens have also been described, including αB crystalline [6] and neuronal proteins such as contactin-2 [38]. Further, auto-reactive CD8 T cell are also observed [15]. Although similar frequencies of auto-reactive T cells are found in MS patients and healthy subjects[15,60], myelin-specific T cell avidity [17] and activation profiles [139] appear to be elevated in MS patients.

Newer technologies such as arrays of protein [58], lipid [64] and gene expression [78] have allowed fresh insight into targets that were heretofore unknown. For example, highly expressed genes in MS plaques compared to non-lesional CNS tissue include osteopontin [28]; however, elevated gene expression does not necessarily imply targeting by the adaptive immune response in MS. Genes encoding myelin proteins actually display reduced expression [78]. Thus, while several of these targets have been validated in EAE [28,75], their involvement in MS has yet to be fully demonstrated. Again, it is likely that multiple CNS antigens exist for T cells during the inflammatory targeting in MS; results from array experiments highlight how diverse and extensive these targets may be.

How these T cells become abnormally activated toward CNS antigens remains unclear. A popular concept invoked to explain how T cells become pathogenic in MS patients is that of molecular mimicry [89,119]. Several infectious agents have been postulated to serve as activation triggers for auto-reactive T cells. The most consistently reported one is Epstein Barr virus (EBV) [113]. Other infectious agents may also serve to trigger cross-reactivity with myelin components [124,126]. One advantage of the adaptive immune response in MS lies in the ability to harness the specificity of T cell responses [122]. As such, very discrete immunologic targets of therapy may be available for treating MS, including vaccines [9] and antigen presenting cell (APC)-mediated delivery of antigens [39] designed to induce antigenspecific tolerance. Clearly, antigen-specific therapeutics for MS must address the potentially wide array of T cell targets as well as HLA-specific presentation of antigens intrinsic to the genetic heterogeneity of individuals with MS.

The role of Th17 cells in MS

Since the identification of IL-17 as a novel cytokine in 1993, an intense inquiry into the role of IL-17 in EAE and MS has taken place. Two members of the IL-17 gene family, IL-17A and IL-17F, are expressed by CD4 T cells [87]. In 2003, Cua and colleagues identified IL-23, and not IL-12, as a critical cytokine regulator of EAE [34]. This quickly led to a new pathway for investigation into the immunologic basis for MS after the discovery that IL-23 regulates IL-17 production by CD4 T cells [34,74,82]. Subsequently, IL-17-secreting CD4 T cells have

emerged as a distinct lineage of T helper cells, termed Th17 cells. In EAE, Th17 cells participate in early infiltration of the CNS [109] and alone induce a unique neutrophil-predominant pathology [70]. However, unlike IL-23, neutralization or genetic deletion of IL-17 in EAE does not result in a complete absence of disease [57]. Hence, the direct contribution of Th17 cells during autoimmune demyelination remains unclear.

Human studies have emerged to bolster the relevance of IL-17 to the pathogenesis of MS. A greater proportion of IL-17-secreting cells, but not IFNγ+ CD4 T cells, is found in the CSF of MS patients compared to those with non-inflammatory neurologic diseases [24]. The percentage of IL-17-producing memory CD4 T cells is elevated in peripheral blood from MS patients experiencing relapses [41], suggesting a prominent role for Th17 cells in MS. Further, IL-17 gene expression is up-regulated in lesions of MS patients [78], and Th17 cells are found in perivascular cuffs and borders of active lesions [130], indicating Th17 cells home to areas of inflammatory demyelination (FIGURE 3). These studies clearly highlight the association between Th17 cells and MS immune pathology.

How might Th17 cells contribute to the pathogenesis of MS? *In vitro* mobilization studies suggest that Th17 cells cross the blood brain barrier (BBB) more efficiently than other T cells. IL-17-secreting CD4 T cells are capable of eliciting damage to the BBB [65], which would promote greater influx of other inflammatory cells. Once present in the CNS parenchyma, Th17 cells are capable of mediating injury via recruitment of PMNs [70] and monocytes, along with co-production of other cytokines, such as IL-22 and IL-21, [120]. Additionally, Th17 cells are more adept at killing human neurons *in vitro* than un-activated T cells, providing enticing data to suggest that Th17 cells are potential mediators of axonal and neuronal damage in MS lesions [65]. These initial studies support the idea that Th17 cells may be critical mediators of immune destruction of myelin and axons during MS.

Recent data highlight the potential for targeting Th17 cells in MS. CD4 T cells from healthy individuals skewed to produce IL-17 are more responsive to one current form of treatment for MS, IFNβ, than those from MS patients [41]. Further, IFNβ can inhibit the differentiation of naive CD4 T cells into Th17 cells [107]. Patient responses to IFNβ may be related to the level of IL-17 prior to the onset of therapy [5]. This result re-emphasizes the complexity and heterogeneity of the disease; in particular, the response to current therapies may signify differences in the pathogenesis of MS between patients [67]. The Th17 pathway offers a new therapeutic target, including the molecular pathways that regulate IL-17 [40]. However, consistent with the complexity of cytokine networks and regulation, a singular stance that IL-17 is purely pathogenic is likely to be an oversimplification. In particular, IL-17 may participate in limiting tissue destruction during an inflammatory response [93]. Overall, evidence is mounting to suggest that Th17 cells are present during the inflammatory destruction of tissue in MS, but questions remain about the direct contribution of this newly identified lineage of CD4 T cells. At this point, it is reasonable to be inclusive of Th17 cells in the pathogenesis of MS (FIGURE 4), but to what degree they are involved remains to be shown.

B cell-mediated CNS damage in MS

Evidence gathered from examination of CNS tissue implicates the role of antibodies during the pathogenesis of MS. As noted, the presence of plasma cells, Ig and complement is a typical feature of the MS plaque [47,79]. Naturally, this observation has prompted consideration over whether Ig present within plaques specifically targets myelin antigens. Molecular features of B cells found within MS plaques demonstrate that B cell receptor genes are modified in a specific way that indicates their evolving response toward specific targets [10,96]. Further specificity of Ig from MS tissue has been described. MBP-reactive

[135,137] and MOG-reactive [92] Ig has been isolated from CNS tissue of patients with MS. Additionally, *in situ* deposition of MOG-specific antibodies has been detected in MS lesions, along with MOG- and MBP-specific Ig complexed with myelin within macrophages [49]. In contrast, detection of Ig appeared non-specifically dispersed throughout cellular constituents of plaques and NAWM, without significant differences compared to control tissues, suggesting that Ig presence may be secondary to white matter injury rather than antigen-specific B cell activation unique to MS [11]. Thus, while controversy persists as to the antigen-specific nature of B cell involvement in MS, the presence of plasma cells and Ig continues to fuel investigation into their involvement during MS plaque development.

As a representation of B cell involvement in CNS parenchymal damage of MS patients, CSF analysis has afforded several clues on the role of Ig in the pathogenesis of MS. The localized intrathecal production of Ig, referred to as oligoclonal bands (OCB), is detected in over 90% of patients with RRMS [46], and their absence is associated with reduced severity of disease [4]. While OCB are thought to be a product of clonally-expanded B cells within the CSF compartment, their potential targets remain elusive. Plasma cells from the CSF of patients with inflammatory demyelinating disease produce antibodies that are capable of binding myelin [134] and recognizing MBP [73]. However, a more recent study of the CSF of patients with MS failed to detect IgG from CSF clonal B cells that bound to MBP, PLP or MOG [95]. Analysis of OCB has also included investigation of IgM, which is found in a subset of MS patients [133]. CSF-restricted IgM isolated from MS patients have been found to target a variety of lipid antigens, predominantly phosphatidylcholine [133]. These persistent lipid-specific IgM OCBs are associated with more aggressive disease [20,133], and might be associated with a poor response to interferon therapy [21]. Taken as a whole, a multitude of potential targets, including those from myelin as well as other CNS antigens[83], have been proposed and investigated in patients with MS (Reviewed by Reindl and colleagues [110]).

Modulation of T cell function may be an equally important function of B cells in the immune dysregulation in MS patients. As noted, anti-CD20 monoclonal antibody targeted depletion of B cells is efficacious for the treatment of RR-MS [59]. This appears independent of antibody effects, since anti-CD20 treatment does not directly target Ig-secreting plasma cells [46]; results in early efficacy in relapse reduction and inflammatory MRI lesions [59]; and does not alter CSF Ig levels [33]. B cells may promote neuro-inflammation in MS via direct and indirect effects on T cells, such as the secretion of the pro-inflammatory cytokines. For example, B cells from MS patients produce more TNF-α and lymphotoxin in the presence of the T cell-derived pro-inflammatory cytokine IFN-γ compared to healthy controls [8]. Following anti-CD20-mediated B cell depletion in RRMS patients T cells produced less IFN-γ and were less proliferative in response to TCR engagement [8]. Conversely, B cells are likely to also have immune-suppressive traits that are important in the immuno-pathogenesis of MS. For example, IL-10 secretion by B cells can serve to limit pro-inflammatory auto-reactive CD4 T cell responses [45].

Finally, recent evidence suggests a role for B cells in the generation of ectopic follicles in MS. Building on observations of Prineas [105], Aloisi and colleagues have described the presence of B cell follicles within the meninges in patients with SPMS [115]. These are characterized by features of germinal centers, including the presence of B cells, follicular dendritic cells (FDCs), and CXCL13 (a chemokine involved in genesis of lymphoid organs). Found in approximately 40% of autopsy specimens only from patients with SPMS [80], the presence of these follicles was purported to be a maker for cortical lesions immediately adjacent to the ectopic follicle within the meninges. This finding has been disputed in subsequent work that failed to detect meningeal follicles in 12 patients with SPMS [68]. Interestingly, there may be an association between the presence of EBV infectious material and secondary lymphoid follicles observed in MS patients [114]. The implication of this

finding is that latent infection of B cells with EBV drives the expansion and maturation of B cells along with ectopic follicle generation within the CNS, promoting intrathecal Ig production and the targeted destruction of underlying myelin in this region [46]. While only observed in patients with SPMS, it is possible that the generation of these lymphoid organ-like structures begins during the relapsing-remitting phase and evolves over time. Whether these structures are inhibited by B cell-depleting therapy in MS remains to be investigated.

Mechanisms of leukocyte entry during MS

Immune access to the CNS is generally considered restricted. In practice, the traditional view of the CNS as an immune privileged site has been replaced with the more appropriate characterization of the CNS as an immune specialized organ [108]. Thus, one key element to immune-mediated damage within the CNS during MS is the process by which immune cells are able to gain access to this specialized compartment. Within the context of universal processes governing immune cell trafficking, there are features that are relatively specific for leukocyte migration to, and within, the brain and spinal cord in health and disease. The molecular components, location, and timing of migration are all important factors during the immuno-pathogenesis of MS.

The BBB serves to actively restrict cellular and macromolecular movement between the blood and CNS tissue. Adequate function of the BBB depends upon several unique anatomic and cellular features, including tight junctions between endothelial cells, specialized expression of molecular transporters, and placement of immune cells within the CNS relative to the vasculature [35]. Only by engaging in a critically timed sequence of events are auto-reactive lymphocytes able to enter the CNS compartment. Initially, leukocytes engage in rolling, activation and arrest to the endothelium of the BBB. This is greatly facilitated by up-regulation of adhesion molecules by the vasculature, including ICAM1 and VCAM1 [102]. While a trigger for vascular change remains unclear in MS, hypothetically changes in the vascular endothelium could result from pro-inflammatory mediators circulating within the vasculature, including TNF and/or LPS. Subsequently, migration of cells through and between endothelial cells takes place [63]. Eventually, concentrated extravasation of immune cells in perivascular cuffs within the CNS parenchyma culminates in a breach of the BBB that is an essential component to the process of inflammatory destruction of white matter in MS [54].

The complex set of molecules that leukocytes depend upon for entry into CNS tissues involves integrins. Integrins are hetero-dimeric cell surface molecules that mediate adhesion between cells. Out of a panel of leukocyte adhesion receptors, the α4 subunit of VLA-4 was identified as a crucial factor for encephalitogenic T cell binding to CNS endothelium. Blockade of α4β1 engagement with one of its binding partners, VCAM-1, successfully abrogated disease in an animal model of MS [138] Clinical trials of a humanized monoclonal antibody targeting the α4 subunit of VLA- 4, called natalizumab, also demonstrated efficacy in the treatment of MS [104,112]. Hence, selective inhibition of specific adhesion molecules are effective at reducing leukocyte entry into the CNS. Of note, natalizumab reduces the influx of a wide range of leukocytes, including T cells and dendritic cells (DCs) [37]. In addition to VLA-4, other trafficking molecules impart specificity of migration into the CNS. Recently, ALCAM-1 was shown to be localized to the BBB and up-regulated in active MS lesions [26]. In experimental animal systems, blockade of ALCAM-1 delayed disease [26]. In addition, osteopontin also serves as a binding partner for VLA-4 and potentially serves as a separate target for reducing leukocyte migration into the CNS of patients with MS [121]. Hence, a multitude of adhesion molecules participate in effective leukocyte trafficking to and within the CNS and serve as potential targets for therapies in MS.

Chemokines, a broad class of cytokines mediating chemotaxis, also contribute to leukocyte migration to the CNS. Several chemokines and their receptors have been implicated in leukocyte influx to the CNS in MS [63]. For example, CXCL12, constitutively expressed in the CNS, is typically localized to the basolateral aspect of the CNS microvasculature and functions to retain leukocytes within the perivascular space. Redistribution of CXCL12 to the luminal aspect of vessels was observed in autopsy specimens from MS patients, which would allow for the dissemination of lymphocytes into the CNS parenchyma [84]. Other chemokines are thought to participate in the recruitment of lymphocytes into the CNS in MS. Recent work in the EAE system has demonstrated that the initial wave of T cell infiltration into the CNS prior to disease onset is CCR6-dependent. [109]. Furthermore, in EAE, the initial wave of inflammatory CD4 T cells express IL-17 and are potentially recruited specifically via CCR6 into the CNS via the choroids plexus that expresses the CCR6 ligand, CCL20. These results have yet to be convincingly replicated in human samples. Several other chemokine and chemokine receptors expressed by various cell types in MS show dysregulation including CCR7, CCL19 and CCL21, CCR5 [97,127]. Thus, there may be a unique chemokine signature at different phases of disease and in different regions of the CNS in order for various leukocytes to localize to the CNS during MS.

The role of antigen presenting cells in MS

Antigen presenting cells (APCs) process and present antigens to T cells, and in the context of MHC, co-stimulatory signals and cytokine secretion, drive adaptive immune responses [30]. Experimental evidence based on animal models has shown that antigen-specific encounters within the CNS between T cells and APCs is crucial to the unfolding of MS. In EAE, without newly generated myelin antigens from the CNS by APCs, inflammatory demyelination does not proceed, even in the presence of myelin-reactive CD4 T cells [116,128]. Thus, CD4 T cell-mediated disease is thought to be a multi-stage process, involving the initial activation of auto-reactive CD4 T cells, as well as reactivation within the CNS immune compartment. DCs are thought to be the major APC during the secondary phase of cognate interactions with CD4 T cells within the CNS [14,56]. Perivascular spaces within areas bordering edges of active lesions contain immnostaining for CD209, a marker for a subset of DCs, suggesting that antigen presentation by DCs at the interface of the BBB contributes to the earliest inflammatory processes promoting lesion formation [61]. Visualization of APC interactions with encephalitogenic CD4 T cells using intravital microscopy in a rat model of MS suggests that primed CD4 T cells actively engage with these perivascular APCs en route to entry within the CNS [13].

In addition to DCs, other APCs likely play an important role in antigen presentation during the pathogenesis of MS. Microglia are hematogenously-derived resident APCs within the CNS. Upon activation, they express greater amounts of MHCII and co-stimulatory molecules [2], signifying a greater capacity to promote pro-inflammatory T cell responses within the CNS. Activated microglia are localized to active plaques [76]. Experimentally, impeding microglial function attenuates EAE [62]. However, relative to other professional APCs, microglia are not as potent at inducing auto-reactive T cell responses [85] and may even down-regulate CD4 T cell functions [94]. Thus, as resident APCs within the CNS, microglia are capable of performing APC functions, but likely are not the lynchpin for driving autoimmunity of the CNS in MS. Another APC potentially involved in driving myelin-reactive CD4 T cells in MS is the B cell. As already mentioned, recent work suggests that B cells play a prominent role in the pathogenesis of MS [46,59], and potentially play important roles in antigen presentation to T cells. In addition to effects on T cells, MHCII-dependent interactions with B cells promotes Ig class switching from IgM to IgG. Thus, antigen-specific interactions between B cells and T cells represent a critical step in the generation of Ig responses in MS. It is important to acknowledge that not all interactions

between APCs and T cells promote inflammation. In addition to the effects of regulatory B cells mentioned above, APCs can also engender anti-inflammatory responses [48]. For example, suppressor myeloid cells are generated after EAE induction and are capable of suppressing T cell function [140]. In MS, the process of myeloid suppression is thought to be regulated in part by TREM-2, a trans-membrane signaling protein expressed by microglial cells, macrophages, monocytes and DCs [101]. This mechanism may be dysregulated by secretion of soluble TREM-2, which could act as a decoy receptor and prevent inhibitory function of transmembrane TREM-2 [101].

Axonal and neuronal damage in MS

Inflammatory CNS injury in MS has increasingly been associated with axonal damage. Although MS has classically been described as a disease marked by the loss of myelin in greater proportion to the loss of axons, axonal damage was noted in the earliest pathological descriptions of MS lesions [29]. Modern techniques have allowed for precise demonstrations of axonal damage. Antibodies directed at amyloid precursor protein show damaged axons in active areas of MS lesions [42]. Representing a major advance in the field of MS pathology, Trapp *et al* [129] were able to directly view and quantify transected axons using confocal microscopy by counting axonal ovoids at the ends of transected axons. The active areas of MS lesions were found to contain more transected axons than inactive areas in more chronic lesions. Of note, comparisons of biopsy and autopsy samples from patients with relapsing-remitting, secondary progressive, and primary progressive MS suggest that axonal pathology is greatest within the first year of disease onset, particularly in patients with RRMS [71]. These studies propose that axonal pathology occurs in areas of active inflammatory demyelination and early during the course of disease.

In addition, a slower rate of axonal damage is also thought to occur and contribute to the clinical decline observed in MS patients. Trapp *et al* [129] and Kornek *et al* [69] showed that axonal ovoids are more common in inactive demyelinated lesions and in NAWM than in the white matter of control patients. However, remyelinated inactive lesions, or shadow plaques, have the same number of abnormal axons as control tissue [69]. Patients with higher levels of motor disability have fewer surviving corticospinal axons traveling through their spinal cord, demonstrating a direct correlation of axonal damage and disease progression [125].

The mechanisms involved in axonal damage in MS are under intense investigation. CD8+ T-lymphocytes can cause axonal damage via the release of cytotoxic granules, induction of apoptosis through activation of surface receptors such as Fas, the release of cytokines such as TNF-α, or direct transection of axons [86,91]. Macrophages/microglia are also found in close proximity to damaged neurons. Release of toxic molecules by these cells such as proteases and reactive nitrogen species can cause oligodendrocyte injury, demyelination, and axonal degeneration, disrupt the blood-brain barrier, and contribute to the loss of axonal conduction. [118]. Axonal damage also occurs by activation of other components of the innate immune response such as Toll-like receptors [43]. Toll-like receptor 2 is over-expressed by oligodendrocytes in MS lesions where it inhibits remyelination [117]. Antibody-mediated injury to axonal components, e.g. neurofascin, can result in axonal and neuronal dysfunction [83]. As a consequence of immune injury to myelin, higher energy demands on demyelinated axons and glutamate-mediated excitotoxicity may further impart unsustainable damage [81 103]. Overall, axonal injury in MS is likely mediated by multiple mechanisms at play in both active and chronic lesions.

Neuronal loss in MS can be severe and occurs throughout the brain. Neuronal loss in the range of 18–35% has been reported in the cortex, hippocampus, thalamus, and spinal cord (reviewed in [18]). Damage to axons and neurons has been evaluated *in vivo* using MRI

techniques such as quantitative proton MR spectroscopy. In patients presenting with their first clinical attack of MS, the amount of NAA in the whole brain is already decreased, indicating early neuronal damage [111]. This reduction is still present one year later and was independent of whether the patients had progressed to develop MS. Other MRI techniques, such as measurement of brain volume or diffusion tensor imaging, have provided more variable results in evaluating axonal and neuronal damage especially in short term studies as factors such as demyelination and edema can confound the results. However, examination of neuronal integrity using OCT shows a loss of macular volume in patients with progressive forms of MS, which correlates with poor visual acuity especially in patients with a history of optic neuritis [25]. Similar to axonal injury, the processes resulting in neuronal loss in MS are likely several-fold. Direct immune injury to the gray matter can result in a loss of neurons, as within gray matter lesions a significant increase in apoptotic neurons was observed primarily in large pyramidal cortical neurons [99]. However, in layer II of primary motor cortex from NAWM, paralbumin interneurons were more affected than other neurons that are relatively spared in MS patients [31]. This differential susceptibility of neurons exposed to the same insult is part of a key consideration in how clinical deterioration, particularly with secondary progression, is related to repeated accumulation of axonal or neuronal damage to various neuronal populations. Further, axonal and neuronal survival may be directly tied to the trophic support provided by myelin, which may be particularly relevant during a high metabolic demand state of neurons exposed to inflammatory stressors [90].

Conclusions

In summary, several new features of cellular and molecular immunity have added to the understanding of the pathology of MS. These include the role of B cells, including antibody-dependent and antibody-independent mechanisms; the extent of axonal and neuronal injury; the contribution of a new lineage of CD4 T cells identified by the production of IL-17; leukocyte trafficking mechanisms to the CNS; and new lymphocyte targets during disease. These stand out among many other recent developments that due to space limitations were not able to be covered in this review. Topics involving resolution of inflammation in MS lesions, suppressor cells (Treg, CD8 T cells, etc.) and remyelination are bound to be important in driving toward a more comprehensive understanding of the pathogenesis of MS. Overall, an attempt has been made not to detail every mechanism involved in the pathology of MS, but rather highlight the features of disease that are under current study (FIGURE 4).

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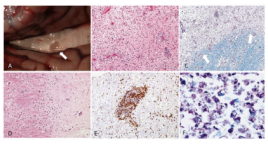

Figure 1.
Gross and histologic features of MS plaques. (A) Gross examination of the brain at autopsy of a 79 year old with RR-MS. A dorsal view of the corpus callosum after separation of the cerebral hemispheres reveals a hardened, discolored area within the body of the corpus callosum (arrow). Photo courtesy of Dr. Robert Schmidt. (B) Acute MS plaque stained with Hematoxylin and Eosin revealing hypercellularity due to the perivascular and parenchymal infiltration of leukocytes. (C) Luxol fast blue/periodic acid–Schiff (LFB-PAS) stained section of a plaque margin reveals a blurred but discrete edge (arrows). (D) Inactive plaque demonstrating borders that are distinct and devoid of inflammation (E) CD3 + lymphocytes clustering in a perivascular cuff within an active lesion area (F) Foamy macrophages characterized by fragments of myelin (arrow) engulfed by macrophages are identified using LFB-PAS at a plaque margin. Images in B-F used with permission; [98] (copyright Elsevier, 2010).

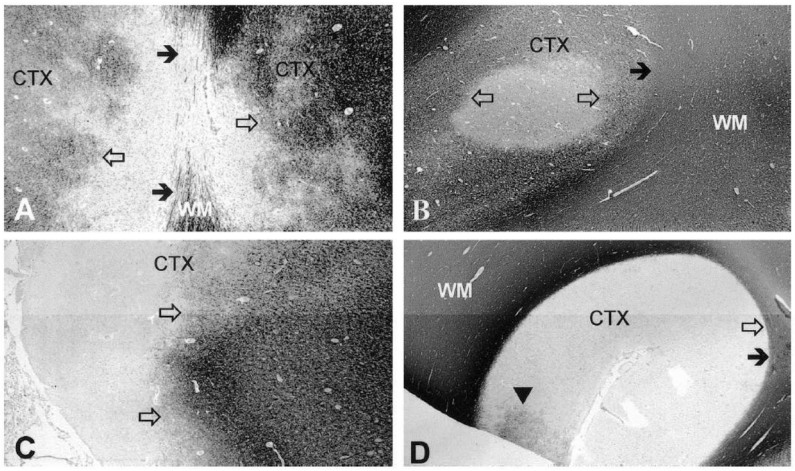

Figure 2.

Cortical lesions in MS revealed by sections immunohistochemically labeled for MBP. (A) Type 1 cortical lesions encompass both white and gray matter. Solid arrows indicate the cortex (CTX)/white matter (WM) border, while the lesion border is delineated by open arrows. (B) Type 2 lesions are contained entirely within the CTX and do not extend to the subcortical WM (solid arrow) or pial surface. The border of this type 2 lesion is indicated by the open arrows. (C) Type III cortical lesions represent cortical demyelination below the pial surface that often cover several gyri and stop at cortical layers three or four. Open arrows indicate the lesion border. (D) Type 4 lesions (border represented by filled arrow) span the entirety of the CTX without involvement of WM (solid arrow). The arrowhead indicates a small area likely undergoing remyelination. Figure from [19] used with permission (copyright Wolters Kluwer Health, 2003).

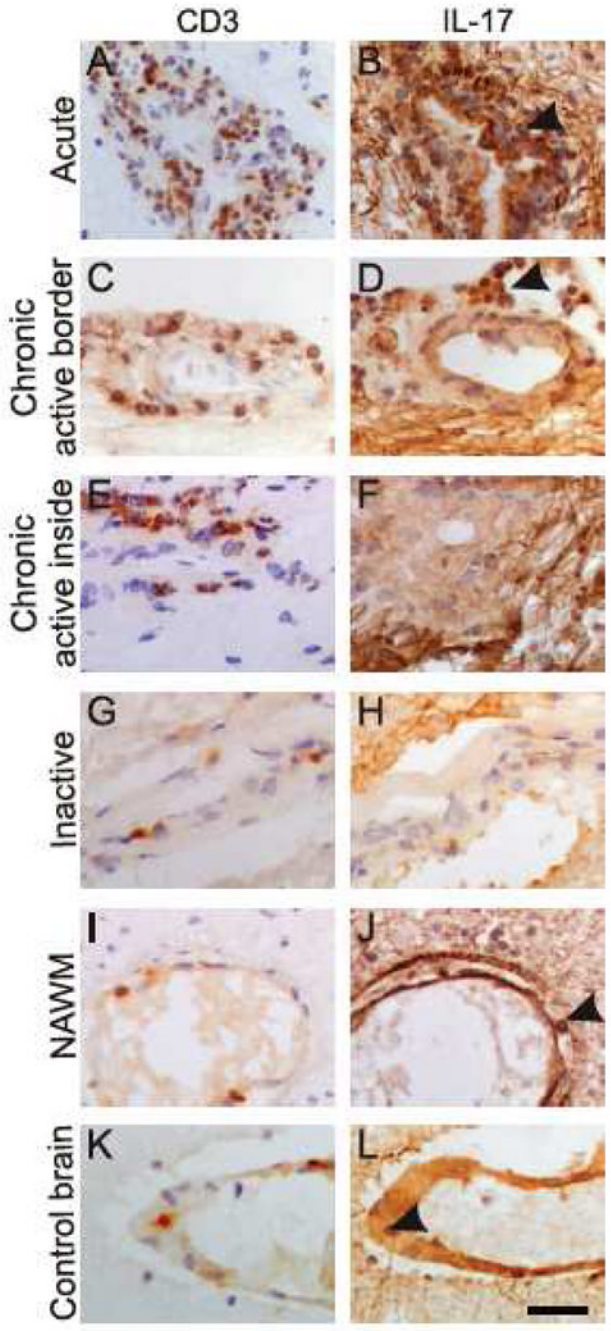

Figure 3.

Involvement of IL-17 in MS lesions. Immunohistochemical identification of CD3 (left column) and IL-17 (right column) in consecutive sections. Abundant IL- 17 staining (arrowhead) is observed in perivascular cells in both acute (A & B) and chronic active (C &D) lesions. (E & F) Whereas CD3+ cells do not co-localize with IL- 17 staining within the internal region of a chronic lesion, the fibrillary pattern of IL- 17 immuno-reactivity is representative of possible astrocyte production of IL-17. Minimal staining is observed in tissue from an inactive lesion (G & H). Sparse Th17 cells are observed in NAWM in MS or within control tissue (arrow heads, I-L). Scale bar = 30 μm. Reprinted from Am J Pathol

2008, 172: 146-155 [130] with permission from the American Society for Investigative Pathology.

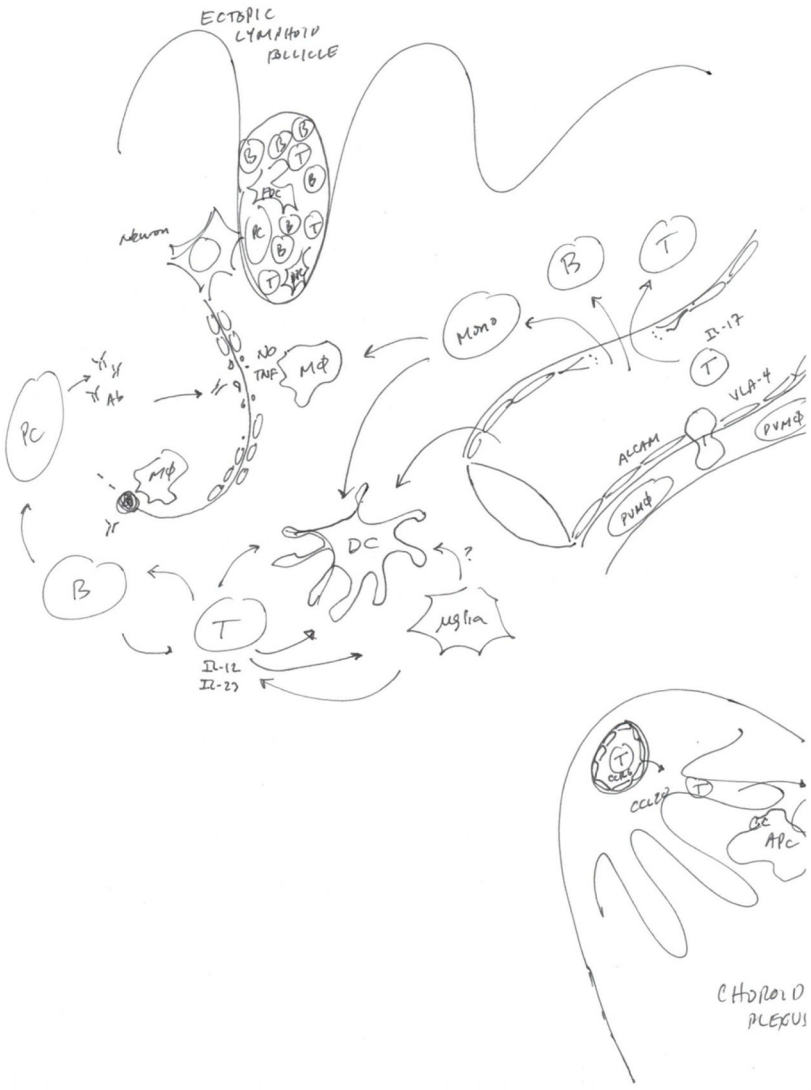

Figure 4.

Cellular and molecular factors involved in the immuno-pathogenesis of MS. Rather than representing an all-inclusive summary of the immuno-pathologic features of MS, this diagram highlights recent advances in the understanding of the neural-immune interactions in MS, including factors involved in leukocyte trafficking, axonal injury and antigen presentation. APC = antigen presenting cell; B = B lymphocyte; T = T lymphocyte; Mφ = macrophage, PVMφ = perivascular macrophage; PC = plasma cell; FDC = follicular DC; μglia = microglia; pDC = plasmacytoid DC.