Pathogenesis of multiple sclerosis: an update on immunology

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Multiple sclerosis is characterized by demyelination and chronic inflammation of the central nervous system. Extensive studies in the animal model experimental autoimmune encephalomyelitis have suggested that multiple sclerosis is an autoimmune disorder mediated by myelin-specific CD4 T cells secreting T helper type 1 cytokines and tumor necrosis factor alpha. This concept has been widely used to develop new experimental therapies. However, recent findings in both experimental autoimmune encephalomyelitis and multiple sclerosis question a simple CD4 T helper type 1 T cell paradigm and provide evidence for the role of various immune cells in the pathogenesis of experimental autoimmune encephalomyelitis and multiple sclerosis. In this paper we review recent progress and discuss the implications for new therapeutic strategies.

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Abbreviations

EAE	experimental autoimmune encephalitis
CSF	cerebrospinal fluid
CNS	central nervous system
MS	multiple sclerosis
NK	natural killer
TCRBV	T cell receptor variable beta chain

1350-7540

Introduction

The clinical and pathological features of multiple sclerosis (MS) were first described more than a century ago. Early studies pointed out the hallmarks of the disease, including demyelination and chronic inflammation in the central nervous system (CNS) white matter. Clinical, imaging and pathological studies have provided insight into possible disease mechanisms operative in MS pathogenesis. They have clearly demonstrated that in most patients, MS is a disease with acute onset accompanied by active inflammation and demyelination. Although axonal degeneration is seen early during the disease, the extent and chronicity of inflammation in MS is clearly different from primary neurodegenerative disorders or acute brain tissue damage. Despite major research efforts within the past few decades, the etiology of MS is still unknown. However, a variety of recent findings in MS and its animal models have contributed to our understanding of disease pathogenesis, and have provided a basis for new therapeutic concepts.

Experimental autoimmune encephalitis paradigms in multiple sclerosis

Experimental autoimmune encephalomyelitis (EAE) has been one of the most valuable autoimmune models, providing insights into many mechanisms during physiological and pathological immune responses [1]. Classical EAE is induced in susceptible animal strains (i.e. Lewis rats) by immunization with myelin antigens and Freund's adjuvant. The disease is usually monophasic with spontaneous remission. EAE has been extensively characterized during the past two decades providing evidence that it can be transferred by CD4 myelin-specific T cells. The immune dynamics *in vivo* have been elucidated through an elegant approach with genetically altered T cell clones in an adoptive transfer rat model [2••]. The study demonstrates that after cell transfer a priming of T cells in lymph nodes and the spleen is necessary before these cells collectively migrate to the brain, mediate inflammation and subsequently undergo cell death.

Although only a minority of myelin-specific T cell clones can transmit disease those cells were thought to belong to the T helper type 1 subtype characterized by the secretion of IL-2, IFN-γ and TNF-α. Furthermore, the secretion of TNF-α, a proinflammatory cytokine, was considered crucial for the encephalitogenicity of the T cell clones [1]. In contrast, blocking TNF pathways and polarizing T cell responses towards a T helper type 2 phenotype (secretion of IL-4, IL-5, IL-10, and IL-13)

was shown to be of therapeutic benefit in most EAE models. This EAE concept has been applied to the human disease classifying MS as a prototypic T helper type 1 disorder and TNF-α as a key player in the cascade of events leading to demyelination [1].

T helper type 1 cells and beyond

From a variety of studies it has recently become evident that this paradigm is oversimplified in EAE and particularly MS. The first questions arose from genetic animal models. IFN-γ knockout mice develop more severe EAE than wild-type mice, and disruption of the IL-4 gene does not affect the disease course [3•]. Findings in genetic models with altered TNF-α expression were more heterogeneous [3•]. The overexpression of TNF-α ?in the CNS mostly results in spontaneous demyelination or at least in a more severe EAE disease course. Depending on the genetic background of the animals, disruption of the TNF-α gene differentially affects disease susceptibility and severity, although EAE induction is possible in all of the knockout models [3•,4].

In parallel, several studies reported on the encephalitogenicity of T helper type 0 T cells [5], which produce both T helper types 1 and 2 cytokines. The possibility to induce EAE with T helper type 2 T cells was formally demonstrated in a transgenic mouse model. In this animal model disease was induced by the transfer of a large number of T helper type 2 T cells into immunosuppressed mice [6]. Although deviation towards T helper type 2 had been considered to be disease alleviating in EAE, this was not true for all experimental conditions. Likewise, Genain and coworkers [7] demonstrated in an outbred monkey model that a T helper type 2 shift induced by peptide therapy may worsen EAE. However, most therapeutic immune intervention strategies in humans have focussed on silencing CD4 myelin-specific T cells, driving immune responses towards a T helper type 2 phenotype or blocking tumor necrosis factor pathways. Although these strategies were highly efficient in classical EAE models, most of them have failed or even worsened the course of MS [8,9].

CD8 T cells: a novel attractive player

Additional aspects arose from recent studies on the role of CD8 T cells in MS and EAE. The early loss of oligodendrocytes and neurons has been difficult to explain by the CD4 T helper type 1 cell concept. Oligodendrocytes and neurons, which only express MHC class I molecules, cannot present antigens to CD4 T cells. It has been hypothesized that microglial cells, the only cell population expressing MHC class II in the CNS, may present myelin antigens to CD4 T cells, which subsequently damage MHC class II-negative targets via bystander mechanisms (e.g. TNF-α). However, T cells that recognize antigens in the context of MHC class I would be more attractive candidates to play a role in MS pathogenesis [10]. Indeed, it has been shown that CD8 T cells can kill oligodendrocytes [11] and neurons [12]. Furthermore, CD8 T cells can attack neurites in an antigen-specific manner, leading to axonal damage with spheroid formation similar to those seen in MS brain lesions [13••]. This finding, although observed *in vitro*, has received attention in the context of two studies addressing the role of CD8 T cells in MS brain lesions and cerebrospinal fluid (CSF). Babbe and colleagues [14] demonstrated by micromanipulation and single-cell polymerase chain reaction the occurrence of clonal T cell receptor V-beta (TCRBV) expansions in CNS lesions of two MS patients. Interestingly, the clonal expansion mainly involved CD8 T cells. Up to one third of all CD8 T cells in the lesions of one of the patients expressed the same TCRBV sequence. The study presented by Jacobsen and colleagues [15] investigated the TCRBV repertoire in the CSF of 36 MS patients. Similarly, TCRBV expression differences between CSF and blood were primarily observed on CD8 T cells and only rarely on CD4 T cells. The TCRBV expression differences were shown to be stable over time, and involved expansion of T cells with highly similar or identical T cell receptor alpha and beta chains. In both studies, the expanded TCRBV chains were not found among peripheral blood T cells. Both studies formally demonstrate a specific clonal and oligoclonal accumulation of CD8 T cells in the CNS of MS patients. Although the role of these cells in the pathogenesis of MS is unknown, it is likely that they target specific antigens in the brain.

The discussion about the role of CD8 T cells in MS has been further stimulated by recent findings in EAE [16]. In the study by Sun and colleagues [17•] progressive and destructive EAE was induced by purified myelin-specific CD8 T cells. Similarly, Huseby and colleagues [18••] induced EAE by the adoptive transfer of CD8 T cell clones specific for a myelin basic protein peptide. Although disease was induced after irradiation of recipient animals, the adoptive transfer of T cell clones proved the encephalitogenicity of myelin-specific CD8 T cells. Interestingly, in this model, lesions were primarily located in the brain, not in the spinal cord as seen in most EAE models induced by CD4 T cells, and characterized by little inflammation, but massive perivascular cell death and demyelination.

The come-back of the B cell

The potential pathogenic role of antibodies has been discussed since the discovery of the intrathecal immunoglobulin synthesis in MS [19•,20]. Indeed, the occurrence of elevated antibody levels and oligoclonal IgG bands in the CSF is still the only valuable immune

parameter in the diagnosis of MS. The antibody response in MS patients seems to be remarkably stable over long periods of time, suggesting that it is focused on few target antigens without major changes during the disease course. This finding is also reflected by studies investigating the local B cell response in MS patients. Several investigators demonstrated the occurrence of clonal B cell accumulation in the CSF or lesions of MS patients by analysing the heavy-chain variable region genes [19•,21]. All studies identified restricted dominant use or the overexpression of certain heavy-chain variable region genes in the diseased organ compartment. Furthermore, the studies demonstrated somatic hypermutations and replacement mutations in the heavy-chain variable region genes compatible with affinity maturation of the antibody response. Such changes were not or were to a much lesser extent identified in the peripheral blood of MS patients [22]. The findings are consistent with the occurrence of an oligoclonal IgG synthesis in CSF, and suggest that the humoral immune response focuses on a few as yet unknown CNS resident antigens. However, similar to the studies on clonal T cell expansion in the CNS, none of the studies has provided evidence that determines whether this is the result of an ongoing active immune response in the CNS or is only simply caused by resident memory cells. Such information can only be provided by serial analysis of CSF or brain tissue to identify the persistence of clonotypes and ongoing antigen receptor maturation of B cells. Nevertheless, the B cell features in MS are very similar to antibody responses seen in chronic infectious diseases of the CNS, in which the local humoral immune response targets disease-associated antigens derived from the causative agent [23••]. One study addressed the specificity of the local antibody response in MS by engineering clonally expanded heavy and light chain genes from lesions into whole human IgG [24]. By screening expression libraries and oligodendroglioma cell lines the authors found reactivity with double-stranded DNA. However, this finding has not been confirmed by other groups nor validated in other MS patients.

The general role of antibodies for disease progression has been addressed in EAE and MS. Iglesias and colleagues [25] have clearly shown that myelin-specific antibodies can enhance disease severity in EAE. Similarly, it seems that high intrathecal antibody production is a negative predictor for disease progression [26]. More recently, Cepok and colleagues [27•] provided evidence for a possible correlation between intrathecal B cell dominance and disease progression.

Regulatory cells and their therapeutic potential

Besides a potentially harmful T and B cell response, it is likely that regulatory immune responses play a significant role in the development of MS. In particular, natural killer (NK) T cells have received attention in studies on MS and EAE. NK T cells constitute a population of T cells that share surface markers and characteristics with NK cells [28]. The cell population expresses a heavily biased T cell receptor repertoire (Vα14-Jα18, Vβ2, 7, or 8.2 in mice and Vα24-Jα15, Vβ11 in humans) and usually recognizes lipid antigens in the context of the CD1d molecule. NK T cells rapidly release large amounts of cytokines including IL-4 and IFN-γ, and play an important regulatory role by skewing immune responses towards T helper type 2 cells. These cells, which are thought to be involved in the pathogenesis of several candidate autoimmune diseases [28], are also found in the peripheral blood [29] and lesions of MS patients [30]. Extensive studies were performed in EAE to address the impact of NK T cells on the disease course [31,32,33••,34•,35•]. Most studies demonstrated that activation of NK T cells by administration of the glycosphingolipid α-galactosylceramide can prevent or ameliorate EAE. However, the timing of administration seems to be important because the co-administration of glycosphingolipid α-galactosylceramide at the time of immunization not affect EAE [34•]. The therapeutic potency of glycosphingolipids is of particular importance because these ligands can also activate human NK T cells, thus providing a promising approach to treat MS. Moreover, following the altered peptide ligand concept with peptide antigens, altered glycosphingolipids have been designed that preferentially induce T helper type 2 cytokines [33••]. Although the concept of treating MS by enhancing regulatory T cells seems attractive, evidence from human studies is necessary to address the role of NK T cells in MS pathogenesis and provide evidence that driving immune responses towards a T helper type 2 response is beneficial.

Mechanisms in the local environment

The introduction of new technologies has allowed dissection of the histology and molecular biology of MS lesions. The discovery of heterogeneity in demyelinating lesions has suggested that different mechanisms may be involved in MS pathogenesis [36]. This observation may be important for future studies on the etiology and therapy of disease [37]. However, the potential to apply these findings to the clinic will rely on the development of technologies that allow the stratification of MS subtypes without being dependent on brain biopsies [27•,38•]. The use of new technologies also allowed dissection of the local immune response in MS brain lesions. The studies contributed significantly to the discovery of clonal T and B cell expansions (see above), and provided information about the local environment in EAE and MS lesions. The first studies using immunohistochemistry and the quantitative polymerase chain reaction technique demonstrated that a

variety of cytokines are upregulated in MS brain lesions, among them T helper types 1 and 2 cytokines [39,40]. The findings were extended by microarray studies on RNA expression levels, comparing lesions with normal appearing white matter in MS and EAE [41–43]. Those studies demonstrated the complexity of gene regulation in MS lesions. Further investigations are necessary and need to be complemented by other approaches to evaluate the role of the identified genes in disease pathogenesis. In addition, microarray technology has reached clinical studies to monitor immune changes after immunomodulatory treatment of MS patients [44•]. In a first study, Chabas and colleagues [45•] used a large-scale sequencing approach on complementary DNA libraries from two MS patient samples and a control sample to search for genes expressed in MS lesions but not in normal white matter. The investigators identified more than 50 genes that were expressed at a significantly higher level in MS lesions, among them osteopontin, a proinflammatory cytokine involved in T cell activation. The presence of osteopontin was confirmed in MS lesions by immunohistochemistry. Interestingly, osteopontin knockout mice were less susceptible to myelin–oligodendrocyte glycoprotein- (MOG) induced EAE than wild-type controls. As the study did not determine whether the upregulation of osteopontin was specific for MS lesions or seen in any CNS inflammatory disease, the results do not enable us to conclude that osteopontin is unique to MS lesions. However, to research in humans with novel technologies and to confirm the biological relevance of candidate molecules in animal models provides a new and highly promising strategy to decrypt the pathology of MS.

Conclusion

Recent studies have provided strong evidence that the immunology of MS is much more complex than previously hypothesized. Although CD4 T helper type 1 T cells may play an important role, a variety of other immune cells including B cells, CD8 T cells and NK T cells, seem to be essentially involved in disease pathogenesis by inducing or controlling the immune response in the CNS of MS patients. Evidence from recent studies suggests that the specific local humoral and cellular immune response is highly focused, which may mean that in individual patients only a limited number of antigens are targeted [14,15,19•]. However, it is still unclear which antigens are recognized and whether this is a primary or a secondary disease event. Further studies involving cutting edge technologies [21,43,45•,46••] will hopefully clarify which antigens are responsible for the chronic inflammatory activity seen in MS patients. The results of these studies in combination with the use of improved animal models [47,48] may provide a better basis for future specific immune intervention strategies in MS.

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