

Potential Role of Humoral Immunity in the Pathogenesis of Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE)

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1. ABSTRACT

The earliest research literature addressing sub-clinical characteristics of Multiple Sclerosis was largely focused on humoral immune components, particularly antibodies in the cerebrospinal fluid of MS patients. However, two decades later, in the 1990's, T cells were established as a major component of the underlying mechanism(s) of MS pathogenesis, especially since EAE, the mouse model of MS, could be readily induced by immunization with myelin derived peptides or passive transfer of encephalogenic T cells. This data has contributed to the concept that the role of humoral immunity in MS pathogenesis may be negligible. However, more recent studies have provided important insights regarding the role of humoral immunity in MS pathogenesis. The goals of this review are to 1) summarize evidence for and against the hypothesis that humoral immunity plays a central role in the pathogenesis of MS, and 2) summarize studies in the EAE model that directly tested the role of humoral immunity in pathogenesis of the disease. With this information, we hope to convince the reader that great strides have been made towards defining a central role of humoral immunity in MS pathogenesis, but that there is a substantial amount of work to be done (especially in the EAE model) to ensure that the contribution of humoral immunity to MS pathogenesis is effectively addressed.

2. HISTORICAL EVIDENCE OF A ROLE FOR B CELLS/ANTIBODY IN MS

Seminal studies of cerebrospinal fluid (CSF) from patients with Multiple Sclerosis (MS) have consistently demonstrated the following: 1) elevated immunoglobulin levels in the CSF of MS patients (1-3), (2) oligoclonal banding (OCB) in the CSF of MS patients (2, 4-6), (3) skewing of the kappa:lambda ratio in the CSF of MS patients (1, 7, 8), (4) the presence of anti-myelin antibodies in the CSF of MS patients (9-18) and (5) the demonstration that antibodies from the CSF of MS patients may contribute to the overall extent of tissue injury in these patients (19-21). Some of these observations were established in the 1970's, but their relevance and impact on MS pathogenesis remains elusive. Nevertheless more recent studies have provided information regarding B cells in the CSF and lesion sites of MS patients that will likely have great impact on our perspective of how these cells may contribute to the pathogenesis of MS.

3. B CELLS IN THE CSF

3.1. Characteristics of CSF B cells

Elevated immunoglobulin (Ig) levels in the CSF of MS patients would suggest that there was either an influx of plasmablasts or plasma cells into the CSF, or a shift in the B cell population within the CSF towards a

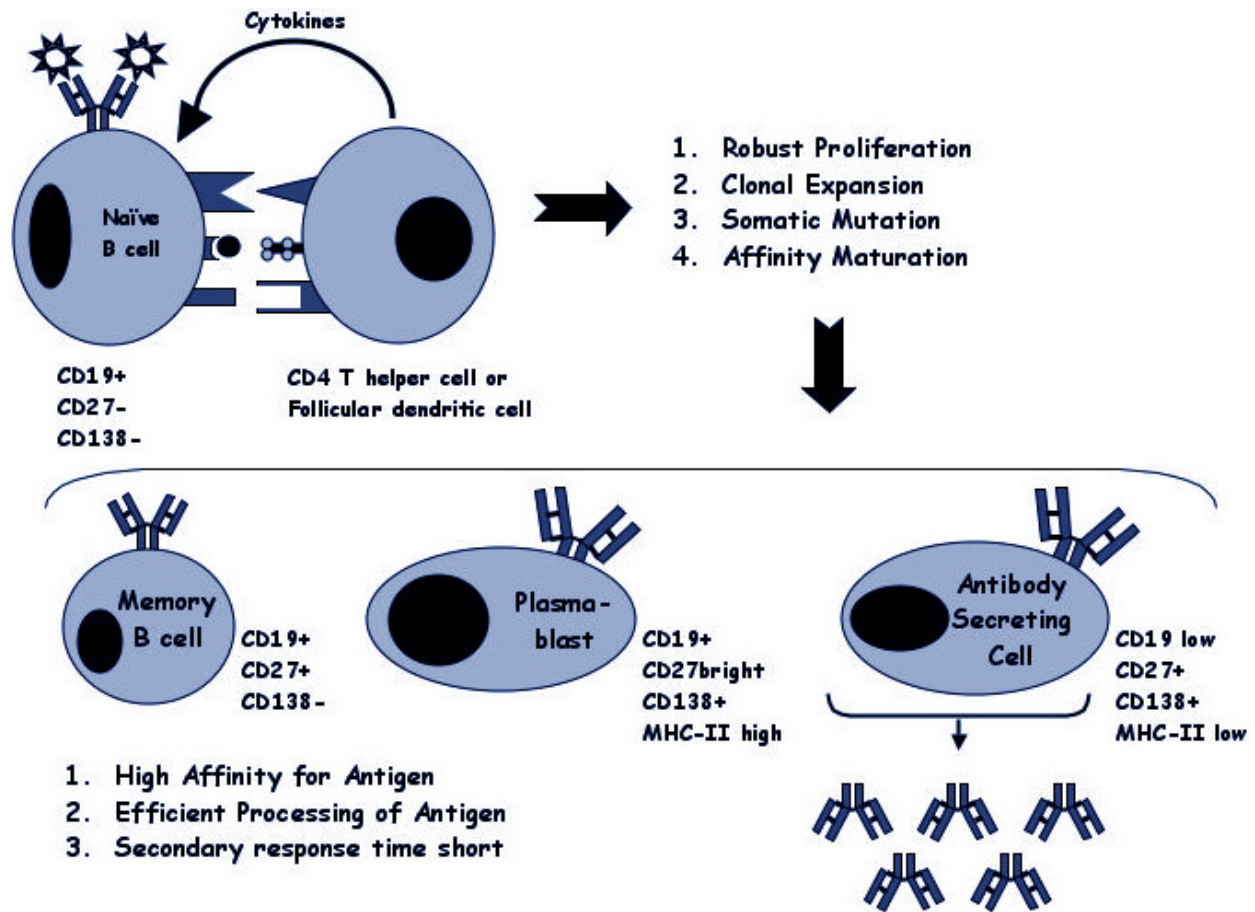


Figure 1. Schematic of B cell differentiation. Naïve B cells receive activation signals through interactions with either CD4⁺ T helper cells, or follicular dendritic cells and undergo activities as listed. These activated B cells can then become either memory B cells, plasmablasts, or antibody secreting cells, as discussed in the text. Markers relevant to this review are listed below each B cell differentiation stage.

more differentiated state (i.e. from largely naïve to memory/plasma cell phenotype, see Figure 1 for summary of B cell differentiation). Indeed, two groups have now performed detailed analysis of the CSF B cell population (22, 23) that substantiates both predictions. Corcione *et al.* obtained CSF and peripheral blood from 16 MS patients and 16 patients with Other Inflammatory Neurological Diseases (OIND), and determined through flow cytometric analysis that the overwhelming majority (~80%) of CD19⁺ B cells in the CSF of both MS and OIND patients were of a memory phenotype (CD19⁺ CD27⁺). This is in direct contrast to peripheral blood from healthy donors, MS patients, or OIND patients, whose B cell population consists largely of naïve (CD19⁺ CD27⁻) B cells. The next largest subset of B cells in the CSF of MS patients were plasma cells characterized by high expression of CD138 and low to no CD19 expression. Interestingly, CSF from 5 MS patients demonstrated higher plasma cell (CD19⁻ CD138⁺) frequency in comparison to CSF from 5 OIND patients (7% vs 1.5%).

Cepok *et al.* obtained CSF from a much larger cohort of 61 MS patients and 21 patients with non-inflammatory neurological disease (NIND), and confirmed

through flow cytometry analysis that the majority of CD19⁺ B cells in the CSF of MS patients were of a memory phenotype (CD19⁺ CD27⁺). However, they further observed that there were two distinct B cell populations based on CD27 expression. The CD19⁺ CD27^{bright} subset co-expressed CD138 and high levels of HLA-DR, identifying them as short lived plasmablasts (PB) (24). Only a small frequency of CD138⁺ cells had no CD19 or HLA-DR expression, indicating that very few mature plasma cells were present in the CSF of this MS group. Interestingly, the frequency of plasmablasts in the CSF of MS patients remained consistent throughout the course of disease, whereas this population fluctuated in response to neuroinfection in the NIND patient population. The authors suggest that this finding is consistent with the concept that MS-associated antigens are continually presented in the CNS, resulting in ongoing activation of the B cell pool. In the setting of neuroinfection, once the antigen is cleared, the humoral response wanes. In order to maintain a constant PB pool in the CNS, there must be continuous maturation of PB precursors, which are memory B cells. It remains controversial whether these PB precursor memory B cells matriculate from the periphery (where relevant antigens can also be presented), or from within the CNS.

Table 1. Analysis of Immunoglobulin Repertoire Overlap in Memory and Plasma Cell CSF Populations from MS Patients¹

| | MS02-19 | | MS02-24 | | MS03-01 | |
|--|-------------------|--------------------|-------------------|--------------------|-------------------|--------------------|
| | PPMS | | SPMS | | CIS | |
| | CD19 ⁺ | CD138 ⁺ | CD19 ⁺ | CD138 ⁺ | CD19 ⁺ | CD138 ⁺ |
| Number of sequences analyzed | | | 42 | 76 | 63 | 78 |
| Number of cells detected | | | 137 | 1151 | 634 | 109 |
| PCR efficiency | | | 50% | 80% | 70% | 80% |
| Number of Cells sorted | | | 84 | 95 | 90 | 98 |
| Percentage of cells analyzed from total number of cells detected | | | 61% | 8% | 14% | 90% |
| Number of clones | 4 | 8 | 2 | 8 | 4 | 13 |
| Number of overlapping clones in both populations | | | | 1 | 0 | 2 |
| Percentage Overlap | | | | 8 (1/12) | 0 (0/10) | 12 (2/17) |
| Number of unique sequences | 21 | 13 | 7 | 12 | 13 | 20 |
| Number of sequences overlapping the other population | 2 | 2 | 6 | 6 | 12 | 12 |
| Percentage Overlap | 10 (2/21) | 15 (2/13) | 86 (6/7) | 50 (6/12) | 92 (12/13) | 60 (12/20) |

¹These data are derived from (25)

One group found that there was minimal overlap in the clonotypes of CD19⁺ and CD138⁺ subpopulations in the CSF of three MS patients (25). This suggests that the memory B cells in the CSF were likely not the main precursors of the antibody producing cells in the CSF, otherwise considerable overlaps in clonotypes would have been detected. Based on this information, Cepok *et al* reasoned that the precursor memory B cells most likely matriculate from the periphery, rather than from memory B cells residing within the CNS. However, it should be noted that the clonal analysis on which this conclusion is based was generated from three patients discordant for their disease type, and that the repertoires analyzed were not exhaustive. For example, one patient (MS02-24) had 137 CD19⁺ CSF B cells, 84 of which were captured for single cell analysis. Of those 84 B cells whose repertoires were analyzed, only 42 of them yielded identifiable sequences. Hence, of the original 137 CD19⁺ B cells in this sampling, only one-third of them were recovered for repertoire summary (42/137, see Table 1 for details), and so it is not surprising that minimal clonal overlap of the resulting repertoire with that of CD138⁺ cells from the same sampling was observed. The second patient (MS03-01) analyzed in this publication had 109 CD138⁺ cells, 78 of which yielded identifiable sequences, such that 71% of the CD138⁺ cells in this sample were recovered for repertoire summary (78/109). However, minimal clonal overlap was again observed, probably because only one-tenth of the CD19⁺ CSF B cells in the sampling were recovered for repertoire summary. Thus, it becomes clear that in order to confidently assess the extent of clonal overlap between the CD19⁺ and CD138⁺ subpopulations, a considerable percentage of both individual populations in a particular sampling must be recovered.

Interestingly, if the two repertoires are compared regardless of which unique rearrangements were part of a clone, the overlap between the CD19⁺ and CD138⁺ subpopulations becomes extensive (Table 1). For example, in MS patient MS02-24, 6 of the 7 unique sequences (86%) found in the CD19⁺ B cells were also detected in the CD138⁺ repertoire, even though only 30.7% (42/137) of the CD19⁺ B cells were recovered for analysis. In MS03-01, 12 out of 13 of the unique sequences (92%) found in the CD19⁺ B cells were also detected in the CD138⁺ repertoire,

even though only 9.9% (63/634) of the CD19⁺ B cells were recovered for analysis. Therefore, at the very least, the underlying non-clonal CD19⁺ and CD138⁺ B cell populations demonstrate extensive overlap in their repertoires.

3.2. Clonal Expansion of B cells in the CSF

Oligoclonal banding (OCB) is a pattern in Isoelectric Focusing (IEF) gels where individual distinct Ig bands are detectable in the pH range of 7-10 (26). CSF samplings that test positive for OCB suggest that the B cell population is not heterogenous in this compartment, but is instead, restricted. This restriction of the B cell population would most likely be reflected in the presence of clonally expanded B cells in the CSF. Five investigators to date have confirmed that clonal expansion of B cells in the CSF of MS patients does occur (25, 27-31). Qin *et al* (31) analyzed CSF cells of 12 MS patients and 15 patients with other neurological diseases (OND's). Heavy chain rearrangements were amplified by standard PCR from cDNA synthesized from bulk CSF cell mRNA, and conventionally sequenced. Sequence analysis of the CDR3s from these heavy chain rearrangements indicated that 10 of the 12 MS patients had significant clonal expansion, most of which were from the VH4 family. Columbo *et al* (28) used similar methodology and found that B cells from the CSF of all 10 MS patients in their cohort exhibited clonal expansion, most of which were from the VH3 and VH4 families. Given that VH3 and VH4 family usage is most prevalent in peripheral blood B cell immunoglobulin repertoires (32), it is not surprising that VH3 and VH4 families would be highly represented in the clonotypes of CSF B cells.

Our laboratory used a different approach, sorting single B cells from the CSF of MS patients, and using random amplification of genomic DNA as an intermediate step to amplifying V(D)J rearrangements from single cells (29). To date, we have analyzed the CSF B cell repertoires from 12 MS patients (6 of which have been published (29)). The CSF Ig repertoires from these patients demonstrate that: 1) clonal expansion of B cells from the CSF of MS patients is readily detectable, even in patients with recently diagnosed disease, 2) these clonally expanded CSF B cells from MS patients are heavily mutated, even

within months of diagnosis, 3) the mutational characteristics of these CSF B cell clones in several cases do not adhere to known targeting phenomenon observed in classical germinal center derived B cells, and 4) evidence of receptor editing, a mechanism by which B cells attempt to escape autoreactivity, is fairly common in the CSF B cell repertoires of MS patients.

CSF B cell repertoire analysis using single cell PCR (SC-PCR) has also been adopted by other investigators (30). Their approach was to use cDNA from single B cells, which would provide them information regarding the isotype of the antibody being generated by each single B cell. Using this approach, they summarized the CSF B cell repertoires from 4 patients with MS, 3 that had been diagnosed within a few years of sampling, and one that had MS for 22 years at the time of sampling. The CSF B cell repertoires from all 4 patients demonstrated clonal expansion, and interestingly, all clonally expanded CSF B cell populations were of the IgG isotype, whereas the majority of those B cells that had not undergone clonal expansion were of the IgM isotype. Although the significance of this observation remains unknown, it is intriguing to speculate that those B cells driven to clonally expand will also undergo class switch. However, it should be noted that others have observed that IgM OCB appears to correlate with the onset of relapses and worsening of the disease course (33, 34).

3.3. Autoreactivity of Clonally Expanded B cells from CSF

Considering that OCB is a hallmark feature of MS, clonal expansion of B cells in the CNS B cell population should not be surprising. However, none of these groups had yet demonstrated that the antibodies produced by these clonally expanded CSF B cells from MS patients contribute to the OCB pattern observed in the CSF of the same patient, which may or may not be relevant depending on whether these antibodies have any pathogenic potential. Determining the antigen specificity of these clonally expanded CSF B cells would likely allow us to dissect their involvement (or lack thereof) in the pathogenesis of MS. In order to address this issue, one group constructed an antibody consisting of the most prominent heavy chain and light chain found in the CSF of an MS patient, re-engineered the rearrangements into a vector that would express these rearrangements as antibody, and tested for antigen specificity (35). Interestingly, this pairing was able to bind DNA.

Qin *et al* have also taken the most prominent heavy and light chain rearrangements found in total RNA from CSF B cells, engineered the rearrangements into a vector that expresses these rearrangements as single chain variable domain fragments, and demonstrated that the resultant single chain Fv was reactive to axons located in brain lesions of an MS patient, but not normal appearing white matter, or brain tissue from a patient with Parkinson's disease (36). This suggests that the antigen to which this antibody reacts is exposed during acute inflammation, but not under normal non-inflammatory conditions. The identity of the axonal protein within the

lesion site that this antibody specifically reacts to has not been revealed to date.

It should be noted that two groups have actually defined antibody repertoires of CSF B cells from MS patients using SC-PCR (29, 30). This technique would ensure that only antibody rearrangements from clonally expanded CSF B cells (versus those B cells with the highest RNA content) are being identified and that the heavy and light chains used to re-engineer antibodies are actually paired in a particular B cell are used.

4. AUTOREACTIVITY OF IMMUNOGLOBULIN FROM CSF

It is quite likely that the main contributor to the activation, differentiation, and eventual autoimmune attack mediated by B cells in the CSF is triggered by antigens associated with the myelin sheath that coats the nerve fibers. Figure 2 is an illustration of a myelin sheath, and the relative levels of various proteins that constitute the myelin sheath. These proteins include proteolipid protein (PLP), myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and myelin associated glycoprotein (MAG) (13, 14, 37-39). Prior to the advent of single cell PCR to amplify individual immunoglobulin rearrangements from CSF B cells, investigators interested in the antigen specificity of antibody residing in the CSF would screen bulk purifications of CSF antibody against particular antigens they considered likely candidates.

One example of this type of analysis that had great impact was published by Sellebjerg *et al.* and demonstrated that anti-MBP and anti-PLP antibodies were detected in approximately 44% of MS patients (n=148) (40). However, this was a weak correlation because anti-MBP and anti-PLP antibodies could also be detected in 38% of non-MS patients. In contrast, Berger *et al.* demonstrated that the presence of serum antibodies against MBP and MOG from patients with clinically isolated syndrome (CIS) indicated a greater likelihood of early conversion to clinically definite MS (CDMS) (41). Furthermore, others have demonstrated that autoantibodies may support remyelination instead of demyelination (42, 43). It is also possible that these autoantibodies simply represent those antibodies most easily extracted by the methodology and may not actually contribute to perpetuation of the disease. Consequently, these investigations have fueled arguments regarding the relevance of autoantibodies in the pathogenesis of MS. Nevertheless, such autoantibodies (regardless of their pathogenic potential) are clearly excellent biomarker candidates, and have prompted development of protein arrays consisting of known and candidate autoantigens to identify patients with positive autoantibody titers, especially in serum samples (44, 45).

5. B CELLS IN BRAIN TISSUE

It is possible that the information obtained from CSF B cells are not translatable to the activity occurring at the sites of brain damage (lesions). Thus, several groups

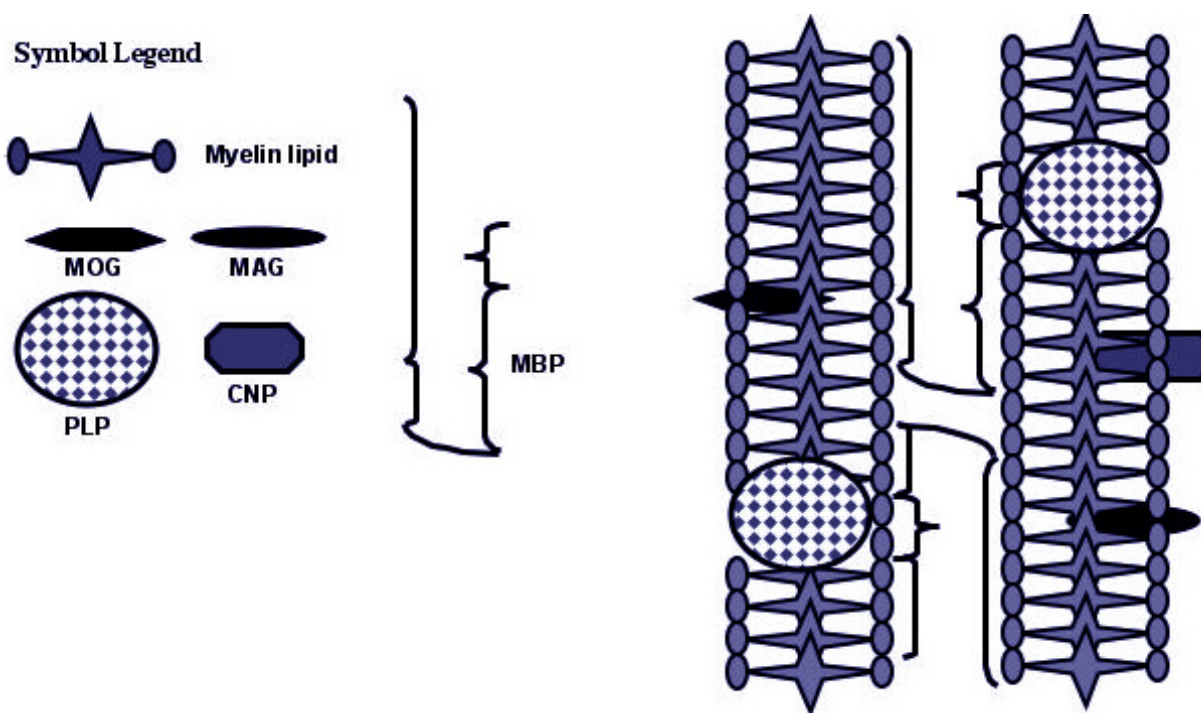


Figure 2. Illustration of myelin and its components. The main components of the myelin sheath including lipid, myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG), proteolipid protein (PLP), 2:3'-cyclic nucleotide-3'-phosphodiesterase (CNP), and myelin basic protein (MBP) are as indicated in the illustration. Each component is depicted in roughly the degree of representation within the entire myelin sheath.

have investigated the presence and characteristics of B cells in brain tissue, and have documented the following: 1) B cell and plasma cell populations are detected in lesions and are more prevalent in active lesions (46-48), 2) antibodies against MBP, CNP and MOG can be isolated from MS lesions (49-54), 3) IgG and complement deposition is readily detected around the demyelinating sites of lesions (55-59), and 3) macrophages within the lesion and in contact with myelin sheaths have immunoglobulin capping (60), indicative of an ongoing Fc receptor mediated phagocytic process.

5.1. Clonal Expansion of B cells in MS Lesions

Three groups have summarized the immunoglobulin repertoire analysis of B cells isolated from lesions of MS patients (36, 61, 62). Owens *et al.* found a prominence of VH4 usage in the cDNA libraries of MS plaques (62), and analysis of the CDR3s also indicated that clonal expansion had occurred (63, 64). Baranzini *et al.* analyzed heavy chain use in the plaque tissue from 10 MS patients and 4 non-MS control samples (61). CDR3 spectratyping of bulk cDNA from these tissues also demonstrated that clonal expansion had occurred specifically in the MS lesions, and that the VH4 family genes 4-34 and 4-39 were utilized most frequently as well as the VH1 family gene, 1-69. More recently, Zhang *et al.* analyzed heavy chain use in 6 lesions and 4 normal appearing gray matter areas from each of 4 patients with clinically definite MS, and also confirmed that clonal expansion had occurred (36). None of these groups have

compared the immunoglobulin repertoires obtained from lesions and CSF of the same patient, most likely for technical reasons.

5.2. Autoreactivity of Clonally Expanded B cells from MS Lesions

Interestingly, these clonally expanded B cells at the lesion sites were often of the IgA isotype, and IgA detected within these same lesions bound to damaged axons as well as areas of inactive demyelination (36). The mechanism by which IgA antibodies contribute to axon damage remains unknown, since they are not complement activators like IgG and IgM isotypes (65). Determining whether the clonally expanded B cells within the lesions actually produce antibody that contributes to demyelination is likely to become a central topic in the near future.

6. CHEMOKINE AND CYTOKINE EXPRESSION IN THE CSF AND CNS

In 2002, Sorensen *et al.* utilized flow cytometry to determine chemokine receptor expression of CXCR3, CCR5 and CXCR5 (any of which would aid migration) by CSF B cells from 12 patients with Optic Neuritis (ON) and 17 patients with CDMS (66), comparing these results with 7 patients with NIND. This analysis demonstrated a significantly higher expression of CXCR3 on CSF B cells from ON and CDMS patients compared to NIND patients (74% vs. 61%, $p < 0.05$). CXCL10 (IP10), which is the ligand for CXCR3, is highly expressed in MS brain lesions,

Table 2. Experimental Autoimmune Encephalomyelitis Characteristics in B cell Sufficient Mouse Strains

| Host mouse strain | EAE induction | Incidence | Disease Onset average days +/- SD or range | Mean Maximal Score | References |
|-------------------|------------------------------------|-----------|--|--------------------|------------|
| uMT/B10.Q+/- | MOG protein, 100 microgram/mouse | 88% | 13 +/- 2 | 4.6 +/- 1.6 | 96 |
| DBA/1 | MOG protein, 50 microgram/mouse | 100% | 13 +/- 1 | 7.6 +/- 1.0 | 96 |
| C57BL/6 | MOG protein, 200 microgram/mouse | 79% | 12.6 +/- 2.2 | 3.4 +/- 0.9 | 95 |
| SJ/L | MBP protein, 200 microgram/mouse | 100% | 15.25 +/- 1.26 | 4 | 88 |
| B10.PL | MBP protein, 200 microgram/mouse | 89% | 23 | 3.6 | 88 |
| BALB/c | Spinal Cord Homogenate, 3 mg/mouse | 70-100% | 11.1-14.7 | 2.7-5 | 120 |

hence identifying this interaction as a likely mechanism by which B cells migrate to the CNS. Interestingly, this same group has demonstrated that the concentration of CXCL10 in the CSF of MS patients who are relapsing correlates with increases in IgG and IgM synthesis in that compartment (67).

Since Corcione *et al.* demonstrated that B cell differentiation can occur in the CNS (centrocytes and centroblasts are readily detected in the CSF, see above section on CSF B cell characteristics and (22)), they were interested in determining if chemokines known to participate in germinal center formation (LT- α , CXCL12 and CXCL13) were present in the CNS. They found that all three of these chemokines were expressed in areas of the brain that have been identified as the main trafficking routes from the blood to the CNS (See Figure 7 in (22) and (68, 69)).

7. IMPACT OF HUMORAL IMMUNITY ON EAE

Experimental allergic encephalomyelitis (EAE), the mouse model of MS, has proven useful in elucidating the role of T cells in this disease, and has the potential to elucidate the role of B cells in this disease, as well. The most common approach to induce EAE in susceptible animals is by active immunization with immunogenic peptides derived from myelin proteins or passive transfer of T cells specific for myelin proteins (70-73). EAE can also be induced experimentally in appropriate mouse strains by injection of protein components of the myelin sheath including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), spinal cord homogenate (SCH) (see Table 2), and proteolipid protein (PLP) (74). However, these approaches are not as commonly utilized because peptide immunizations impressively mobilize T cells, which have historically been the primary encephalogenic lymphocyte under investigation. As a consequence, the impact of B cells and the antibodies they produce in the development and pathogenesis of EAE (and consequently, MS) has not been thoroughly investigated. Nevertheless, recent studies suggest a central role of humor immunity in the pathogenesis of EAE.

7.1. Potential Role of Antibodies in EAE induction

Evidence for the direct role of antibodies in the development of EAE was suggested by induction of demyelination in primary CNS cultures with serum from rabbits immunized with whole myelin protein (75). In addition, myelin specific antibodies could readily be detected in the serum of animals with EAE (76) as assessed by radioimmunoassay and several serum antibodies have

been identified that can potentiate EAE (77-81). For example, serum from animals with chronic EAE showed greater multifocal demyelination in immunohistochemical analysis than serum from animals with acute EAE. Interestingly, anti-MOG antibodies were more prevalent in the chronic stage of EAE than the acute phase (82), suggesting that anti-MOG antibodies in particular may cause extensive demyelination in brain hemispheres, optic nerve and spinal cord. In addition, more severe EAE and demyelination is observed in a transgenic mouse backcrossed to C57BL/6 background whose B cells express and secrete anti-MOG antibody in comparison to wild type controls (83). Interestingly, these mice inherently produce high titers of anti-MOG antibody, but do not develop EAE until immunized with MOG, indicating that the presence of potentially pathogenic B cells and antibodies may be inconsequential until encephalitogenic T cells cross the blood brain barrier and CNS inflammation has been initiated. These observations suggest that transgenic anti-MOG B cells are not actively tolerized, but rather persist due to clonal ignorance to antigen sequestered in the CNS. Morris-Downes, *et al.* have demonstrated in the EAE mouse model that injection of anti-MOG antibodies into mice at disease onset could augment both clinical neurological disease and CNS demyelination (80) as defined by increased clinical score and severity of histopathological changes. These observations strongly suggest that autoantibodies can contribute to autoimmune disease directly.

Interestingly, removing or neutralizing antibody (using intravenous immunoglobulin (IVIG) treatment for example) reduces EAE symptoms in rats or mice that have been actively induced by immunization with SCH or MBP (84-86). These results support the hypothesis that antibodies directed against particular CNS antigens contribute to EAE. However, B cells also have the capacity to promote T cell activation, and so the contribution of autoreactive B cells in EAE may not be limited to autoantibody production. Interestingly, others have recently demonstrated that both antigen presentation and antibody production by B cells are critical for induction of severe autoimmune arthritis in mice (87).

7.2. Potential Role of B cells in EAE induction

One approach to assess the potential role of B cells in EAE would be to determine whether EAE could be induced in animals deficient in mature B cells. For example, SJ/L mice immunized with either MBP or PLP derived peptides present EAE with one or more episodes of paralysis followed by recovery, which is similar to the disease course observed in multiple sclerosis patients (88,

Table 3. Experimental Autoimmune Encephalomyelitis Characteristics in B cell Deficient Mouse Strains

| Host mouse strain | EAE induction | Characteristics | Disease Onset (days) | Mean Maximal Score | References |
|-----------------------|----------------------|--|----------------------|--------------------|------------|
| uMT/C57BL/6 | MOG peptide 33-55 | Developed inflammatory lesions with primary demyelination in the spinal cord, brain and optic nerves. Similar incidence of disease, mortality, average day of onset, and average maximum disease severity when compared with C57BL/6 controls. | 11.3 +/- 0.7 | 3.2 +/- 0.2 | 91 |
| uMT/C57BL/6 | MOG peptide 33-55 | EAE susceptible with no detectable differences in disease incidence, onset, mean maximal clinical grade and relapse rate. | 18.1 +/- 4.7 | 2.4 +/- 1.2 | 95 |
| uMT (B10.PLxSJL/J) F1 | MBP peptide Ac1-11 | Relapsing remitting disease course with similar day of onset and day of first relapse than wild type controls. Reduced early T cell proliferative response after in vitro restimulation. | 20.1 +/- 1.1 | N.R. | 90 |
| uMT/B10.PL | MBP peptide Ac1-11 | Chronic sustained EAE instead to monophasic acute disease course. | 15.4 +/- 0.92 | 2.66 +/- 0.20 | 121 |
| uMT/B10.Q | MOG protein | Decrease clinical disease severity and incidence when compared with heterozygous littermates. Decrease in extent of CNS infiltrates and demyelination. | 12 +/- 1 | 3.5 +/- 2.1 | 96 |
| Xid/DBA/1 | MOG protein | Decreased EAE incidence and severity. | 13 +/- 1 | 5.3 +/- 2.3 | 96 |
| uMT/DBA/1 | MOG protein | Similar EAE incidence and decrease severity, with occasional clinical recovery. Similar influx of inflammatory cells, but decreased demyelination. | 14 +/- 1 | 3.7 +/- 1.3 | 96 |
| uMT/C57BL/6 | MOG protein | Resistant to protein-induced EAE. Significant less inflammation, demyelination and axonal loss, with atypical EAE lesions. | N.A. | N.A. | 95 |
| uMT/C57BL/6 | MOG+ B cell transfer | Similar EAE disease incidence with later disease onset and decreased severity than C57BL/6 controls. | 18 | 2 | 122 |
| uMT/C57BL/6 | MOG-primed serum | EAE development of similar incidence, onset, and severity. Restored typical EAE lesions, inflammation, demyelination and axonal loss. | 19.0 +/-1.0 | 3.5 +/- 0.5 | 122 |

89). However, when B cells were depleted from this strain by backcrossing with mice deficient in mature B cells, the EAE disease course (which was induced by MBP Ac1-11 peptide) was not altered in comparison to the SJL/J control mice with respect to incidence of disease, day of onset, day of first relapse, severity and duration of first episode, and rate of relapse (90) (also see Table 3). This report suggested that B cells are not required for the relapsing course observed in the SJL EAE model. Similar results were found in experiments performed in C57BL/6 B cell-deficient uMT mice when immunized with MOG peptide. B cell deficient mice developed characteristic demyelination and CNS infiltration pathology similar to patterns observed in C57BL/6 controls (91), again suggesting that B cells were not required for the primary demyelination observed in C57BL/6 mice.

However, it has been well established that B cell activation is dependant on internalization of the whole antigen/B cell receptor complex (65, 92-94). Peptides do not elicit an initiating activation response by naïve B cells. Thus, it becomes likely that the B cell depletion experiments in the SJL and B6 mouse strains did not result in alteration of EAE because peptides were used as the immunogens, rather than whole protein preparations. To confirm this concept, Lyons *et al* demonstrated that passive transfer of *in vitro* activated MOG-specific B cells into naïve B cell deficient mice made them susceptible to EAE with clinical and histological measurements similar to that observed in wild type counterparts (95). Similarly, passive transfer of serum from wild type mice enriched for anti-MOG antibodies into naïve B cell deficient mice reconstituted disease induction, with comparable CNS lesions, incidence, onset and severity of EAE when

compared with controls (Table 3). These data emphasized that B cell mediated acceleration of disease onset and aggravation of the neurological deficit in B cell deficient mice was dependent on the nature of the immunogen such that B cell deficient mice immunized with peptide were susceptible to EAE, but B cell deficient mice immunized with whole protein were not (95). These data also established a critical role of antibodies in EAE pathogenesis, and further highlighted the importance of Ag-specific B cell activation for disease induction.

To further emphasize this concept, we have immunized wild type B10.PL mice with Ac1-11, the common MBP peptide used to induced EAE in this strain, or whole MBP to assess whether these two forms of immunization elicit compatible EAE scores (Figure 3). Both the Ac1-11 and whole MBP immunized mice developed EAE with 100% incidence, with comparable maximum EAE scores. However, there was a slight delay of EAE onset in mice immunized with whole MBP, most likely because processing of whole MBP by B cells and activation of B cells in response to MBP requires additional time. Indeed, B cell activation was greatly enhanced in MBP immunized mice in comparison to wild type controls, as assessed by CD69 expression (compare 3% CD69 expression in wild type controls to 13% CD69 expression in MBP immunized B10.PL mice) (Figure 4), and splenic B cells from MBP immunized mice underwent proliferation in response to the main encephalogenic peptide of MBP, Ac1-11 (Figure 5). Finally, EAE lesions were readily detected in the spinal cord of mice immunized with whole MBP, with large amounts of meningeal and perivascular infiltrates (Figure 6). Thus, both peptide and whole protein immunization protocols (Table 2) can induce EAE in the

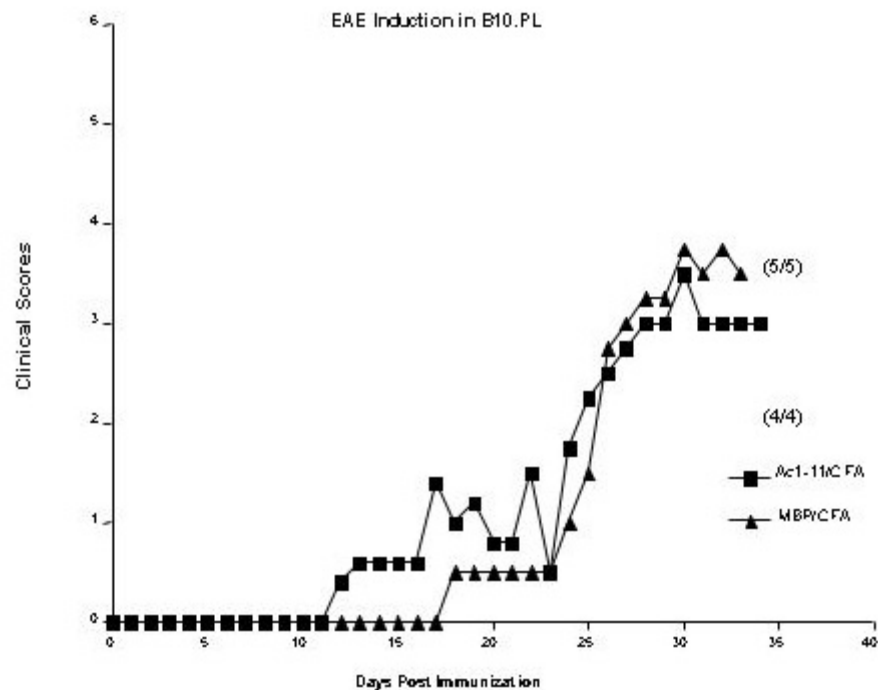


Figure 3. EAE induction with whole MBP is delayed in comparison to Ac1-11, but with the same incidence and disease severity as mice immunized with Ac1-11. Two groups of mice were immunized with 50 microgram MBP/CFA or 400 microgram MBP/CFA at day 0. Mice received 200ng i.p. injections of pertussis toxin on day 0 and 2. Mice were examined daily for clinical signs of disease and a mean clinical course assigned based on the following scale: 1, a limp tail; 2, moderate hind limb weakness; 3, severe hind limb weakness; 4, complete hind limb paralysis; 5, quadriplegia or moribund and 6, death due to EAE. The experiment was terminated at day 34. In parenthesis: incidence of clinical disease.

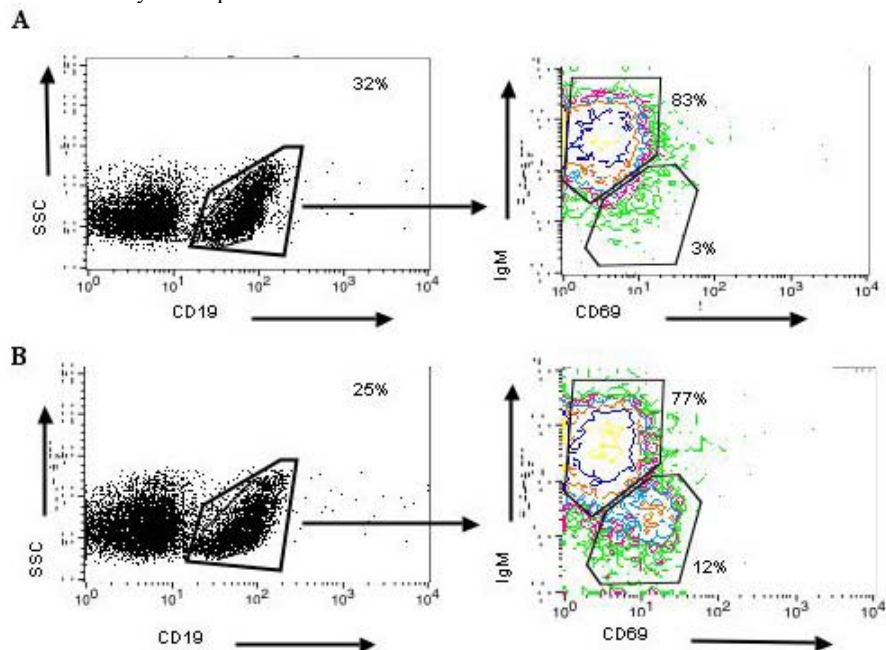


Figure 4. Analysis of the B cell activation profile from the spleen of MBP-immunized B10.PL mice. CD19⁺ cells were examined for the expression of IgM and the early activation marker CD69. Four populations of cells could be defined according to their expression of these markers as follows: CD69⁻ IgM⁺ naïve, CD69⁺ IgM⁺ activated naïve, CD69⁻ IgM⁻ memory and CD69⁺ IgM⁻ activated memory. (A) Unmanipulated control. (B) Splenocytes were prepared from MBP-immunized mice 60 days post immunization with maximum clinical grade 3, clinical grade 1 (recovery) at time of sacrifice. Numbers at the right of each region represent the percentage of cells present in the total population. Note the increase in the population of memory cells present in the EAE induced sample.

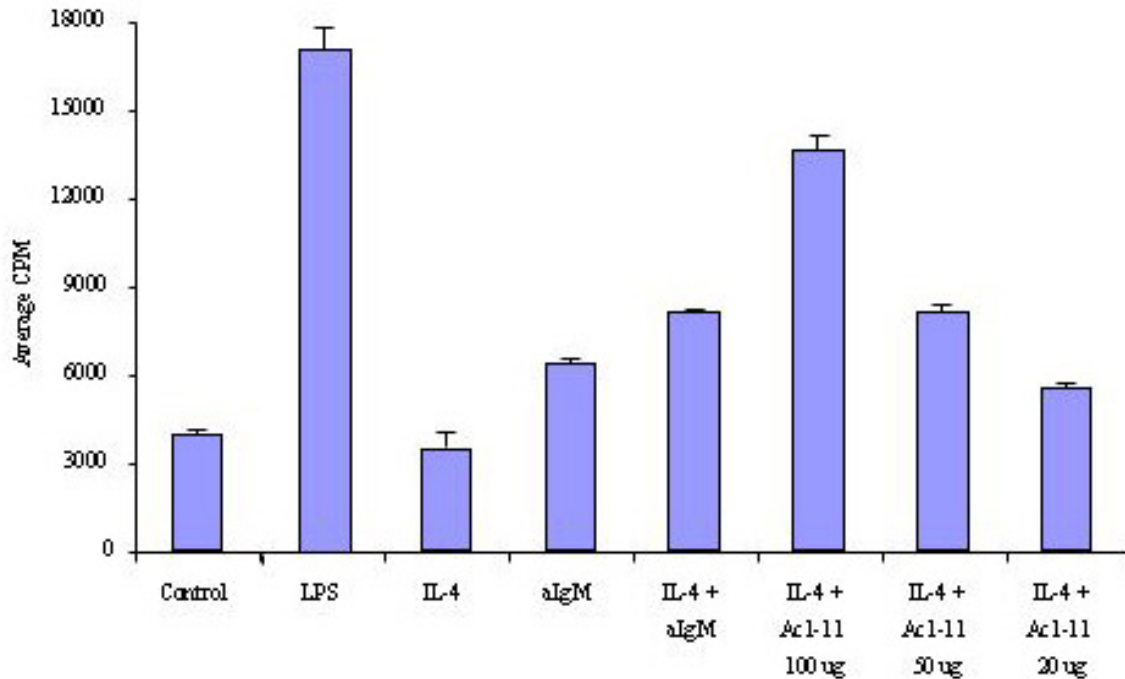


Figure 5. MBP Ac1-11-specific proliferation of B cells. Purified B cells (5.0×10^5) from the spleens of whole MBP-immunized B10.PL mice (3 month post immunization, max. clinical grade 3, clinical grade 0 (recovery) at time of sacrifice, n=6 mice) were stimulated for 3 days with various concentrations of MBP AC1-11 peptide. Cells were harvested 18h post H^3 -Thymidine treatment.

proper mouse strains, but EAE induced by whole protein immunizations are more likely to be B cell dependant.

To further characterize the role of B cells in MOG induced EAE, Svensson *et al* used two different mouse strains with B cell deficiencies (96). One was the uMT C57BL/10 mouse strain, which lacks mature B cells due to targeted disruption of the transmembrane region of the IgM heavy chain. The second strain was the X-linked immunodeficiency DBA/1 mice, which have a reduced B cell response in part due to the absence of peritoneal cavity B1 cells, which are considered the main components of the autoreactive pool. These EAE models do not require the use of pertussis toxin (in contrast to the frequently used MOG 35-55 EAE in C57/BL6 models) for the induction of EAE which might influence disease susceptibility due to the activation of other innate immune pathways, such as the TLR4 pathway (97, 98). In this system, the authors demonstrated that B cells are not critical for the development of MOG induced EAE, but that B cells contribute to severity since both strains of B cell deficient mice exhibited a milder phenotype and decreased extent of demyelination. B cell-deficient uMT mice on different genetic backgrounds (C57BL/10 and DBA/1 strains) developed EAE, although with a reduced clinical severity. Histological analyses revealed decreased demyelination in the CNS while infiltration of inflammatory cells was similar or only slightly reduced as compared to wild type mice. These results obtained in multiple strains confirmed the important role for B cells in EAE pathogenesis and

strongly suggests that the effects observed are related to the B cell deficiency rather than to genetic susceptibility (96).

7.3. Potential Role of B cells on Resolution of EAE

In contrast to the studies mentioned, other groups have shown that B cells may ameliorate EAE severity. For example, establishment and persistence of inflammatory lesions associated with EAE has been attributed to a $CD4^+$ T cell mediated Th1 polarized response, whereas high concentrations of IL-10 (a Th2 cytokine) can mediate recovery. B cells are a main cellular source of IL-10 production, and so it is possible that the lack of a recovery phase in MOG-induced B cell deficient mice, is attributable to the lack of B cells making IL-10 (99-101).

B cells could also be used as a therapeutic source for antigen-specific tolerance induction. Xu and Scott demonstrated that mice pre-treated or treated with retrovirally-transduced B cells expressing MBP or MOG can delay disease onset and ameliorate EAE severity (102). The molecular mechanism of tolerance induction utilizing this gene therapy protocol have not been fully elucidated, but do highlight the relevance of B cells in modulating the immune response in EAE.

7.4. Chemokines in EAE

Chemokines may also play a critical role in the immune response as regulators of lymphocyte trafficking and activation. The CNS has transpired as a site where neuroinflammation is sustained through ectopic lymphoid structures. CCL19 and CCL21 (as well as their common

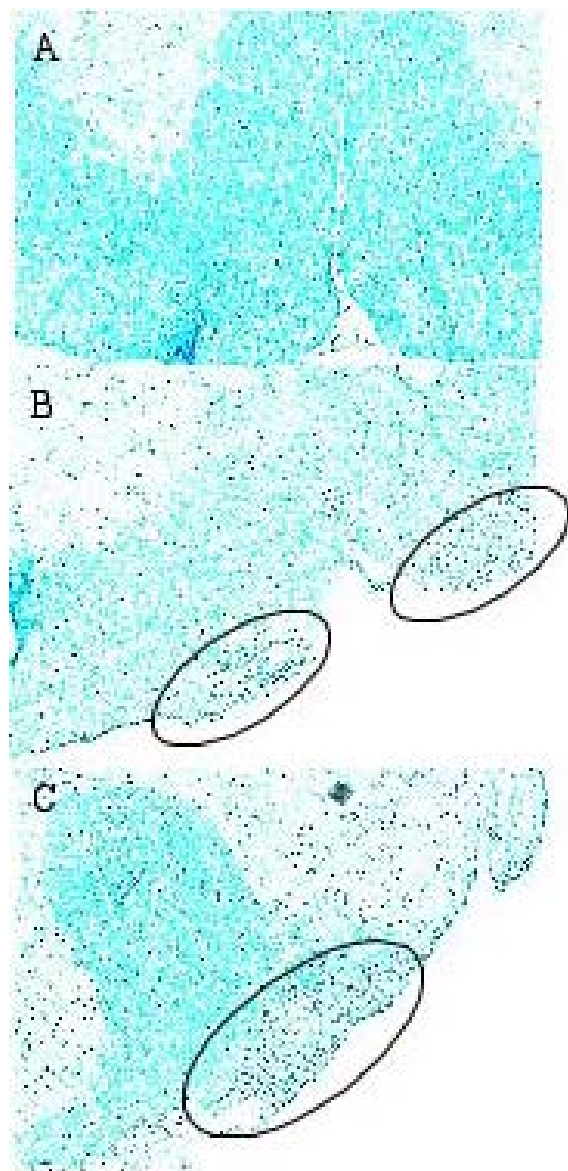


Figure 6. EAE lesions in B10.PL MBP-immunized mice. Sections (7 μ m) from the spinal cords of mice immunized with MBP were stained with luxol fast blue. (A) WT mouse, non manipulated. Normal spinal cord anatomy with no pathological characteristics. (B and C) B10.PL mouse, 30 days post immunization, clinical grade 5 at time of sacrifice. Note the large amount of meningeal and perivascular inflammation (delineated with black lines).

receptor, CCR7), are chemokines that recruit dendritic, T- and B-cells. Interestingly, these chemokines are induced in the CNS, likely resulting in a continuous local antigenic stimulation (103, 104). In addition, Magliozzi, *et al* detected the formation of lymphoid follicle-like structures consisting of B cells, lymphoid homing chemokine CXCL13⁺ cells and follicular dendritic cells within the meninges of a proportion of EAE mice (105). These findings point to the relevance of B cell recruitment by the

expression of relevant homing lymphoid chemokines and formation of ectopic lymphoid structures.

7.5. Potential Role of Complement in EAE

Complement activation by the classical pathway is a major mechanism of the effector functions of humoral immunity. Complement dysregulation or activation in response to abnormal stimuli (such as antibodies against self antigens or immune complexes deposited in tissues) may significantly contribute to the pathology of EAE. There are several reports in the EAE literature that explore the importance of complement in the development of inflammatory autoimmune response, tissue injury and repair. For example, demyelination was reduced by depletion or inhibition of complement using cobra venom factor or soluble complement receptor in rats that had MOG-induced EAE (106-109). In this same EAE model, complement fixing anti-MOG antibodies were found to be essential for induction of demyelination.

However, studies using mice deficient in several complement components have led to contradictions regarding the role of complement in EAE pathology. Boos *et al* reported that MOG peptide-induced EAE in mice deficient in C4, a protein required for full activation of the classical pathway, does not significantly alter histopathology, onset or severity of EAE (110). Yet C3 deficient mice had substantially reduced clinical scores with less inflammation when compared with controls, suggesting that C3 exacerbates demyelination associated with EAE induced by MOG (111). However, higher MOG inoculums negated these differences such that clinical scores were similar to control animals (112). These differences may be contributed to other mechanisms of demyelination that may occur under this EAE induction regimen and/or genetic susceptibility between strains.

A more complicated role for complement activation was revealed in myelin induced EAE C5 deficient mice. C5 deficient mice exhibited similar EAE disease onset, recovery phase and chronic courses as compared to controls (113). However, there were marked differences in histopathology, such that CNS inflammatory cell infiltration and tissue damage were more extensive and diffuse in wild-type control mice, indicating that C5 deposition is central to the establishment of the inflammatory lesion. In contrast, C5 deficient mice that had a chronic form of EAE demonstrated increased axonal depletion and severe gliosis, indicating that C5 is required for axonal survival and more efficient remyelination. Thus, C5 may play two roles in EAE, first as an enhancer of inflammatory demyelination in acute EAE and second, as a promoter of remyelination during recovery. The mechanisms underlying the influence of complement components on demyelination and remyelination have not been elucidated.

8. PERSPECTIVE

The studies discussed in this review provide a deeper understanding of the complex role of humoral immunity (B cells, antibodies and complement) in MS and EAE. However, at least three areas require further

elucidation that must be addressed to define the role of humoral immunity in human MS. These include 1) full characterization of B cells with respect to subpopulation phenotypes and regulatory elements in different compartments and at different disease stages, 2) identification of antigenic specificity of antibodies produced and the clarification or categorization of beneficial or detrimental antibodies, and 3) role of chemokines and complement factors on recruitment of lymphocytes and subsequent damage. The EAE model will be critical in effectively developing potential MS therapies directed against humoral immune components. However, successfully utilizing this model to address the effectiveness of novel therapies in MS will require substantial investigation of how humoral immunity influences EAE development and exacerbation. Such studies are more than timely considering that many agents in current consideration as therapeutics of MS directly affect B cells, such as Rituximab, a B cell depleting therapeutic (114-119).

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