New Immunopathologic Insights into Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system. Although the immune system seems to play an important role in the pathogenesis of disease, target antigens are still uncertain and pathways leading to tissue destruction have not been fully elucidated. Recent studies have significantly contributed to a better understanding of the disease process and broadened our view on possible scenarios of disease initiation and progression. We review the role of the immune system for the manifestations and evolution of MS and discuss different pathogenetic concepts. We conclude with an outlook on future strategies to identify the cause of MS.

Risk Factors
Both genetic and environmental factors influence susceptibility and the course of MS. The prevalence of MS varies strongly, depending on the genetic background [6]. Prevalence is high in whites, but MS is rare in Asians and Africans. Family members of MS patients are at greater risk, which ranges from 250-fold higher in monozygotic twins to 10-fold higher in children of MS patients [2,7]. In contrast, the prevalence in spouses and adopted children is not increased. Multiple genome screens and family studies recently completed indicate that MS follows a polygenetic trait, which involves a large number of genes with each contributing little to the overall risk [2,7,8]. Furthermore, the studies also provide evidence for significant heterogeneity of susceptibility genes. The role of genetic factors is even more complex because they appear also to impact on disease course. As a result of the polygenetic and heterogeneous genetic predisposition, positional cloning and candidate gene approaches have been largely unsuccessful or yielded inconclusive results [8]. Only the human leukocyte antigen (HLA) class II alleles DRB1*1501 and HLA-DQB1*0601 have consistently been associated with MS in whites (relative risk of 2 to 4) [9]. Although this association was established more than 20 years ago, it is still unknown how these HLA alleles confer greater susceptibility of contracting MS.
Pathology of Multiple Sclerosis Lesions

Multiple sclerosis is a disease that predominantly, although not exclusively, affects CNS white matter and leads to demyelinating lesions [13]. Most lesions are located around the ventricles with relation to small vessels. In acute MS lesions, demyelination of axons, activation of microglia, and infiltration of immune cells are key features. The infiltrates mostly consist of T cells and macrophages. B cells and plasma cells are also found, but at lower numbers. Extensive antibody deposition is seen in part of the patients. Eosinophilic granulocytes are sometimes encountered, but other immune cells, such as granulocytes, γ/δ T cells or natural killer T cells, are largely absent from lesions. Among T cells, CD8+ T cells outnumber CD4+ T cells in the parenchyma, whereas the later ones are found more frequently in cuffs and meninges [14,15]. An array of different lymphokines, chemokines, and proteases is expressed in acute MS lesions [16–18].

Work performed by Lucchinetti et al. [19] suggests that acute demyelinating lesions significantly differ in terms of oligodendrocyte pathology, the presence or absence of inflammatory and demyelinating changes, and the extent of remyelination. Two patterns have been noted most frequently, one characterized by significant antibody deposits and remyelination, the other by oligodendrocyte loss without remyelination. A high degree of heterogeneity is also demonstrated on the cerebrospinal fluid (CSF) cytology of MS patients. Although in particular the extent of the humoral immune response appears to be stable over time in MS patients, it varies significantly inter-individually [20]. Together with the high variability of the clinical phenotype and in disease progression, it is tempting to speculate whether different pathogenic pathways and even etiologies underlie those histologically defined subtypes.

MS is, however, not only characterized by its inflammatory, but also by its neurodegenerative changes, which are already prominent early in the course of disease [21–23]. In acute lesions, the extent of axonal damage correlates with inflammation, especially invasion by macrophages and CD8+ T cells, suggesting that both cell populations are directly involved in causing axonal loss [23].

Much less is known about the immunopathology of the chronic active or silent lesions. Chronic active lesions feature ongoing inflammation, demyelination, and axonal degeneration, although inflammation is usually less vigorous than in acute lesions. Gliosis and permanent neuronal and oligodendroglial damage with variable degrees of demyelination occur in the silent lesions with little cellular infiltrates and activation of immune mediators [13,24].

The Classic Experimental Autoimmune Encephalomyelitis Concept

A role of the immune system in the pathogenesis of MS was first suggested by observations of acute demyelinating episodes that followed rabies vaccination. The vaccine was contaminated with myelin antigens, raising the possibility that the disease was induced by an antmyelin immune response. This hypothesis was confirmed in animal models. Immunization with myelin antigens and Freund’s adjuvant gives rise to CNS inflammation in susceptible animals. This animal model was termed experimental autoimmune encephalomyelitis (EAE) [25]. Target antigens, extent of demyelination, presence and degree of inflammation, and disease course are dependent on the animal strain and genetic background [26]. The role of an autoimmune response in this model was confirmed by adoptive transfer experiments, which demonstrated that predominantly CD4+ T cells from diseased animals can transmit disease to naive animals. CD4+ T cells secreting T-helper (Th) 1 cytokines (e.g., interferon γ [IFNγ]), tumor necrosis factor β (TNF-β) and interleukin (IL)-2, and the proinflammatory cytokine TNF-α were more potent in transferring disease than other myelin-specific T cells [27]. T cells secreting Th-2 cytokines (IL-4, IL-5, IL-10, and IL-13) conversely seem to protect from or ameliorate EAE [28]. Although T cells are the disease-transferring population, they rely on innate immunity in the CNS. Microglia cells provide the proinflammatory milieu required for efficient T-cell recognition of autoantigens [29]. All together, these findings established EAE as a prototypic autoimmune disorder and created the widely accepted paradigm that a Th-1 T-cell response to myelin antigens is destructive, whereas a Th-2 response is protective [30].

The Autoimmune Hypothesis of Multiple Sclerosis

Based on the observation that administration of the Th-1 cytokine IFNγ exacerbates MS [31], the EAE concept was extrapolated to the human disease (Figs. 1,2). This was fostered by numerous efforts to characterize immune responses to myelin antigens in MS patients. Antibodies against myelin antigens are detected in serum, CSF, and the CNS of MS patients [32,33]. Similarly, CD4+ T cells specific for a variety of myelin antigens are present in the blood of MS patients.
[34]. Recent studies demonstrated that these T cells can recognize and respond to a large number of different antigens, among them a variety of self and foreign antigens including peptides derived from microbes [35,36]. However, the ability to react with a large number of different antigens is not confined to autoreactive T cells, but probably an intrinsic feature of T cell recognition [37,38]. Nevertheless, the high degeneracy in T-cell recognition observed provides a possible explanation of how autoreactive T cells may be activated by exogenous infectious agents and initiate a first demyelinating episode (molecular mimicry). After the first destructive event, myelin antigens are released that may further prime a chronic polyreactive autoimmune process (epitope spreading) [39].

Although the autoimmune hypothesis generated in the EAE model involving both molecular mimicry and epitope spreading is attractive to explain many aspects of MS, experimental support for this hypothesis is still limited. Likewise, CD4+ T cells specific for myelin antigens are not only retrievable from MS patients, but also from healthy donors, indicating that autoreactive T cells are part of the normal T-cell repertoire and not necessarily harmful [34]. So far, studies have not provided conclusive results that myelin-specific T cells differ in terms of antigen recognition or phenotype between MS patients and control subjects [34,40]. Similarly, myelin-specific antibodies are not confined to MS, but can be detected in different neurologic diseases and even in healthy control subjects [32,33].
Broadening the Autoimmune Concept
During the past decade, many experimental immunotherapies in MS were based on the EAE model. These intervention strategies included global immunosuppression, inhibition of proinflammatory cytokines, or shifting the immune response from Th-1 to Th-2 [30]. However, studies in the EAE model quickly raised questions of whether the Th-1 concept can be applied without modifications to all EAE models and, more importantly, to human disease. The first objections came from studies on genetically modified animals [41]. In some experimental settings, animals lacking IFNγ or TNF-α develop similar or even more severe EAE than their wild-type littermates [41]. In contrast, disruption of the IL-4 gene does not affect the disease course. In a transgenic mouse model, it was even possible to induce EAE with myelin-specific Th-2 T cells [42]. Finally, a therapeutic approach based on a myelin peptide that induced a Th-2 shift unexpectedly resulted in severe relapses in an EAE monkey model [43].

At variance with the classic EAE dogma, myelin-specific CD8+ T cells may even evoke EAE under certain conditions. In these models, lesions are restricted to the brain and characterized by extensive demyelination and cell death [44,45].

Although EAE cannot be adoptively transferred by B cells, antibodies are also undoubtedly important for the disease course. In some models, EAE severity is significantly enhanced by co-administration of myelin-specific antibodies after induction of disease [46].

Clinical trials in MS patients further strengthened the view that the EAE Th-1 concept can not simply be applied to human disease. Treatment of MS patients with a TNF-α blocking antibody or soluble TNF receptor precipitated acute attacks [47]. Global depletion of CD4+ T cells did not have an impact on the disease course of MS [48]. Antigen-based therapies, such as tolerance induction by oral application of myelin or application of altered peptides derived from myelin antigens, were inefficient or even worsened disease [49,50,51]. Many other immunomodulatory and immunosuppressive drugs failed in clinical trials [51]. To date, only three drugs have been approved for the treatment of RR-MS and SP-MS. Novantrone, a cytotoxic drug with immunosuppressive properties, seems to reduce relapse rates and progression in MS [52]. Glatiramer acetate, a randomly synthesized polypeptide mixture based on four amino acids that are contained at high levels in myelin proteins, also seems to decrease relapse rates [1]. Among the postulated therapeutic effects are its immunomodulatory and neuroprotective properties. So far, the most robust data are available for IFNβ, which strongly suppresses MRI activity, decreases relapse rates, and also seems to affect disease progression [1]. The drug exhibits both antiviral and immunomodulatory effects, although the mode of action in MS is still not entirely understood. Given the inconclusive results on the role of antimyelin responses in MS and the disappointing outcome of a number of clinical trials, a rethinking of the pathogenetic scenarios accumulating in inflammation, demyelination, and destruction of CNS tissue is needed.

Alternative Pathogenetic Scenarios
Inflammation and demyelination are observed not only in autoimmune conditions, but also following infection or even primary neurodegenerative events (Figs. 1,2). The idea that MS is caused by a neurotropic agent has been sup-
ported by the identification of causative viruses in subacute sclerosing panencephalitis (SSPE) or human T-cell leukemia virus-1 (HTLV-I)–associated myelopathy [53]. In mammals, a variety of viruses can elicit acute or chronic CNS demyelination and inflammation (eg, Theiler’s murine encephalomyelitis virus and mouse hepatitis virus) [53,54]. In these models, predominantly CD8+ but also CD4+ T cells are crucial to control the virus in the acute phase [53,54], whereas B cells and antibodies seem to be more relevant during the chronic disease phase [55]. Although T-cell and B-cell responses are clearly important to contain the infection, they may also contribute to tissue damage [56]. The possible negative impact of immune system activation in primary infectious CNS disorders is particularly impressive in experimental Borna virus disease. Untreated animals may develop a severe immune-mediated encephalomyelitis, whereas tolerized or immunosuppressed animals may only develop subtle behavioral abnormalities [57].

Many features of MS are compatible with a chronic CNS infection, but the search for an infectious agent has been utterly unsuccessful. Although many microbes have been associated with disease, up to now evidence is lacking that any of them play a definite role in the pathogenesis of MS. Few pathogens are still the subject of intense investigation as Chlamydia pneumoniae and different herpes viruses [58,59].

Immunology of the Multiple Sclerosis Lesion
One of the first immunologic observations in MS was the finding of high immunoglobulin G (IgG) levels in the CSF, apparently caused by a local oligoclonal IgG response and mainly entailing IgG1 and IgG3 isotypes [65]. The IgG response involves a limited number of clonotypes being responsible for the oligoclonal IgG banding pattern in CSF. Indeed, the occurrence of an oligoclonal intrathecal antibody response is still the only reliable immunologic test in the diagnosis of MS, although it is not specific and is similarly found in a variety of other predominantly infectious diseases of the CNS (eg, SSPE, neurosyphilis, neuroborreliosis). In these disorders, the antibodies comprised in the oligoclonal bands recognize antigens from the infectious agents [66]. The pattern of intrathecal antibody production in MS does not change significantly during the course of disease, suggesting that the same antibodies are secreted over a long period of time [67]. These findings were recently complemented by B cell repertoire analyses in CSF and CNS of MS patients [68–71]. All studies demonstrated a preferential use of specific heavy chain genes or clonotypic accumulation of B cells in the local compartment. B cells in the CNS lesions display extensive replacement mutations clustered in the hypervariable region of B-cell receptor (BCR) genes. Comparable BCR maturation is only seen after repeated exposure of memory B cells to the same antigen.

Similar findings have been obtained concerning the T-cell response in the CNS of MS patients. By analyzing single cells from CNS lesions or CSF of MS patients, two groups demonstrated clonal accumulation of T cells in the local compartments. Clonal expansion predominantly of CD8+ and to a much lesser extent of CD4+ T-cell populations was noted [15,72]. In the lesions of one patient, up to 30% of all T cells were derived from a single CD8+ T cell as evidenced by the analysis of the molecular structure of their rearranged T-cell receptor [15]. These T cells were identified only at low numbers in the blood of these patients, suggesting specific migration to and accumulation in the CNS compartment [72].

Immunologic Clues to Multiple Sclerosis Etiology
Two main findings characterize the recent advances in our understanding of MS immunology. MS is a heterogeneous disease with respect to clinical phenotype, its pathologic changes, and its inheritance. This level of complexity is contrasted by the highly focused local immune response in the brain of MS patients. A significant number of the T cells in lesions originate from single cells. Similarly, B cells are clonotypically accumulated in the brain of MS patients and their BCRs are antigen maturated.

Although the primary event, which drives the immune response in the CNS, is still unknown, it is highly likely that the initiation and perpetuation takes place in the lym-
and could thus directly damage the antigen-expressing cell binding to cell surfaces activate the complement cascade. Specific recognition of the antigens results in clonal expansion of both T and B cells. After acquisition of effector functions, these cells circulate through the body and enter the CNS. The mechanism of transendothelial migration is mediated by the complex interplay of cellular adhesion molecules, chemokines, and matrix metalloproteinases [74]. Within the CNS, they encounter their target antigens presented by CNS cells. CD8+ T cells will respond to antigen presented by HLA-class I-expressing CNS cells, among them neurons and glia cells. Upon recognition of the specific HLA-peptide complex, the cells may release cytokines and directly damage the antigen presenting cells. In vitro, CD8+ T cells can lyse neurons and oligodendrocytes (Fig. 3) [75,76] and induce neurite damage in an antigen-dependent fashion [77]. Given the broad expression of HLA-class I molecules in the brain, the accumulation of CD8+ T cells, and the extent of axonal loss and demyelination in acute lesions [22], it is likely that CD8+ T cells play a central role in the inflammatory process in the CNS of MS patients.

CD4+ T cells require presentation of antigens in the context of HLA-class II molecules. The major source of endogenous HLA-class II expression in the CNS is activated microglia cells. Upon reactivation, CD4+ T cells initiate effector functions and synthesize cytokines and chemokines. The release of proinflammatory molecules recruits other inflammatory cells, such as macrophages, to the lesion (Fig. 3). Although CD4+ T cells play a central role in EAE, their function in MS is less clear. The cells are predominantly found in the meninges and do not seem to be of clonal origin [14,15]. Both findings do not exclude a central role of CD4+ T cells in MS because the capacity of CD8+ T cells to expand clonally is much higher than for their CD4 counterpart [78]. CD4+ T cells could still target defined disease-associated antigens, but in a much broader fashion. On the other hand, CD4+ T cells in the brain of MS patients have the capacity to release neurotrophic factors such as brain-derived neurotrophic factor (BDNF) [79]. Thus, it is tempting to speculate that some of these cells are important for neuroregeneration and protection, as observed in the axotomy model [63].

Finally, the humoral immune response also seems to play an essential role in disease pathogenesis. This view is supported by the occurrence of a persistent intrathecal IgG response and the clonal accumulation of B cells in the CNS of MS patients. In contrast with T cells, antibodies are not dependent on presentation or HLA expression and can recognize both soluble and bound proteins. IgG1 antibodies binding to cell surfaces activate the complement cascade and could thus directly damage the antigen-expressing cell (Fig. 3). However, similar to CD4+ T cells, antibodies may not only mediate detrimental effects, but also promote regeneration [80]. According to the animal studies, the humoral immune response seems to be most important in the chronic phase of disease. Several studies have investigated the target of the local humoral immune response in MS. Using expression or phage display libraries, antigen mimics were identified, although as yet their pathogenetic role in MS has not been established [81,82].

Besides the acquired immune response, both macrophages and microglia also seem to be essential for demyelination and axonal loss (Fig. 3) [23]. In the context of active ongoing demyelination, a number of toxic molecules may be generated in an inflammatory cascade: glutamate, nitric oxide, matrix metalloproteinases, calpain, and so forth [60,83]. The vigorous inflammatory response may thereby antigen-nonspecifically inflict damage on the axon. It is widely assumed that the final common pathway is calcium overflow facilitated by up-regulation of N-type calcium channels, consequent calpain activation, and eventually cytoskeleton disintegration. Finally, loss of neurotrophic support may compromise axonal and neuronal survival.

The Focus of the Immune Response

The immune response in the CNS of MS patients during the inflammatory phase of the disease appears to be highly focused, involving CD8+ and CD4+ T cells and B cells. The occurrence of a conserved and persistent intrathecal IgG1 and IgG3 antibody secretion is consistent with an ongoing immune response against proteins. The dominance of a CD8+ T cell response argues that at least part of the target antigens are derived from endogenous proteins that are synthesized within CNS cells. Given the recent broadening of the EAE concept by demonstrating encephalitogenicity of CD8+ T cells, both self antigens and antigens from neurotropic pathogens are possible candidate target antigens in MS. The focus of the immune response to the CNS limits the number of autoantigens to those that are exclusively expressed in the CNS or which occur in a unique modification in the CNS (e.g., splice-variants). Alternatively, the proteins could be derived from an intracellular pathogen. This pathogen must enter the CNS and persist there without being associated with any life-threatening diseases. Given the worldwide distribution of MS and the fact that significant epidemics have not occurred in areas where MS had been endemic before, such a pathogen must be ubiquitously present. Most of the current candidate pathogens fulfill these requirements.

At this point, it remains uncertain what accounts for the heterogeneity in clinical phenotype and pathology. Theoretically, two scenarios are possible. In case of MS being caused by one defined pathogen/autoantigen, the heterogeneity is most likely a result of the individual genetic mix-up that governs the extent and phenotype of immune responses, vulnerability of the different CNS cells, as well as their neuroprotection and neuroregeneration.
Alternatively, the causative pathogen/autoantigen may vary in individual patients, thus being the main driving force for the variability in clinical phenotype and pathology.

Conclusions
The key players in the immunopathology of MS have now been defined. Recent studies suggest that B cells and T cells that clonally accumulate in the lesion are driven by defined protein antigens, independently of whether the response is causative or protective. One of the main goals over the next few years will be to define which antigens attract the acquired immune response to the CNS. Techniques are now available that allow determination of ligands for both antibodies [84] and T cells [85]. For both approaches it will be essential to identify and isolate the relevant antibodies and T cells from the organ compartment. The success of approaches to identify the target antigens will largely depend on tools that incorporate all possible ligands with the naturally occurring modifications. These studies have to be supplemented by the use of new high-throughput techniques that allow dissection of the MS lesions in order to determine the expression profile of...
genes and proteins [86,87•,88•]. The success of these approaches will in large part depend on the quality of samples and the rigorous use of appropriate controls to determine which expression pattern is unique to MS and not only related to CNS inflammation. Although we are just at the beginning of these studies, refocusing research on the human disease and the initial events leading to the manifestation of MS may finally provide new insights in the etiology and pathogenesis of MS. We may then have the chance to design and employ therapies that significantly impact on the course of this disabling disease.

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References and Recommended Reading
Papers of particular interest, published recently, have been highlighted as:

- Of major importance
- Of importance

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG: Multiple sclerosis. N Engl J Med 2000, 343:938–952.
2. Compton A, Coles A: Multiple sclerosis. Lancet 2002, 359:1211–1213.
3. Confavreux C, Vukusic S: Natural history of multiple sclerosis: implications for counselling and therapy. Curr Opin Neurol 2002, 15:257–266.
4. Losseff NA, Wang L, Lai HM, et al.: Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. Brain 1996, 119:2009–2019.
5. Brex PA, Ciccarelli O, O’Riordan JI, et al.: A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. N Engl J Med 2002, 346:158–164.
6. Rosati G: The prevalence of multiple sclerosis in the world: an update. Neurol Sci 2001, 22:117–139.
7. Ebers GC, Dyment DA: Genetics of multiple sclerosis. Semin Neurol 1998, 18:295–299.
8. Oksenberg JR, Baranzini SE, Barcellos LF, Hauser SL: Multiple sclerosis: genomic rewards. J Neuroimmunol 2001, 113:171–184.
9. Olerup O, Hillert J: HLA class II-associated genetic susceptibility in multiple sclerosis: a critical evaluation. Tissue Antigens 1991, 38:1–15.
10. Buljjevac D, Flach HZ, Hop WC, et al.: Prospective study on the relationship between infections and multiple sclerosis exacerbations. Brain 2002, 125:952–960.
11. Gale CR, Martyn CN: Migrant studies in multiple sclerosis. Prog Neurobiol 1995, 47:425–448.
12. Kurtzke JF: Epidemiology of multiple sclerosis. Does this really point toward an etiology? Leckio Doctoralis. Neuroil Sci 2000, 21:383–403.
13. Lassmann H, Raines CS, Antel J, Prineas JW: Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. J Neuroimmunol 1998, 86:213–217.
14. Gay FW, Drye TJ, Dick GW, Esiri MM: The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis. Identification and characterization of the primary demyelinating lesion. Brain 1997, 120:1461–1483.
15. Babbe H, Roers A, Waisman A, et al.: Clonal expansions of CD8+ T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 2000, 192:393–404.
16. Cannella B, Raine CS: The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 1995, 37:424–435.
17. Baranzeini SE, Elfstrom C, Chang SY, et al.: Transcriptional analysis of multiple sclerosis brain lesions reveals a complex pattern of cytokine expression. J Immunol 2000, 165:6576–6582.
18. Trebst C, Ransohoff RM: Investigating chemokines and chemokine receptors in patients with multiple sclerosis: opportunities and challenges. Arch Neurol 2001, 58:1975–1980.
19. Lucchinetti C, Bruck W, Parisi J, et al.: Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000, 47:707–717.
20. Cepok S, Jacobsen M, Schook S, et al.: Patterns of cerebrospinal fluid pathology correlate with disease progression in multiple sclerosis. Brain 2001, 124:2169–2176.
21. Trapp BD, Peterson J, Ransohoff RM, et al.: Axonal transaction in the lesions of multiple sclerosis. N Engl J Med 1998, 338:278–285.
22. Kuhlmann T, Lingfeld G, Bitsch A, et al.: Axial axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 2002, 125:2202–2212.
23. Bitsch A, Schuchardt J, Bunkowski S, et al.: Axial axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain 2000, 123:1174–1183.
24. Prineas JW, Kwon EE, Cho ES, et al.: Immunopathology of secondary-progressive multiple sclerosis. Ann Neurol 2001, 50:646–657.
25. Wekerle H, Kojima K, Lannes-Vieira J, et al.: Animal models. Ann Neurol 1994, 36:S47–S53.
26. Olsson T, Dahlman I, Wallstrom E, et al.: Genetics of rat neuroinflammation. J Neuroimmunol 2000, 107:191–200.
27. Zamvil SS, Steimam L: The T lymphocyte in experimental allergic encephalomyelitis. Ann Rev Immunol 1990, 8:579–621.
28. Rocken M, Rake M, Shevach EM: IL-4-induced immune devia- tion as antigen-specific therapy for inflammatory autoimmune disease. Immunol Today 1996, 17:225–231.
29. Becher B, Durell BG, Miga AV, et al.: The clinical course of experimental autoimmune encephalomyelitis and inflammation is controlled by the expression of CD40 within the central nervous system. J Exp Med 2001, 193:967–974.
30. Steimam L: Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 1996, 85:299–302.
31. Panitch HS, Hirsch RL, Schindler J, Johnson KP: Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology 1987, 37:1097–1102.
32. Archelos JJ, Storch MK, Hartung HP: The role of B cells and autoantibodies in multiple sclerosis. Ann Neurol 2000, 47:694–706.
33. Cross AH, Trotter JL, Lyons J: B cells and antibodies in CNS demyelinating disease. J Neuroimmunol 2001, 112:1–14.
34. Martin R, McFarlan HE, McFarlin DE: Immunological aspects of demyelinating diseases. Annu Rev Immunol 1992, 10:153–187.
35. Wucherpfennig KW, Strominger JL: Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995, 80:693–705.
36. Hemmer B, Fleckenstein BT, Vergelli M, et al.: Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone. J Exp Med 1997, 185:1651–1659.
37. Kersh GJ, Allen PM: Essential flexibility in the T-cell recognition of antigen. Nature 1996, 380:495–498.
38. Hemmer B, Vergelli M, Pinilla C, et al.: Probing degeneracy in T-cell recognition using peptide combinatorial libraries. Immunol Today 1998, 19:163–168.
Demyelinating Disorders

39. Lehmann PV, Sercarz EE, Forshuber T, et al.: Determinant spreading and the dynamics of the autoimmune T-cell repertoire. *Immunol Today* 1993, 14:203–208.

40. Haller DA, Saadeh MG, Kuchroo VK, et al.: TCR usage in human and experimental demyelinating disease. *Immunol Today* 1996, 17:152–159.

41. Owens T, Wekerle H, Antel J: Genetic models for CNS inflammation. *Nat Rev Neurosci* 2001, 2:161–166.

42. Lafaille JJ, Keere PV, Hsu AL, et al.: Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. *J Exp Med* 1997, 186:307–312.

43. Genain CP, Abel K, Belmar N, et al.: Late complications of immune deviation therapy in a nonhuman primate. *Science* 1996, 274:2054–2057.

44. • Sun D, Whitaker IN, Huang Z, et al.: Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 2001, 166:7579–7587. Study demonstrated the encephalitogenic potency of CD8+ myelin-specific T cells in experimental autoimmune encephalomyelitis.

45. • Huseby ES, Liggitt D, Brabb T, et al.: A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *J Exp Med* 2001, 194:669–676. Study demonstrated the encephalitogenic potency of CD8+ myelin-specific T cells in experimental autoimmune encephalomyelitis.

46. Linting C, Braldi M, Lassmann H, et al.: Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am J Pathol* 1988, 130:443–454.

47. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology* 1999, 53:457–465.

48. van Oosten BW, Lai M, Hodgkinson S, et al.: Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody CM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology* 1997, 49:351–357.

49. • Bielekova B, Goodwin B, Richert N, et al.: Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med* 2000, 6:1167–1175. This paper addresses the possible negative impact of peptide therapy on the course of multiple sclerosis by correlating immunologic changes and magnetic resonance imaging activity in a small group of multiple sclerosis patients during therapy.

50. Kappos L, Comi G, Panitch H, et al.: Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group. *Nat Med* 2000, 6:1176–1182.

51. Wiendl H, Hohlfeld R: Therapeutic approaches in multiple sclerosis: lessons from failed and interrupted treatment trials. *Bio Drugs* 2002, 16:183–200.

52. Hartung HP, Gonsette RE, König N, et al., and Mitoxantrone in Multiple Sclerosis Study Group (MMMS): Mitoxantrone in progressive multiple sclerosis: a placebo-controlled double-blind, randomized, multicentre trial. *Lancet* 2002, 360:2018–2025.

53. Stohlman SA, Hinton DR: Viral induced demyelination. *Brain Pathol* 2001, 11:92–106.

54. Haring I, Perlman S: Mouse hepatitis virus. *Curr Opin Microbiol* 2001, 4:462–466.

55. Ramakrishna C, Stohlman SA, Atkinson RD, et al.: Mechanisms of central nervous system viral persistence: the critical role of antibody and B Cells. *J Immunol* 2002, 168:1204–1211.

56. Dankedar AA, Wu GE, Pernie L, Perlman S: Axonal damage is T cell mediated and occurs concomitantly with demyelination in mice infected with a neurotropic coronavirus. *J Virol* 2001, 75:6115–6120.

57. Carbone KM, Rubin SA, Nishino Y, Pletnikov MV: Borna disease: virus-induced neurobehavioral disease pathogenesis. *Curr Opin Microbiol* 2001, 4:467–475.

58. Soldan SS, Berri R, Salem N, et al.: Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nat Med* 1997, 3:1394–1397.

59. Seman S, Stratton CW, Yao S, et al.: Chlamydia pneumoniae infection of the central nervous system in multiple sclerosis. *Ann Neurol* 1999, 46:6–14.

60. Allan SM, Rothwell NJ: Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2001, 2:734–744.

61. Barone FC, Feuerstein GZ: Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999, 19:815–834.

62. del Zoppo G, Ginis I, Hallenbeck JM, et al.: Inflammation and stroke: putative role for cytokines, adhesion molecules and INOS in brain response to ischemia. *Brain Pathol* 2000, 10:95–112.

63. Moalem G, Leibowitz-Amir R, Yoles E, et al.: Autoimmune T cells protect neurons from secondary degeneration after central nervous system atrophy. *Nat Med* 1999, 5:49–55.

64. Gold R, Hartung HP, Lassmann H: T-cell apoptosis in autoimmune diseases: termination of inflammation in the nervous system and other sites with specialized immune-defense mechanisms. *Trends Neurosci* 1997, 20:399–404.

65. Losy J, Mehta PD, Wisebe MS: Identification of IgG subclasses' oligoclonal bands in multiple sclerosis CSF. *Acta Neurol Scand* 1990, 82:4–8.

66. Vartdal F, Vandvik B, Michelsen TE, et al.: Autoimmune T cells in multiple sclerosis: Results of a phase II clinical trial. *J Neurol* 1999, 246:1292–1298.

67. Walsh MJ, Tourtellotte WW: Temporal invariance and clonal uniformity of brain and cerebrospinal IgG, IgA, and IgM in multiple sclerosis. *J Exp Med* 1986, 163:41–53.

68. Qin Y, Duquette P, Zhang Y, et al.: Clonal expansion and somatic hypermutation of V(H) genes of B cells from cerebrospinal fluid in multiple sclerosis. *J Clin Invest* 1998, 102:1045–1050.

69. Owens GP, Kraus H, Burgoon MP, et al.: Restricted use of VH4 germline segments in an acute multiple sclerosis brain. *Ann Neurol* 1998, 43:236–243.

70. Baranzini SE, Jeong MC, Butroni C, et al.: B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. *J Immunol* 1999, 163:5133–5144.

71. Colombo M, Mone M, Cazzola P, et al.: Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. *J Immunol* 2000, 164:2782–2789.

72. • Jacobsen M, Cepok S, Quak E, et al.: Oligoclonal expansion of memory CD8+ T cells in the cerebrospinal fluid from multiple sclerosis patients. *Brain* 2002, 125:538–550. Study demonstrates clonal accumulation of CD8+ T cells in lesion and cerebrospinal fluid of multiple sclerosis patients.

73. Csern H, Knopf PM: Cervical lymphatics, the blood–brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* 1992, 13:507–512.

74. Kieseier BC, Seifert T, Giovannoni G, Hartung HP: Matrix metalloproteinases in inflammatory demyelination: targets for treatment. *Neurology* 1999, 53:20–25.

75. Jurewicz A, Bididdon WE, Antel JP: MHC class I-restricted lysis of human oligodendrocytes by myelin basic protein–specific CD8 T lymphocytes. *J Immunol* 1998, 160:3056–3059.

76. Neumann H, Medana IM, Bauer J, Lassmann H: Cytotoxic T lymphocytes in autoimmunity and degenerative CNS diseases. *Trends Neurosci* 2002, 25:313–319.

77. Medana I, Martinić MA, Wekerle H, Neumann H: Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. *Am J Pathol* 2001, 159:809–815.

78. Foulds KE, Zenerowicz LA, Sheardown DJ, et al.: Cutting edge: CD4 and CD8 T cells are intrinsically different in their proliferative responses. *J Immunol* 2002, 168:1528–1532.
79. Kerschensteiner M, Gallmeier E, Behrens L, et al.: Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 1999, 189:865–870.

80. Bieber AJ, Warrington A, Pease LR, Rodriguez M: Humoral autoimmunity as a mediator of CNS repair. Trends Neurosci 2002, 24:39–44.

81. Cortese I, Capone S, Luchetti S, et al.: CSF-enriched antibodies do not share specificities among MS patients. Mult Scler 1998, 4:118–123.

82. Archelos JJ, Trotter J, Previtali S, et al.: Isolation and characterization of an oligodendrocyte precursor-derived B-cell epitope in multiple sclerosis. Ann Neurol 1998, 43:15–24.

83. Bjartmar C, Trapp BD: Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. Curr Opin Neurol 2001, 14:271–278.

84. Gilden DH, Burgoon MP, Kleinschmidt-DeMasters BK, et al.: Molecular immunologic strategies to identify antigens and b-cell responses unique to multiple sclerosis. Arch Neurol 2001, 58:43–48.

85. Jacobsen M, Cepok S, Oertel WH, et al.: New approaches to dissect degeneracy and specificity in T cell antigen recognition. J Mol Med 2001, 79:358–367.

86. Whitney LW, Becker KG, Tresser NJ, et al.: Analysis of gene expression in multiple sclerosis lesions using cDNA microarrays. Ann Neurol 1999, 46:426–428.

87. Chabas D, Baranzini SE, Mitchell D, et al.: The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science 2001, 294:1731–1735.

First approaches applying microarray and large-scale sequencing technology to dissect gene expression in multiple sclerosis lesions.

88. Lock C, Hermans G, Pedotti R, et al.: Gene microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002, 8:500–508.

First approaches applying microarray and large-scale sequencing technology to dissect gene expression in multiple sclerosis lesions.