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Symposium on Multiple Sclerosis: New dimensions in basic research and their clinical applications

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Aims of the symposium

The permanent acquirement and clinical application of exponentially growing new scientific discoveries are demanding tasks for academic neurologists. To facilitate the overview of recent developments in basic and clinical sciences of inflammatory demyelination we invited seven internationally recognized experts in multiple sclerosis (MS) to discuss their most recent works. These lectures allowed the audience to formulate a modern concept of MS by integrating data from studies on molecular pathogenesis, genetics, MRI characteristics and clinical trials. The audience was invited from the broader medical community including graduate and medical students, neurology residents, private practitioners, academic neurologists, clinical and research specialists of MS.

Overview: The autoimmune hypothesis of Multiple Sclerosis

While the exact cause of MS remains unknown, both environmental and genetic factors are implicated in the development of pathology. In accordance with genetic epidemiologic studies genomic scans confirm that multiple susceptibility loci are involved, each probably with minor effect (Ebers *et al*, 1995, 1996). The autoimmune hypothesis of MS implies that activated monocytes and autoreactive lymphocytes migrate from the peripheral circulation via the blood-brain barrier (BBB) into the central nervous system (CNS), where either directly or through their soluble inflammatory products contribute to demyelination and axonal degeneration. The activation of a CD4+ T lymphocyte is initiated by the engagement of the T cell receptor (TCR) with its specific peptide presented by the MHC II molecule (Richert *et al*, 1989; Hemmer *et al*, 1997). Several self and microbial antigens with putative pathogenic significance in MS have been inter-related by molecular mimicry or by a degenerative TCR-antigen recognition (Hemmer *et al*, 1997). Recent data raise the possibility that modified self molecules differentially expressed in MS brains rather than an abnormality in the immune response are responsible for the development of the dysimmune process (Voskuhl *et al*, 1993; Martin *et al*, 1994; Wood *et al*, 1996; Becker *et al*, 1997; Archelos *et al*, 1998). Importance of accessory molecules is now recognized not only in T cell activation and differentiation, but also in a multitude of immune interactions. Differentiated T helper (TH) 1 and TH2 lineages with distinct cytokine profiles are thought to promote or inhibit the development of MS, respectively. Critical events in the pathogenesis of MS occur at the BBB where a group of adhesion molecules, matrix metalloproteinases and chemokines control the entry of MNCs in the CNS (Yong *et al*, 1998). Many of these cell surface and secretory molecules involved in immune interactions are targeted by new experimental treatment strategies, and also found to be regulated by drugs (e.g. beta-interferons) controlling the relapse and progression rate of the relapsing-remitting form of the disease (Lublin *et al*, 1996; Issazadeh *et al*, 1998; Comabella *et al*, 1998; Yong *et al*, 1998). Magnetic resonance imaging (MRI) has been an invaluable tool to describe the natural history and to monitor efficacy of treatments in MS. MRI may also serve to clarify a long debated question as to whether MS is a systemic autoimmune condition with secondary demyelination in the CNS, or a CNS restricted abnormality with secondary infiltration by MNCs (Sriram and Rodriguez, 1997). Most but not all of serial MRI studies using traditional sequences (Gadolinium enhanced T1 and T2 images) alone or in combination with more recent methods (magnetization transfer ratio) suggest that the break down of BBB precedes or coincides with the development of new lesions in MS (Kermode *et al*, 1990; Katz *et al*, 1993; Filippi *et al*, 1998; Silver *et al*, 1998). Demyelination in the CNS has been related to activated immune cells and to their soluble products. Both new MR and

histologic methods improve our understanding of the mechanism and temporal development of this process. Axonal degeneration occurs in association with inflammatory demyelination in the CNS (Trapp *et al*, 1998). As axonal degeneration is the major pathological correlate of clinical disability, development of neuroprotective strategies may open a new period in the treatment of MS.

References

Archelos JJ, Trotter J, Previtali S, Weissbrich B, Toyka KV, Hartung HP (1988). Isolation and characterization of an oligodendrocyte precursor-derived B-cell epitope in multiple sclerosis. *Ann Neurol* **43:** 14–24.

Becker KG, Mattson DH, Powers JM, Gado AM, Biddison WE (1997). Analysis of a sequenced cDNA library from multiple sclerosis lesions. *J Neuroimmunol* **77:** 27–38.

Comabella M, Balashov K, Issazadeh S, Smith D, Weiner HL, Khoury SJ (1989). Elevated interleukin-12 in progressive multiple sclerosis correlates with disease activity and is normalized by pulse cyclophosphamide therapy. *J Clin Invest* **102:** 671–678.

Ebers GC, Sadovnick AD, Risch NJ, the Canadian Collaborative Study Group (1995). A genetic basis for familiar aggregation in multiple sclerosis. *Nature* **377:** 150–151.

Ebers GC, Kukay K, Bulman DE, Sadovnick AD, Rice G, Anderson C *et al*, (1996). A full genome search in multiple sclerosis. *Nat Genet* **13:** 472–476.

Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G (1998). Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. *Ann Neurol* **43:** 809–814.

Hemmer B, Fleckenstein BT, Vergelli M, Jung G, McFarland H, Martin R, Weismuller KH (1997). Identification of high potency microbial and self ligands for a human autoreactive class-II restricted T cell clone. *J Exp Med* **185:** 1651–1659.

Issazadeh S, Navikas V, Schaub M, Sayegh M, Khoury S (1998). Kinetics of expression of costimulatory molecules and their ligands in murine relapsing experimental autoimmune encephalomyelitis in vivo. *J Immunol* **161:** 1104–1112.

Katz D, Taubenberger JK, Canella B, McFarlin DE, Raine CS, McFarland HF (1993). Correlation between magnetic resonance imaging findings and lesion development in chronic, active multiple sclerosis *Ann Neurol* **34:** 661–669.

Kermode AG, Thompson AJ, Tofts P, MacManus DG, Kendall BE, Kinsley DPE, Moseley IF, Rudge P, McDonald WI (1990). Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. *Brain* **113:** 1477–1489.

Lublin FD, Whitaker JN, Eidelman BH, Miller AE, Arnason BGW, Burks JS (1996). Management of patients receiving interferon beta-1b for multiple sclerosis: report of a consensus conference. *Neurology* **46:** 12–18.

Martin R, Whitekar JN, Rhame L, Goodin RR, McFarland HF (1994). Citrulline-containing myelin basic protein is recognized by T-cell lines derived from multiple sclerosis patients and healthy individuals. *Neurology* **44:** 123–129.

Richert JR, Robinson ED, Deibler GE, Martenson RE, Dragovic LJ, Kies MW (1989). Evidence for multiple human T cell recognition sites on myelin basic protein. *J Neuroimmunol* **23:** 55–66.

Silver NC, Lai M, Symms MR, Barker GJ, McDonald WI, Miller DH (1998). Serial magnetization transfer imaging to characterize the early evolution of new MS lesions. *Neurology* **51:** 758–764.

Sriram S, Rodriguez M (1997). Indictment of the microglia as the villain in multiple sclerosis. *Neurology* **48:** 464–470.

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998). Axonal transection in the lesions of multiple sclerosis. *New Eng J Med* **338:** 278–285.

Yong VW, Chabot S, Stuve O, Williams G (1998). Interferon beta in the treatment of multiple sclerosis. Mechanism of action. *Neurology* **51:** 682–689.

Voskuhl RR, McFarlin DE, Tranquill LR, Deibler G, Stone R, Maloni H, McFarland HF (1993). A novel candidate autoantigen in a multiplex family with multiple sclerosis: prevalence of T-lymphocytes for an MBP epitope unique to myelination. *J Neuroimmunol* **46:** 137–144.

Wood DD, Bilbao JM, O'Connors P, Moscarello MA (1996). Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. *Ann Neurol* **40:** 18–24.

Differential gene expression in MS immune cells

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We used differential display to screen for differential gene expression in peripheral blood mononuclear cells (PBMCs) from identical twins discordant for MS. An mRNA detected only in the healthy twin codes for the transcription factor Sp3. Using RT-PCR, Sp3 was detected in 88% of control subjects, including those with RA and SLE, but was found in only 18% of MS patients ($P<0.001$). These results were confirmed by Rnase protection assay. The lack of Sp3 mRNA could not be explained on the basis of abnormally rapid degradation, as treatment of PBMCs with cyclohexamide under conditions that increased detectable levels of c-fos mRNA in the same cells and Sp3 mRNA in control cells failed to produce detectable Sp3 mRNA in the MS PBMCs. Sp3-specific primers did amplify bands of appropriate size from chromosomal DNA from all subjects tested and Southern blot analyses were identical in both groups of subjects. Normal CD4+ and CD8+ T cells, B cells, and macrophages expressed comparable amounts of Sp3 message. No Sp3 expression could be detected in normal NK cells nor in any of these cell types from Sp3-negative MS patients. We propose that transcription of the Sp3 gene is blocked in immune cells from most MS patients, leading to altered expression of one or more gene products involved in the development of CNS inflammation.

Understanding molecular aspects of T cell recognition and effector functions. Implications for molecular mimicry and novel immunotherapies of multiple sclerosis

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The etiology of multiple sclerosis (MS) is still unknown, but autoreactive T lymphocytes directed against myelin antigens are considered relevant for its pathogenesis. During recent years, we have dissected in detail the fine specificity, activation requirements and functional repertoire of myelin basic protein (MBP)-specific CD4+ T cells in MS patients and controls. The analysis of the recognition of the immunodinant MBP peptide (83-99) in DR2+ MS patients and controls provided the basis for the development of an altered peptide ligand (APL) which presently is in phase II testing. Furthermore, studies with mutated peptides and combinatorial peptide libraries shed new light on molecular mimicry and its potential role for the induction of autoimmune responses. Finally, targeting enzymatic pathways in T lymphocytes and mononuclear cells may provide interesting treatment strategies in T helper 1 mediated autoimmune diseases such as MS.

Experimental therapies in MS

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Several lines of evidence from experimental and clinical research suggest that multiple sclerosis is an immune mediated disease affecting central nervous system myelin. Patients with chronic progressive MS have an increased production of IL-12. We investigated the immunologic changes in MS patients treated with immunosuppressive therapies, and found that cyclophosphamide monthly boosters downregulate the expression of IL-12 in monocytes of patients with progressive MS. The mechanisms of this finding and potential use of other drugs that downregulate IL-12 will be discussed.

The rationale for many of the therapies in multiple sclerosis (MS) is generally based on findings in the EAE model. In EAE, the disease is initiated by antigen-specific encephalotigenic CD4+ T cells. T cells require 2 signals for full activation: the first signal is provided by engagement of the T cell receptor with the antigenic peptide plus major histocompatibility complex on antigen-presenting cells, and the second 'costimulatory' signal is provided by binding of specific receptors on T cells with the ligand/s on APCs. The best characterized costimulatory pathway is that provided by CD28 on T cells binding to B7-1 and B7-2 on professional APCs. Blocking costimulatory signals has been reported by several laboratories including ours to protect from EAE. We will discuss experimental approaches to impart specificity to this therapy, and potential synergy between costimulatory pathway blocking agents.

Genetics of Multiple Sclerosis

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Multiple Sclerosis (MS) is a common chronic central nervous system disease in young adults. Relative familiar risk appears determined largely by genes while population risk is strongly influenced by environmental factors. This is supported by genetic epidemiological studies which also suggest an oligogenic inheritance of susceptibility. The HLA DRB1*1501, DQA1*0102, DQB1 0602 haplotype is associated with the disease but HLA contributes only modestly to overall susceptibility. The results of three genomic searches are concordant with the genetic epidemiology and imply a number of genes with interacting effects will be found. Importantly, no single region has been identified with a major influence on familiar risk.

Axonal transection in the lesions of multiple sclerosis

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This presentation will review data that support the hypothesis that irreversible axonal pathology, including axonal transection, occurs as a consistent consequence of inflammatory demyelination in MS lesions, and importantly, that axonal transection begins at the time of disease onset, and is later increasingly accompanied by axonal degeneration. We argue that irreversible axonal injury represents the underlying pathogenic process responsible for development of irreversible neurologic disability, and specifically for conversion from RR-MS to SP-MS. The therapeutic corollary to this concept is that axon preservation should be considered as a primary strategy for future clinical trials in MS patients. Further, neuroprotective strategies need to be applied early in the disease course, during periods of apparent quiescence.

The role of MRI in defining the natural history of multiple sclerosis and in monitoring experimental therapies

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The results of studies of multiple sclerosis (MS) using MRI over the past decades have brought a dramatic new understanding of the disease. Evidence of a treatment effect has played an important role in the approval of the first drug, interferon beta 1b, for the treatment of MS. Currently MRI is used as either a primary or secondary outcome measure in almost every recent trial of new therapies. In addition to the value of MRI in monitoring new therapies, it has yielded a new understanding of the disease. MRI measures of disease activity indicate that approximately ten times more activity is seen as compared to clinical measures of disease activity. More specifically, serial studies of patients with elapsing remitting MS relatively early in the course of disease indicates that nearly two-thirds have evidence of active disease as seen on contrast enhanced MRI. Patients followed for 6 months or longer show considerable variation in the number of enhancing lesions from month to month but the average lesion frequency over any 6-month period remains relatively constant; some patients will have a consistently low frequency of activity while others may have an average of 20 or more lesions per month. MRI techniques other than those used conventionally to monitor disease are now providing additional insights. The strongest correlation with clinical disability is with the burden of disease as measured by hypointensities on T1 weighted images. Magnetization transfer imaging may provide a valuable tool for more specifically analyzing demyelination. It is hoped that these new imaging techniques will provide valuable tools for

Current clinical trials in MS

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Currently, most pivotal trials underway are assessing additional indications for the agents that have already been approved for treatment of relapsing-remitting MS. These include studies on secondary progressive and primary progressive patients as well as mono-symptomatic patients, who have not yet met the diagnostic criteria for MS. In addition studies are starting to assess the effects of combination therapy. Earlier phase studies are underway on a number of immunomodulatory agents, including adhesion molecular blockade.

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