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from
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in collaboration with
Neuroimmunology Unit, Medical School, University of Tampere,
Tampere, Finland

Multiple Sclerosis

MRI Diagnosis, Potential Treatment and Future
Potential for Nanoparticle Applications

Xingchen Wu, M.D.

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Abstract

Multiple Sclerosis is an inflammatory demyelinating disease of the central nervous system with unknown etiology and without fully effective treatment. It is the leading cause of neurological disability among young adults. The majority of patients initially experience a relapsing-remitting clinical course with transient symptoms followed by a secondary progressive course characterized by gradual progression of disability and neurodegeneration. Multiple Sclerosis continues to be diagnosed on a clinical basis, although this is facilitated by supportive laboratory and radiological examinations. Magnetic resonance imaging is an important tool for diagnosing of Multiple Sclerosis and monitoring its evolution of pathology in vivo.

Over the past decade, several disease-modifying therapies for Multiple Sclerosis have become available. The introduction of interferon (IFN)-β therapy has altered the natural course of the disease. In relapsing-remitting form of Multiple Sclerosis a clear dose response effect has been demonstrated, indicating that a higher dose is more effective. IFN-β-1a has been shown to be effective in the treatment of Multiple Sclerosis, but little is known as to how discontinuation of treatment affects the subsequent disease course. Therefore we investigated the effect of low-dose low-frequency and high-dose high-frequency IFN-β-1a in patients with secondary progressive Multiple Sclerosis, and also after discontinuation of treatment. Serial volumetric magnetic resonance imaging and clinical assessments were carried out at the same time points. The longitudinal study showed that a single weekly dose of 22 µg IFN-β-1a had a mild beneficial effect visualized on magnetic resonance imaging, but this dosage and frequency of administration was insufficient in controlling clinical disease activity. Discontinuation of IFN-β-1a was associated with an acceleration of neurological disability and brain lesion development. The observations stress the importance of high-dose high-frequency IFN-β-1a administration and the maintenance of treatment in patients with active secondary progressive Multiple Sclerosis.

The current disease-modifying therapies for Multiple Sclerosis have had a significant impact, although being partially effective in halting the disease process. There is unfortunately no treatment currently available that is capable of preventing relapses or disease progression. A major hurdle in the research of neuroinflammatory disorders of the central nervous system is the inaccessibility of the organ. To overcome such limitation approaches have been developed using animal models to study pathological mechanisms and to design rational therapeutic strategies. Experimental autoimmune/allergic encephalomyelitis (EAE) is an animal model that closely mimics key features of human Multiple Sclerosis. We induced rat and mouse EAE by immunization with myelin oligodendrocyte glycoprotein (MOG), or peptides of myelin basic protein (MBP), proteolipid protein (PLP), or MOG. We studied EAE-regulatory genes and explored novel individualized therapeutic strategies in these EAE models.

Multiple Sclerosis is governed by multiple genes exerting small individual effect. Genetic analyses aim to identify primary mechanisms that mediate disease susceptibility. Difficulties arise from genetic heterogeneity, insufficient sample sizes, and small effect of each disease-regulating gene in Multiple Sclerosis. Positional cloning of genes in experimental models of the disease may circumvent some of these difficulties. A number of inbred rat strains differ in their relative susceptibilities to EAE.
a genetic predisposition. The availability of such strains of rats, the discovery of polymorphic genetic markers, as well as the development of genetic and physical maps provided an opportunity for mapping of disease-regulating genes in EAE. An advanced intercross line (AIL) is obtained by random intercrossing of two inbred strains for several generations. This regime generates genetically unique offspring individuals with a dense mixture of founder chromosomal fragments, in which a genomic region involved in regulation of a disease is called a quantitative trait locus (QTL). We fine-mapped QTLs that regulate MOG-induced EAE on rat chromosome 10 in the 7th generation of an AIL. AIL mapping was shown to be an efficient approach to narrow down QTLs and to resolve closely situated QTLs. The identification of EAE-regulatory genes will hopefully improve the understanding of Multiple Sclerosis pathogenesis and further unravel more efficient therapeutic targets.

Multiple Sclerosis is of putative autoimmune etiology. Autoimmune diseases are characterized by loss of tolerance to self-determinants, and therapeutic approaches aim to re-establish this tolerance. Dendritic cells (DC) are antigen-presenting cells specialized to regulate immune responses. DC not only control immunity, but also maintain tolerance to self-antigens, thereby minimizing autoaggressive immune responses. The recent identification of tolerogenic subsets of DC and their generation in culture may provide a rationale for designing immunotherapeutic strategies in Multiple Sclerosis. We investigated the therapeutic potential of IFN-γ modified DC and CD8α+ DC, respectively, in rat and mouse EAE models. Severity of clinical disease signs was dramatically inhibited in animals injected with IFN-γ modified DC or CD8α+ DC, also showing no lesions of the spinal cord and brain on magnetic resonance imaging. Infiltration of inflammatory cells within the spinal cords was also reduced in actively treated animals as compared to control groups. These approaches may provide a cellular basis for a novel individualized immunotherapy using autologous, in vitro modified DC in Multiple Sclerosis.

In conclusion, this thesis studied Multiple Sclerosis from clinical perspective, genetic pathogenesis to therapeutic strategies using magnetic resonance imaging and EAE animal models as tools. The data from the clinical studies support the concept that IFN-β treatment should be administrated at high-dose high-frequency, and be maintained in patients with active secondary progressive Multiple Sclerosis. The therapeutic studies on EAE models may provide a cellular basis for a future individualized immunotherapy as a complement to current treatment options in Multiple Sclerosis.

Given the availability of current partially effective treatments, it is important to make the early diagnosis and treatment for patients with Multiple Sclerosis to prevent the accumulation of disability. The next step is to improve the specificity and sensitivity of magnetic resonance imaging to further unravel the pathogenesis of Multiple Sclerosis, to explore more targeted therapeutic strategies, and to transfer experimental agents or strategies from the laboratory bench to bedside via of magnetic resonance molecular imaging and nanotechnology.

Key words: dendritic cell, experimental autoimmune encephalomyelitis, interferons, magnetic resonance imaging, multiple sclerosis, nanoparticles, therapy.
Original Papers

This thesis is based on the following papers, which are referred to by their Roman numbers.


<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>ACI</td>
<td>A×C 9935 Irish</td>
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<tr>
<td>AIL</td>
<td>advanced intercross line</td>
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<tr>
<td>ALS</td>
<td>amyotrophic lateral sclerosis</td>
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<td>AM</td>
<td>adhesion molecule</td>
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<td>APC</td>
<td>antigen-presenting cells</td>
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<td>B6</td>
<td>C57BL/6</td>
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<td>BBB</td>
<td>blood-brain barrier</td>
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<td>BN</td>
<td>Brown Norway</td>
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<td>BPF</td>
<td>brain parenchyma fraction</td>
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<tr>
<td>CCL</td>
<td>CC chemokine ligand</td>
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<tr>
<td>CIS</td>
<td>clinically isolated syndrome</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>DA</td>
<td>Dark Agouti</td>
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<td>DC</td>
<td>dendritic cells</td>
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<td>DW</td>
<td>diffusion-weighted</td>
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<tr>
<td>EAE</td>
<td>experimental autoimmune/or allergic encephalomyelitis</td>
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<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<tr>
<td>FA</td>
<td>Freund’s adjuvant</td>
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<td>FLAIR</td>
<td>fluid-attenuated inversion recovery</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>FSE</td>
<td>fast spin echo</td>
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<tr>
<td>GA</td>
<td>glatiramer acetate</td>
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<td>GEFI</td>
<td>gradient echo fast imaging</td>
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<tr>
<td>Gd</td>
<td>gadolinium</td>
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<tr>
<td>Gd DTPA</td>
<td>gadolinium-diethylenetriamine pentaacetic acid</td>
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<tr>
<td>HLA</td>
<td>human leukocyte-associated antigen</td>
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<td>IDO</td>
<td>indoleamine 2,3-dioxygenase</td>
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<td>IFN</td>
<td>interferon</td>
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<td>Ig</td>
<td>immunoglobulin</td>
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<td>interleukin</td>
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<td>IVIG</td>
<td>intravenous immunoglobulin</td>
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<td>MAP</td>
<td>myelin associated protein</td>
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<td>Mb</td>
<td>megabase</td>
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<td>MBP</td>
<td>myelin basic protein</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>MION</td>
<td>monocrystalline iron oxide nanoparticles</td>
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<td>MMP</td>
<td>matrix metalloproteinase</td>
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<td>MOG</td>
<td>myelin oligodendrocyte glycoprotein</td>
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<td>mPEG</td>
<td>methoxy poly-ethylene glycol</td>
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<td>MR</td>
<td>magnetic resonance</td>
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<td>MRI</td>
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<td>MRMI</td>
<td>MR molecular imaging</td>
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<td>MRS</td>
<td>MR spectroscopy</td>
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<td>MS</td>
<td>multiple sclerosis</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MSFC</td>
<td>MS Functional Composite</td>
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<tr>
<td>MT</td>
<td>magnetization transfer</td>
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<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
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<tr>
<td>Nab</td>
<td>neutralizing antibody</td>
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<td>NAGM</td>
<td>normal-appearing grey matter</td>
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<tr>
<td>NAWM</td>
<td>normal-appearing white matter</td>
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<tr>
<td>NO</td>
<td>nitric oxide</td>
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<tr>
<td>OCB</td>
<td>oligoclonal band</td>
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<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PC</td>
<td>personal computer</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PD</td>
<td>proton density</td>
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<td>PLLA</td>
<td>poly-L-lactic acid</td>
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<td>PLP</td>
<td>proteolipid protein</td>
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<tr>
<td>PPMS</td>
<td>primary progressive MS</td>
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<tr>
<td>PRMS</td>
<td>progressive-relapsing MS</td>
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<td>PVG</td>
<td>Piebald Virol Glaxo</td>
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<td>QTG</td>
<td>quantitative trait locus</td>
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<tr>
<td>RARE</td>
<td>rapid acquisition with relaxation enhancement</td>
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<td>RA</td>
<td>rheumatoid arthritis</td>
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<tr>
<td>RF</td>
<td>radio frequency</td>
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<tr>
<td>rMOG</td>
<td>recombinant MOG</td>
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<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>RR</td>
<td>relapsing-remitting</td>
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<td>RRMS</td>
<td>relapsing-remitting MS</td>
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<tr>
<td>SE</td>
<td>spin echo</td>
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<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
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<tr>
<td>SPION</td>
<td>superparamagnetic iron oxide nanoparticles</td>
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<td>SPMS</td>
<td>secondary progressive MS</td>
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<td>T</td>
<td>Tesla</td>
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<tr>
<td>TE</td>
<td>echo time</td>
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<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
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<tr>
<td>TGF</td>
<td>transforming growth factor</td>
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<tr>
<td>Th</td>
<td>T help</td>
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<tr>
<td>TIMP</td>
<td>tissue inhibitor of metalloproteinase</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<tr>
<td>UVR</td>
<td>ultraviolet radiation</td>
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<td>VEP</td>
<td>visual evoked potentials</td>
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<td>VLA</td>
<td>very late antigen</td>
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<td>3D</td>
<td>3-dimensional</td>
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1 INTRODUCTION
1.1 GENERAL BACKGROUND

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) with unknown etiology. The disease typically manifests between 20 and 40 years of age (1, 2), and an increasing number of MS patients are being identified among children and adolescents (3). MS is usually not life shortening. However, it is the major cause of neurological disability in young adults, and therefore it has substantial personal, social, and economic costs.

In MS, myelin sheath, the fatty substance that surrounds and insulates nerve fibers, is focally damaged (demyelination) in multiple areas, leaving scar tissue called sclerosis. These damaged areas are also known as plaques or lesions (Fig 1). Sometimes the nerve fiber (axon) itself is also damaged. Conduction of the nerve impulse is then slowed or halted, producing neurological signs and symptoms. Symptoms of MS are unpredictable and vary from patient to patient and from time to time in the same patient. For example, one patient may experience abnormal fatigue, while another might have severe vision problems. One could have loss of balance and muscle coordination making walking difficult; another one could have slurred speech, tremor, dizziness, sensory disturbances and bladder problems. While some symptoms will come and go over the course of the disease, others may be more lasting. The symptoms of MS vary depending in part on the location of plaques within the CNS.

MS is characterized by its highly variable clinical course and lesions disseminated both in time and space within the CNS, although some areas, such as the optic system, periventricular and subcortical white matter, cerebellum, brain stem, and spinal cord are

![Fig 1. Cartoon of a demyelinated neuron and corresponding multiple brain lesions shown by MRI in a patient with relapsing-remitting MS](image-url)
sites of predilection. There may be recurrences at unpredictable intervals, affecting the
same or different parts of the CNS. Pathological hallmarks are mononuclear cell
infiltration, demyelination, gliosis and axonal degeneration (4). Axonal damage occurs
in addition to demyelination and may be the cause of later permanent disability (5, 6).
Distinct pathological subtypes may differentiate among patients with MS (7, 8). The
clinical heterogeneity as well as the finding of different pathological patterns suggests
that MS may be a spectrum of diseases that may represent different pathological
processes.

1.2 CLINICAL COURSE OF MULTIPLE SCLEROSIS

It is generally very difficult to predict the course of individual MS patient. Natural
history study shows that the median duration of MS is about 30 years (9). Four different
clinical courses (or subtypes) of MS have been defined (10) (Fig 2). The severity of
these subtypes may vary from mild to severe.

About 85% of MS patients initially experience a relapsing-remitting (RR) clinical
course, which is characterized by one or more self-limited attacks of neurological
dysfunction, known as relapses, or exacerbations followed by complete or partial
recovery, and there is a 2:1 predominance in women over men in relapsing-remitting
MS (RRMS) (11-13). During this phase, patients are clinically stable between relapses.

![Different Clinical Courses of Multiple Sclerosis](image)

**Fig 2. Different Clinical Courses of Multiple Sclerosis**
Adapted from Seminars in Neurology 2003; 23 (2):133-146
**Fig 3. The evolution of MS: from RRMS to SPMS**

*From top:* Schematic depiction of the clinical evolution by EDSS score (*black curve*); brain volume indicative of brain atrophy (*blue curve*); T2 lesion load documenting overall tissue damage by MRI (*red curve*); the frequency of inflammatory events by MRI – Gadolinium-enhancing lesions on T1-weighted image showing blood-brain barrier (BBB) destruction (*black arrows*); main therapies target to the pathological changes; typical MRI characteristics: Left panel showing lesions by T2-weighted image and right panel showing cerebral atrophy by T1-weighted image. *Pathological evolution:* from inflammatory demyelination to axonal degeneration (grey triangle).
Within 10 years, about 50% of RRMS patients experience gradual progression of disability with or without superimposed relapses. These patterns are called secondary progressive MS (SPMS). Approximately two-thirds of patients with RRMS eventually transitioned to SPMS (Fig 3). The remaining 15% of patients experience a clinical course that is progressive from onset, with or without relapses – patterns known as primary progressive (PPMS) and progressive-relapsing MS (PRMS), respectively. However, others have suggested that PRMS is, in fact, a variant of PPMS (14). Aside from the clinical course, PPMS demonstrates different demographic, pathological and clinical features from RRMS/SPMS, i.e. more men are affected than women, with greater age of onset, relatively lack of inflammation, and predominantly with spastic paraparesis (15, 16).

1.3 DIAGNOSIS OF MULTIPLE SCLEROSIS

MS continues to be diagnosed on a clinical basis, although this is facilitated by supportive laboratory and radiological examinations (17, 18). All diagnostic criteria are based on evidence of neurological dysfunction disseminated in time (at least three months apart) and space (lesions in different locations of the CNS). Early diagnostic criteria depended entirely on history and physical examination. Later the classical Poser diagnostic criteria for MS was made upon the occurrence of two or more distinct attacks and objective evidence of two or more lesions with magnetic resonance imaging (MRI), cerebrospinal fluid (CSF), and visual evoked potentials (VEP) analyses as diagnostic aids (19).

An international panel of MS experts has recently published new diagnostic criteria (20). The new criteria for MS focus on the objective demonstration of lesions disseminated in time and space. MRI has been integrated into the criteria, which allow earlier diagnosis of patients at their first episode – clinically isolated syndrome (CIS) and of patients with progressive forms of MS even before clinical dissemination has occurred. A diagnosis of MS requires that no other disease can better explain the symptoms besides meeting these criteria. No single neurological or laboratory test can confirm or exclude MS.

CSF abnormalities may include mildly elevated CSF white blood cell count, elevated immunoglobulin (Ig)G in CSF compared to serum (IgG index), and identification of two or more unique oligoclonal bands (OCBs) by CSF protein electrophoresis – indicative of intrathecal IgG production. OCBs are characteristic of but not specific for MS.

The Expanded Disability Status Scale (EDSS) (Fig 4), which is based on standard neurological examination of eight functional systems, has conventionally been used to evaluate the degree of neurological disability of MS patients (21). The EDSS is an ordinal clinical rating scale ranging from 0 (normal neurological examination) to 10 (death due to MS) in half-point increments. A recently new outcome measure is the MS Functional Composite (MSFC), which is based on quantitative tests of ambulation, arm function and cognitive function and is believed to be more sensitive to neurological disability change than the EDSS (22).
Fig 4. Evaluation of clinical disability by EDSS in patients with MS

1.4 PROGNOSIS OF MULTIPLE SCLEROSIS

The prognosis of MS is widely variable. The natural history of the disease is that the majority of MS patients will exhibit a progressive deterioration and with substantial clinical disability. Approximately 50% of MS patients will require the use of a cane to ambulate safely (an EDSS score 6) within 15 years after onset (2, 23) (Fig 4). In addition to sustained physical disability, 45% to 65% of patients with MS experience cognitive dysfunction that is usually irreversible (24). The observed progression from RRMS to SPMS represents the accumulation of permanent damage to the CNS (25, 26) (Fig 3). There are some guidelines that may be used to infer prognosis. Good prognostic indicators include: female gender, younger age of onset, optic neuritis, pure sensory attacks, complete recovery from attacks, few attacks in the first several years after onset, long intervals between attacks, absence of OCBs in CSF, and a low MRI lesion load. Patients who have cerebellar involvement or insidious onset of a motor deficit as first symptom, who have frequent attacks with incomplete recoveries, more lesions on MRI early on, or older age at onset, male sex, tend to have a more progressive disease course (27, 28). The prognosis of PPMS has been considered to be poorer compared with RRMS/SPMS (15).

1.5 EPIDEMIOLOGY OF MULTIPLE SCLEROSIS

The epidemiology of MS has been intensively studied. MS is probably an etiologically heterogeneous entity, in which genetic and environmental factors act together to cause disease.
1.5.1 Geographical distribution

Differences in the risk of MS by region have been reported. Worldwide, MS occurs with much greater frequency in higher latitudes away from the equator, than in lower latitudes, closer to the equator (29). Kurtzke has collated existing surveys and defined bands of MS prevalence as three frequency zones (30) (Fig 5). High prevalence (>30 per 100 000) areas include northern Europe, northern USA and Canada, southern Australia, and New Zealand. Medium prevalence (5–30 per 100 000) areas include southern Europe, southern USA, and northern Australia. Low prevalence (<5 per 100 000) areas include Asia and South America.

Fig 5. Worldwide prevalence of multiple sclerosis per 100 000 population.
Adapted from: Lancet Neurology 2004 (12):709-18

Environmental factors, genetic factors, or both can explain the geographical distribution of MS. Several ecologic studies have reported an inverse correlation between solar ultraviolet radiation (UVR) and MS risk (31), which is a possible explanation for the geographic distribution. Studies of the US Army veterans identified a north-south gradient of decreasing risk that reflects both the distribution of Scandinavian ancestry throughout the USA and a colder climate effect (32, 33). There are exceptions to the north-south gradient and will be briefly reviewed.

1.5.2 Genetic predisposition

MS is not hereditary in a strict sense. However, MS develops with a genetic predisposition. The hereditary tendency of MS is indicated by both an increased risk in individuals who have a biological first-degree relative with MS (3-5%) compared with the general population (0.1%) (34), and an increased concordance rate in monozygotic twins (25~30%) compared with dizygotic twins (~5%) (35, 36). Half-siblings of affected persons have roughly half the risk of full siblings of developing MS (37), and adopted siblings have no greater risk than the general population (38). These findings
confirmed that familial aggregation is significantly influenced by genetic factors rather than shared family environment.

MS is more common among Caucasians, particularly those of northern European ancestry, than other ethnic groups (39). Moreover, there are distinct resistant ethnic groups living in medium-to-high risk areas, such as Lapps in Norway (40), Native Americans (41), Maoris in New Zealand (42). These also suggest an influence of genetic factors in determining disease.

Monozygotic twins have a greater concordance rate than dizygotic twins (36), which suggest a polygenic nature of the disease. A strategy to study the genetics of complex disease is to first perform a whole genome screen and then to test established candidate genes within the identified regions. The results of genome screening studies provide strong evidence for exclusion of a major locus in MS. There are, however, many genes with small individual effects that seem to be associated with MS, consistent with a polygenic disease (43).

An increasing number of children and adolescents with MS are being identified (3). The risk of developing early onset MS was lowest in unrelated MS patients and higher in concordant monozygotic twins as compared to concordant non-twin siblings (44), thus early onset MS patients carry a greater genetic load for MS.

The strongest and most consistent evidence for a susceptibility gene in MS is within the major histocompatibility complex (MHC) on chromosome 6q21.3 (45). It has been suggested that interactions between different genes could contribute to an increase in susceptibility (46). Therefore, the contribution of genetic factors is complex. The total genetic susceptibility attributed to the human leukocyte-associated antigen (HLA) locus in MS is estimated between 15% and 50% (47), indicating a significant role of non-HLA genes.

### 1.5.3 Environmental factors

Although it seems clear that there is a genetic component in MS etiology, the fairly low concordance rate among identical twins (25%~30%) indicates that non-genetic (environment, stochastic) factors play a strong role in influencing the risk (36). Support for viral involvement of MS comes from the notion that viral infections often antecede the disease onset (48) and up to 25% of relapses are triggered by virus (49, 50). A reduced risk in migrants moving from high-risk to low-risk areas during the first two decades of life (51) implicates the effect of environmental factors in MS etiology. Migrants from high to lower risk areas retain the MS risk of their birth place only if they are older than age 15 at migration, with susceptibility extending from about age 11 to 45 (30). Therefore, MS is ordinarily acquired in early adolescence with a lengthy latency before symptom onset. Potential environmental risk factors of MS include infectious agents, vaccinations, stress, occupational exposures, cold climate, and diet.

There is, as yet, no definite proof that diet actually influences the incidence of MS (52). Whereas there is some evidence that a high intake of saturated fat increases the incidence of MS (53). Epidemiological studies imply that unsaturated fatty acids may
have a beneficial effect on the course of MS (54). However, the results of controlled studies are ambiguous (55). Supplementation of Vitamin D is associated with a lower incidence of MS (56). The effects of various minerals, trace elements, antioxidants, fish oil, and other vitamins remain speculative.

The environmental component appears to be ubiquitous and acts at a population level rather than in the familial microenvironment. This far, no single environmental risk exposure has been consistently identified as a causal factor in MS. Thus, environmental factors promoting the outburst MS has to be evaluated as statistical risk factors on top of the genetic predisposition.

1.6 THE CENTRAL NERVOUS SYSTEM – THE CENTRAL PLAYER

The central nervous system has long been considered as an immunologically privileged site (57, 58). (i) The CNS parenchyma is separated from the systemic circulation by the specialized blood-brain barrier (BBB). (ii) The general absence of immune cells, and there is a relative lack of lymphatic drainage from the CNS which would allow antigen-presenting cells (APC) to migrate to immune organs and trigger a full immune response; (iii) MHC determinants are absent in the mature CNS, preventing the presentation of antigenic peptide to specific T cells; (iv) professional APC are missing in the CNS. However, it is now clear that the CNS does not display absolute immunological privilege as activated lymphocytes can cross the BBB, certain cells such as microglia may have an APC capacity, and there is lymphatic drainage from the CNS into the deep cervical lymph nodes (59). Thus immune reactions do occur in the CNS, but take on a distinctive character. The CNS parenchymal tissue may represent an immunosuppressive environment, and the CNS should more accurately be viewed as an immunologically specialized site.

1.6.1 The blood-brain barrier

The blood-brain barrier (Fig 6) is composed by three cellular elements of the brain capillary – endothelial cells, astrocyte end-feet, and smooth muscle cells (also called pericytes). Tight junctions, present between the cerebral endothelial cells, form a diffusion barrier that strictly regulating the entry of plasma proteins and blood borne cells into the nervous tissue. The endothelium proper is complemented by a basement membrane that separates it from the CNS parenchyma, and apposition of astrocyte end-feet to the basement membrane adds another dimension to the BBB (60).

Astrocytes and microglia play a significant role in host defense as well as in the pathogenesis of infectious and autoimmune diseases of the CNS. They ordinarily protect the CNS, but in pathologic circumstances can amplify inflammation and mediate cellular damage (60). Disruption of the BBB is one of the initial key steps operative in MS pathogenesis, which follows massive infiltration of T cells and the formation of demyelinating foci (61, 62). The dynamic properties of the BBB make it a promising site for direct immunomodulatory therapies in MS (63).
1.6.2 The myelin sheath

Myelin is a substance rich in lipid (70–75%) and protein (20–30%), which forms layers around the nerve fibers – myelin sheath (Fig 7). The CNS myelin, believed to be the...
major target of MS pathology, is produced by oligodendrocytes (In the peripheral nervous system, Schwann cells form myelin). The myelin sheath is segmented regularly by nodes of Ranvier that are naturally unmyelinated. Saltatory conduction of electrical current along the nerve occurs by jumping from node to node. Thus, myelin sheath insulates and enhances the conduction of nerve impulse through axons. Several components of myelin have been demonstrated to have encephalitogenic potential in animals and will be briefly reviewed here. The major contents of myelin are proteolipid protein (PLP) and myelin basic protein (MBP), making up 50% and 30% of the myelin proteins respectively. Minor components include myelin associated protein (MAP) (less than 1%) located at the inner surface of the myelin sheath opposing the axon surface, and the CNS-specific myelin oligodendrocyte glycoprotein (MOG) constituting only 0.05% of the total myelin proteins. MOG belongs to the Ig superfamily with a single extracellular Ig-like domain (64). MOG is expressed on the outermost surface of the myelin sheath, which makes it directly accessible and vulnerable to antibody attack (65).

1.7 EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS

A major hurdle in the research of neuroinflammatory disorders of the CNS is the inaccessibility of the organ. Thus investigation is limited to end-stage disease and systemic changes that occur during disease progression, neither of which may reflect the pathological process in the CNS. To overcome such limitations approaches have been developed using relevant animal models to study pathological mechanisms as well as the design of rational therapeutic strategies.

Experimental autoimmune encephalomyelitis (EAE), also called experimental allergic encephalomyelitis (EAE), an animal model of human MS, can be induced either by active immunization or by adoptive transfer of myelin specific T cells (66). Immunization is performed with myelin component together with Freund’s adjuvant (FA), which enhances immune responses to emulsified antigens (67). Most commonly used antigens are whole spinal cord homogenate, purified CNS myelin antigens such as MBP, PLP, MOG, or their peptides. Depending on the animal strain, antigen used, and type of immunization, acute monophasic or chronic relapsing-remitting disease courses can emerge, and different clinical courses simulate various subtypes of MS. For example, SJL/J mice immunized with a peptide of PLP (PLP139–151) or injected with PLP139–151-specific T cells exhibit a relapsing-remitting course reminiscent of the most common form of MS. By contrast, C57BL/6 (B6) mice actively immunized with a peptide of MOG (MOG35–55) develop a progressive form of EAE characteristic of later stages of MS. EAE has been induced in a number of animal species including rats, mice, guinea pigs, rabbits, and monkeys (68). Mice and rats are the most commonly used species since they are easy to maintain and handle.

EAE is considered to be primarily T cell-mediated autoimmune disorder, and CD4\(^+\) antigen-specific T cells within the CNS appear crucial in the development of EAE (69). This is proofed by the induction of EAE via passive transfer of encephalitogenic CD4\(^+\) T cells into a naïve animal – “transfer EAE” (70). Most studies of EAE have focused on the role of CD4\(^+\) T helper 1 (Th1) cells and many therapeutic strategies have been directed toward ameliorating the activity of this subset of T cells. Indeed, many recent advances in MS therapeutics, including the introduction of glatiramer acetate (GA) and
antibodies (natalizumab) against very late antigen (VLA)-4, arose from EAE animal model studies (68).

1.8 IMMUNOLOGY OF MULTIPLE SCLEROSIS

1.8.1 Autoimmunity of MS

The concept of autoimmunity means that the immune system mistakenly attacks the body’s own tissue. It is generally believed that MS is an autoimmune disease, in which a genetically susceptible individual when exposed to an environmental factor or factors trigger an autoimmune response directed against the CNS myelin, the oligodendrocytes (26). There is increasing evidence supporting the autoimmune nature of MS. First, predominance of women is affected, similar to autoimmune disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and myasthenia gravis (71). Second, there is a transient amelioration of disease activity during pregnancy, a relatively immunosuppressed state (72). Third, susceptibility to the disease is linked to various regulatory genes of immune response (73). Fourth, there are clinical and immunopathological similarities of MS with the animal model EAE, which is known to be autoimmune in nature (6). Fifth, increased quantities of immune cells and antibody are found in the CNS lesions and CSF of MS patients (8, 74). CSF OCBs are found in the majority of MS patients at some time during the disease course (26). Sixth, other autoimmune diseases occur with increased frequency in MS patients and in their families, it suggests that MS patients have a genetic predisposition to autoimmunity in general (75). Finally, it has been shown that immunotherapy can modify the disease course, at least in the short term (76).

1.8.2 Immunopathogenesis of MS

Regarding MS pathogenesis, although not yet formally proven, the current consensus is that MS pathogenesis comprises an initial autoimmune inflammatory phase, followed by a phase of selective demyelination and at last, a neurodegenerative phase (77, 78) (Fig 3).

Present theories about the pathophysiology of MS have largely developed from EAE models in rodents. Inflammatory demyelination in MS involves a spectrum of immunological mechanisms, and myelin-specific CD4⁺ T cells play a central role. The prevailing hypothesis is that autoreactive T cells within the peripheral circulation are activated by an unknown mechanism, probably by molecular mimicry (79). Once activated, T cells have the capability to transmigrate across the BBB into the CNS by upregulation of adhesion molecules (AMs) and release of matrix metalloproteinases (MMPs) (62, 80). Within the CNS parenchyma, the myelin-reactive T cells reactivated once they encounter their specific myelin epitope presented by resident APC: microglia cells or perivascular macrophages (81). Reactivated in the CNS, CD4⁺ T cells proliferate and secrete pro-inflammatory cytokines and chemokines. These will facilitate the further recruitment of T cells, B cells and macrophages, thus contributing to the amplification of the immune inflammatory response (82). The pro-inflammatory cytokines enhance macrophage and microglial activity that may destruct of oligodendrocytes and myelin sheath by secreting toxic mediators such as glutamate, reactive oxygen species (ROS), nitric oxide (NO), and tumor necrosis factor (TNF)-α.
Additional mechanisms may include complement-dependent antibody-mediated damage (85) and a direct attack on oligodendrocytes by CD8$^+$ cytotoxic T cells (86, 87). Microglia and macrophages are also the chief debris-removing cells that eliminate damaged myelin, resulting in the widespread loss of axonal covering in the CNS white matter (88).

Demyelination affects the conduction of nerve impulse, and it can also lead to “cross-talk” between nerves – abnormal “nerve-to-nerve signaling”, which may also produce symptoms. During the pro-inflammatory response, there is concurrent opposing down-regulation of inflammation (76). In some instances, mediators of pro-inflammatory response may also be involved in tissue repair, such as TNF-α may limit the extent and duration of severe CNS damage (89), the regulatory functions of interferon (IFN)-γ as an anti-T cell proliferative cytokine, its potential role in the induction of T cell apoptosis and subsequent termination of the immune response within the CNS (90). Autoimmune T cells, under certain circumstances, can exert a beneficial effect by protecting injured neurons from spread of the damage (91). Microglia and macrophages appear to play a decisive role in the induction of remission, as well as resistance to the induction of the disease (92, 93). Spontaneous myelin sheath repair allows efficient saltatory conduction of nerve impulses, despite the thinner myelin sheath characteristic of remyelinated axons (94).

Different mechanisms may play a role in perpetuation of the disease. For example, dysregulation in the apoptotic mechanisms of T cells would lead to accumulation of autoreactive cells in the periphery or in the CNS (95, 96). Another possible mechanism is the progressive diversification of autoreactive T cells and antibodies, a phenomenon known as “epitope spreading” that is most likely a result from ongoing demyelination leading to the release of previously inaccessible myelin components (82, 97). Epitope spreading has been observed in EAE and may limit the success of antigen specific therapies (98).

Epitope spreading might explain the transition from a relapsing-remitting to a secondary progressive course of MS. Autoreactivity targeting of myelin antigens might lead to a relapsing-remitting course with clinical recovery due to remyelination, whereas spread of autoreactivity targeting of axonal antigens might lead to a progressive course because axonal regeneration is limited in the CNS. This hypothesis can account for many characteristics of MS (96, 99). Alternatively, relapses might be due to predominantly T cell-mediated autoimmune attack, whereas gradual progression of disease might be due to predominantly antibody-mediated autoimmune attack on the CNS (96, 99).

1.8.3 Cytokine pattern in MS and EAE

Cytokines play an important role in the pathogenesis of MS and EAE. Effective treatments for both diseases have been shown to alter cytokines in the CNS and in activated mononuclear cells (100). Depending on the set of cytokines produced, CD4$^+$ T cells can be subdivided into T helper 1 (Th1), Th2, and regulatory Th3 subsets (101). Th1 cells produce Th1 cytokines that include TNF-α, IFN-γ, interleukin (IL)-2 and IL-12; these tend to be pro-inflammatory and function mainly in cell-mediated immunity.
Th2 cells produce Th2 cytokines such as IL-4, IL-5, and IL-10, which tend to be anti-inflammatory and support humoral immune responses (102). It is generally assumed that Th1 and Th2 cytokine are mutually inhibitory at T cell levels (103). The Th3 cytokine, transforming growth factor (TGF)-β, has been shown to suppress the production of Th1 cytokines (104). In EAE, production of Th1 cytokines by myelin-specific T cells has been shown to correlate with encephalitogenicity (69). In contrast, the expression of Th2 and Th3 cytokines in the CNS has been associated with disease recovery (105). These observations suggest that EAE is a Th1-mediated disease; subsequent studies demonstrated that Th2 myelin-specific lines or clones suppress disease in EAE (106). There are however, a few notable exceptions to the Th1/Th2 paradigm (107). Similar to EAE, in MS there appears to be an elevation of Th1 cytokines, and a diminution of Th2 cytokines, preceding and during relapse (108-110). Thus, some of the aims of therapy would be to decrease the activation of autoreactive Th1 cells through antigen presentation, and to skew the T cell response from a pro-inflammatory Th1 milieu towards an anti-inflammatory Th2 cytokine profile.

1.9 PATHOLOGY OF MULTIPLE SCLEROSIS

Gross examination of brain tissue of individuals with MS reveals multiple sharply demarcated plaques in the CNS white matter with a predilection to the optic nerves and white matter tracts of the periventricular regions, brain stem, and spinal cord.

MS is characterized morphologically by multifocal areas of inflammation, demyelination, gliosis and, axonal damage. The hallmark of MS lesion is focal areas of myelin destruction. It occurs on a background of an inflammatory reaction consisting of T cells, a few B cells and plasma cells, and extensive activated macrophage/microglial. This pathological feature is similar to that found in EAE, an experimental paradigm of Th1 cell-mediated autoimmune model (111).

The classic view of MS pathology is that it is characterized by demyelination with relatively preservation of axons. However, recent studies showed that in addition to demyelination, axonal injury, which causes irreversible disability, occurs early in the disease course (112, 113). It is unclear whether axonal damage is the consequence of a primary active destructive process (114), or a pathophysiological response that occurs secondarily to demyelination based on increased vulnerability and loss of trophic support (5, 115).

Recently four pathologic categories of lesion were defined in MS patients. Two patterns (I and II) showed close similarities to T cell-mediated or T cell plus antibody-mediated autoimmune encephalomyelitis, respectively. Other two patterns (III and IV) were highly suggestive of a vasculopathy or primary oligodendrocyte dystrophy, reminiscent of virus- or toxin-induced demyelination rather than autoimmunity (8). It was of interest that the patterns of lesion were heterogeneous between patients, but were homogenous within multiple lesions from a single patient (8). Therefore, it is possible that different pathogenic mechanisms of demyelination may operate in different subgroups of MS patients. This has obvious consequences for therapy development.
Neurological deficits in MS patients have two pathogeneses: acute inflammatory demyelination and axonal degeneration (Fig 3). Disability caused by inflammatory demyelination clinically dominates the early stages of RRMS and is reversible. Axonal transection occurs at sites of inflammation and begins at disease onset but is clinically silent in RRMS because the CNS compensates for neuronal loss. Once a threshold of axon loss is exceeded, MS patients enter an irreversible secondary progressive stage. In SPMS, axonal degeneration is caused by chronic demyelination and may be irreversibly progressive (115, 116). This view of MS provides a conceptional framework that explains conversion of RRMS to SPMS and provides a rationale for early aggressive anti-inflammatory and neuroprotective therapies.

Episodes of reversible clinical symptoms during RRMS are primarily associated with acute inflammatory demyelinating lesions in related areas of the CNS. Resolution of the inflammation, redistribution of axolemmal sodium channels, remyelination, and/or compensatory cortical adaptation contribute to clinical remission (115, 116). Adaptive cortical changes, possibly involving reorganization of functional pathways, contribute to maintained function after axonal damage during early stages of MS (117).

Most MS patients develop progressive irreversible functional impairment 8–15 years after disease onset (Fig 4). The extent of axonal loss in SPMS patients with long disease duration (118, 119), the reduction of levels of the neuronal/axonal marker N-acetyl aspartate (NAA) in the CNS of MS patients over time (120), and the correlations between NAA levels and functional impairment (121, 122), all support the hypothesis that permanent neurological disability develops when a threshold of axonal loss is reached and the CNS compensatory resources are exhausted (115, 123). The time-point when a patient reaches this threshold varies among individuals, and probably reflects a number of factors such as location of lesions, disease activity, genetic susceptibility, and medication.

An initial silent stage of neuronal cell loss is a characteristic of all neurodegenerative diseases. For example, in amyotrophic lateral sclerosis (ALS) and in Parkinson’s disease, it has been estimated that 50% to 80% of target neurons, respectively, may be lost before these patients present with neurological symptoms (124, 125). Quantitative correlations between tract specific axonal loss and disability are difficult to obtain in MS patients. In addition, such correlations may not be straightforward since axonal loss threshold levels probably vary between different tracts of the CNS. However, the hypothesis that cumulative loss of axons determines disability in patients with MS is supported by experimental study in a chronic relapsing-remitting EAE mouse model (126).

Axonal injury begins at disease onset and that cumulative axonal loss provides the pathological substrate for permanent disability in patients with MS (127). The concept of MS as an inflammatory neurodegenerative disease has several important implications. Since different mechanisms may contribute to axonal damage during different stages of the disease, it is crucial to clarify the pathophysiology of neurodegeneration in MS. Inflammation may cause continuous tissue damage with no
clinical manifestations during RRMS. Therefore, inflammation remains the major therapeutic target during early stage of MS. Another possibility is the development of neuroprotective therapies that will spare axons from transection during various stages of MS.

1.11 MAGNETIC RESONANCE IMAGING AS A DIAGNOSTIC TOOL

Magnetic resonance imaging (MRI) provides an objective and noninvasive method of evaluating pathological evolution in vivo. Since its introduction early in the 1980s, MRI has become the most important diagnostic tool of MS (18, 19). MRI is five to ten times more sensitive than clinical evaluation of MS disease activity (128), therefore it has altered the understanding of MS by revealing its dynamic nature, in which lesions progress and resolve undetected clinically. MRI allows in vivo assessment of several aspects of EAE- or MS-related pathological processes, and different MRI sequences detect various aspects of the disease process (129). MRI is currently regarded as the gold standard for evaluation of disease status, and used to monitor MS natural history and its modification by treatment (130).

1.11.1 Conventional Magnetic Resonance Imaging

Conventional magnetic resonance imaging of the brain, which includes T2-weighted, T1-weighted imaging and gadolinium (Gd)-enhanced T1-weighted imaging, are routinely used for the diagnosis and longitudinal monitoring of MS because of their high sensitivity in detection of lesions (131).

T2-weighted images provide a nonspecific measure of the overall macroscopic tissue injury (Fig 8a and 8b). T2 hyperintense lesions reflect increased “water” content, indicating different pathologic substrates ranging from edema, inflammation and demyelination to irreversible Wallerian degeneration (axonal degeneration distal from the sites of transection) and axonal loss (131, 132), and therefore could represent different clinical outcomes. T2-weighted images are used to monitor short-term disease activity by counting the number of new or enlarged T2 lesions visible on monthly scans, and to assess the overall disease burden by measuring the total hyperintense lesion volume on yearly scans (130). A correlation has been identified between the MRI measures of disease activity or lesion burden in T2-weighted images and clinical disability (EDSS), but its strength is modest (133, 134).

T2-weighted lesion assessment is the most widely used MRI technology with high sensitivity (130). However, periventricular lesions are often indistinguishable from the adjacent CSF. Fluid-attenuated inversion recovery (FLAIR) imaging holds great promise for the detection of lesions in the periventricular region, which is a common site for MS lesions (135) (Fig 8b). The long inversion time of FLAIR pulse sequence suppresses the signal from CSF and the long echo time (TE) produces very heavy T2 weighting, enabling anatomical detail to be seen, particularly in areas close to CSF (136, 137). This sequence is also superior at detecting cortical/juxtacortical lesions. FLAIR is therefore a commonly used MR sequence when MS has been raised as a possible clinical diagnosis. The only drawback is inferior quality lesion detection in the posterior fossa and spinal cord where proton density (PD) and T2 weighting are preferred.
Fig 8a. T2-weighted MRI by 4.7 T scanner: transversal spinal cord slices of MOG-induced EAE rat showing sparse signal enhancement in the lumbar region (left panel) and hyperintensity CSF in the cauda equina region (right panel).

Fig 8b. MRI of axial brain slices of a 62 year old patient with secondary progressive MS showing multiple periventricular lesions with (A): T2-weighted image and (B): FLAIR image
Hypointense lesions on T1-weighted (T1) images know as “black holes” (Fig 9) represent tissue damage including demyelination, edema, and axonal loss; and chronic black holes are caused by permanent axonal loss (138). Axonal loss is a major pathologic process responsible for irreversible disability in MS (5). Although strong correlation was initially described between the T1-hypointense lesion volume and neurological disability (139), this has not been confirmed by later studies based on much larger samples of patients (140, 141).

T1-weighted images demonstrate the CNS anatomy and are used to measure brain volume (indicating of atrophy) in MS. Axonal damage may eventually lead to loss of tissue and CNS atrophy. Axonal injury has been identified as the major determinant of irreversible neurological disability in MS (127). Since brain white matter bulk consists predominantly of axons (46%), axonal loss can be assessed in vivo by measuring of brain volume change (142). Brain and spinal cord atrophy (143, 144) is an important new surrogate marker of MS which is associated with clinical disability. Atrophy reflects destructive pathological processes in MS, and is closely associated with T1 hypointense lesion load (145). Brain atrophy appears useful in demonstrating treatment effects in controlled clinical trials (146). Brain atrophy can be measured in two ways – either by estimating the brain volume or by estimating the volumes of CSF spaces (147).

Fig 9. Axial brain slices of a patient with RRMS by different MRI sequences showing multiple periventricular lesions: (A) T2-weighted image; (B) T1-weighted image; (C) FLAIR image; (D) T1-weighted gadolinium enhanced image showing enhancing lesions.
Gd-enhanced T1-weighted images allow visualization of lesions with increased BBB permeability associated with acute inflammatory activity of MS (18, 148) (Fig 9). New lesions appear enhanced, and usually persist for 3.07 weeks on average (149). The number and volume of Gd-enhancing lesions at a single examination are strong predictors of subsequent clinical disease activity (150), and used as a useful marker for monitoring disease activity. Such lesions play an important role in indicating dissemination in time within the new diagnostic criteria. Triple dose gadolinium can increase active lesion detection further but are not required in clinical practice.

1.11.2 Newer Magnetic Resonance Imaging techniques

In patients with MS, postmortem studies reveal the presence of diffuse damage in brain tissues that seem normal on gross pathological examination and conventional MRI – the normal-appearing white matter (NAWM) and normal-appearing grey matter (NAGM) (122, 151, 152). Conventional MRI is widely used for diagnosing of MS and for monitoring its activity and evolution. However, the correlation between conventional MRI and clinical findings of MS is limited, possibly due to the low pathological specificity of the abnormalities seen on conventional MRI scans and to the inability of conventional MRI to quantify the extent of the damage of the NAWM and NAGM (153). Structural and metabolic magnetic resonance (MR)-based techniques with increased specificity to the heterogeneous pathological substrates of MS have the potential to improve our understanding of how MS evolves. Magnetization transfer (MT) (154) and diffusion-weighted (DW) (155) MRI can quantify the extent and pathological severity of microstructure changes of the brain tissue occurring within and outside conventional MRI visible MS lesions. Proton MR spectroscopy (MRS) can add information on the biochemical nature of such changes (156) (Fig 10), and in this way resemble a “noninvasive biopsy technique”.

A number of reports have demonstrated that NAA, a marker for neuronal and axonal integrity, is reduced in MS plaques as well as in NAWM (122), indicating that even what was considered “healthy tissue” is already marked by early axonal loss. An inverse correlation between NAA and disability has been demonstrated: the lower the NAA levels the more severe the patient’s disability (119). Finally, functional MRI (fMRI) aids in the mapping of regions of brain activation during motor, sensation, and cognitive tasks, and can provide new insights into the role of cortical adaptive changes (117). Metrics derived from these newer MRI techniques should be implemented to achieve reliable specific in vivo quantification of MS pathology. Besides enhancing our understanding of the pathologic mechanisms in MS, these newer MRI techniques may also be useful in identifying patients with suspected MS. None of the available MR techniques are optimal for MS analysis. Thus there is a compelling need to define more sensitive and specific MRI measures in the monitoring of MS pathogenesis.
1.11.3 Magnetic Resonance Molecular Imaging

At present, molecular imaging in clinical use is only possible by positron emission tomography (PET) and related techniques; this imaging modality requires use of high-energy ionizing radiation. MRI is emerging as an advantageous technique for molecular imaging, given its high spatial resolution and unique capability to elicit both anatomic and physiologic information simultaneously. MR molecular imaging (MRMI) reveals the location of target molecules in living organ by non-invasive, high-resolution in vivo imaging (157). It is expected to open new perspectives in medical diagnostics by selectively imaging the molecules involved in causing disease, and it is likely to become an essential tool in tracking drug distribution dynamically in living organism during targeted drug delivery. MRMI involves the use of contrast agents linked to nanoparticles that are small enough to penetrate physiological diffusion barriers in the body and selectively target predetermined cells or tissue effectively (158).

1.11.4 Nanotechnology and application of nanoparticles

During last decade, nanotechnology has developed to such an extent that it has become possible to fabricate, characterize and specially tailor the functional properties of nanoparticles for biomedical applications and diagnostics (159-162). Several approaches have been used for magnetically labeling cells with superparamagnetic iron oxide nanoparticles (SPION) for MRMI (163-166). SPION comprised of a magnetic...
core formed of iron oxide (Fe$_3$O$_4$ or Fe$_2$O$_3$) crystals and a macromolecular shell as surface coating (Fig 11A). The surfaces of these nanoparticles could be modified through the creation of few atomic layers of polymer (Fig 11B), which is suitable for further functionalization (166, 167). Biological molecules such as antibodies, proteins, targeting ligands, etc., may be bound to the polymer surfaces of nanoparticles to make them target specific. Another possible and most promising application of these magnetic nanoparticles is as carriers of drug for site-specific delivery (Fig 11B). SPION are unique MR contrast agents and are of great interest because of their multiple potentials.

Fig 11A. Colloidal Templating of Nanoparticles with Core and Shell Structure

![Diagram of core and shell nanoparticles](image)

Fig 11B. Transmission electron microscope images of nanostructures representing second and third generation of advanced nanoparticles

(a). TEM image showing magnetite and protein loaded biodegradable polymer (PLLA-mPEG).

(b). Protein loaded PLLA-mPEG (poly-L-lactic acid/methoxy poly-ethylene glycol) nanoparticles.

(c). Protein loaded PLLA-mPEG nanoparticles with magnetite.

(d). TEM image of a gold nanoshell nanoparticle construction on a copolymer PLLA-mPEG core.

(e). A schematic representation of target-oriented drug release by drugs encapsulated in polymeric nanocapsules represented as building blocks.
Cells or tissue magnetically labeled with SPION produce a susceptibility artifact and shorten the tissue nuclear MR relaxation times (T2 and T2*), resulting in a decrease in signal intensity that is detectable on MRI (167, 168) (Fig 12 and Fig 13). Gradient echo fast imaging (GEFI) sequence is more sensitive in detecting SPION than T2 weighted imaging, but might overestimate the anatomical localization of the nanoparticles (Fig 12 and Fig 13).

Fig 12. One hour after injection of iron oxide nanoparticles (Endorem) in the striatums of a living rat brain: left (1µg, 0.5µl) and right (5µg, 0.5µl), showing contrast enhancing areas with different MRI sequences by a 4.7 Tesla scanner.

Fig 13. The dynamic changes over time of monocrystalline iron oxide nanoparticles (MION) injected into the striatum of a living rat brain by a 4.7 Tesla MRI scanner: a, b, and c with RARE sequence; d, e, and f with GEFI sequence.
1.12 CURRENT DISEASE-MODIFYING THERAPIES OF MULTIPLE SCLEROSIS

Over the past decade, several disease-modifying therapies for RRMS and SPMS have become available (Table 1), and their beneficial effect on relapse rates, MRI outcomes and, in some cases, relapse-related disability have been shown in numerous clinical studies (169, 170). However, these treatments are only partially effective in halting the disease process, and there is no treatment currently available that is capable of preventing relapses or disease progression in MS. Current disease modifying agents are directed at resolving acute relapses (corticosteroids), reducing relapses and slowing disability progression (immunomodulatory drugs). Mitoxantrone (immunosuppressive drug) may reduce relapses and slow disability progression in worsening form of RRMS and SPMS, but its use is limited by the risk of cardiotoxicity (171); it may also be a second-line treatment for patients who do not respond to immunomodulatory drugs. There are currently no effective treatments for PPMS. Nevertheless, symptomatic treatment remains of crucial importance in the management of MS patients.

Table 1. Current treatment strategies in various clinical subtypes of MS

<table>
<thead>
<tr>
<th>Clinical subtype</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute relapse</td>
<td>Pulsed high dose corticosteroids</td>
</tr>
<tr>
<td>Clinically isolated syndrome</td>
<td>IFN-β</td>
</tr>
<tr>
<td>Relapsing-remitting MS</td>
<td>First line: IFN-β, glatiramer acetate</td>
</tr>
<tr>
<td></td>
<td>Second line: IVIG, azathioprine</td>
</tr>
<tr>
<td></td>
<td>With severe relapses and progression: Mitoxantrone</td>
</tr>
<tr>
<td>Secondary progressive MS</td>
<td>With relapses: IFN-β</td>
</tr>
<tr>
<td></td>
<td>Progressive: Mitoxantrone</td>
</tr>
<tr>
<td></td>
<td>Second line: Cyclophosphamide</td>
</tr>
<tr>
<td>Primary progressive MS</td>
<td>No established therapy available</td>
</tr>
<tr>
<td>Progressive-relapsing MS</td>
<td>Mitoxantrone</td>
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</tbody>
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Adapted from Seminars in Neurology 2003; 23 (2):133-146

The introduction of beta-interferons (IFN-β) and glatiramer acetate (GA) into MS therapeutics has altered the natural course of the disease. IFN-β and GA are the two main groups of immunomodulatory drugs used in the treatment of MS.

1.12.1 Interferon-β

Interferons are naturally occurring cytokines, with antiviral, anti-inflammatory and immunomodulatory effects (172). IFN-β belongs to Type I IFNs, and is the first drug to be approved for the treatment of RRMS on the basis of its effect on relapse rates and inflammatory disease activity determined by MRI (173, 174). Over the past decade,
IFN-β has become an important milestone in the treatment of MS. Three different recombinant IFN-β preparations are currently available for the treatment of MS, two IFN-β-1a products (Avonex® and Rebif®) and one IFN-β-1b (Betaferon®/Betaseron®). These products differ with respect to the recommended dose, dosage regimen, and injection route. Their biological effects are quite similar (172, 175). Of these, with the longest experience is IFN-β-1b. Approximately 12 years ago in 1993, IFN-β-1b was the first approved IFN-β for the treatment of RRMS. The 5-year trial with IFN-β-1b (173, 174) was the first study to provide evidence for the efficacy of IFN-β in the treatment of MS (176). Since then, dose-dependent effects of IFN-β in MS have been extensively discussed. Such effects had already been observed in the pivotal trial and were followed by dose comparison trials with IFN-β-1a (177, 178). The data from these clinical trials also suggest that, while dose is an important determinant of efficacy, the frequency of administration may be of even greater importance (179, 180). Five years later in 1998, the therapeutic efficacy of IFN-β-1b could also be demonstrated in SPMS patients (181). IFN-β-1b became the first available IFN-β with proven efficacy in the treatment of SPMS. We learnt from further studies that benefit from IFN-β in SPMS seems to be most pronounced in those patients still having active disease manifested by superimposed relapses or clear progression (182-184). The data would support the concept that IFN-β is most effective in RRMS, and is also effective in the early phase of SPMS when the disease still has inflammatory activity mirrored by clinical exacerbations.

IFN-β treatments were safe and generally well tolerated. The most common side effects were flu-like symptoms, injection-site reactions, and laboratory abnormalities, such as elevation in liver function tests or lymphopenia (173, 177). However, these side effects are generally mild, tend to disappear within the first month of treatment, and thus do not necessitate discontinuation of treatment.

In several studies neutralizing antibodies (NAbs) in a proportion of MS patients under IFN-β treatment have been found. The presence of these NAbs may be associated with a reduction in clinical efficacy (178); however, the existing data are ambiguous and the biological effect of the NAbs is unclear at present (185). Based on the experience of 12 years, IFN-β belongs to the first line therapeutics in RRMS and SPMS (186).

IFN-β has been shown to be effective in the treatment of MS. The precise mechanism of action remains unclear. However, several effects on the immune cascade have been demonstrated such as regulation of MHC expression, inhibition of T-cell activation and transmigration of these cells into the CNS by down-regulating the expression of AMs and inhibiting the activity of MMPs, inhibition of T cell production of proinflammatory cytokines: IFN-γ and TNF-α (102, 109, 187). Regarding if interferon-β cause a Th2 shift from a Th1 milieu, the literature has been extremely confusing (109, 188).

1.12.2 Glatiramer acetate

Glatiramer acetate (GA) is a synthetic polypeptide made up of four amino acids in a specific molar ratio. It initially was designed to mimic MBP and induce EAE. However, it was not encephalitogenic, but instead, suppressed EAE in a number of species (189) which led to trials in MS. There was evidence that GA reducing relapse
rate and showing a beneficial effect on disability progression in RRMS (190). Its immunological mechanisms of action are not completely understood. Several studies indicate that GA causes an immune deviation from a Th1 to a Th2 phenotype, induces antigen-specific T-suppressor cells that cross-react with putative autoantigens in the CNS, and inhibits antigen presentation (102, 191). Side effects of GA are generally mild and injection site reactions are usually minimal (192).

Notably, while both IFN-β and GA ultimately decrease the CNS inflammation, but by different mechanisms. IFN-β has potent activity at the BBB and inhibits the trafficking of inflammatory cells into the CNS. In contrast, GA has negligible effect at the BBB, allowing GA-specific Th2 lymphocytes to enter the CNS to decrease inflammation through bystander suppression – the phenomenon of antigen-specific T cells that suppress the immunological response induced by other antigens (193). As a result, independent of the autoantigen predominant (PLP, MBP, MOG, or other) at the actual stage of the disease the release of anti-inflammatory cytokines suppresses disease activity (194).
2 AIMS OF THE THESIS

The overall aim of the thesis is to establish an experimental and clinical platform of MS, from where further species independent improvement of MRI sensitivity and specificity can be done for the early diagnosis and for in vivo monitoring of the pathogenesis and therapeutic effects. The final goal is to explore individualized, antigen-specific targets of MS therapy to maximize therapeutic efficacy and to minimize adverse effects in MS.

Specific aims:

1. To determine the efficacy of treatment by low-dose low-frequency and high-dose high-frequency IFN-β-1a, and the subsequent changes after discontinuation of IFN-β-1a treatment in SPMS patients, evaluated clinically and by volumetric MRI.

2. To identify target genes of relevance in EAE which could lead to testing of new therapeutic principles in MS patients by fine-mapping MOG-induced EAE quantitative trait loci in an advanced intercross line.

3. To noninvasive evaluate the potential therapeutic effect of novel individualized therapeutic strategies using in vitro modified dendritic cells in EAE, an experimental animal model of MS.

4. To lay the ground of transferring experimental agents or strategies from the laboratory bench to clinical bedside of MS patients by using a species independent technique, MRI, in combination with molecular imaging and nanotechnology.
3 MATERIALS AND METHODS

3.1 TREATMENT STUDIES ON MS PATIENTS (PAPER I, II)

3.1.1 Protocol of clinical MS studies

The clinical study (Paper I) consists of 28 definitive secondary progressive MS (SPMS) patients (14 males and 14 females, mean age 46 years) with initial EDSS score 3.5 to 6.5 and an average 16 years clinical course. These patients (a Finnish subgroup) were included in the Nordic SPMS study – a multicenter, randomized, double blind, placebo controlled, phase III study on clinical efficacy of IFN-β-1a (195). We extended the Nordic SPMS study by performing volumetric analyses of focal and atrophic changes on MRI and measuring the levels of MMP-9 in serum. A total of 14 patients received once weekly, 22 μg, subcutaneous IFN-β-1a (Rebif) and 14 patients received placebo for 3 years. A neurological examination including evaluation of relapses and determination of disability by EDSS was performed yearly; serial cranial MRI measurements, determination of serum MMP-9 levels and clinical evaluations were performed at the same time points. After the clinical trial, (Paper II) 26 of the 28 patients were recruited to receive subcutaneous IFN-β-1a, 44 μg, three times weekly for one year. The patients were studied both clinically and by MRI before and after the one-year treatment. The therapy was discontinued after 1-year of high-dose high-frequency IFN-β-1a administration, but we continued to follow the remaining 21 patients without treatment for an additional year.

3.1.2 Magnetic Resonance Imaging

The serial cranial MRI follow up was measured on the same 0.5 Tesla (T) MRI unit (Vectra GE, Wisconsin, USA) throughout the study using the same MRI protocol anatomical landmarks for orientation. From the MRI protocol, axial 3-dimensional (3D) T2 fast spin echo (FSE), T2 spin echo (SE) and T1 SE sequences were used for volumetric analyses of plaques and cerebral atrophy. Gd-enhanced T1-weighted images were used to detect active lesions (Gd DTPA was administered intravenously as a bolus of 0.2 mmol/kg). The individual skull size is not uniform and therefore we calculated brain atrophy by a ratio to normalize individual intracranial volume. In Paper I, Brain parenchymal fraction (BPF) was calculated by total brain parenchyma volume/total intracranial volume, and used as a surrogate marker of brain atrophy. In Paper II, CSF fraction was calculated by total intracranial CSF volume/total intracranial volume, and was used as a marker of brain atrophy.

3.1.3 Computerized volumetric segmentation

Semiautomatic segmentation of volumetric analysis (Fig 14) was performed using the Anatomatic™ software operating in PC/Windows environment (196). Segmentation procedure is semiautomatic and consists of several image-processing methods, such as image enhancement, amplitude segmentation, region growing, manual editing, and decision trees (197, 198). An intuitive graphical user interface was developed to enable the efficient use of image processing. The volumetric accuracy of the Anatomatic™ program was demonstrated by known volume phantom test in MRI. The relative error of the total volume measurement was 1.5% (197). Also the repeatability was tested by...
inter- and intra-observer studies and showed that the computer segmentation was accurate enough for both the MS plaque and atrophy estimation (197, 199).

Fig 14. Segmentation of MS cerebral lesions and atrophy on MRI: (A) and (B) Original T1- and T2-weighted images; (C) and (D) Corresponding segmented T1 and T2 lesions; (E) Corresponding segmented brain parenchymal volume on T1 image and (F) Corresponding segmented CSF spaces on T2 image.

3.2 GENETIC STUDY IN MOG-INDUCED EAE (PAPER III)

3.2.1 Congenic strains and advanced intercross line (AIL)

Inbred DA rat (Dark Agouti) is a susceptible strain to MOG-induced EAE, whereas both ACI (A×C 9935 Irish) and PVG.1AV1 (Piebald Virol Glaxo) rat strains are EAE-resistant (200). A congenic strain is an inbred strain in which one part of the genome has been transferred from one strain (donor) to the other (recipient) by repetitive backcrossing to the recipient strain and by selection of animals having the region of interest. An advanced intercross line (AIL) is obtained by random intercrossing of two inbred strains for several generations, avoiding brother-sister mating (201). This regime generates genetically unique offspring individuals with a dense mixture of founder chromosomal fragments. A genomic region involved in regulation of a disease is called a quantitative trait locus (QTL).

Intercrossing causes accumulation of recombination events leading to an expansion of the genetic map, which allows more precise QTL mapping. In this study the seventh generation (DA x PVG.1AV1) AIL (F₇) was used to fine-mapping previously detected QTL – designated Eae18 on rat chromosome 10.
3.2.2 Induction of EAE and phenotyping

Recombinant rat MOG was expressed in *Escherichia coli* and purified to homogeneity by chelate chromatography (202). A total of 1068 F7 rats containing equal numbers of females and males were immunized by a single intradermal injection at the dorsal base of the tail with 200 µl of inoculum containing recombinant MOG (rMOG) 40 µg emulsified (1:1) with incomplete FA.

Rats were weighed and monitored daily for clinical signs of EAE until day of sacrifice at day 31–40 post-immunization. Clinical onset is preceded one-to-two days by conspicuous loss of body weight. Affected rats displayed different degrees of severity and a variety of disease courses (i.e., RR, monophasic, or chronic progressive EAE). Phenotype reflects severity of disease based on an ordinal scoring scale. The clinical scoring criteria were as follows: 0, no clinical signs of EAE; 1, tail weakness or tail paralysis; 2, hind leg paraparesis or hemiparesis; 3, hind leg paralysis or hemiparesis; 4, tetraplegy or moribund; and 5, death.

3.2.3 Genotyping and linkage analysis

The immunization resulted in 15% disease incidence. A total of 152 definitive clinically affected rats and 162 randomly selected unaffected rats were genotyped. Eae18 region was genotyped with 56 microsatellite markers. DNA was extracted from tail tip of the rats. After PCR amplification the products were size fractionated on 6% polyacrylamide gels and visualized by autoradiography. All genotypes were evaluated manually.

In order to understand and dissect the regions of the genome that affect complex traits, we have used mapping techniques that allow statistical analyses of the association between phenotype and genotype. Linkage analysis was done by a multiple QTLs model test using R/qtl software (203). Data were analyzed implementing the nonparametric model for maximum EAE score, cumulative EAE score, duration and onset of EAE, and the binary model for incidence of EAE. Permutation tests were performed to determine the threshold levels for significant linkage. To further evaluate the identified QTLs, we have implemented a multiple QTL model test using R/qtl software. The region was also tested for epistatic interactions by implementing a two-dimensional scan with a two-QTL model. The results were confirmed by nonparametric pointwise analysis (JMP, 4.0.2; SAS Institute, Cary, NC).

3.3 TREATMENT STUDIES ON EAE ANIMAL MODELS (PAPER IV, V)

3.3.1 Induction of EAE and clinical evaluation

In Paper IV, Female Lewis rats were immunized in footpads with 200 µl inoculum containing 25 µg MBP_{68–86} emulsified in complete FA containing 2 mg *mycobacterium tuberculosis*. To induce chronic EAE, Female SJL/J and B6 mice were immunized along their back with 100 µl inoculum containing 150 µg PLP_{139–151} or 50 µg MOG_{35–55} and 0.4 mg *mycobacterium tuberculosis*. Immediately after immunization and 2 days after immunization the mice received an intravenous injection of 200 ng of pertussis toxin in 200 µl PBS. In Paper V, Female Lewis rats were immunized at the base of the
tail with 25 µg MBP$_{68-86}$ emulsified (1:1) in 100 µl complete FA containing 2 mg *mycobacterium tuberculosis*.

Rats were used at 6–8 weeks of age and mice were used at 8–12 weeks of age. Animals were weighed and evaluated for clinical signs daily after immunization. Clinical symptoms can reach a peak on day 14 post-immunization in MBP$_{68-86}$-induced Lewis rat EAE. Clinical scores of EAE were graded according to the following criteria: 0, asymptomatic; 1, complete loss of tail tone; 2, hind limb paraparesis; 3, complete hind limb paralysis; 4, hind limb paralysis with forelimb involvement; and 5, moribund/dead.

### 3.3.2 Preparation, modification and injection of dendritic cells (DC)

In Paper IV, Splenic dendritic cells (DC) were prepared from healthy Lewis rats, and SJL/J and B6 mice for different EAE models. DC were exposed to recombinant rat or mouse IFN-γ (100 U/ml) (IFN-γ-DC) or to medium (naive DC) for 48 h at 37°C. DC were then injected subcutaneously into the animals that had been immunized 5 days earlier with MBP$_{68-86}$ (in Lewis rats; 2 x 10$^6$ DC/rat) or 7 days earlier with PLP$_{139-151}$ or MOG$_{35-55}$ (in both SJL/J and B6 mice; 0.5 x 10$^6$ DC/mouse).

In Paper V, Splenic DC were prepared from EAE rats on day 14 post-immunization. For the preparation of CD8$\alpha$+ subset, the DC were separated using positive selection columns and CD8$\alpha$ MicroBeads. After cell fractionation, the CD8$\alpha$+ fraction (CD8$\alpha$+ DC) was made up of approximately 95% CD8$^+$ cells. Total DC and CD8$\alpha$+ DC were injected subcutaneously into Lewis rats that had been immunized 5 days earlier with MBP$_{68-86}$ (1 x 10$^6$ DC/rat).

### 3.3.3 Magnetic Resonance Imaging

MRI recordings were performed on a 4.7 T magnet with a bore diameter of 40 cm (Biospec Advance 47/40 spectrometer; Bruker, Karlsruhe, Germany). For the MRI experiments, rats were anesthetized with isoflurane. Rat body temperature was recorded via rectal probe thermometer and maintained at 37.5°C by warm air. A RF (radio frequency) coil birdcage resonator, with 35-mm in diameter, was used for the brain, and MRI was measured using a rapid acquisition with relaxation enhancement (RARE) protocol. A homemade surface coil (Fig 15) with a length of 12 cm was used for spinal cord, and MRI was measured by RARE protocol in Paper IV and T2 map in Paper V.

![Fig 15. Spinal Cord Coil: pane A shown the surface where the animal is placed on and panel B shown the electronic side that basically consists of 2 capacitors.](image)
4 RESULTS AND COMMENTS

4.1 TREATMENT STUDIES ON MS PATIENTS (PAPER I, II)

In Paper I, a substudy of the Nordic SPMS study, we observed the efficacy of weekly 22 µg IFN-β-1a on the extent of brain lesions and atrophic changes on MRI and the level of serum MMP-9 in SPMS. It appeared that IFN-β-1a slowed T2 lesion development slightly. We did not observe any clinical effect of IFN-β-1a on either EDSS or relapse rate, nor was any effect on level of serum MMP-9, the number of Gd-enhancing lesions, the volume of T1 lesions, or BPF indicative of brain atrophy.

In Paper II, we observed that during one-year IFN-β-1a 44 µg three times weekly treatment both clinical and MRI measures remained stable. One year after treatment discontinuation, however, the disability as measured by EDSS and the volumes of T2 and T1 lesion increased significantly. No apparent effect on the CSF fraction indicative of brain atrophy was seen either during treatment or after its discontinuation.

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the CNS. Matrix metalloproteinases (MMPs), a group of endopeptidases with the ability to degrade the protein components of extracellular matrix, are upregulated in MS (204-206). The activity of MMPs is tightly regulated (207). Tissue inhibitors of metalloproteinases (TIMPs) inhibit the enzymatic activity of activated forms of MMPs (208). Despite these controls, excessive MMPs production and activation is thought to be a key feature of inflammatory pathogenesis.

There is accumulating evidence indicating that MMP-9 involved in the major steps of MS pathogenesis (208). MMP-9 plays an important role in the migration of activated T cells and macrophages from the intravascular compartment into the CNS parenchyma, which is a key event in MS pathogenesis (204, 209, 210). High levels of serum MMP-9 correlate with new Gd-enhancing lesions on MRI and IFN-β therapy reduces serum MMP-9 activity in relapsing-remitting (RR) MS (211, 212). However, the reported effects of IFN-β on the level of MMP-9 vary in secondary progressive (SP) MS (213, 214).

IFN-β has been shown to be effective in the treatment of MS. In RRMS, a clear dose response effect of such therapy has been demonstrated on both clinical and MRI outcomes, indicating that a higher dose is more effective (177, 178, 215-217). The data from these clinical trials also suggest that, while dose is an important determinant of efficacy, the frequency of administration may be of even greater importance.

In SPMS, three large-scale studies of IFN-β showed consistent treatment benefits on relapse rate, MRI assessed disease activity, and partially on disability progression especially in those cases still with relapses indicating of inflammation (181-184, 218). In contrast to doses of IFN-β-1a 22 µg or 44 µg three times weekly in the SPECTRIMS study in SPMS (183), the Nordic SPMS study of weekly 22 µg IFN-β-1a did not show any benefit on either disability or relapse outcomes (195). These results add a point to the dose-response spectrum of IFN-β therapy in SPMS.
The effect of weekly 22 µg IFN-β-1a was analyzed for the first time in the OWIMS study on patients with RRMS (179). This study showed a reduction in new T2 lesion count and total T2 lesion burden, but revealed no significant effect on clinical outcomes. In the ETOMS and CHAMPS studies the treatment of patients who had experienced their first demyelinating episode (clinically isolated syndrome) both weekly 22 µg subcutaneous IFN-β-1a and weekly 30 µg intramuscular IFN-β-1a delayed the emergence of clinically definitive MS (219, 220). In the present study with SPMS, low-dose low-frequency IFN-β-1a delayed T2 lesion development slightly, this being most likely related to an anti-inflammatory effect of IFN-β-1a (26, 172). This dosage and frequency of administration, however, appeared to be insufficient in controlling the destructive pathogenesis of chronic demyelination and axonal loss indicated by T1 lesions or BPF (18, 221). The present results are thus in agreement with those of the OWIMS study and confirm that a single weekly dose of 22 µg IFN-β-1a is insufficient to suppress clinical disease activity in definitive MS, particularly in advanced phases.

Gd-enhancing lesions observed on MRI reflecting BBB damage constitute a MRI marker of acute disease activity (131). The lack of effect of IFN-β-1a on the level of MMP-9 or the number of Gd-enhancing lesions in this study is most likely explained by the advanced phase of SPMS, the very small number of active lesions and the low dose of IFN-β-1a.

Although some studies have shown that IFN-β may slow the progression of MS, little is known as to how discontinuation of treatment affects the subsequent disease course. This question has only been addressed in relatively small studies performed on RRMS patients (222, 223). Paper II is to our knowledge the first to evaluate clinical and MRI effects following discontinuation of IFN-β-1a in SPMS.

Since T2 lesions indicate the overall extent of macroscopic tissue damage and T1 lesions correspond to axonal degeneration (18, 224), an increase in the volumes of these lesions after discontinuation of IFN-β-1a treatment is consistent with accumulation of inflammatory and degenerative changes in the brain, and parallels the accumulating neurological disability detected in this study. Brain atrophy on MRI is a surrogate marker of tissue damage including axonal loss, the pathologic process leading to irreversible disability in MS (225). In this study, no apparent effect on the CSF fraction indicative of brain atrophy was seen either during treatment or after its discontinuation. This could be explained by the anti-edema and anti-inflammatory effect of IFN-β-1a, as well as an insufficient neuroprotective effect (142).

In conclusion, this double-blind, placebo-controlled longitudinal study showed that weekly 22 µg IFN-β-1a had a mild subclinical beneficial effect visualized on MRI, but this dosage and frequency of administration seems to be insufficient in controlling clinical disease activity in SPMS. Discontinuation of IFN-β-1a 44 µg three times weekly in SPMS patients may lead to acceleration of neurological disability and brain lesion development. The results stress the importance of high-dose high-requency of IFN-β-1a administration and the maintenance of the treatment in active SPMS.

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4.2 GENETIC STUDY IN MOG-INDUCED EAE (PAPER III)

Genetic analysis of MS aims to identify primary mechanisms which mediated disease susceptibility and to define candidate targets, enabling the design of more efficient and targeted therapies. Genetic dissection of relevant animal models is of value for defining genes and/or mechanisms operating in diseases pathogenesis. It is highly likely that certain genes, especially those conserved during evolution, will be shared between species. Initial steps to map genes using linkage analysis in F2 intercross or backcross populations, however, result in broad QTLs containing hundreds of genes. In Paper III, we fine-map Eae18 on rat chromosome 10 in MOG-induced EAE using an AIL.

Previously, linkage analysis performed in a (DA × ACI) F2 intercross identified a QTL on chromosome 10, Eae18. A congenic, DA.ACI-Eae18, was established and the phenotypical effect of this locus on MOG-induced EAE was demonstrated (226). This disease-regulatory locus has been further narrowed down to ~30-Mb large region in the recombinant congenic strain DA.ACI-R5. Further congenic fine-mapping was hampered by the lack of polymorphic microsatellite markers between the DA and the ACI strains (227). The DA x PVG.1AV1 strain combination provides a substantially higher degree of polymorphism for high-resolution mapping in an AIL. Fine-mapping was then performed in an AIL consisting of a (DA x PVG.1AV1)F7 intercross, resulting in two highly significant EAE-regulating QTLs designated Eae18a and Eae18b. Eae18a and Eae18b are syntenic to human chromosome 17p13 and 17q11, respectively, which both display linkage to MS.

The 3-Mb Eae18b region contains 10 genes, including a cluster of chemokine genes, CC chemokine ligand 1 (CCL1), CCL2, CCL7, and CCL11. These genes are particularly attractive candidates in EAE and MS, because chemokines are important mediators of leukocyte migration to the CNS parenchyma. These polymorphisms were suggested to alter the chemokine activity and the capability of binding to tissue. Blocking of chemokine receptors has been a successful therapeutic approach in EAE and is now also tested in MS (228, 229). Any polymorphisms in a certain chemokine gene that can be linked to EAE susceptibility will provide a more specific therapeutic target. The 5.5-Mb interval Eae18a situated within a very gene-dense genome region, consisting of ~130 genes that including several relevant candidate genes.

Both Eae18a and Eae18b display an interesting overlap with loci identified in MS and EAE. Eae18b overlaps with two QTLs, which displayed linkage to murine EAE (230-233). Moreover, Eae18b is syntenic to human 17q11.2-q12, which has displayed linkage to MS (234-236). Eae18a overlaps with Eae3 that was identified in a Lewis x BN (Brown Norway) strain intercross (237). Furthermore, it is syntenic to the human chromosome 17p13.1–17p13.2, which displayed linkage in the whole genome scan performed in MS materials (234, 235, 238). Eae18a and Eae18b also overlap with regions that displayed linkage to other autoimmune diseases. Accordingly, Eae18a and Eae18b are an example of clustering of genes of importance for different inflammatory diseases (239).

In conclusion, an AIL is successfully used to define two highly significant, closely situated QTLs. Furthermore, we demonstrate synteny between the identified MOG-
induced EAE regulatory QTLs in rat and MS QTLs on human chromosome 17. The overlap between Eae18a and Eae18b and the regions implicated in human MS linkage studies support the notion that susceptibility alleles in MS are evolutionarily conserved between species. Eae18b comprises only 10 genes, which makes possible both positional cloning attempts using recombinant congenic strains, expression and sequence polymorphism analysis of genes within the region, and association studies in large cohorts of MS patients.

4.3 TREATMENT STUDIES ON EAE ANIMAL MODELS (PAPER IV, V)

The main finding of Paper IV is that injection of IFN-\(\gamma\)-DC (derived from health rats or mice) demonstrates therapeutic potential on acute EAE in Lewis rats and chronic EAE in mice. This was accompanied by the lack of MRI lesions in the brain and spinal cord and by reduced numbers of macrophage and CD4\(^+\) T cell infiltration within the spinal cord. IFN-\(\gamma\)-DC triggered an antigen-specific IFN-\(\gamma\) production, and induced apoptosis of CD4\(^+\) T cells possibly through DC expressing indoleamine 2,3-dioxygenase (IDO\(^+\) DC) and/or an IFN-\(\gamma\)-dependent pathway.

Our results (Paper V) confirm that rat splenic CD8\(\alpha^+\) DC (derived from rats with EAE) suppress the development of clinical EAE in Lewis rats compared to total DC. This was accompanied by the lack of MRI lesions in the brain and spinal cord and by reduced numbers of inflammatory cells within the spinal cord. Injection of CD8\(\alpha^+\) DC leads to inhibit T cell proliferation, increase of IFN-\(\gamma\) secretion and NO production.

Autoimmune diseases such as MS are characterized by the loss of tolerance to self-determinants, activation of autoreactive lymphocytes and subsequent damage to single or multiple organs. The mechanisms by which autoimmune responses are triggered, and how activation of autoreactive lymphocytes is initiated and maintained, are not fully understood (240). Therapeutic approaches in autoimmune diseases have so far concentrated on antigens and T cells to re-establish tolerance to self components (241). The recent identification of tolerogenic subsets of DC and their generation in culture may provide a rationale for designing immunotherapeutic strategies in autoimmune diseases.

DC are specialized antigen-presenting cells (APC) that capture antigen, migrate from the periphery to lymphoid organs and present antigens to naive T cells. They not only activate lymphocytes, but also tolerate T cells to self-antigens, thereby minimizing autoaggressive immune responses (242). The tolerogenic properties of DC are linked to their maturation state. Immature DC subcutaneous injection can lead to peripheral tolerance by differentiation of regulatory T cells (243, 244). Thus, the concept of ‘tolerogenic’ DC reflects an additional property of these important APC, which might be useful in autoimmune diseases (245). Recent studies demonstrate that DC exposed \textit{in vitro} to IFN-\(\gamma\) resulted in T cell tolerance \textit{in vitro} to self-antigen by initiating T cell apoptosis (246).

In view of the established Th1-mediated autoimmune pathogenesis of EAE, the therapeutic effect of IFN-\(\gamma\)-DC is unexpected. However, the traditional view has been challenged by a number of studies describing unexpected disease-ameliorating effects
by IFN-γ in EAE (93, 247-249). In autoaggressive immunity, it has been proposed that pro-inflammatory cytokines may be required at an early stage to induce self-responses by priming Th1 responses. The late expression of the same cytokines could drive the terminal differentiation and death of T cells (250). In IFN-γ−/− mice, 10- to 16-fold more activated CD4+ T cells were accumulated in the CNS during EAE than in wild-type mice (247), providing evidence that IFN-γ may limit the severity of EAE by inducing apoptosis of activated CD4+ T cells. Intrathecal delivery of IFN-γ inhibited EAE by inducing apoptosis of infiltrating T cells (249). IFN-γ also mediates apoptosis of activated CD4+ T cells via NO induction (93, 251, 252). Thus, antigen-induced IFN-γ production in rats injected with IFN-γ-DC may play an important role in inducing T cell apoptosis and preventing cell infiltration.

Another view is IFN-γ induced IDO+ DC can inhibit T cell responses. Stimulation with IFN-γ promoted DC to generate functional IDO (253), which may control autoreactive T cells by the depletion of tryptophan (254). It is of interest that IFN-γ induces IDO expression of DC and that IFN-γ-induced IDO+ DC cause peripheral tolerance by initiating apoptosis of antigen-specific CD4 T cells (255). In in vitro experiments, we observed that IFN-γ induced IDO mRNA expression of DC, and that IDO+ DC exhibited an IDO-dependent proliferative suppression and apoptosis of T cells. These data represent a possible mechanism that IDO+ DC may trigger apoptosis of CD4 T cells in vivo by tryptophan metabolites.

Our data support that injection of IFN-γ-DC induced an antigen-specific immune response in vivo. In animals injected with IFN-γ-DC, antigen-induced IFN-γ production was significantly increased, as compared to spontaneous IFN-γ production. As discussed above, IFN-γ can result in apoptosis in multiple cells by different pathways, including by the promotion of caspase-8-dependent apoptosis (256), up-regulating Fas (257), and inducing NO production (258). Thus it is proposed that injection of IFN-γ-DC induce antigen specific IFN-γ production, which influences expansion and infiltration of activated T cells by initiating apoptosis of T cells. Our further experiments indicate that IFN-γ-DC can cause apoptosis of MBP-reactive T cells in the presence of antigen. In addition to an apoptotic mechanism mediated by IFN-γ, it may be consistent with the idea that treatment of DC with IFN-γ increases IDO expression and confers tolerogenic properties of DC, which may induce antigen-specific T cell tolerance (259).

In Paper IV rat or mouse splenic IFN-γ-exposed DC used to treat ongoing EAE were derived from healthy animals. However, if DC were derived from EAE animals, IFN-γ-exposed DC did not suppress the development of clinical EAE, thereby limiting the option that autologous DC might be used to treat MS. The activation state of DC is also crucial for the functional outcome of the DC – T cell interaction. Non-activated DC tolerize or delete T cells, whereas activation converts the DC to a stimulatory state that results in T cell activation and memory (260). We hypothesize that DC under steady state tolerize T cells and that activated DC from EAE rats promotes T cell activation, suggesting that this may be the reason why IFN-γ-DC from EAE rats did not suppress severity of clinical EAE.
Recent evidence demonstrates that murine CD8α⁺ DC are responsible for inducing peripheral tolerance (261, 262). It was proposed that CD8α⁺ DC may mainly activate CD8 T cells, whereas activated CD8α⁻ DC may activate both CD8 and CD4 T cells (263). Our results of Paper V confirm that rat splenic CD8α⁺ DC suppress the development of clinical EAE in Lewis rats compared to total DC. Injection of CD8α⁺ DC leads to inhibit T cell proliferation, increase of IFN-γ secretion and NO production. IFN-γ can result in apoptosis in multiple cells by different pathways (264). IFN-γ is also a powerful stimulus of NO production that inhibits T cell proliferation (265). DC induce cell apoptosis which is mediated partially by NO (266). In our studies, NO production was enhanced in CD8α⁺ DC injected rats, indicating that the IFN-γ -NO pathway may be related to inhibition of T cell proliferation. We do not exclude another possibility, namely that IFN-γ can up-regulate the expression of IDO by DC, which in turn inhibits T cell function through tryptophan catabolism (267). Peripheral CD8α⁺ DC have been shown to actively suppress immune responses in vivo via IDO, suggesting that this subset of CD8α⁺ DC may utilize multiple mechanisms to limit T cell responsiveness (268). T regulatory cells represent another mechanism by which tolerogenic DC mediate peripheral tolerance. T regulatory cells suppress immune responses via cell-to-cell interactions and/or the production of IL-10. Our data show that injection of CD8α⁺ DC induced the expansion of CD4⁺ T cells expressing IL-10.

The hypothesis that CD8α⁺ DC have tolerizing capacity was challenged by subsequent reports that CD8α⁺ DC induce a Th1 response, whereas CD8α⁻ DC lead to Th2 differentiation (269). One possibility is that CD8α⁺ DC may be subdivided further into immunogenic and tolerogenic subsets. It is more probable that tolerance induction is the default outcome, and that additional stimuli associated with foreign antigens are responsible for their immunogenicity (270). Other studies support the notion that the CD8α⁺ DC can be converted to an immunogenic form by CD40 signalling (271), indicating that the immunogenicity of these DC can be influenced by environmental signals. This dual role for a DC subset should be noted in designing immunotherapeutic strategy for autoimmune diseases.

In conclusion, our observations provide the possibility that autologous DC, from the individual MS patient, might be modified in vitro and then re-infused to the patient for therapeutic purposes. These approaches may provide a cellular basis for a novel individualized immunotherapy as a complement to current treatment options in MS.
5 GENERAL DISCUSSION

5.1 THE CLINICAL TREATMENT STRATEGIES OF MS

5.1.1 The importance of early treatment

The goals of disease-modifying treatment in MS are to prevent clinical relapses and, more importantly, to prevent irreversible damage and consequent disability. Although current immunomodulatory agents (IFN-β and GA) show clear efficacy on a number of short-term outcome measures, they have limited efficacy in retarding the accumulation of disability, particularly during secondary progressive phase of MS wherein much of the disability develops (272). Therefore, **MS treatment should be initiated at the earliest possible time in order to prevent or delay the development of functional disability.** The rationale for the early treatment of MS is predominantly based on the pathological evidence that both axonal loss and brain atrophy may begin early in the disease process, when little disability is present (113, 273). This is also supported by the association of Gd-enhancing lesions early in the disease course with cumulative damage of presumably irreversible nature (274). There is growing hope but as yet only limited evidence, that early use of the currently available, partially effective drugs may modify the course of MS in the long-term.

5.1.2 Individualized treatment and treatment optimization

There has been tremendous progress in the immunomodulatory treatment of RRMS and SPMS, and the introduction of IFN-β and GA has had a significant impact on those who are living with MS. However, not all patients with MS respond well to such treatments. This may largely be a consequence of disease heterogeneity. From a clinical perspective, patients with different disease courses show different treatment responses. Patients with RRMS are more likely to respond to immunomodulatory therapy than those with SPMS. So far, there has been no positive study of immunomodulatory therapy in patients with PPMS.

MS may be a spectrum of diseases that exhibits profound heterogeneity in clinical course, lesion pathology, involvement of susceptibility gene loci, and response to therapy. In order to optimize outcomes it is necessary to modify the treatment of an individual patient according to the pathological subtype. At least from a theoretical point of view, some therapeutic approaches appear very attractive (275). In order to remove antibodies, plasmapheresis and/or intravenous immunoglobulin (IVIG) could most plausibly be the best approach for the immunopathological subtype of MS, which is characterised by antibody and complement deposition. The subtype of MS that is associated with heavy macrophage activation, T cell infiltration and expression of Th1 cytokines, may be most likely to respond to immunomodulation by IFN-β or GA. Other subtypes of MS in which viral infection or oligodendrocyte degeneration appear to play a role. It is possible that these could benefit from antiviral therapy, oligodendrocyte protection or oligodendrocyte transplantation, although therapies based on these latter approaches have yet to be developed.
5.1.3 Combination of treatments and neuroprotective agents

Heterogeneity underlies all aspects of MS. The modest efficacy of current approved treatments is likely at least in part to be a result of applying a single therapy across a heterogeneous population of MS patients. Theoretically, some combination therapies seem promising due to assumed synergistic or complementary mechanisms of action in the future (76, 276).

Results from clinical trials indicate that immunomodulating and immunosuppressive treatments for MS lack substantial efficacy in preventing the development of brain atrophy, despite the marked effects of these treatments on clinical and MRI outcomes of disease activity (277, 278). A modest but significant treatment effect on brain atrophy has been reported for patients with RRMS only in one trial of intramuscular IFN-β-1a (Avonex) (279) and in another study of long-term corticosteroid therapy (280). Such failures indicate that treatments to be effective in reducing MS-related inflammation might be insufficient on the neurodegenerative process of the disease. Another possible explanation is that new active lesions with inflammation and edema, the disappearance of which causes an apparent reduction in brain volume (277).

As the pathology of MS becomes increasingly understood, it has become clear that this is not only an inflammatory demyelinating disease, but also a degenerative one (77). Thus, useful therapies must combine immunomodulatory actions with neuroprotective ones. Clearly, prevention of inflammatory tissue damage is a form of neuroprotection. However, protecting neural tissue from secondary degeneration following inflammatory injury is another facet of neuroprotection. The most potential therapeutic approach may be to combine anti-inflammatory agents with neuroprotective drugs (194). Promoting remyelination at an early stage of MS will restore conduction and, more importantly, may be neuroprotective since chronic demyelination can cause axonal degeneration. Possible avenues to obtain remyelination include enhancing endogenous CNS cells to repopulate and remyelinate lesions, or transplanting stem or progenitor cells into lesions. Bone marrow cells that can give rise to neuronal cells may provide an effective source of donor cells for transplantation. Stem cells may assume an important place in both stages of treatment (281).

The goal is to find optimal combinations that simultaneously address different targets, such as relapse inhibition, delay of disease progression, prevention of axonal loss and improvement of remyelination. Possible future therapeutic cocktails may combine IFN-β and GA, or either of them with neuroproective and/or remyelination-promoting therapies, according to the disease subtype and degree of activity. At present, all of these approaches are still at an experimental stage. It has to be kept in mind however, that treatment combinations might produce unpredictable adverse events.

5.1.4 From the laboratory bench to bedside

Effective immune manipulations in EAE frequently fail to translate into effective treatments in MS. Indeed, some therapeutic strategies, such as the administration of IFN-γ and blockade of TNF-α, ameliorate EAE (249) but make MS worse (282-284). This discrepancy may be explained by the occurrence in MS of defects in immunoregulatory mechanisms, the integrity of which is essential for the efficacy of
these treatments in EAE (285). Other likely reasons are the differences between well-defined EAE models in inbred animal strains and the complexity of human MS (276, 285), and therapies based on biological reagents may be species-specific. This implies that reagents developed for treatment of humans should be tested in a valid preclinical model that is more closely related to the human disease. Non-human primates with significant genetic and immunological similarity to humans, provide an excellent model that approximates chronic MS by the clinical and neuropathological presentation (286).

5.2 EAE ANIMAL MODELS

Many aspects of MS are still unclear, the use of an experimental model is critical to determine the mechanisms of the CNS damage that may be operating in MS, as well as to provide the tools to evaluate the possible therapeutic strategies. An ideal animal model would reproduce all aspects of MS such as susceptibility, clinical aspects and histopathology.

EAE animal model, an acquired inflammatory demyelinating autoimmune disease of the CNS, mimics certain aspects of the pathophysiology of human MS. EAE is not a single disease in a single species, but different EAE models represent different features of MS. As with humans and MS, not all rats or mice will have a natural propensity to acquire EAE, and different strains will develop different forms of EAE which mimic different subtypes and stages of MS.

A number of inbred rat and mouse strains differ in their relative susceptibilities to EAE using the same induction protocol, demonstrating a genetic predisposition. The availability of such strains, the discovery of polymorphic genetic markers, as well as the development of genetic and physical maps provided an opportunity for mapping QTLs in EAE. The used dose of rMOG in paper III had been titrated in order to obtain a detectable clinical difference between susceptible and resistant strains. A too high dose would kill off susceptible rats and resistant rats would display an increased incidence. Generally no rat or mouse strains are 100% resistant to EAE. In contrast, a too low dose in susceptible strains would result in a lower incidence and severity. There is thus a threshold effect for disease induction that is crucial for detecting differences in different strains.

Lewis rat EAE is the most classical model, and has been commonly used to study the immunopathogenesis of EAE as well as different treatment strategies of possible use in MS. It is easily induced, highly reproducible. A single injection of MBP peptides together with complete FA in Lewis rat induced EAE at an incidence of almost 100%. It is characterized by an acute, monophasic severe disease course with spontaneous recovery, and after recovery, rats are resistant to re-induce the disease (66). Severity of EAE is related to the dose of mycobacterium in an inoculum. MBP combined with high dose of mycobacterium induced severe disease, whereas only mild disease was developed upon immunization with MBP and low dose mycobacterium. MBP-induced Lewis rat EAE is mediated by CD4$^+$ T cells exerting a proinflammatory phenotype (287). The histopathological features include macrophage and T cell infiltration in the CNS, almost without primarily demyelinating lesions. This is in contradiction to MS, in which demyelination is the primary feature.
EAE has been transferred by CD8\(^+\) T cells recently, showing interesting similarities to MS, in particular, in lesion distribution (more inflammation in the brain relative to the spinal cord), and demyelination was noted (288, 289). Thus, the potential importance of CD8\(^+\) T cells has begun to emerge. The possible role for CD8\(^+\) T cells, a lymphocyte subset that has long been underrated in MS and should now be considered in new therapeutic approaches. The limited efficacy of T cell-directed therapies (290) suggest that the T cell-mediated immune reaction may not be the only pathogenic immune response involved in MS (111). EAE can not be transferred by antibodies. Subsequent studies revealed that injection of autoantibodies against a surface-exposed myelin antigen, MOG, can amplify the T cell attack by inducing large-scale demyelination (291, 292).

The whole spectrum of MS pathology was closely reflected in MOG-induced EAE in susceptible rat strains. MOG is the only antigen described so far that induces both a T cell inflammatory response and a demyelinating antibody response in EAE animal models (82, 292-294). Moreover, MOG-induced EAE phenotype is stable, reproducible and rather standardized. Disclosed mechanisms and genes in MOG-induced EAE are likely to be more relevant for human MS. MOG is encephalitogenic in most studied species (295). Immunization with MOG leads to EAE with a variety of clinical courses, including chronic, relapsing-remitting, acute lethal, or monophasic, depending on the rat strain and the type of immunization (296-298). Disease is typically characterized by perivascular inflammation, scattered focal demyelination, axonal damage, glial scar formation and remyelination (297); thereby representing a suitable model for testing axon-protective therapies in inflammatory demyelinating conditions (299).

EAE in mice shares many features with human MS since it can have a chronic relapsing-remitting course and also shows histological evidence of demyelination (300, 301). However, they are generally difficult to induce by requiring strong adjuvants and/or repeated immunizations.

While many animal models are available, the use of relevant animal models that mimic either the different subtypes of MS or the spectrum of MS are critical to examine genes or proteins that are of pathogenic relevance and can be used as therapeutic targets, as well as the novel therapeutic strategies (302).

### 5.3 MAGNETIC RESONANCE IMAGING

#### 5.3.1 The clinical-radiological paradox in MS

The use of MRI has had a major impact on the early diagnosis of MS (20). Conventional MRI is widely used for diagnosing of MS and evaluating MS clinical trials. However, there is a poor correlation between the clinical disability and total lesion volume on MRI. There are several possible explanations for the clinical-radiological paradox. First, the low pathological specificity of conventional MRI and the inability to quantify the extent of damage of the normal-appearing white matter, which is contributing to neurological disability (153). Second, the lesion localization in the CNS, especially in regard to spinal cord lesions which are not routinely investigated by MRI, but are more likely to result in neurological disability contributing to the
EDSS compared with lesions in the brain (303). Third, the EDSS is nonlinear and heavily weighted toward ambulatory deficits (304). Finally, cortical reorganization (305) could contribute to functional deficits. These compensatory cortical adaptive changes are another factor potentially limiting the strength of the relationship between MRI measures of pathology and clinical measures of disability (117, 306). The increased application of modern quantitative MRI techniques should help to clarify the riddle of the clinical-radiological paradox in MS.

5.3.2 Magnetic resonance molecular imaging and nanotechnology for improved diagnostics and target specific drug delivery

Current MRI techniques only provide limited information on the actual extent and mechanisms of inflammatory activity and irreversible tissue loss. Gd-enhanced MRI detects the BBB injury, however, it provides no information about the extent and severity of the inflammation (307, 308), the constitution of its cellular components (130, 309), or the resultant tissue damage (310, 311). MR molecular imaging and nanotechnology have been proposed to increase the potential of MRI analysis of biologic processes at the cellular and molecular level.

Vascular adhesion and transmigration of mononuclear cells are key prerequisites in the new lesion formation of MS and EAE, and likely to be important contributors to subsequent lesion evolution (62). Superparamagnetic iron oxide nanoparticles (SPION), a new contrast agent, can be used to label various cells of the immune system for in vivo trafficking studies (166, 309, 312). Cells such as macrophages can be labeled in vivo by introducing the contrast agent into the bloodstream, with the uptake of SPION occurring by phagocytosis, which has been used to image inflammatory processes (312-314). Histopathologic analysis has revealed the presence of macrophages at the sites where SPION-enhancing abnormalities were seen (309, 313). Macrophages with iron burden would be detectable by MRI within the CNS at sites of inflammatory cellular activity (312, 315). SPION-enhanced MRI shows a higher sensitivity for the detection lesions than that of conventional T2-weighted and Gd-enhanced images (309, 312). Therefore, MRI-based in vivo macrophage tracking clearly highlights inflammatory processes in the CNS and can be an important complement to the conventional MRI approaches (314).

Active targeting refers to ligand-directed, site-specific accumulation of SPION (316, 317). In order to improve the specificity, the next step is to label the second generation “smart” nanoparticles - functionalized with antibodies, ligands, or probes. The labeled nanoparticles would conjugated to the target cells, and the target cells could be visualized by MRI (318). Importantly, targeted paramagnetic nanoparticles can serve as a unique platform for diagnosis, treatment, and monitoring of therapy in MS and other diseases.
6 CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, this thesis studied MS from genetic pathogenesis to therapeutic strategies using MRI and EAE animal model as tools. The data from the clinical studies support the concept that IFN-β treatment should be administrated at a high-dose high-frequency and be maintained in the long-term in patients with active SPMS. The therapeutic studies on EAE models provide a cellular basis for future individualized treatment of MS using autologous, *in vitro* modified DC.

The diagnosis and therapy of MS have been improved considerably over the past decade because of the availability of MRI and partially effective immunomodulating therapies. The limited efficacy of immunomodulating drugs in the neurodegenerative stage of MS highlights the importance of developing remyelinating and neuroprotective strategies for the disease. Given the availability of current partially effective therapies, it is important to make the early diagnosis and therapy for MS patients to prevent the accumulation of disability. The future direction is to improve MRI specificity and sensitivity to unravel the mechanisms operating in MS pathogenesis, to explore more targeted therapeutic strategies, and to transfer experimental agents or strategies from the laboratory bench to bedside via of Magnetic Resonance Molecular Imaging and Nanotechnology.
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