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Introduction

Although the cause of MS is still unknown, it seems likely that the disease is the result of an interaction between certain susceptibility genes and environmental factors. About one year ago, a vast array of studies did focus on the role of viruses; for a review see 9. Although firm evidence for a viral etiology of the disease has been obtained, recently, a virus, poxvirus, multiple subacute-sclerosing panencephalitis (MSPV), formerly known as rAVMR, was repeatedly isolated from MS patients. In addition, conflicting reports have been published on the association between human herpes virus 6 (HHV-6) and multiple sclerosis (MMR). Finally, it has been postulated that MS might be caused by an initial infection with a common "MS-vaccine" followed by an infection with the Epstein-Barr virus and an adulthood or later in life.

Another major theory concerning the cause of MS is the activation of autoimmune. This is discussed against CNS antigens attack normal fetal tissue, which is therefore MS, the autoimmune disease. The theories on viral and autoimmune etiology can be unified in the concept of molecular mimicry. Through the concept of molecular mimicry, normal fetal antigens, viral antigens, and viral infection may result in a process in which the body's immune system attacks and destroys localized inflammatory responses. The etiology of MS probably is auto-immune to the pathogenesis of MS is still unclear. The most likely candidate for the cause of MS is the myelin basic protein (MBP), the major component of the brain's insulation and many other antigens including proteolipid protein (PLP), myelin basic protein, and galactocerebroside (GalC), proteolipid protein glycoprotein (GPG), and the extracellular matrix components (ECM) have been proposed.
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Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that affects especially young adults in the northern hemisphere. The incidence in North America and Europe ranges from 0.1-0.2 % in the overall population, and about twothird of the patients is female. A characteristic feature of the disease is the breakdown of myelin which is a major component of the sheath around central axons. The architecture of this sheath is essential for the rapid conduction of action potentials along the nerves. Therefore, breakdown of myelin results in neurological disturbances, and eventually patients progress to a mild or severe state of disability. The presence of multifocal lesions within the CNS is one of the main characteristics of the disease. Within these lesions signs of inflammation and demyelination can be found. Recently, magnetic resonance imaging (MRI) techniques have become available that can visualize these lesions in vivo. These techniques have proven to be a valuable tool in diagnosis and measurement of disease progression (for a recent review see )

Although the cause of MS is still unknown, it seems likely that the disease is the result of an interplay between certain susceptibility genes and environmental factors. About ten years ago a vast array of studies did focus on the role of viruses (for a review see ), but no firm evidence for a viral etiology of the disease has been obtained. Recently, a novel, so-called, multiple sclerosis-associated retrovirus (MSRV), formerly known as LM7, was repeatedly isolated from MS patients. In addition, conflicting reports have been published on the association between human herpes virus 6 (HHV-6) and relapsing-remitting MS (RRMS). Finally, it has been postulated that MS might be caused by an initial infection with a common ‘MS-retrovirus’ followed by an infection with the Epstein-Barr virus in early adulthood or later in life.

Another major theory concerning the cause of MS is that activated autoreactive T cells directed against CNS antigens attack normal brain tissue, which characterizes MS as an autoimmune disease. The theories on viral and autoimmune etiology can be united by the concept of molecular mimicry. Through similarity between certain viral epitopes and autoantigens, viral infection may result in a process in which autoreactive cells become activated and mediate localized inflammatory responses. The identity of the presumable auto-antigen in the pathogenesis of MS is still unclear. The most likely candidate for a target of autoreactivity is myelin basic protein (MBP), the major component of the myelin sheath, but many other antigens including proteolipid protein (PLP), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), the heat-shock protein αB-crystallin and the oligodendroglial enzyme transaldolase (TAL) have been proposed.
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Peptide fragments of these proteins are presented to the T cell receptor (TCR) on CD4^pos^ T cells in the groove of major histocompatibility complex (MHC) class II molecules on antigen presenting cells (APC). MHC class II molecules are highly polymorphic cell surface glycoproteins, that belong together with the MHC class I molecules to the human leukocyte antigen (HLA) system, and bind antigenic peptides with different affinities. Many studies have focused on a possible association of MS with certain HLA class-II haplotypes. However, apart from a (weak) association with the HLA-DR2 haplotype in Caucasians, no major associations have been found.

In marked contrast to well-defined animal models for experimental autoimmunity, e.g. experimental autoimmune encephalomyelitis (EAE), studies on human autoimmune diseases such as MS, are complex where it concerns defining etiology and unraveling pathogenic mechanisms. One of the reasons for this problem is that, when patients with presumed autoimmune disease (e.g. MS or Rheumatoid Arthritis (RA)) present themselves for the first time to an out-patient department their disease is already chronic in nature. This seriously impedes studies on the pathogenesis of human autoimmune disease for several reasons. First, due to epitope- and antigen-spreading, T-cells that have been involved in the initiation of disease are extremely difficult to trace. Second, at the sites of inflammation chronic autoimmune disease will induce localized changes in the vascular system (for instance upregulation of adhesion molecules on high endothelial venules (HEV)). As a result, apart from disease-specific immune cells, also other activated leukocytes will home to the site of inflammation. Due to inflammatory processes, changes through both positive and negative feedback mechanisms will occur in immunoregulatory networks. It has been extremely difficult to dissect which of these changes is(are) essential to the disease process.

Nevertheless, numerous attempts have been made to get more insight into immunopathogenic mechanisms in MS.

Evidence for an activated immune system in MS

The possible role of the immune system in the (aetio)pathogenesis of MS has been reviewed extensively, but analogous to many human diseases with a putative autoimmune origin, conclusive evidence confirming this hypothesis is still lacking. Still, lymphocytes and macrophages can be demonstrated in the perivascular inflammatory cuff, which is supposed to be the earliest histologically identifiable CNS lesion. Especially CD4^pos^ T cells are present in high numbers at these sites. Most studies provide evidence for an enhanced state of activation of these helper T cells. On the other hand, contradictory findings have been reported on systemic immune activation. Elevated levels of interleukin(IL)-2 and IL-2 receptor (IL2R) have been demonstrated in serum of patients with progressive
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disease\textsuperscript{68,71,72,77,85}, but not in serum of RRMS patients\textsuperscript{50,53}. Also, in peripheral blood of MS patients a decrease in the number of CD45RA\textsuperscript{pos} cells, which are supposed to represent the 'unprimed' T-cell population\textsuperscript{89}, and an increase of CD45R0\textsuperscript{pos}, i.e. 'primed', T cells has been reported\textsuperscript{59,90,91}. But, these observations could not be confirmed in other studies\textsuperscript{39,73,83}. Expression of the T-cell specific activation antigen CD26 (Tal) was found to be elevated especially in MS patients with progressive disease\textsuperscript{27,96}. More recently, enhanced expression of CD40 ligand (CD40L/CD154) was found on CD4\textsuperscript{pos} T cells from MS patients\textsuperscript{9}. These kind of phenomena are not specific for MS, because enhanced activation of CD4\textsuperscript{pos} T cells has also been reported in diseases induced by viruses like human immunodeficiency virus type 1 (HIV-1)\textsuperscript{47}, in malignancies where tumor infiltrating lymphocytes (TIL) show enhanced expression of activation markers\textsuperscript{18}, and in other autoimmune diseases such as systemic lupus erythematosus (SLE)\textsuperscript{17,19,21} and rheumatoid arthritis (RA)\textsuperscript{13,60,100}. Surprisingly, the in vitro proliferative response of circulating T cells in bulk cultures in all of these diseases is very low in comparison with healthy controls\textsuperscript{46,56,63,75,76,86-88}. This could be due to a diminished expression of the TCR-\(\gamma\) chain, which has been demonstrated for TIL\textsuperscript{49} in patients with colorectal carcinoma, and for T cells present in HIV\textsuperscript{25}, RA\textsuperscript{5,14} and SLE\textsuperscript{4}, but has not yet been tested in MS. Is it conceivable that this low functional response might be caused by an increase in the frequency of antigen-specific T cells that proliferate poorly upon restimulation in vitro, possibly related to activation in vivo? On one hand this may not seem likely, because based on limiting dilution analysis (LDA) assays the precursor frequency of these antigen-specific T cells is too low to explain such major effects. Moreover, using proliferation assays as a measure of myelin antigen T cell autoreactivity no or only minor differences have been found between MS patients and controls\textsuperscript{78,84,95}. On the other hand, in line with recent findings on the magnitude of T-cell expansion following acute viral infection\textsuperscript{6}, studies by Link and coworkers have shown that upon antigenic stimulation the number of IFN-\(\gamma\) secreting cells is much higher than that in control groups\textsuperscript{74}. Therefore, it is possible that we underestimate the contribution of antigen-specific T cells in these diseases.

Immunoregulatory disturbances in MS

A number of observations suggest that self tolerance, i.e. tolerance against autoantigens, does not solely result from clonal deletion (occurring in the thymus and periphery), but that it depends on a number of active homeostatic mechanisms that control potentially autoreactive T cells. First, as was already mentioned above, myelin-reactive T cells are present in MS patients as well as in healthy donors. Second, a study on the animal model for MS, experimental autoimmune encephalomyelitis (EAE), in which T-cell receptor (TCR) transgenic mice specific for MBP were used, showed that CD4\textsuperscript{pos} anti-MBP T cells in the
absence of any other lymphocytes can induce EAE. Importantly, the presence of only small numbers of normal T and B cells with possible regulatory functions are able to suppress the development of EAE.

The nature of these regulatory mechanisms have more recently been the focus of a lot of studies. Differentiated helper T cells can be subdivided into two subpopulations, known as Thelper(H)1 cells and Thelper(H)2 cells. Th1 cells typically secrete interferon(IFN)-γ, tumor necrosis factor(TNF)-α and TNF-β, and are primarily involved in cell-mediated immunity, while Th2 cells secrete interleukin(IL)-4, IL-5 and IL-10, and regulate humoral immune responses. A balance between these two helper T cell subsets is maintained by crossregulatory mechanisms. IL-4 downregulates development of Th1 type cells, and vice versa IFN-γ suppresses the Th2 type response. It has been hypothesized that a relative overexpression of Th1 type cytokines might play an important role in autoimmune diseases like MS. One of the first and most important observations in humans supporting this hypothesis is that systemic administration of IFN-γ to MS patients results in an increased exacerbation rate. An increase in the number of cells expressing IFN-γ has been found in cerebrospinal fluid (CSF) as well as in peripheral blood, and lymphocytes from MS patients have been shown to produce high levels of IFN-γ in vitro. Using histochemistry, TNF-α and TNF-β have been detected in MS lesions. Several studies suggest that especially TNF-α may be implicated in active disease. On the other hand, in general expression of IL-10 in peripheral blood is low in MS patients, but enhanced in patients with stable disease.

Antigen presenting cells (APC) such as dendritic cells (DC) or monocytes/macrophages are involved in the priming of helper T cells. By means of the secretion of cytokines they are supposed to play an important role in skewing the immune response in either Th1 or Th2 direction. Basically, IL-12 induces a Th1 type response, while IL-10 secretion may counteract this mechanism. IL-12 expression has been demonstrated in acute plaques of MS patients but not in inflammatory infarcts, suggesting that it might play a role in the initiation of the disease. Two other studies provide evidence for upregulation of IL-12 in (chronic) progressive MS patients, that may result from enhanced expression of CD40L on T cells.

**Immunotherapy as a tool to study (aetio)pathogenesis in human autoimmune disease**

Solutions for the methodological problems inherent to the study of the (aetio)pathogenesis of human autoimmune disease will be hard to find. In contrast to experimental autoimmune disease, it will especially be difficult to get insight into the initiating events of human autoimmune disease. Even with the further improvement of diagnostic possibilities, e.g. by
the use of MRI techniques, it is questionable that - given the variety of early symptoms and patient- and doctor-delay - a substantial cohort of MS patients can be studied in the early phase of the disease. Because of this it is necessary to follow patients participating in experimental immunotherapy trials. If under these carefully controlled experimental conditions changes in the immune system correlate with changes in disease activity, new insights in pathogenic mechanisms may be obtained and these may subsequently lead to development of novel therapeutic strategies.

The first part of our studies deals with the functional and phenotypic characterization of the circulating T-cell population in untreated MS patients. We set out to investigate whether peripheral blood T cells from MS patients differ from healthy controls in expression of activation and differentiation markers. Next, the capacity of circulating T cells to proliferate \textit{in vivo} was tested in accessory-cell independent and in accessory-cell dependent assays. To investigate whether the dysfunction that was observed in accessory-cell dependent activation systems might be due to abnormalities in circulating monocytes in MS, we compared the cytokine secretion pattern of circulating monocytes from MS patients with that of healthy donors and neurological controls.

In the second part of our studies immune functions of MS patients treated in various experimental therapy trials were investigated. With respect to the immunological monitoring of these patients, the following three issues are addressed in this thesis:

1. Can immunological laboratory parameters be identified that correlate with disease activity in MS?
2. What are the effects of the treatment protocols on immune functions and do these effects correlate with clinical efficacy?
3. Do interindividual variations in the response to immunomodulatory drugs \textit{in vitro} show a relationship with \textit{in vivo} responses to these drugs?
References


