Immune functions in untreated and treated multiple sclerosis patients
Rep, M.H.G.

Citation for published version (APA):
Chapter 9

Summary & general discussion
Summary and general discussion

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). Studies on the (etio)pathogenesis of MS have indicated that the disease might be mediated by autoaggressive immune cells that react with CNS antigens. As was already outlined in the introduction of this thesis, defining etiology and unraveling pathogenic mechanisms in human autoimmune disease has proven to be very difficult. In studying MS and other autoimmune diseases we have to consider that pathogenic mechanisms in the early stages of the disease and in established disease are not necessarily the same. For instance, it has been shown that (viral) infections trigger of exacerbations, but this does not mean that these infectious agents are the cause of the disease. Whether the immune system in MS patients is primarily at fault is an issue that has not been resolved yet, but there is circumstantial evidence that MS patients have functional aberrations in their T-cell population, and it has been suggested that immunoregulatory changes might play a role in disease progression.

In our studies, functional properties of peripheral blood T cells were analyzed in untreated and treated MS patients. The first aim of this thesis was to analyze whether MS patients differ from healthy donors in phenotype and function of circulating T cells and to find possible explanations for the differences found. The second aim of this thesis was to correlate parameters of disease activity with immune activity in patients treated in various experimental therapy protocols. Finally, we investigated whether in vitro immune parameters can be used in the selection of patients for specific treatments.

In vivo immune activation and in vitro immune dysfunction: common features of MS?

Activation of CD4^{pos} T cells seems to play an important role in MS. More careful evaluation of studies on this subject leads to the conclusion that this may vary with the type of patient and/or with the stage of the disease. A decrease in the number and percentage of circulating 'naive' CD4^{pos}CD45RA^{neg} T cells, and an increase in percentage of CD26 (Tal) expressing T cells seem to be more or less confined to patients with progressive disease. Indeed, using a heterogeneous group of MS patients we did not find significant changes in expression of markers of T-cell activation compared to normal controls (chapter 2). Results of some other case-control studies show that especially patients in an active phase of the disease show signs of T-cell activation, thereby suggesting that at least some of these findings are related to the stage of the disease. Longitudinal studies have been performed to investigate the temporal relationship between disease activity and changes in parameters of T-cell activation. Unfortunately, these studies suffer from major methodological problems. Statistical analysis of results is often difficult or not possible at all due to the small number of patients used. Also, objectivity is not always guaranteed because of the use of clinical parameters of disease activity. More accurate and objective measurement of disease activity can now be achieved by the use of MRI techniques. Studies done so far suggest that enhanced disease activity defined by MRI is somehow related to parameters of T-cell activation. In chapter 8 we describe a longitudinal study on MS patients enrolled in a clinical trial in which...
the relationship between both clinical and MRI parameters of disease activity and various immunological parameters was evaluated. In agreement with the findings mentioned above, we found that the percentage of circulating unprimed CD4\textsuperscript{pos} T cells declines before a clinical exacerbation and in the two months before the occurrence of an active MRI lesion. Our findings suggest that activation of immune cells could play a role in the transition from subclinical to clinical disease activity and are in favor of the hypothesis that activation of T cells in the peripheral lymph nodes is followed by the entrance of activated T cells in the CNS.

Patients with diseases characterized by activation of the cellular immune system (i.e. viral infections, autoimmunity), show \textit{in vitro} diminished proliferative responses of T cells to polyclonal stimulation (see also chapter 1). In MS it is not clear whether there are differences in these responses between different groups of patients. Diminished reactivity has been found in RRMS patients\textsuperscript{37}, but also in CPMS patients\textsuperscript{63}. From our studies it arises that diminished T-cell proliferative responses depend on the stimulation conditions used. Low responses were found in MS when circulating T cells were stimulated with CD2 antibodies\textsuperscript{63}, a condition in which T-cell reactivity depends on the presence of accessory cells such as monocytes\textsuperscript{72}. In line with this, our studies show that diminished T-cell responsiveness in MS patients only occurs in accessory cell dependent activation systems (chapter 2). Consequently, we investigated whether functional abnormalities exist in peripheral blood monocytes from MS patients. Earlier studies have indicated that monocytes from MS patients, especially in the active phase of the disease, were not able to stimulate responder cell proliferation either in allo-MLR or in auto-MLR\textsuperscript{75}. In addition it has been reported that HLA-DR expression on monocytes is decreased in patients with MS\textsuperscript{65,68}, but that this can be restored \textit{in vitro} by the addition of IFN-\gamma or LPS\textsuperscript{61}. Although in our studies no defects were found in accessory function of monocytes in T-cell proliferation assays (chapter 2), additional experiments revealed that the capacity of monocytes to produce IL-6, IL-10, and IL-12 \textit{in vitro} is significantly lower in MS patients compared with healthy controls (chapter 3). Similar findings have been obtained in autoimmune diseases such as rheumatoid arthritis (van der Pouw Kraan, unpublished observations), and systemic lupus erythematosus\textsuperscript{33}. Because monocytes can also be activated by T cells or their products, as yet no definite answer can be given to the question whether monocytes from MS patients have an inherent defect or that functional disturbances are caused by T cells.

\textbf{Immunoregulatory disturbances in MS}

A popular scenario concerning the immunopathogenesis of MS is the following: T cells specific for myelin antigens somehow become activated, most probably in the peripheral lymph nodes, migrate to the central nervous system, and mediate a local inflammatory process eventually resulting in damage to the myelin sheath. T cells specific for myelin antigens can not only be demonstrated in MS patients but also in healthy controls\textsuperscript{67,73,79}. Apparently, these T cells have not been deleted in the thymus, but most of the time, due to either ignorance, peripheral clonal inactivation ('anergy') and/or active suppression, they do not cause pathology. Evidence exists that activated T cells are able to migrate into the CNS. This has
been demonstrated for instance by the fact that also in other (inflammatory) diseases of the central nervous system, and even in healthy controls, the majority of the cerebrospinal fluid T cells shows signs of previous activation\textsuperscript{65-71}. Hence, mere expression of activation markers does not necessarily mean that these T cells are involved in local inflammatory processes in the CNS. There is evidence that suppression mechanisms in the CNS could be responsible for the fact that these T cells normally do not cause damage\textsuperscript{23,50}.

As has been discussed in the introduction of this thesis it is extremely difficult to get insight into the initiating events leading to MS. In EAE evidence has been provided that overexpression of \( \text{T}_{\text{H}1} \) vs. \( \text{T}_{\text{H}2} \) cytokines might play a role\textsuperscript{17}. This, in combination with a locally insufficient production of anti-inflammatory cytokines such as TGF-\( \beta \), IL-4 or IL-10, might then lead to inflammatory processes in the brain. In humans it is far from clear whether these mechanisms might play a role in the initiation of the disease process. Proinflammatory cytokines have been detected in the CNS of MS patients\textsuperscript{6,27,45,64}, but also anti-inflammatory cytokines can be found\textsuperscript{21,48}. Our studies provide indirect evidence for a disease-promoting role of \( \text{T}_{\text{H}1} \) type cells in ongoing disease. In chapter 7 immunological effects of treatment with the chimeric CD4 mAb cM-T412 are described in the context of a randomised, double-blind, placebo-controlled, Magnetic Resonance Imaging (MRI)-monitored Phase II trial. Although the mAb strongly reduced the number of circulating T cells, it had only a minimal effect on IFN-\( \gamma \) secreting T cells. This effect coincided with the absence of an effect of this treatment on either clinical or MRI measures of disease activity\textsuperscript{22}. In addition, treatment with pentoxifylline, (a phosphodiesterase inhibitor), which is probably only marginally effective in MS\textsuperscript{13,29}, did not result in significant changes in \textit{in vitro} secretion of \( \text{T}_{\text{H}1} \) and \( \text{T}_{\text{H}2} \) type cytokines (chapter 4). Stronger evidence for a disease promoting role of \( \text{T}_{\text{H}1} \) cells could of course be provided by treatments that do have a significant effect on disease activity. Few years ago, beneficial effects have been reported on treatment of RRMS patients with the type I interferon IFN-\( \beta \)\textsuperscript{24,40,53,57}. Most human cells have receptors for IFN-\( \beta \)\textsuperscript{49}. Consequently, treatment with this cytokine has pleiotropic effects, and it seems difficult to determine which of these are responsible for the treatment effects. Results from both \textit{in vitro}\textsuperscript{28,31,43} and \textit{in vivo}\textsuperscript{11,31} studies seem to suggest that enhanced expression of the anti-inflammatory cytokine IL-10 and reduced expression of proinflammatory cytokines like IFN-\( \gamma \) and TNF-\( \alpha \) might explain (some of) the treatment effects of IFN-\( \beta \). Our data on \textit{in vitro} (chapter 5) and \textit{in vivo} (chapter 6) effects of IFN-\( \beta \) are to a large extent in agreement with the studies mentioned above, and in addition we show that enhanced expression of IL-10, that preferentially downregulates production of \( \text{T}_{\text{H}1} \) type cytokines, indeed coincides with a predominant decrease in the percentage of IFN-\( \gamma \) producing T cells in the circulation (chapter 6).

Our studies did not provide evidence for an important role of TNF-\( \alpha \) in MS. Treatment with IFN-\( \beta \) did not affect TNF-\( \alpha \) secretion by PBMC (chapter 6), nor did we find an association between TNF-\( \alpha \) secretion and changes in disease activity (chapter 8). In addition, anti-TNF-\( \alpha \) therapy has not been very successful, as will be discussed later. In the acute EAE model it has been reported that TNF-\( \alpha \) is not necessary for induction of disease\textsuperscript{18}, and that it may even have anti-inflammatory potential in established EAE\textsuperscript{7}. Whether this also applies to human disease remains to be seen.

As mentioned above functional aberrations may also exist in antigen presenting cells.
IL-12 is a cytokine produced by APC that is known for its capacity to induce Th1 responses. Others found enhanced production of this cytokine in MS, but our studies (chapters 3 and 6) did not provide evidence for an important role of IL-12 in ongoing disease.

Another mechanism that could be involved in the pathogenesis of MS is activation-induced cell death or apoptosis. Recently, it has been proposed that a genetically determined failure of activation-induced cell death of autoreactive T cells in the CNS might cause the disease. Although this seems to be an attractive hypothesis - some findings in MS can indeed be nicely explained by it - experimental evidence, especially for a causative role of a failure of activation-induced cell-death, has yet to be obtained. In a recent study a lower proportion of apoptotic cells, consisting of lymphocytes and monocytes, was found in CSF of MS patients as compared to patients with non-inflammatory neurological diseases. We have shown that CD95 (Fas) expression on circulating T cells might play a role in ongoing disease (chapter 6). It could be that the beneficial effect of IFN-β is attributable to an enhanced expression of CD95 on autoaggressive T cells. Further studies are needed to find out whether this also makes these cells more sensitive to activation-induced cell-death. In addition, it would be interesting to know whether IFN-β also effects expression of CD95 ligand on relevant cells in the CNS.

The use of immunological parameters as markers for disease activity
Based on the involvement of the immune system in MS, several attempts have been made to correlate changes in immunological parameters with changes in disease activity. One of the main goals of these studies is to identify immunological laboratory parameters that could be used as (surrogate) disease markers. Consequently, this would enable one to predict forthcoming relapses and would help physicians in arranging appropriate treatment protocols. Also it may increase our understanding of the disease dynamics in MS. As was already briefly mentioned above, most of the studies done so far on the relation between disease activity - often defined as the absence or presence of clinical relapses - and immune parameters were cross-sectional. In addition, in longitudinal studies no accurate markers of disease were available. Nowadays MRI techniques are being used, that can measure disease activity more accurately and objectively, and have greatly improved our understanding of the disease process. Recently, in a review problems associated with correlating immunological markers and MRI markers of disease activity in MS have been discussed. The authors also stress the weak association of these markers with clinical disease progression as measured by EDSS score.

Because MS primarily is a central nervous system disease, immune reactions in the brain might be expected to correlate best with the formation or enhancement of lesions. However, also here specific problems exist. Furthermore, for practical reasons it is more advantageous to look for markers in peripheral blood. Studies on the relationship between changes in circulating T-cell subsets and changes in disease activity have been discussed above. Although, as we have also described in chapter 8, there are indications that changes in some subsets are related to changes in disease activity, the correlation is not impressive, and therefore these parameters cannot be used as predictive markers. In other studies MRI activity was related to expression of soluble factors like adhesion molecules (soluble intercellular
adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1)) \(^{19}\), cytokines \(^{1,12,44}\) or cytokine receptors \(^{52}\) in the circulation. In most studies a positive, but weak association was found between proinflammatory markers and MRI activity. In contrast, in our studies the capacity of circulating T cells to secrete cytokines of either T\(_{H1}\) or T\(_{H2}\) phenotype did not show any relationship with the number of active lesions nor with clinical exacerbations (chapter 8). Using flowcytometric measurement of intracellular cytokines, that allows an enumeration of T\(_{H1}\) (IFN-γ\(^{pos}\)) and T\(_{H2}\) (IL-4\(^{pos}\)) subsets, we also failed to demonstrate a relation between production of proinflammatory cytokines and MRI measures of disease activity. Importantly, the ratio T\(_{H1}\) vs. T\(_{H2}\) type cells increased in the two months before a clinical relapse. However, due to the relatively small changes also this parameter is of little value as a predictive marker. One attempt has been made to assess the prognostic value of TNF-α secretion by peripheral blood mononuclear cells\(^{12}\), but failed to find it. If, as we have hypothesized in chapter 8, peripheral T-cell activation precedes lesion formation and/or enhancement in the CNS, and we would be able to define a predictive T-cell activation marker, this would make treatment of relapses more feasible.

Other possible applications of immunological markers are early diagnosis of MS, the selection of patients for specific treatment protocols and adjusting therapy regimens. Oligoclonal bands in CSF are being used in the diagnosis of MS, but are not specific for this disease \(^{56}\). To our knowledge other markers have not been tested yet, and because this subject falls beyond the scope of this thesis, it will not be further discussed. Considering the fact that currently various types of treatment are available in MS, and that presumably not all (types of) patients will respond to these treatments, it would be very helpful if immunological laboratory markers could be used in the selection of patients for specific treatment protocols. A first attempt to find such a marker was made in chapter 6. Enhanced serum levels of IL-10 are an important effect of treatment with IFN-β, and may well be indicative of clinical efficacy. Although we were not able to relate mitogen-induced IL-10 secretion \(in vitro\) directly to measures of disease activity, we did demonstrate that patients responding well \(in vitro\) to IFN-β (measured by induction of IL-10 secretion) also had high levels of IL-10 in plasma. Future studies are warranted to get more insight into the relationship of these and other immunological parameters with clinical efficacy.

Success and failure of rational approaches to immunotherapy in MS

Immunological analysis of MS patients enrolled in various clinical trials probably is one of the main approaches, or even the only feasible approach as has been pointed out above, to get more insight into the disease process. From another point of view it may also be helpful in explaining why certain therapeutic protocols are successful while others are not.

The development of most therapies in human autoimmune disease is based on experimental models, in case of MS on experimental autoimmune encephalomyelitis (EAE). The extrapolations from this animal model to human disease are, however, difficult. Thus, it is not surprising that therapeutic protocols in MS based on studies in EAE either did not work at all or even lead to aggravation of the disease. The classical example of the latter is IFN-γ therapy. Although at this moment there is no experimental evidence for the discrepancy in
these results, it was hypothesized that a failure in activation-induced apoptosis in MS might play a role\(^9\).

Several attempts have been made to eliminate or counterattack the action of proinflammatory cytokines. Pentoxifylline, an inhibitor of the production of TNF-\(\alpha\), prevented onset of disease in the rat EAE model\(^{58}\), and has been shown to cause immune deviation towards a more \(T_H1\) type profile in rats. In agreement with studies of others it is demonstrated in chapter 4 in a pilot study that treatment with pentoxifylline did not lead to significant changes in TNF-\(\alpha\) secretion by PBMC, nor did influence the secretion of \(T_H1\) and \(T_n2\) type cytokines. In another study pentoxifylline diminished expression of \(T_H1\) type cytokines and enhanced expression of \(T_H2\) type cytokines in PBMC\(^{30}\). Nevertheless, clinical efficacy seems to be limited to the reduction of early side-effects of treatment with IFN-\(\beta\)\(_-\).\(^{13,26}\) Other anti-TNF-\(\alpha\) therapies also have not been successful. Treatment of rheumatoid arthritis patients with monoclonal antibodies directed against TNF-\(\alpha\) showed promising results in phase II clinical trials\(^{46}\), but worsening of disease was seen in two MS patients treated in open-label phase I trial with the same antibody\(^{34}\). In addition, recent trials with linomide, which apart from its inhibiting effect on programmed cell death of peripheral blood T cells\(^{47}\) inhibits TNF-\(\alpha\) production\(^{55}\), have also met with severe side-effects (unpublished results).

Because CD4\(^{pos}\) T cells are supposed to play a major role in MS, several therapies have been aimed at trying to eliminate or trying to interfere with the function of these cells. In pilot trials the former approach, using depleting CD4 mAb, looked promising, but in placebo-controlled phase II trials no effect was seen on disease activity\(^{22}\), a result which was also seen in other autoimmune diseases such as rheumatoid arthritis\(^{42,59}\). In our opinion the results of the studies described in chapter 7 are an illustration of how immunological analysis can contribute to understanding therapeutic success or failure. These studies demonstrate that depleting CD4 mAb preferentially delete circulating ‘naive’ CD4\(^{pos}\)CD45RA\(^{pos}\) T cells, while \(T_H1\) type ‘memory’ cells remain relatively unaffected. In conclusion lack of clinical efficacy coincides with the observation that cells thought to be involved in the disease process are not eliminated. This type of treatment is principally also based on experimental models\(^{37,78,64,45}\).

As is discussed in chapter 7, we feel that the success of this type of treatment in animal models can be partly explained by the fact that treatment here often starts before the induction of disease, and therefore involves priming of ‘naive’ T cells that are affected by the treatment. This leads us to an important general point of consideration in the development of new therapies, namely that in the human situation we are dealing with already established disease. A similar line of reasoning can be applied to antigen-specific therapies, because while in animal models disease can be induced by a single antigen, due to epitope- and antigen-spreading it is difficult to select appropriate antigens in human disease. However some of these therapies seem to be based on the induction of ‘bystander suppressor cells’\(^{56}\), and may involve enhanced secretion of anti-inflammatory cytokines like IL-4, IL-10 and TGF-\(\beta\)\(^{35,36}\). As will be discussed below, the latter is probably also an important mode of action of IFN-\(\beta\).

In the light of the results of therapies based on a rational approach described above it is striking that other therapies for which the rationale was less clearly defined have shown beneficial clinical effects. Certainly, a rationale exists for all novel therapies (reviewed in\(^{20}\),}
but the examples of IFN-ß and copolymer-1 (Cop-1) show that the actual mode of action of a drug in vivo may turn out to be somewhat or even completely different. The application of IFN-ß was initially based on the antiviral capacities of this cytokine, but evidence is growing that in vivo effects may be caused by its influence on immunoregulation. In particular enhanced expression of the anti-inflammatory cytokine IL-10 (chapters 5 and 6) could be an important mode of action of this agent, although other mechanisms, e.g. an increase in activation induced cell-death of autoaggressive T cells (chapter 6), downregulation of MHC class II expression or inhibition of migration across the blood-brain barrier either through the decrease of very late antigen-4 (VLA-4) expression on peripheral blood lymphocytes or effects on endothelial cells, or abrogation of the secretion of matrix degrading enzymes like matrix metalloproteinase (MMP)-9. Some of these mechanisms actually might interact with each other, but more importantly all of these findings suggest that IFN-ß acts by dampening the immune response. Cop-1 is a synthetic basic copolymer originally designed to mimic the activity of MBP in inducing EAE, but unexpectedly in several animal experiments it was found to suppress EAE. It has been suggested that Cop-1 is a competitive antagonist of MBP, PLP and MOG for binding to MHC class II molecules, but the exact mode of action is still unknown. Nevertheless, beneficial results have been obtained in the treatment of MS patients.

In conclusion, our studies provide evidence for functional abnormalities in peripheral blood T cells. Several questions remain to be answered. Is this primarily a defect in the T-cell population or do APC play a role in this phenomenon? Do regulatory (‘suppressive’) T cells play a role? Are there differences between certain subtypes of MS? Do these disturbances have a relationship with disease activity? Is it so that we first have to get more insight into pathogenic mechanisms in MS (for instance by studying EAE) before we are able to develop new immunotherapies?

To a certain extent it may be true that our knowledge of pathogenic mechanisms in MS is not sufficient for the development of new therapies, but our studies show that longitudinal analysis of immunological parameters in treated patients might increase our understanding of pathogenic mechanisms in ongoing disease. Although based on the poor results obtained with anti-CD4 therapy some have expressed their doubt as to whether T cells actually are important in MS and other autoimmune-like diseases, our data on IFN-ß therapy indicate that enhanced expression of anti-inflammatory cytokines in combination with downregulation of TH1 type cells and enhanced expression of CD95 on T cells, probably leading to an increase of activation-induced apoptosis of these cells, coincide with clinical efficacy. Future studies with larger patient groups and longer treatment periods, and also in vitro studies especially on the effects of IFN-ß on CD95 expression and sensitivity to apoptosis will hopefully give more insight into these mechanisms. The importance of the balance between TH1 and TH2 type cells was further substantiated by our finding that clinical relapse in MS is preceded by an increase in the balance between TH1 subsets and TH2 subsets. We have shown that changes in disease activity are related to changes in immunological parameters, but thus far no predictive markers have been found. The use of immunological laboratory parameters in the selection of patients for various treatment protocols and in the adjustment of treatment protocols is a
future perspective, and, although they are preliminary, our data on the relation between *in vitro* production of IL-10 and *in vivo* IL-10 levels in IFN-β treated patients indicate that we may cherish optimistic thoughts about this.
References


Summary & general discussion


