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Studies on the CNS Histopathology of EAE and Its Correlation with Clinical and Immunological Parameters

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1. Introduction

Multiple sclerosis (MS) is one of the most difficult to diagnose neurological diseases because its clinical manifestations are highly variable and the disease course also shows unpredictable individual patterns. We are far from understanding the complexities that underlie this variability, but certain patterns clearly emerge. First, it has become clear that different genetic backgrounds will lead to different manifestations of an autoimmune T cell attack on the central nervous system (CNS) (Hoppenbrouwers and Hintzen, 2010). It is also clear that differences in the CNS antigen-specificity of the T cell response can result in a differential involvement of anatomical regions of the CNS (Berger et al., 1997; Kuerten et al., 2007). Differences in lesion localization are a typical feature of MS, termed dissemination in space, and are likely to cause heterogeneity in clinical symptoms. There is evidence for the prevalence of either T cell-/macrophage- or antibody-/complement-mediated CNS demyelination versus a primary oligodendrogliopathy in MS patients (Lucchinetti et al., 2000). While the patterns of demyelination remain the same in individual patients over time, heterogeneity is evident when comparing different patients (Lucchinetti et al., 2000). In addition to these rather defined parameters of CNS histopathology (termed "pattern I-IV" by Lucchinetti et al., 2000) there are dynamic elements of the inflammatory cascade that can result in interindividual variations of disease progression. Among these are the extent of antigen determinant spreading (Lehmann et al., 1992; McRae et al., 1995), the prevalence of antigens in different CNS regions to which the spreading occurs (Targoni et al., 2001) as well as the rate at which regulatory or compensatory reactions of the immune system surface to counterregulate the damage of the target organ (Kasper et al., 2007).

Due to the impossibility of obtaining CNS tissue samples from individual patients repeatedly over time, studies as to the pathogenesis of the human disease need to rely on suitable animal models. To study pathologic features of MS three main animal models are used: disease induction by toxic agents, viral models, and finally different types of experimental autoimmune encephalomyelitis (EAE). Toxic agents like the copper chelator

cuprizone cause demyelination in the relative absence of inflammation or axonal damage. Lesions induced in the cuprizone model typically resemble primary oligodendrocyte dystrophy in MS patients, while lacking the characteristic T cell infiltrate. The cuprizone model has no autoimmune component. Still, it is well-suited to investigate principle features of de- and remyelination in the CNS (Kipp et al., 2009). Intracerebral inoculation of Theiler's murine encephalomyelitis virus (TMEV) is used to investigate how viral infections can induce CNS autoimmunity. After an early, subtle disease period, susceptible mouse strains develop brain and spinal cord inflammation, demyelination and axonal damage. The clinical course resembles that of chronic, progressive MS (Tsunoda et al., 2010). However, EAE remains the most intensively studied animal model of MS.

2. Experimental Autoimmune Encephalitis (EAE)

EAE was introduced by Thomas Rivers and his colleagues in the early 1930s (van Epps, 2005). Since then, it has been subject to elaborate studies (reviewed in Goverman and Brabb, 1996; Steinman, 1999; Hemmer et al., 2002). Animals studied initially included guinea pigs and rats, in particular the Lewis rat, but later also involved marmoset monkeys and mice – the latter being the dominant model organisms used nowadays (Gold et al., 2006). EAE can either be induced by active immunization with CNS antigens in adjuvant (active EAE) or by the passive transfer of encephalitogenic T cells (adoptive/passive EAE). In addition, spontaneous EAE models relying on transgenic animals exist (Fig. 1).

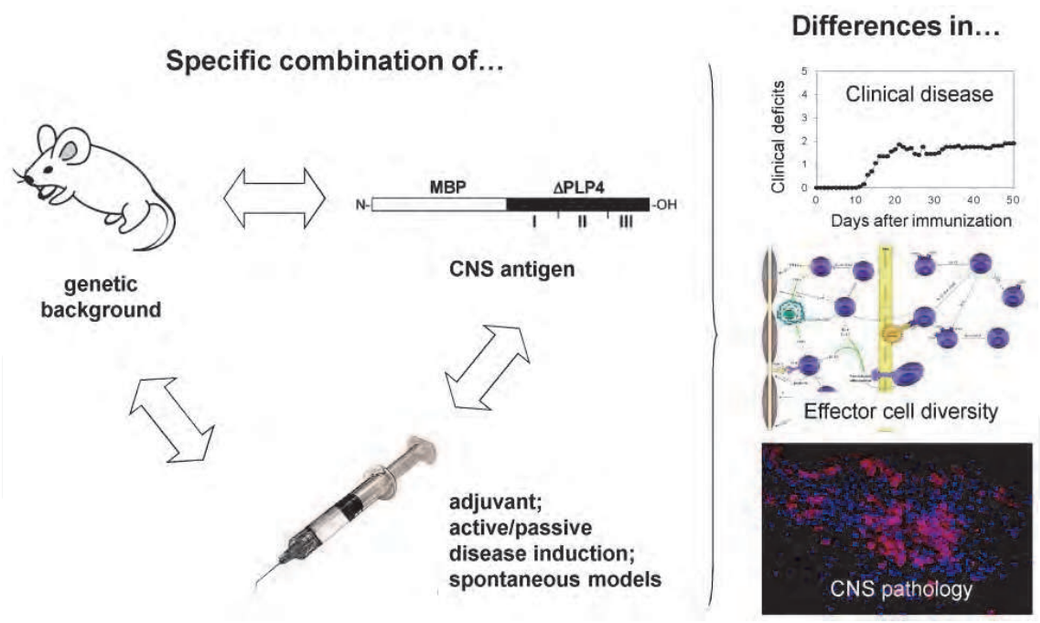


Fig. 1. The interplay between genetic background, disease triggering antigen and the mode of disease induction results in differences in EAE outcome.

Originally, whole spinal cord homogenate (SCH) (Einstein et al., 1962; Bernard & Carnegie, 1975; van Epps, 2005) was used for disease induction, before specific target antigens were defined. Early efforts to characterize the encephalitogenic antigen in SCH identified myelin basic protein (MBP) (Einstein et al. 1962; Martenson et al., 1970; Hashim et al., 1975)

comprising approximately 30 – 40% of the proteins in the myelin sheath. H-2^u mice, in particular the B10.PL and PL/J strains are highly susceptible to MBP- or MBP peptide-induced EAE (Fritz et al., 1983 and 1985), while most common mouse strains, including C57BL/6 (B6) mice are resistant to MBP-induced disease (Bernard, 1976; Fritz and Zhao, 1996; Gasser et al., 1990; Skundric et al., 1994). The in-depth characterization of the B10.PL and PL/J model revealed highly restricted T cell responses to MBP involving a single immune dominant determinant and a limited usage of T cell receptor (TCR) chains (Zamvil et al., 1988; Urban et al., 1988; Kumar and Sercarz, 1994; Radu et al., 2000). Since similar findings were made in the Lewis rat (Burns et al., 1989), hopes emerged that such features could also apply to MS, providing therapeutic possibilities. These perspectives faded as diverse T cell repertoires were found in the proteolipid protein (PLP):139-151-induced EAE of SJL mice (Kuchroo et al., 1992) and after realizing that autoimmune T cell repertoires undergo determinant spreading (Lehmann et al., 1992; McRae et al., 1995; Jansson et al., 1995; Yu et al., 1996; Tuohy et al., 1999). Recent studies of antigen-specific autoantibodies in EAE have also provided for the diversification of the autoimmune response (Stefferl et al., 2000; Cross et al., 2001). PLP constitutes approximately 50% of the myelin proteins. As with MBP-induced EAE, only few strains were found to be susceptible to PLP-induced EAE. C57BL/6 mice were reported to be resistant (Tuohy, 1993; Mendel et al., 1995; Fritz and Zhao, 1996; Klein et al., 2000). The search for additional encephalitogenic antigens identified myelin oligodendrocyte glycoprotein (MOG) (Lebar et al., 1986; Mendel et al., 1995 and 1996; Schmidt, 1999), myelin associated glycoprotein (MAG) (Schmidt, 1999; Morris-Downes et al., 2002; Weerth et al., 1999), myelin oligodendrocyte basic protein (MOBP) (Schmidt, 1999; Holz et al., 2000; de Rosbo et al., 2004), oligodendrocyte-specific glycoprotein (OSP) (Morris-Downes, 2002), 2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNPase) (Schmidt, 1999; Morris-Downes et al., 2002), β-synuclein (Mor et al., 2003) as well as S100β, which is not only expressed on astrocytes, but also in many other tissues including the eye, thymus, spleen and lymph nodes (Kojima et al., 1997; Schmidt, 1999).

Each combination of antigen with the respective susceptible strain and also considering the mode/protocol of disease induction results in a characteristic form of EAE (Goverman & Brabb, 1996; Steinman, 1999; Schmidt, 1999) (Fig. 1). The different EAE models show fundamental differences, however. For example, the MBP-induced disease in B10.PL and PL/J mice is monophasic: the mice completely recover after a single episode of short acute disease and become resistant to re-induction of EAE (Waxman et al., 1980). PLP peptide 139-151-induced EAE in SJL mice is remitting-relapsing (Hofstetter et al., 2002), while the disease elicited by MOG:35-55 in C57BL/6 mice is chronic (Eugster et al., 1999). In addition, the different EAE models involve differences in CNS histopathology and the role of antigen-specific antibodies, which will be described in detail below.

There have been extensive discussions regarding which antigen/strain combination provides the "best" EAE model for MS. The prevalent view is that none of them individually, but all of them jointly are best (Schmidt, 1999; van Epps, 2005; Hafler et al., 2005). MS does not seem to be a single disease entity, but rather involves a profound heterogeneity. As Vijay Kuchroo (Harvard University) once pointed out "each EAE model recapitulates a small piece of the human disease", thus facilitating the analysis of each single step disrupting immune competence leading finally to a severe autoimmune disease (van Epps, 2005; Steinman and Zamvil, 2006). EAE is an appropriate model for studies of basic mechanisms that underlie autoimmune pathology because, unlike in spontaneous

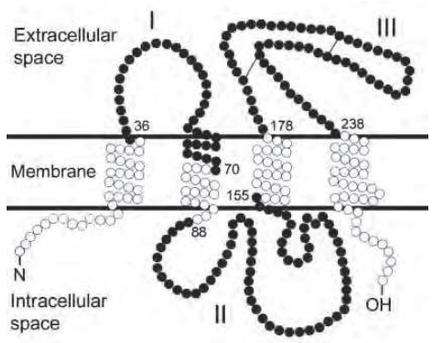
autoimmune diseases, the autoantigen, the time point and the site of the ensuing autoimmune response is known, and the type of cytokine differentiation of the induced T cells can be directed (Forsthuber et al., 1996; Yip et al., 1999). Being able to control the above parameters as well as the ability to monitor the autoantigen-specific T cells in the course of the disease renders EAE suitable for studies aiming at defining the mechanisms of therapeutic interventions. Genetically-manipulated mice have been and will continue to gain increasing importance for such studies.

Traditionally, mechanistic studies have relied on the use of antibodies and on complex manipulations of mice. However, such treatments that can be applied to essentially any EAE model, do not necessarily permit unambiguous conclusions. For example, when a cell surface marker-specific antibody is injected to study the role of that molecule in EAE, the antibody might have a clinical effect on the disease, but it could be due to a multitude of mechanisms. The antibody could deplete the marker positive cells via the activation of complement, antibody-dependent cell-mediated cytotoxicity (ADCC) or apoptosis (Cebecauer et al., 2005), which in turn may be associated with a varying degree of inflammation causing unaccounted effects. Alternatively (or in addition) antibodies can inactivate or activate the marker positive cells, with variable bystander cell involvement. When antibodies are injected to study the role of a cytokine, the clinical effect seen can result from the neutralization of the cytokine, or on the contrary, from the prolongation of the half-life of that cytokine. For such reasons, the use of antibodies for mechanistic studies has frequently resulted in contradictory, inconclusive data (Dittmer and Hasenkrug, 1999; Silvera et al., 2001). The use of genetically-targeted mice, along with adoptive transfers of cells that express/do not express molecules of interest is increasingly becoming indispensable for mechanism-oriented studies, and the more this "tool box" expands the more powerful it will become. Most gene knock-out/knock-in mice have been generated on the 129 (*H-2*^{b}) background and backcrossed to *H-2* congenic C57BL/6 mice. Instead of having to move each new member of this ever expanding "toolbox" to the background of each EAE susceptible strain, it is much more effective to be able to study EAE in C57BL/6 mice. This is why MOG:35-55-induced EAE in C57BL/6 mice is increasingly becoming essential for mechanism-oriented studies – and why at the same time it is problematic to rely on this single EAE model for MS. To this end, we set out to establish and characterize additional EAE models for C57BL/6 mice. PLP protein-induced EAE has not been extensively studied; unlike the hydrophilic MBP molecule, PLP is highly hydrophobic and thus as a protein very difficult to utilize (Tuohy, 1993). PLP as an antigen for EAE induction established itself only after the encephalitogenic PLP peptide 139-151 had been identified for SJL mice (Kuchroo et al., 1992; Lehmann et al., 1992). Only recently, PLP peptide 178-191-elicited disease in C57BL/6 mice has been introduced (Tompkins et al., 2002), but this model still awaits thorough characterization. Encompassing most potential determinants of the two major myelin antigens, MBP and PLP, the MP4 fusion protein was generated as a drug candidate for MS (Elliott et al., 1996). MP4 contains the three hydrophilic loops of PLP (domains I, II and III; Fig. 2A), while the four hydrophobic transmembrane sequences have been excised. These hydrophilic domains constitute ΔPLP4 that has been linked to the 21.5 kD isoform of human MBP (Fig. 2B).

In SJL mice it has been shown that, when given under tolerogenic conditions, MP4 can prevent and revert EAE induced by MBP- and PLP-specific T cells (Elliot et al., 1996). It has also been shown that MP4 can induce EAE in SJL/J mice, and in another report (Jordan et

al., 1999) MP4 was found to be encephalitogenic in marmoset monkeys. Our studies later demonstrated that MP4 was also capable of inducing EAE in C57BL/6 mice, thus introducing a much needed alternative to the MOG:35-55 and PLP:178-191 peptide model (Kuerten et al., 2006). Overall, there are few systematic studies as to whether different EAE models can reproduce distinct features of MS histopathology. One typical problem is that – as mentioned above – the induction of EAE requires the specific combination of genetic strain and CNS antigen. Yet, it is difficult to compare results obtained in different models since it is unclear, which outcome can be ascribed to the antigen and which one depends on the genetic background (Kuerten et al., 2009). It is therefore crucial to modify only one variable at a time, that is either the antigen or the genetic background. With the introduction of the MP4 model on the C57BL/6 background, the spectrum of models on this background covered all main antigens known from MS pathology: MOG, MBP and PLP. In addition, this background offers the possibility of performing genetic modifications, facilitating mechanistic studies.

In the following the characteristic histopathological features of MOG:35-55-, MP4- and PLP:178-191-induced EAE on the C57BL/6 background will be discussed and critically evaluated in the context of MS pathology considering the three hallmarks of MS pathology inflammation, demyelination and axonal damage.



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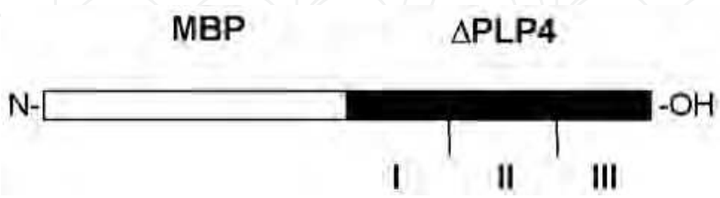

B

Fig. 2. The molecular structure of the MBP-PLP fusion protein MP4. (A) Structure of PLP. PLP is a transmembrane protein that consists of two extracellular (I and III) and an intracellular (II) hydrophilic domain, and four hydrophobic transmembrane sequences. (B) Structure of MP4. The three hydrophilic PLP domains have been fused to create ΔPLP4, which has been linked to the 21.5 kD isoform of human MBP.

3. Studies on the CNS histopathology of EAE and its correlation with clinical and immunological parameters

3.1 The CNS lesion topography and composition depends on the antigen used for immunization in models of C57BL/6 EAE

Inflammation is a feature of MS pathology that can essentially be reproduced in any EAE model. In principle, pathology is initiated when autoreactive T cells enter the CNS. Before, these cells need to be primed in the secondary lymphoid organs. In MOG:35-55- and PLP:178-191-induced EAE the antigen used for immunization is a single peptide and the autoreactive T cell response is directed against this peptide, while determinant spreading does not occur, which could include further determinants into the autoimmune response. The MP4 model, in contrast, is characterized by a multideterminant-specific $CD4^+$ T cell response and we have shown that there is no single dominant determinant being recognized in mice immunized with MP4 (Kuerten et al., 2006). Rather, the response seems to randomly target different determinants of the MP4 protein with interindividual variation in individual mice. The advantage of multideterminant specificity in the MP4 model resides in the fact that it may be used to better mirror the heterogeneity of the T cell response present in MS patients. It has been shown that there is not a single determinant targeted by the autoimmune response in MS. Differences do not only exist between individual patients, but also develop as disease progresses, since new determinants can be engaged into the immune response through determinant spreading (Tuohy et al., 1998). In patients this is a random process, which is highly unpredictable and can at least in part account for the kinetics of disease progression.

We assume that differences in the peripheral antigen-specific response have major implications on the subsequent CNS histopathology. In all models that we analyzed, infiltration of the cerebrum with focus on the meninges close to the hippocampal region occurred already in acute disease. In addition, in MP4- and PLP peptide-induced EAE inflammation of the spinal cord meninges was evident, while in the MOG peptide model inflammation extended into the parenchyma. Cerebellar infiltration was absent in the former two models, but pronounced in the latter in the acute stage of the disease. In chronic EAE (50 days after immunization), lesion distribution shifted towards the spinal cord and cerebellar parenchyma in the MP4 model, while it decreased remarkably in the cerebrum. In MOG peptide- and PLP peptide-induced EAE the lesion topography was comparable to the acute stage. Overall, CNS inflammation was time-dependent and dynamic only in the MP4 model, shifting from the cerebrum to the spinal cord and finally involving the cerebellum, thus allows the staging of the disease (Kuerten et al., 2007). MS is characterized by lesion dissemination in time and space, for which the MP4 EAE could serve as a valuable model. In contrast, the PLP and MOG model showed rather static inflammatory patterns that remained unchanged throughout the disease.

Next to differences in lesion topography we found differences in the cellular composition of CNS lesions. In particular, these differences pertained to the numbers of CNS infiltrating B cells.

3.2 The development of tertiary lymphoid organs (TLOs) in MP4-induced EAE of C57BL/6 mice

Studying the MP4, MOG peptide and PLP peptide model systematically early and late after immunization, we found that B cell infiltration was a common feature of the MP4 model,