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MP4- and MOG:35–55-induced EAE in C57BL/6 mice differentially targets brain, spinal cord and cerebellum[◇]

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Abstract

Mechanism-oriented studies of EAE rely mostly on gene-modified mice on the C57BL/6 background. Here we report that MP4-induced EAE displays characteristic differences in CNS pathology as compared to MOG peptide 35–55-elicited disease. While in the latter, the topology of CNS infiltration remained unchanged throughout the disease, in MP4-induced EAE it was dynamic and stage-dependent shifting from the brain to the spinal cord and finally to the cerebellum. Unlike in the MOG peptide model, the frequencies and sizes of CNS lesions in MP4-induced disease showed a clear correlation with clinical disease severity. These characteristic features of MP4-induced EAE may contribute to modelling the complex spectrum of disease manifestations seen in MS.

Keywords

Autoimmunity; EAE/MS; Histopathology; Neuroimmunology Staging

1. Introduction

Progress in understanding the immune pathology of multiple sclerosis (MS) is still largely dependent on suitable animal models (Steinman, 1999). Studies of the human disease itself have been hampered by limited access to central nervous system (CNS) tissue at various stages of MS. In addition, there is insufficient understanding of whether MS is indeed mediated by autoreactive T cells and if so, which neuroantigens are actually targeted by these cells (Sospedra and Martin, 2005; Gold et al., 2006; Barnett and Sutton, 2006). There is persuasive evidence that MS is not a single disease entity, but that it could instead result from different types of

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immune responses and patterns of CNS histopathology (Lassmann and Ransohoff, 2004; Sospedra and Martin, 2005; Berger et al., 1997; Sun et al., 2003).

In contrast, animal models - in particular, variants of experimental autoimmune encephalomyelitis (EAE) - have permitted the use of an experimental approach to MS (reviewed in Goverman and Brabb, 1996; Martin and McFarland, 1995; Wekerle et al., 1994). Immunizations of susceptible animal strains with CNS antigens - originally with spinal cord homogenate (SCH) (Kabat et al., 1950; Freund et al., 1950) - induce lesions that resemble many features of MS (Goverman and Brabb, 1996; Martin and McFarland, 1995; Schmidt, 1999; Steinman, 1999; Wekerle et al., 1994). Importantly, different EAE models have been shown to reflect different courses of MS (Lassmann and Ransohoff, 2004). The classical myelin basic protein (MBP)-induced disease in the B10.PL mouse shows an acute and monophasic clinical outcome (Waxman et al., 1980). In contrast, proteolipid protein (PLP):139–151-triggered EAE in SJL mice is chronic relapsing (Tuohy et al., 1989; Hofstetter et al., 2002). Apparently, there is no single EAE model that would be best suited to study MS. Instead different EAE models seem to be needed to cover the spectrum of pathology seen in subtypes of MS.

EAE models are known to be well-suited tools for experimentally addressing mechanisms that cause autoimmune pathology of the CNS and for studying therapeutic principles for preventing or curing the disease. Increasingly, EAE in the C57BL/6 (B6) mouse has become the most desired model to work with since gene knock-out (KO) and knock-in strains are predominantly created on this background, thus providing congenic strains for rapid progress with mechanistic studies. B6 mice, however, were initially considered to be non-EAE susceptible. They have been reported to be resistant to MBP- (Bernard, 1976; Gasser et al., 1990; Skundric et al., 1994; Fritz and Zhao, 1996) and PLP-induced EAE (Fritz and Zhao, 1996; Klein et al., 2000; Tuohy et al., 1988). Only the discovery that myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (MOGp) was able to induce EAE in the B6 mouse (Mendel et al., 1995) made them join the EAE field. However, it remains unclear to what extent MOGp-induced EAE reflects the diverse disease courses and manifestations as well as the immune pathomechanisms involved in MS.

In an effort to develop an alternative EAE model for B6 mice, the PLP peptide 178–191-(PLPp) induced disease has been introduced in 2002 (Tompkins et al., 2002). This model still awaits closer characterization. Except for the description of the pathogenicity of this peptide, and evidencing the presence CD4⁺, I-A^b- and F4/80-expressing cells in the spinal cord 20 days after immunization, there is no detailed published information available on the immune pathology of this model and on how it relates to MOGp-induced EAE of the B6 mouse.

Also, in an attempt to diversify the EAE model portfolio on the B6 background, we recently introduced the MBP-PLP fusion protein (MP4)-induced disease (Kuerten et al., 2006). Our initial characterization of the MP4 model provided in that publication showed fundamental differences to the two other models available for B6 mice, the MOGp- and PLPp-induced EAE. Thus, the onset of the disease was more rapid in MP4-induced EAE; its development was dependent on B cells and CD8⁺ cells (in addition to CD4⁺ cells), and autoantigen-recognition entailed multi-determinant specificity.

Here, we provide the first systematic histopathological characterization of the MP4 model while comparing it to MOG:35–55- and PLP:178–191-induced EAE. Defining the characteristic differences in disease course and pathology in each of these three models on the B6 background may represent a unique opportunity for studying new aspects of disease pathogenesis. B6 mice are of particular interest for mechanism-oriented studies since an increasing repertoire of gene-modified strains is available on this background. Considering

that MS itself has multiple manifestations it should be important to be able to selectively induce some of them in the B6 mouse.

2. Materials and Methods

2.1. Mice

Female wild-type (WT) C57BL/6 mice (6 – 8 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained at the animal facilities of Case Western Reserve University, Cleveland, OH. All treatments complied with the institutional guidelines.

2.2. Induction and clinical assessment of EAE

MP4/Apogen was obtained from Alexion Pharmaceuticals (Cheshire, CT). PLP:178–191 (FNTWTTCQSIAPPS), MOG:35–55 (MEVGWYRSPFSRVVHLY RNGK) and hen egg lysozyme (HEL) protein were purchased from Princeton Biomolecules (Langhorne, PA). Incomplete Freund's adjuvant (IFA) was prepared as a mixture of Mannide Monooleate (Sigma-Aldrich, St. Louis, MO) and Paraffin Oil (EMScience, Gibbstown, NJ), Complete Freund's adjuvant (CFA) was obtained by mixing *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Franklin Lakes, NJ) at 5 mg/ml into IFA. For disease induction, groups of 8 mice were immunized s.c. in both sides of the flank with either 150 µg MP4, 100 µg MOG:35–55 or 200 µg PLP:178–191 in CFA. Mice immunized with 200 µg HEL protein in CFA in addition to unimmunized mice served as negative controls. 200 ng pertussis toxin (PTX; List Biological Laboratories, Hornby, ONT, Canada) in 500 µl sterile PBS were given on the day of immunization and 48 hours later.

Clinical assessment of EAE was performed daily according to the following criteria: (0), no disease; (1), floppy tail; (2), hind leg weakness; (3), full hind leg paralysis; (4), quadriplegia; (5), death. Mice that were in between the clear-cut gradations of *clinical signs* were scored intermediate in increments of 0.5.

2.3. Histological evaluation

On days 7, 9, 13, 16, 25 and 50 after disease induction via immunization with the respective neuroantigens, mice were euthanized under deep anesthesia. Brain, brainstem/cerebellum and spinal cord were obtained and fixed in 4% paraformaldehyde (PFA) for at least 48 hours. Following fixation, brains were placed into a mouse brain matrix (World Precision Instruments, Berlin, Germany) and dissected. As a rostral border, we took the midpoint of the olfactory bulbs (bregma 2.7 mm) and as dorsal border, the beginning of the *vermis cerebelli*. (bregma - 4.2 mm). The cryo-protected (sucrose-infiltrated) tissue (brain, spinal cord and brainstem/cerebellum) was then cut into 20 µm thick sections on a Reichert-Jung Cryocut CM1850 cryotome (Leica, Deerfield, IL) and consecutively mounted on SUPERFROST/Plus slides (Roth, Karlsruhe, Germany).

For each animal, 10 equidistant coronal sections through the brain (i.e. every 35th), 10 equidistant longitudinal sections through the spinal cord (i.e. every 3rd) and 10 equidistant longitudinal sections through the brainstem/cerebellum (i.e. every 10th) were selected and stained with haematoxylin and eosin (HE) according to the standard protocol. The number of infiltrates, differentiated into meningeal versus parenchymal/perivascular, was evaluated in each section on a Zeiss Axioskop 50 microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) at 10x magnification. The “total infiltration” in Fig. 7A to D was calculated as the sum of both cumulative meningeal and parenchymal infiltration numbers in all mice analyzed. Infiltrates consisting of less than 20 aggregated cells were neglected. The Image-Pro Plus acquisition software (Media Cybernetics, Silver Spring, MD) was used to evaluate lesion sizes. Results in Fig. 7E to H are depicted as mean meningeal or parenchymal lesion sizes individually

or as the mean between the two (i.e. the “mean total lesion sizes”). Images were captured at a $10\times$ magnification and the software was calibrated accordingly to measure the area of each infiltrate in the dimension of μm^2 . Results in Fig. 7 are shown in the dimension of mm^2 .

Ten additional longitudinal equidistant sections through the spinal cord of each animal were selected for Luxol Fast Blue (LFB) staining, also according to the well-established method. Spinal cord demyelination was scored as follows: (1) traces of subpial demyelination; (2) marked subpial and perivascular demyelination; (3) confluent perivascular or subpial demyelination; (4) massive perivascular and subpial demyelination involving one half of the spinal cord with presence of cellular infiltrates in the CNS parenchyma; and (5) extensive perivascular and subpial demyelination involving the whole cord section with presence of cellular infiltrates in the CNS parenchyma.

The examination of all histopathology, i.e. assessment of infiltration and demyelination was done by an observer blinded as to the sensitization and clinical status of the animals.

2.4. Statistical analysis

SigmaStat software (Version 7.0; SPSS, Chicago, IL) was used to assess the strength of correlation between clinical scores and lesion frequencies/sizes in MP4- and MOG:35–55-induced EAE. The coefficients of determination (r^2 -values) were calculated to evaluate the percent of data closest to the line of best fit and thereby to show how well the regression model described the data.

3. Results

3.1. Rapid onset of clinical EAE in the MP4 model

B6 mice were immunized with MP4 protein, MOG peptide 35–55 or PLP peptide 178–191 respectively, each in doses that had been established before as maximally pathogenic (see *Materials and Methods*). The course of disease elicited by these immunizations is shown in Fig. 1. For mice immunized with MP4, *clinical signs* of EAE frequently developed as early as day six (Fig. 1A). In contrast, most mice immunized with MOG peptide 35–55 did not develop disease until day 10 (Fig. 1B). In both models acute EAE was followed by a monophasic non-relapsing disease course. PLP peptide 178–191-triggered EAE showed an onset around day 18 after which the disease took a chronic course (Fig. 1C).

3.2. CNS lesion topology in acute EAE: absence of parenchymal, spinal cord, and cerebellar infiltration in MP4-immunized mice

In all three models, acute EAE was defined as the time period between the onset of the first *clinical signs* and the transition to the chronic stage, after the disease scores reached maximal value. Thus, the acute phase for MP4-induced EAE encompassed CNS material obtained on days 7, 9, 13 and 16, for MOG:35–55-induced EAE on days 9, 13 and 16, and for the PLP:178–191-triggered disease, on days 16 and 25, respectively. At these time points, the brain, spinal cord and cerebellum were subjected to histological analysis. A summary of the cardinal features of CNS lesion topology in acute EAE observed in all three models is shown in Table 1. Representative examples showing the typical CNS lesion topology in acute MP4-, MOG:35–55- and PLP:178–191-induced EAE are provided in Fig. 2.

Studies of the cortical histopathology did not show major differences for the acute stage of the disease in the three models. In all of them, mice showed heavy infiltration of the cerebral meninges, which prevailed in the region of the hippocampus involving the lateral and the third ventricle (arrows in Fig. 2A, B and C).

Studies of the spinal cord also did not show major differences with regard to the involvement of the spinal meninges (see arrows in Fig. 2D, E and F). However, while all MOG:35–55 immunized mice developed severe parenchymal infiltration in the spinal cords on day 9, day 13 and day 16 (7/7 mice for each time point; indicated by arrows in Fig. 2E), no effect on this region was seen in MP4-induced EAE; the spinal parenchyma of these mice was free of infiltrates in 8/8 mice on day 7, 8/8 on day 9, 7/7 on day 13 and 7/7 on day 16. In PLP:178–191-triggered disease infiltrates were also seen, but at a lower incidence. In addition, in this model spinal cord inflammation followed hardly recognizable patterns. Whereas three of 14 mice showed infiltration of both the parenchyma and meninges, in the same number (three of 14), only the meninges were affected. In eight of 14 mice inflammation was entirely absent in these two CNS regions.

Studies of the cerebellar white matter for MOG:35–55-induced EAE showed infiltrates in all mice on days 13 and 16 (8/8 and 7/7 mice, respectively, an example is given in Fig. 2H). In contrast, in MP4-immunized mice (which underwent severe acute EAE with severe infiltrations in the brain and the spinal cord meninges), no detectable inflammation was observed in the cerebellum (in 0/8 mice on day 7, 0/8 mice on day 9, 0/7 mice on day 13 and 0/7 mice on day 16). Immunization with PLP:178–191 induced cerebellar infiltration only in 2/14 mice (Table 1). Therefore, MP4-induced EAE clearly differs from the MOG peptide-elicited EAE by the involvement of the cerebellum and the spinal cord parenchyma during the acute phase of the disease.

3.3. CNS lesion topology in chronic EAE: infiltration of the cerebellum and spinal cord parenchyma develops in MP4-immunized mice while cortical lesions disappear

A summary of the cardinal features of CNS lesion topology in chronic EAE observed in all three models is shown in Table 2, and representative slides exemplifying CNS lesions are presented in Fig. 3. In MOG:35–55-induced disease, the infiltrates seen during the acute stage in the brain, spinal cord and cerebellum persisted in the chronic stage of the disease (Fig. 3B, E and H). By this time, MP4-immunized mice (that had not shown infiltrates in the cerebellum and the spinal cord parenchyma during acute EAE) also developed lesions in the cerebellum and the spinal cord parenchyma resembling those in the MOG peptide model (arrows in Fig. 3A, D and G). However, during chronic EAE, infiltrates were not present in the brain (both *in the meninges* and *the ventricles*) of MP4-immunized mice while such infiltrates persisted in the MOG:35–55-induced disease (compare Fig. 3A to B). PLP:178–191-triggered EAE behaved like the latter showing cortical infiltrations, however most frequently in the absence of cerebellar lesions (only in one of 10 mice infiltration of the cerebellum was evident) (Fig. 3C to I). Spinal cord infiltration was present in all MP4 and MOG peptide immunized mice, but regularly absent in PLP peptide-induced EAE. Likewise, the location of the infiltrates within the spinal cord was variable. Fifty percent (5/10) of the PLP peptide-immunized mice developed infiltrates in the spinal cord meninges – in two of these five mice the inflammation was restricted to the meninges while in the three other infiltrates extended to the parenchyma (Table 2).

Because of the differences seen between MP4- and MOGp-induced EAE, we examined in greater detail the kinetics of the CNS inflammation in both models. Since PLP:178–191-induced EAE did not show clear-cut patterns of infiltration in spinal cord and cerebellum it was not included into these analyses.

3.4. Characteristic lesion progression in MP4-induced EAE

Fig. 4 shows the extent of *total* infiltration in the various CNS compartments over time for MP4- and MOG:35–55 induced EAE. MOG:35–55-induced EAE showed synchronous and static CNS histopathology throughout the disease; infiltration of the spinal cord, brain and

cerebellum developed simultaneously during acute disease and persisted to a similar extent throughout the observation period of 50 days (Fig. 4A). On the contrary, in MP4-elicited disease, infiltration of the brain was the first to appear; it peaked by day 13 and close to completely vanished by day 50 (Fig. 4B). Showing a reverse kinetic, the infiltration of the cerebellum in MP4-induced EAE did not develop until very late in the disease, being initially visible on day 25 and increasing by day 50 (Fig. 4B). Therefore, MP4-induced disease showed a characteristic progression in which anatomical regions were targeted in a successive order.

3.5. Chronic demyelination occurs in MP4-induced EAE, but is absent in MOG:35–55- and PLP:178–191-induced EAE

In addition to inflammatory infiltrates, demyelination is a hallmark of MS that however, only some EAE models can reproduce (Schmidt, 1999; Gold et al., 2006). We performed LFB staining in CNS sections of all aforementioned mice to study the loss of myelin at different time points after immunization. These results are summarized in Fig. 5 evaluating the demyelination scores and in Fig. 6 showing representative images. In acute EAE, spinal cord demyelination was seen in both MP4- and MOG peptide-immunized mice (starting on day 9 in the former and on day 13 in the latter). It was more pronounced in MOG:35–55-immunized mice and reached the highest levels on day 16 after immunization (Fig. 6C vs. G). Subsequently, however, demyelination was absent in MOG:35–55-immunized mice on day 50 (Fig. 6H) although cellular infiltration persisted (Fig. 4A). On the contrary, demyelination in MP4-immunized mice persisted at plateau levels (Fig. 6D). PLP:178–191-injected mice did not develop detectable demyelination (Fig. 6I to L). Therefore, the severity and persistence of demyelination additionally distinguished MP4-, MOG:35–55- and PLP:178–191-induced EAE in the B6 mouse.

3.6. Severity of MP4-induced disease (but not of MOG peptide-elicited disease) correlates with lesion frequency and size

The ability to correlate functional damage (clinical disease severity) and structural damage (histological disease manifestations) in EAE would largely facilitate EAE/MS research. However, previous reports failed to show such a correlation (Kerschensteiner et al., 2004; Berger et al., 1997). For MOG:35–55-induced EAE, our data confirm those reports; neither lesion frequency in the brain ($r^2 = 0.29$) and the spinal cord ($r^2 = 0.66$) nor lesion size in brain ($r^2 = 0.18$) and spinal cord ($r^2 = 0.36$) correlated clearly with clinical disease severity (Fig. 7B, D, F and H). In MP4-induced EAE, however, an excellent correlation between clinical scores and lesion frequency ($r^2 = 0.83$ in the brain and $r^2 = 0.93$ in the spinal cord) as well as size ($r^2 = 0.93$ in the brain and $r^2 = 0.97$ in the spinal cord) was seen (Fig. 7A, C, E and G). For each group the mean meningeal infiltration (open circles), ventricular (in the brain) or parenchymal infiltration (open triangles) and the total infiltration (solid squares) is shown.

4. Discussion

Histological correlates of autoimmune disease provide important information on the underlying autoimmune pathology. Milestone histological studies of EAE date back into the 1970s involving different species (guinea pigs, rabbits, rats and mice) and within these species frequently different strains and different antigens were studied (Lassmann and Wisniewski, 1979; Levine et al., 1975; Mitsuzawa and Yasuda, 1976; Raine et al., 1977; Sensharma and Singh, 1975; Snyder et al., 1975). Fundamental differences were seen in these models but the genetic and antigenic differences between the EAE models studied (Bernard, 1976; Berger et al., 1997) made it difficult, however, to delineate the key factors that accounted for these dissimilarities. While the B6 mouse has emerged as a particularly attractive model for EAE studies there is only a limited amount of information available on the histological correlates of the “classical” MOG peptide-induced disease (Berger et al., 1997; Oliver et al., 2003; Shao

et al., 2004; Sun et al., 2001). With regard to PLP peptide 178–191-induced EAE the information available is even more limited to the description of the pathogenicity of this peptide, and to showing CD4⁺, I-A^b- and F4/80 expressing cells in the spinal cord 20 days post immunization (Tompkins et al., 2002). Thus far, there are no information on the CNS histopathology of MP4-induced EAE in B6 mice. This study represents the first systematic comparison of the MP4-, MOG:35–55- and PLP:178–191-induced EAE of the B6 mouse. Importantly, the results have even been obtained comparing B6 mice from the very same mouse colony. The use of gene knock-in and knock-out mice will facilitate the dissection of the immune mechanisms that account for these differences eliminating the genetic variability and transferring histological correlates into a mouse strain in which mechanism-oriented studies can be conducted.

One of the most compelling differences between MP4- and MOG:35–55-induced EAE pertained to the sequence in which anatomical regions of the CNS were targeted. In MOG peptide-induced EAE all the three anatomical regions that we have studied (i.e. brain, spinal cord and cerebellum) were attacked simultaneously, and to an extent that remained static throughout the course of the disease. In contrast, in MP4-induced disease, infiltrates were first detectable in the brain, but after an initial wave of inflammatory activity, they became abortive in this region. Spinal cord inflammation developed later in MP4-immunized mice, but persisted throughout the observation period. Also, unlike in MOGp-induced disease, in MP4-triggered EAE the cerebellum was targeted only at a late stage of the disease. Therefore, in the MP4 model (unlike in MOG:35–55-induced disease), the differential involvement of these different regions with time allows for staging of the disease with respect to disease history.

The exact reasons remain unclear why the targets of the autoimmune attack differed between the two models. Although it has been described before that different EAE models are characterized by different patterns of CNS histopathology (Schmidt, 1999) in those studies not only did the autoantigens differ, but frequently also different mouse strains, or even other species were used. This diversity in genetic background could have conceivably contributed to the different disease manifestations. In our study, the differences occurred in a single mouse strain and even the same mouse colony, thereby eliminating genetic and environmental variables. Different solubility and bioavailability of the antigens is one of the possible explanations for their different encephalitogenic activities. Adoptive transfer experiments addressing this possibility have been performed by the Lassmann group (Berger et al., 1997). They studied passive EAE induced by different neuroantigens in the Lewis rat and found major differences in lesion topography depending on the neuroantigens used for the priming of the transferred T cells. Therefore, the antigen specificity of the T cells rather than the biophysical properties of the antigens was responsible for the different EAE manifestations. Since these biophysical properties of the neuroantigens are independent of the test organism, we assume that this conclusion applies for the B6 mouse as well.

In addition to the topology of CNS lesion formation, we also observed differences in the patterns of demyelination. Demyelination was not evident in PLP peptide-induced disease and was transient in MOG peptide-triggered EAE. In contrast, chronic demyelination was seen in MP4-immunized mice. It is unclear whether this difference resides in the nature of the antigen. MP4 contains the PLP:178–191 determinant and PLP-induced EAE in other mouse strains is indeed characterized by massive chronic demyelination (Tsunoda et al., 2005). The fact that we did not observe demyelination using the isolated PLP:178–191 peptide might be related to this peptide being a cryptic determinant in the B6 mouse. When we studied the fine specificity of the MP4-induced T cell response to its PLP constituents, we found that multiple PLP peptides were targeted; however, PLP:178–191 was not one of them (Kuerten et al., 2006). In attempting to induce EAE with various PLP peptides in our previous work (Kuerten et al., 2006) we

achieved rather weak and non-demyelinating disease with several of these PLP peptides, similar to EAE induced by PLP:178–191 in this study.

Further studies will be required to establish whether the demyelination that occurs in the MP4 model and that persists into the chronic stage is irreversible and possibly mediated by axonal damage/transection or whether it represents only inflammation-mediated *functio laesa*. If the former proves true, the MP4 model should be an important extension of EAE studies in the B6 mouse, permitting one to address mechanisms of transient *functio laesa* type of demyelination vs. permanent axonal loss. The relative inaptitude of PLP peptides to cause demyelination versus the only *transiently demyelinating* disease course of MOG:35–55-induced disease and the chronically demyelinating MP4-induced disease might provide a useful model system for studies of demyelination and remyelination in B6 mice.

A third striking difference between MOGp- and MP4-induced disease pertained to the correlation between lesion frequencies/sizes and disease severity (i.e. clinical score), which was absent in the former model, but convincing in the latter. A concern about “disseminated” EAE models such as the MOGp-elicited disease has indeed been the absence of such a correlation (Kerschensteiner et al., 2004), making it difficult to evaluate the success of therapeutic strategies. Without the possibility of linking functional and structural damage in MS/EAE, assessing clinical disease severity would be the primary readout for disease activity. Introducing a second readout (i.e. measurements of lesion size and frequency in the MP4 model) may facilitate studies that aim at quantifying disease activity by an independent parameter.

The data presented here clearly show that MP4-induced EAE bears histopathological features that are distinct from the MOG:35–55-induced disease. In addition, our previous work suggests that different immune mechanisms are involved in these two EAE models of the B6 mouse. Since MS occurs in a wide spectrum of manifestations and seems to involve a variety of mechanisms, we believe that the introduction of MP4-induced EAE will facilitate studies that aim at experimentally dissecting mechanisms involved in EAE/MS.

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Abbreviations

B6

	C57BL/6
EAE	experimental autoimmune encephalomyelitis
HE	haematoxylin-eosin
HEL	hen egg lysozyme
KO	knock-out
LFB	Luxol Fast Blue
MBP	myelin basic protein
MP4	MBP-PLP fusion protein
MOG	myelin oligodendrocyte glycoprotein
MOGp	MOG peptide 35–55
MRI	magnetic resonance imaging
MS	multiple sclerosis
PFA	paraformaldehyde
PLP	proteolipid protein
PLPp	PLP peptide 178–191
PTX	pertussis toxin
SCH	spinal cord homogenate
WT	wild-type

Fig. 1.

The clinical disease courses in MP4-, MOG:35–55- and PLP:178–191-induced EAE. B6 mice were immunized with either MP4 (150 μ g) (A), MOG:35–55 (100 μ g) (B) or PLP:178–191 (200 μ g) (C) in CFA. The course of disease, assessed daily, is shown for eight individual mice by the thin lines - the mean clinical score for each group of mice is indicated by the bold line. The time point at which 50% of all mice had contracted the disease in each group is indicated by the hatched vertical line. The data are shown from one representative experiment out of three performed.

Fig. 2.

Lack of parenchymal spinal cord and cerebellar infiltration in acute MP4-induced EAE.

Representative histopathologic images are shown for acute MP4-induced EAE (days 7, 9, 13 and 16 after immunization) in the panels on the left (A, D, G), MOG:35–55-induced EAE (9, 13 and 16 days after immunization) in the center (B, E, H) and PLP:178–191-induced disease (16 and 25 days after immunization) on the right (C, F, I). The upper panels show the cerebrum focusing on the region of the hippocampus (HC), the center panels the spinal cord (meninges and parenchyma) and the lower panels the cerebellum. Infiltrates are indicated by arrows in each panel. All images are at 160 × magnification. HE staining.

Fig. 3.

Emergence of infiltrates in the spinal cord parenchyma and cerebellum and disappearance of infiltrates in the brain of MP4-immunized mice in chronic EAE. The panels are arranged as in Fig. 2: representative images are shown for the chronic MP4-induced EAE (25 and 50 days after immunization) in the panels on the left (A, D, G), MOG:35–55-induced EAE (25 and 50 days after immunization) in the center (B, E, H) and PLP:178–191-induced disease (50 days after immunization) on the right (C, F, I). The upper panels show the brain focusing on the region of the hippocampus (HC) and the lateral ventricle (LV), the center ones the spinal cord (meninges and parenchyma) and the lower panels show the cerebellum. Infiltrates are indicated by arrows in each panel. All images are at $160\times$ magnification. HE staining.

Fig. 4.

Characteristic lesion progression in MP4-induced EAE. Brain, spinal cord and cerebellum were dissected on days 7, 9, 13, 15, 25 and 50 after immunization with MOG:35–55 (A) or MP4 (B), and stained with HE. The cumulative *total* lesion frequency was evaluated in each of these sections. The mean cumulative number of *total* lesions per animal is shown for a total of 10 cerebral (hatched line), spinal cord (solid line) and cerebellar sections (dash-dotted line) per animal respectively, with eight mice evaluated in each group. SD were typically < 20%.

Fig. 5.

Chronic demyelination is present in MP4-induced EAE, but absent in MOG:35–55- and PLP:178–191-induced EAE. Spinal cord sections were obtained on days 7, 9, 13, 16, 25 and 50 after immunization with MP4, MOG:35 or PLP:178–191 and stained with LFB. In each of these sections the demyelination score was evaluated (described in detail in *Materials and Methods*). Mean and SD are given for each group and each time point after immunization. Triangles represent results for MOG peptide-, circles for MP4- and squares for PLP peptide-immunized mice.

Fig. 6.

The course of demyelination in MP4-, MOG:35–55-, and PLP:178–191-induced EAE.

Representative images are shown for MP4-induced EAE in panels A to D, for the MOGp model in panels E to H and for PLP:178–191-triggered disease in panels I to L. Demyelinated areas are indicated by arrows. All images are at $160\times$ magnification. LFB staining.

Fig. 7.

Severity of MP4-induced disease (but not of MOG peptide-induced disease) correlates with lesion frequency and size. Mice were immunized with MP4 or MOG:35–55 as described in the legend to Fig. 1. In each animal and depending on the clinical score, we evaluated lesion frequencies (A to D) and sizes (E to H) in 10 cerebral (A, B and E, F) and 10 spinal cord (C, D and G, H) sections per animal with 45 mice per group. In panels A to D the correlation of infiltrates with either the time elapsed after immunization, or the clinical score, the cumulative meningeal infiltration (open circles), cumulative ventricular (in the brain) or parenchymal infiltration (in the spinal cord) (open triangles) and the cumulative total infiltration (solid squares) is shown (the latter represents the sum of both meningeal and ventricular/parenchymal infiltration). In panels E to H the lesion sizes are correlated with either the time elapsed after

immunization or the clinical score -- mean lesion sizes for meningeal infiltration (open circles) or ventricular/parenchymal infiltration (open triangles) are depicted individually. In addition, the mean “total” lesion sizes (i. e. the average of mean meningeal and mean ventricular/parenchymal infiltration) are depicted by the solid squares. The SD is not graphed but shown as a number above each data point for the total infiltration. The r^2 -values shown in the upper right corners of the panels also refer to total infiltration.

Table 1Topolgy of CNS inflammation in acute MP4-, MOG:35–55- and PLP:178–191-induced EAE^a

Infiltration of:		Acute EAE induced by:		
		MP4 ^b	MOG:35–55 ^c	PLP:178–191 ^d
Cortex	meninges	30/30 [*]	23/23	14/14
	parenchyma	0/30	0/23	0/14
	ventricles	30/30	23/23	14/14
Spinal cord	meninges	30/30	23/23	6/14
	parenchyma	0/30	23/23	3/14
Cerebellum	parenchyma	1/30	16/23	2/14

^a WT B6 mice were immunized with either MP4, MOG :35–55- or PLP :178–191 in CFA as described in Fig. 1. CNS tissue was obtained during acute EAE as defined in the text, stained with HE and the presence of meningeal, parenchymal and ventricular infiltration was assessed.

^b Acute MP4-induced EAE was evaluated on days 7, 9, 13 and 16 after immunization.

^c Acute MOG :35–55-induced EAE was evaluated on days 9, 13 and 16 after immunization.

^d Acute PLP :178–191-induced EAE was evaluated on days 16 and 25 after immunization.

^{*} The number of mice showing infiltration out of the number of mice studied is shown whereby infiltration was defined as the presence of aggregations containing more than 20 adjacent lymphatic cells.

Table 2Topology of CNS inflammation in chronic MP4-, MOG:35–55- and PLP:178–191-induced EAE^a

Infiltration of:		Chronic EAE induced by:		
		MP4 ^b	MOG:35–55 ^c	PLP:178–191 ^d
Cortex	meninges	0/15*	23/23	8/10
	parenchyma	0/15	0/23	0/10
	ventricles	2/15	23/23	10/10
Spinal cord	meninges	30/30	23/23	5/10
	parenchyma	0/30	23/23	3/10
Cerebellum	parenchyma	1/30	16/23	1/10

^a See legend to Table 1 except that CNS tissue was studied during the chronic stage of the respective EAE model as defined in the text.

^b Chronic MP4-induced EAE was evaluated on days 25 and 50 after immunization.

^c Chronic MOG :35–55-induced EAE was evaluated on days 25 and 50 after immunization.

^d Chronic PLP :178–191-induced EAE was evaluated on day 50 after immunization.