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Models of chronic experimental allergic encephalomyelitis

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Based on recent neuropathological and immunological data, a hypothetical model of experimental allergic encephalomylitis (EAE) and experimental allergic neuritis (EAN) is presented. The discussed pathogenetic mechanisms suggest that the full picture of inflammatory demyelination is induced by a complex interaction of cellular and humoral immune rections. Furthermore, they show that in principle several different antigens of the CNS and PNS can mediate autoimmune inflammation or demyelination respectively.

Key words: experimental allergic encephalomyelitis, experimental ellergic neuritis, multiple sclerosis, inflammatory demyelination.

Introduction

Detailed clinical and pathological descriptions of neurologic complications following rabies and other vaccinations in the early decades of our century indicated that autoimmune reactions against neural antigens may induce an inflammatory or inflammatory demyelinating disease of the nervous system [32, 52, 85]. This concept was then formally proven by the induction of experimental allergic encephalomyelitis [62] and experimental allergic neuritis [88] by active sensitization of susceptible animals with tissue of the central and peripheral nervous system, respectively. The animal models of EAE and EAN have then been extensively studied and have lead to considerable new insights in basic problems of immunology and autoimmunity, as well as to more specific aspects of the pathogenesis of demyelinating diseases, including multiple sclerosis.

Studies in EAE models started from what at that time was believed to be a rather simple problem. Since active sensitization with neural tissue lead to disease in a broad variety of animal species and also in humans [73], the two key questions at that time were:

1. What is the antigen, against which the immune reaction is directed?
2. What immunological mechanisms lead to disease?

Interestingly, both problems are not yet solved today.

These two questions seemed to be answered in the middle of this century, when myelin basic protein (MBP) was found to be an encephalitogenic antigen [31] and when the disease was transferred to naive recipient animals by lymphocytes from EAE animals [59]. However, soon after these discoveries it became evident that EAE pathogenesis is much more complicated than expected before.

First, disease induction was in general more effective and the clinical and pathological manifestation more complete, when the whole CNS tissue was used for sensitization instead of MBP. This indicated that besides MBP other CNS antigens may be involved in the pathogenesis of the disease. Furthermore, not a single peptide epitope of MBP is responsible for disease induction, but different MBP epitopes are effective in different animal species and even in different strains of a given species [1].

Secondly, EAE susceptibility is under genetic control. The genetic control not only involves the T-cell immune reaction against MBP, but apparently also immune responses against other CNS antigens, as well as factors governing the susceptibility of the target tissue.

Thirdly, extensive studies using various different sensitization protocols showed that even subtle changes in the immunization procedure may profoundly change the disease. This suggests that complex immunoregulatory mechanisms are involved in disease induction and that additional immunological (e. g. antibodies) and non-immunological factors (e. g. neuropeptides or vasogenic amines) may modify the disease.

All these studies have lead to a large number of different models of autoimmune encephalitis and neuritis, with variable relevance for human inflammatory demyelinating diseases. In the following text it will be tried to clarify some principal aspects of EAE and EAN pathogenesis and to explore their relevance for research in human inflammatory demyelinating diseases.

Pathology of experimental allergic encephalomyelitis and neuritis

As mentioned before, EAE and EAN are inflammatory demyelinating diseases. This already characterizes the main pathological features:

Inflammation consists of perivenous infiltrates of inflammatory cells, which in active disease also disperse within the surrounding tissue (Fig. 1). They are mainly composed of lymphocytes and monocytes / macrophages, but in very severe disorders an additional considerable number of polymorphonuclear leucocytes can be present in the lesions. In early phases most of the lymphocytes are T-cells, phenotypically characterized as "helper / inducer" T-cells (Table 1) [26, 80, 90]. Later during the disease (in the recovery phase), "suppressor / cytotoxic" T-cells dominate (Table 1) [27, 39]. The majority of cells in the lesions, however, are stained by monoclonal antibodies against macrophage antigens (Fig. 4*a*) and express Class II (Ia) histocompatibility antigens (Table 1; Fig. 1 *c, e, f, i*); [26, 39, 75]. The composition of inflammatory infiltrates is in principle similar in acute EAE (Fig. 1), active lesions of chronic EAE [39] (Fig. 2), acute EAN [56] and PNS lesions of EAE [40].

The second main feature of EAE pathology is demyelination (Figs 2*c*, 3, 4*b*). Demyelination in EAE and EAN in general is a selective process, leading to

Table 1. Quantitative evaluation of T-cell subsets and Ia-antigen in the spinal cords of SD-rats at different stages of acute and chronic EAE

	Unsensit. controls	Early disease	Peak of disease	Acute recovery	Chronic progressive	Remission
Dps	0	15	18–24	28–37	28–36	52
clin	0	1,2 ± 0,2	3,0 ± 0,6	1,5 ± 0,7	3,5 ± 0,5	0
n	8	5	4	5	4	4
W3/13	0,22 ± 0,16**	28,6 ± 37,4*	52,1 ± 32,5^{ns}	58,8 ± 26,7^{ns}	82,6 ± 26,1	3,0 ± 2,3*
O × 8	0,18 ± 0,15**	5,4 ± 6,8*	16,7 ± 6,9^{ns}	29,6 ± 12,8*	18,0 ± 1,8	2,1 ± 1,6*
O × 6	0,02 ± 0,02**	54,0 ± 74,3*	51,7 ± 23,9*	33,2 ± 11,6*	303,3 ± 194,3	0,3 ± 0,3*
%O × 8	68,4 ± 43,8**	25,5 ± 19,7^{ns}	36,9 ± 11,3^{ns}	55,1 ± 15,6*	23,6 ± 8,0	83,3 ± 59,1^{ns}

W3/13: numbers of W3/13$^+$cells/mm (total T cells)
O × 8: numbers of O × 8$^+$ cells/mm^2 ("suppressor/cytotoxic" T cells)
O × 6: numbers of O × 6$^+$ cells/mm^2 (Ia$^+$ cells)
%O × 8: percentage of O × 8$^+$ cells compared to total (W3/13$^+$) T-cells

means
±SD

dps: days after sensitization
clin: clinical score
n: numbers of animals

Note: Statistical analysis was performed by using the U-test according to Mann and Whitney. The values of the "chronic progressive" group were compared with all other values; levels of significance are indicated as follows: ns = not significant, * = p 0,05; ** = p 0,01.

destruction of myelin sheats with sparing of axons (Fig. 3*b*) and, when present, neurons. Myelin sheaths can be destroyed by several ways [33]. Most frequently macrophages with their processes invade between the myelin lamellae (myelin stripping). In some models of EAE also attachment of myelin fragments to coated pits of macrophages is noted [17], a pattern similar to that described in chronic multiple sclerosis lesions [61]. In other lesions whole myelin segments are transformed into vesicular material (vesicular disruption of myelin [33]). Reactive gliosis and subsequent astroglia scar formation parallels with inflammation and demyelination. This pattern of tissue damage results in perivenous sleeves of demyelination or in the formation of large, confluent demyelinated plaques (Figs 3, 4). In general, only minor loss of myelin sheaths is noted in the nervous system of animals with acute EAE and EAN, whereas demyelination is much more pronounced in models of chronic disease.

The structure of chronic EAE lesions and the extent of demyelination, however, vary from model to model: in guinea pigs selective primary demyelination is pronounced, leading to large confluent plaques (Fig. 3*a*), closely resembling those found in multiple sclerosis [36]. On the contrarty, SJL mice and rats with chronic EAE show relatively sparse demyelination [12, 34]. In mice, larger confluent lesions are associated with extensive inflammation and considerable unspecific tissue destruction. In rats, large demyelinated foci in the spinal cord may show additional pronounced destruction of astrocytes [34]. Subsequent remyelination is carried out by Schwann cells in these cases. Interestingly, a similar pathology with destruction of astrocytes has recently been described in Japanese multiple sclerosis patients [29]. Overall, remyelinating activity is much higher in EAE as compared to multiple sclerosis.

Summarizing the present knowledge on the pathology of EAE and EAN, the similarities to the alterations in human inflammatory demyelinating diseases, including multiple sclerosis, are striking [36]. Although this does not allow the conclusion that the etiology of these diseases is the same, it indicates that very similar pathogenetic mechanisms are responsible for the initiation and propagation of the lesions.

Basic pathogenetic principles of EAE and EAN lesions

As discussed above, the main alterations in the nervous system in EAE and EAN are the inflammatory process and demyelination. Since there are apparent differences in the mechanisms leading to inflammation and demyelination respectively, they will be discussed separately.

Inflammation

T-cell mediated immune reactions against MBP

The transfer of EAE by intravenous injection of lymphocytes from sensitized donors [59] suggested that T-lymphocytes are involved in the induction of the disease. More recently, monospecific MBP reactive T-lymphocyte lines and clones have been raised, which are able to transfer EAE to healthy rats and mice [2, 100]. Similarly, EAN can be induced by intravenous injection of T-cell lines reactive with P2-protein [44]. This new technology now allows to address the following questions: how many cells are the minimum requirement to induce disease, how do these cells reach the norvous system, how are they locally activated and how do they induce tissue damage?

Titration experiments with MBP reactive T-cell lines showed that, regardless of the animal species, 10^5-10^6 encephalitogenic T-cells are required to induce clinical disease [3, 67, 100]. However, careful pathological analysis of the CNS of such transfer animals revealed that as few as 10^3 MBP reactive T-cells give rise to focal, clinically silent inflammatory infiltrates in the CNS (Lassmann et al., in preparation). Furthermore, in vivo stimulation of such T-cells by simultaneous intravenous injection of recombinant interleukin 2 potentiates clinical disease in a dose-dependent manner [68]. These studies thus show that extremely few specific MBP reactive T-cells are required to start autoimmune inflammation in the CNS. This may partly explain why it is so difficult to detect a correlation between autoreactive T-cells and disease activity in chronic relapsing EAE [18] and multiple sclerosis.

It is controversial at present how encephalitogenic T-lymphocytes reach the brain. One concept postulates a soluble pool of MBP in the brain extracellular space, which is transported through the blood-brain barrier and presented to MBP reactive T-cells on the luminal surface of cerebral endothelial cells. In principle, it has been shown that intrathecally injected proteins, including MBP can be transported through the blood-brain barrier to the vascular lumen [86]. In EAE, perivascular accumulation of MBP and "endothelial" staining has been reported [74, 81], although convincing ultrastructural evidence is lacking. In our own material we were not able to find MBP reaction product by light and electron microscopic immunocytochemistry in or around vessel walls in acute and chronic EAE lesions.

A second requirement for antigen recognition by T-cells in cerebral vessels would be expression of class II (Ia) histocompatibility antigens on endothelial cells. Indeed, induction of Ia-expression on cerebral endothelial cells by stimulation with gamma-interferon was suggested from in vitro studies [51]. A large number of light microscopic immunocytochemical studies have documented Ia-reaction product associated with the wall of cerebral vessels [15, 39, 50, 76, 80, 81, 87]. Whereas in some studies Ia-expression on endothelial cells is described [76, 80, 81], all studies on rat CNS tissue did not report endothelial Ia-staining [15, 39, 49, 50, 87, 90]. Similarly, by ultrastructural immunocytochemistry, Ia-antigen was found on the luminal surface of guinea pig cerebral endothelial cells [77] but not on those of rats [39, 87]. It has, however, to be considered that the amount of antigen, as well as of Ia-determinants which is needed for T-cell activation, is very small and may be far below the level of detectibility by immunocytochemical techniques.

There are, however, some observations which argue against the concept of antigen presentation and recognition on the surface of cerebral endothelial cells. These aspects have recently been summarized by Wekerle et al. [91]. If cerebral endothelial cells present autoantigens in normal animals, resting MBP reactive T-cells should recognize their antigen, become locally activated and start the disease. However, transfer studies with MBP-reactive T-line cells showed that only activated cells are able to initiate EAE, whereas resting cells, even when injected in extremely high numbers, do not even enter the CNS compartment. Furthermore, not only MBP-reactive activated T-cells may reach the brain tissue, but also activated T-blasts directed against irrelevant antigens like ovalbumin. These data suggest that the primary T-cell migration through the blood-brain barrier and immune surveillance of the CNS depends on peripheral T-cell activation and is antigen-independent [91]. Whether specific antigen recognition on the endothelial cells of cerebral vessels plays an additional role in augmenting the inflammatory response has yet to be determined.

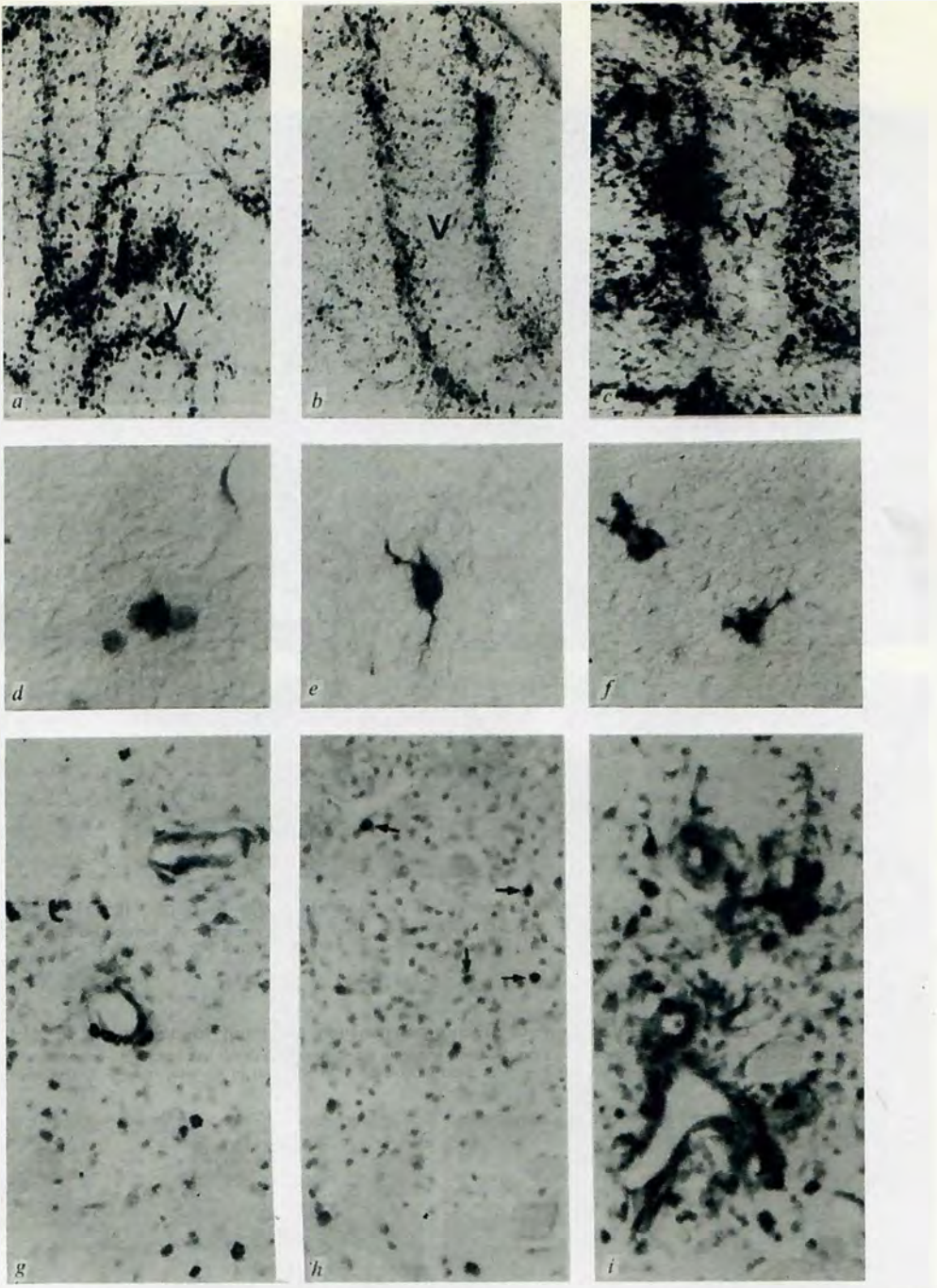

Fig. 1. T-lymphocytes and Ia-antigen in inflammatory lesions of acute rat EAE

a — 15 days after sensitization (dps): many W3/13$^+$ cells (total T-cells) in the meninges, mainly in perivenous position; v — vene. Isolated leptomeninges, × 65; *b* — 15 dps: 0 × 8$^+$ (suppressor/cytotoxic) T-cells in similar distribution as W3/13$^+$ mononuclear cells. Isolated leptomeninges, × 65; *c* — 15 dps: intensive expression of Ia — (0 × 6) antigen in the meninges. Isolated leptomeninges, × 65; *d* — incubation period, 8dps: W3/13$^+$ mononuclear cell in the spinal cord parenchyma. Paraffin section, × 650; *e, f* — incubation period, 8dps: 0 × 6$^+$ cells in the spinal cord white matter with slender, ramifying proceses. Paraffin section, × 650; *g* — acute, inflammatory EAE lesion, 15 dps: many W3/13$^+$ mononuclear cells in perivascular position and in the parenchyma. Paraffin section, × 650; *h* — same lesion as in Fig. 1*g*: 0 × 8$^+$ cells (arrows) are present in a similar distribution, although in lower numbers as compared to W3/13$^+$. Paraffin section, × 260; *i* — same lesion as in Fig. 1*g*: massive expression of 0 × 6 antigen on cells in perivascular position and in the parenchyma. Paraffin section, × 260

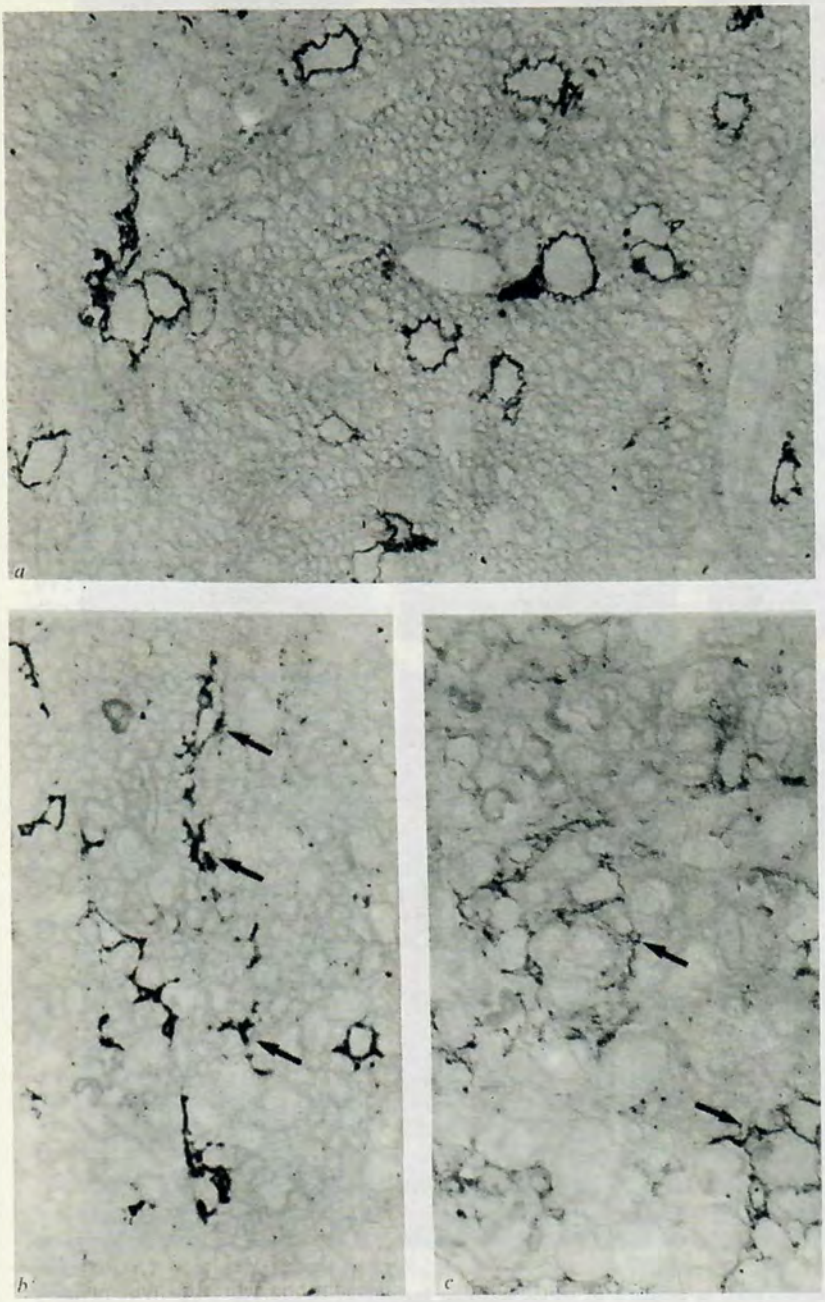

Fig. 2. Inflammatory infiltrates in mouse and rat EAE

a — Lyt1$^+$ T-cells dispersed within the relatively well preserved spinal cord white matter. SJL mouse, 310 dps, plastic section, preembedding staining method, × 1170; *b* — strongly Ia$^+$ cells with stellate shape (arrows) at the edge of a demyelinating lesion; SD rat with chronic progressive disease course, 35 dps; plastic section, preembedding staining method, × 1170; *c* — Ia antigen in an actively demyelinating lesion from the same animal as in Fig. 2*b*; 0 × 6$^+$ cell processes (arrows) engulfing disintegrating myelin sheaths; plastic section, preembedding staining method, × 1170

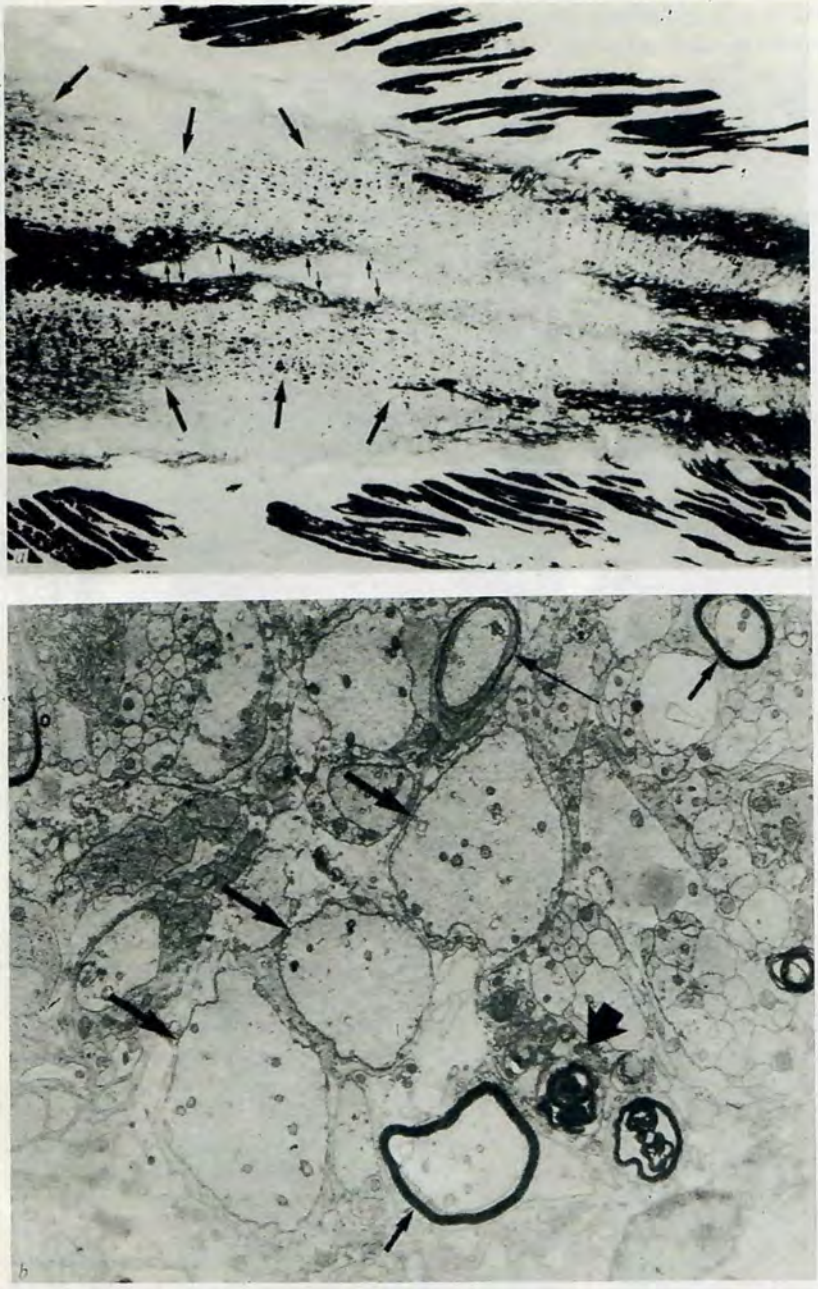

Fig. 3. Demyelination in EAE

a — large, confluent perivenous (small arrows) and subpial (large arrows) demyelinated plaques in the medullary conus of a guinea pig with chronic EAE; 386 dps, paraffin section, Klüver's myelin stain, × 35; *b* — demyelinated lesion in the spinal cord of a rat injected with encephalitogenic T line cells and a monoclonal antibody to a myelin / oligodendrocyte glycoprotein (MOG): a macrophage process with myelin debris (short thick arrow) among demyelinated axons (long thick arrow), two well-preserved nerve fibers (shorter arrow) and a remyelinated axon (long thin arrow), 4 days after antibody injection. EM, × 6000

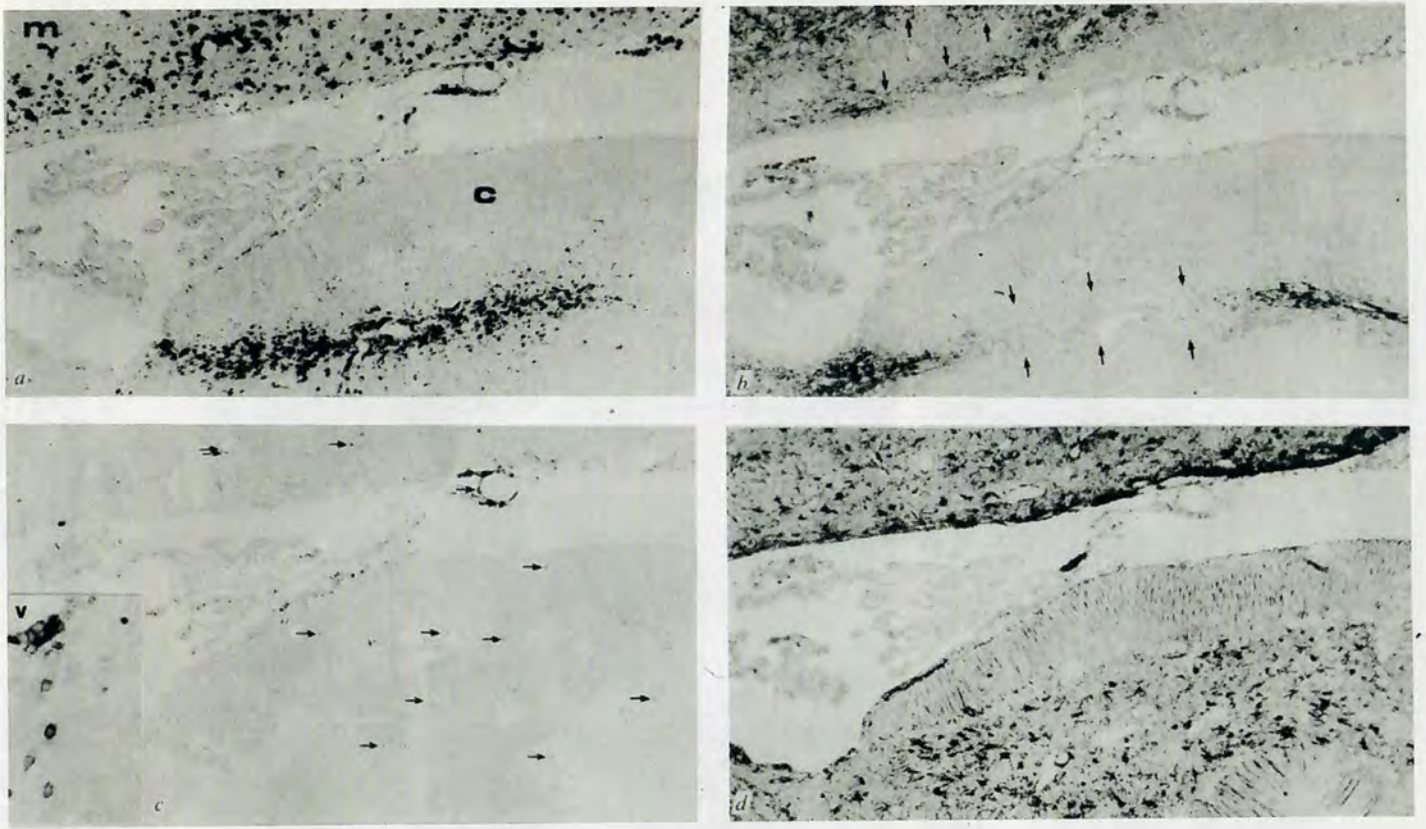

Fig. 4. MBP-reactive T line cells plus monoclonal antibody: a model to produce demyelination in acute EAE

a *d*: cerebellum (c) and medulla oblongata (m) of a Lewis rat injected with 4×10^6 encephalitogenic T line cells and 5 mg monoclonal antibody to MOG 4 days later. Perfusion 6 days after antibody injection. Serial paraffin sections, immunostained with a macrophage marker 2ED1, *a*), anti MBP (*b*), a T cell makrer (W3/13, *c*), and anti GFAP (*d*). × 100
a — numerous macrophages infiltrating the nervous tissue; *b* — large, party confluent zones of demyelination, corresponding to macrophage invasion (borders indicated by arrows); *c* — only few perivascular and infiltrating T cells (some are indicated by arrows); insert: higher magnification of inflammatory T cells (v — vascular lumen; × 400); *d* — *reactive glosis parallels with inflammation and demyelination*

As discussed above, activated autoaggressive T-lymphocytes play an essential role in the induction of the disease and probably also in the propagation of the lesions. At least a high percentage of T-lymphocytes in established EAE lesions carry activation antigens (Lassmann, et al., unpublished). It is thus not surprising that therapy of EAE with monoclonal antibodies against T-cell activation antigens is effective and superior to that with antibodies against total T-cells or the "helper / inducer" T-cell subset [69, 70]. Considerable efforts during the last years have been devoted to the question, how encephalitogenic T-lymphocytes are activated and how their function is regulated within EAE lesions. Since CD4+ T-lymphocytes recognize their antigen only in conjunction with Class II (Ia) histocompatibility antigens, the presence and distribution of Ia-antigens within the CNS and especially in EAE lesion gives clues on possible sites of T-cell activation in the CNS. Indeed, EAE as well as EAN lesions show dramatic expression of Ia-antigens in the tissue [26, 56, 75] (Fig. 2*b, c*). Abundant Ia-reactivity is present on macrophages and lymphocytes in the lesions and on stellate, microglia like cells in the surrounding tissue [50, 87] (Fig. 2 *b,c*). Interestingly, however, the number of Ia-positive cells was found to be considerably higher as compared to the number of cells stained with monocyte markers [15, 75; Lassmann et al., unpublished]. Since astrocytes have been shown to express Ia-antigens in vitro [19, 20], some of the stellate Ia-positive cells in EAE lesions could be astrocytes. Indeed, some authors have described Ia-positive astrocytes in EAE lesions by light microscopic immunocytochemical double staining, using glia fibrillary acidic protein as a marker for astrocytes [28] or by classifying Ia-positive cells by their morphology [80, 81]. Definite identification of Ia-positive astrocytes in light microscopic sections is, however, still problematic, even in double-staining experiments. By ultrastructural immunocytochemistry Ia-positive astrocytes have not been observed in acute EAE [87]; however, some weak Ia-reaction product has been found on astrocyte processes in chronic EAE lesions in rats [39]. No convincing evidence for Ia-expression on oligodendrocytes, myelin and neurons is available.

With recovery from acute EAE Ia expression in the lesions declines parallel with a decrease of "helper / inducer" T-cells and a relative increase of the "suppressor / cytotoxic" T-cell population (Table 1) [39]. It is not clear at present whether antigen-specific suppressor mechanisms downregulate the inflammatory reaction during recovery.

Another important question in EAE pathogenesis is how encephalitogenic T-cells interact with local tissue components and to what extent they by themselves exert tissue damage. In vitro, some demyelinating effects of EAE lymphocytes have been described in myelinated tissue cultures [5, 98]. More recently, demyelinating effects in vitro have also been reported to be induced by MBP reactive T-cell lines [47]. However, in these experiments the T-cell lines contained a considerable fraction of cells not reactive with T-cell markers. Furthermore, demyelination was also observed after exposure of cultures to lines directed against mycobacterial antigens [47]. A specific interaction of MBP reactive T-line cells with autoantigen-presenting astrocytes, leading to lysis of the presenter cells, has been described by Sun and Wekerle [78].

In vivo, T-cell line mediated acute EAE is a nearly exclusively inflammatory disease, with minimal or absent demyelination. In some models of chronic T-cell line mediated EAE demyelination may be present [79, 100]; it is, however, not yet excluded that during the chronic course of the disease additional immune responses are induced by endogenous liberation of antigens in the encephalitic

process. Direct injection of activated MBP reactive T-cell lines into the spinal cerebrospinal fluid did not induce structural lesions or demyelination in the adjacent white matter [91]. Thus, the present evidence suggests that MBP reactive T-cells induce vasculitis without significant direct damage to the CNS parenchyma. Clinical signs of T-cell mediated EAE are rather a consequence of vasculitis (edema, ischemia, etc.) than of immune mediated destruction of CNS tissue components like myelin.

The situation appears to be different in the peripheral nervous system. Contrary to the corresponding CNS disease, T-cell line mediated acute EAN is associated with significant demyelination [30]. This suggests that peripheral myelin sheaths or Schwann cells could be destroyed by a direct cell-mediated mechanism. Interestingly, Schwann cells in contrast to oligodendrocytes are able to express Ia-antigens in vitro and to present MBP to MBP reactive T-cell lines [92]. Thus, it is possible that Schwann cells during T-cell mediated EAN are destroyed in the course of antigen presentation in a similar manner as described in vitro for astrocytes [78].

Other nervous system antigens and other immunological mechanisms involved in the pathogenesis of inflammation in EAE and EAN

Recently, evidence accumulated that MBP is not the only antigen which may induce EAE. Although already the early studies by W a k s m a n [89] suggested that proteolipid protein (PLP) may be encephalitogenic, only recently several models have been developed, which clearly document induction of acute or chronic EAE by highly purified PLP [13, 25, 48, 82, 95, 99]. Similar to MBP-induced EAE, the disease can be transferred to naive recipient animals by lymphocytes [96]. However, contrary to MBP-induced EAE, chronic disease courses are frequent [13, 82, 99], associated with widespread demyelination in the CNS. Another difference to MBP-induced EAE is that antibodies play a role in the pathogenesis of the disease [99], although no clear-cut correlations between clinical course and cellular or humoral immune responses were observed [82, 99].

In addition to PLP, other autoantigens have been described to induce disease by active sensitization. For the central nervous system they include gangliosides [14] and a yet not clearly defined membrane fraction of cerebral endothelial cells [84].

In the peripheral nervous system P2 protein is the main neuritogenic antigen [11], disease induction has also been reported by sensitization with galactocerebroside [65] and gangliosides [53].

In the central nervous system a cellular (T-lymphocyte mediated) immune response seems to be required to start an inflammatory or inflammatory demyelinating desease. At the present moment, there is no convincing evidence available that other immune reactions may start CNS disease in EAE. On the contrary, in the peripheral nervous system in addition to cellular immune reactions autoantibodies, either alone or in combination with immune complexes, may initiate lesions [65, 83].

Demyelination

Cellular immune reactions

Although, as discussed above, there is some demyelination in chronic models of MBP reactive T-cell line mediated EAE, there is overall little evidence for direct antigen-specific cellular immune responses leading to demyelination. This is further supported by ultrastructural immunocytochemical studies on the

interaction of inflammatory cells with tissue components during demyelination in central and peripheral nervous system lesions of EAE [39, 40, 77, 87]. These sudies did not find evidence for an interaction of T-lymphocytes with myelin sheaths during active demyelination. Demyelination was rather accomplished by Ia-positive cells with ultrastructural characteristics of monocytes and macrophages. It is thus not surprising that depletion of macrophages or inhibition of macrophage enzymes can inhibit demyelination in EAE [8, 9].

On the other hand, a T-cell mediated immune response could induce primary demyelination by an antigen unspecific way, in the sense of a bystander reaction [94]. In this situation monocytes and macrophages, activated in the course of the inflammatory reaction, secrete proteolytic enzymes, which could attack myelin sheaths (and other structures) in the surrounding. However, the importance of bystander demyelination, at least in the peripheral nervous system, has recently been questioned [23, 60]. In these experiments significant demyelination was only found, when in addition to an unspecific inflammatory reaction antibodies against epitopes located on the myelin surface were circulating. Similar studies in the central nervous system are lacking. The exact mechanism of myelin damage in EAE induced by transfer of MBP reactive T-cell lines is thus not determined. Furthermore, it is important to note that in these models significant demyelination is only observed after long-standing chronic disease course. It has thus to be considered that in the course of chronic CNS inflammation additional autoimmune responses are induced, which then are responsible for demyelination.

The lack of evidence for a direct, demyelinating T-cell cytotoxicity against MBP is not surprising, as MBP is located on the cytoplasmic side of the myelin membrane [58] and is thus not directly accessible for the immune system. Immune recognition of MBP can only take place when it is either processed and presented like in the peripheral nervous system [92], or when it is liberated into the extracellular space.

Antigen recognition in the CNS and subsequent demyelination is more likely for antigens located on the surface of myelin sheaths like PLP, galactocerebroside (GC) or gangliosides. Although T-cell mediated destruction of oligodendrocytes via galactocerebrosides has been described in vitro [54] and cellular immune reactions against gangliosides have been observed in EAE animals [55], their role in demyelination in active EAE lesions is not yet determined.

Demyelinating antibodies

Demyelinating activity of sera from EAE animals has first been described by Bornstein and Appel [4]. Complement dependent demyelinating activity was found in purified IgG fractions, as well as in IgG depleted fractions. This indicates that also IgM and (or) IgA may be involved in demyelination [24]. A similar demyelinating activity was also found in sera from animals with EAN [97]. More recently, demyelination was also reported to occur after injection of EAE sera into the cerebrospinal fluid [35], into the CNS tissue [93] or into the vitreous of the rabbit eye [7]. Similarly, EAN sera may induce demyelination when injected into peripheral nerves [64].

The immunological mechanisms by which demyelinating antibodies mediate demyelination in vivo are not fully elucidated. In the rabbit eye model, only IgG fractions are effective [24] and complement does not seem to play a role [7]. Demyelination is most likely mediated by an interaction of specific antibodies with macrophages, activated by lymphokines [7]. After injection into the cerebrospinal fluid or directly into peripheral nerves IgG, as well as IgM fractions are effective and the complement is at least partly involved in the demyelinating process [37, 66]. However, again in these models interactions with activated macrophages may play a role in demyelination [37].

An interesting feature of demyelinating antibodies is that they are not directed against the main encephalitogenic antigen, the MBP molecule [72]. This is not surprising, since MBP is located on the cytoplasmic side of the myelin membrane [58] and is thus not directly accessible for the immune system.

The main requirement for a myelin antigen as a target in antibody-mediated demyelination is its localization on the extracellular surface. Up to now, several such antigens have been identified. They include galactocerebroside [16] gangliosides GM1 and GM4 [63, 71], myelin/oligodendroglia glycoprotein (MOG) [41, 43] and possibly myelin-associated glycoprotein (MAG) [42]. Antibodies against epitopes of PLP which are located on the surface of myelin have not yet been tested for demyelinating activity.

Role of demyelinating antibodies in the pathogenesis of EAE

EAE animals develop a humoral immune response against myelin antigens, which is most pronounced in the chronic stage of chronic relapsing EAE [21, 57]. In general, serum-demyelinating activity is highest in chronic disease, when large plaques of demyelination are formed in the CNS [35, 38], and in some models a correlation between myelination-inhibiting serum activity and demyelination in the CNS has been observed [6]. Furthermore, experimental induction of focal inflammatory infiltrates in the vitreous of the rabbit eye in galactocerebroside-sensitized animals induced primary demyelination in the retina [10, 22].

Most of the studies on antibody-mediated demyelination have been performed either with EAE sera or with antisera directed against galactocerebroside. However, in chronic relapsing EAE in guinea pigs, which is at present the model with the most widespread demyelination, the immune response against galactocerebroside is inconsistent and weak, and many demyelinating antisera from these animals do not contain measurable antibody titres against galactocerebroside [71]. More recently, however, we were able to show that demyelinating activity of guinea pig EAE sera correlate well with antibody titres against a novel myelin/oligodendroglia glycoprotein (MOG) [47], an antigen which is located in the surface of myelin sheaths and is a target for antibody-mediated demyelination in vivo [41].

We have used a monoclonal antibody against MOG to modulate the disease course and pathology of T-lymphocyte mediated EAE in rats [46] (Figs. 3*b*, 4): when anti-MOG antibodies are intravenously injected at the onset of EAE, induced by MBP-reactive T-cell lines, the severity of clinical disease is augmented and large confluent plaques of demyelination are formed, which closely resemble those found in models of chronic relapsing EAE. Furthermore, different disease patterns, reflecting the whole spectrum of inflammatory demyelinating diseases, can be reproduced by varying the balance between the number of encephalitogenic T-cells and monoclonal antibodies.

A similar augmentation of demyelination was also found, when anti-galactocerebroside monoclonal antibodies were injected in animals, in which EAE or EAN was induced by MBP or P2 protein reactive T-cell lines.

Summary

Based on recent neuropathological and immunological data, a hypothetical model of EAE and EAN pathogenesis is presented. The discussed pathogenetic mechanisms suggest that the full picture of inflammatory demyelination is induced by a complex interaction of cellular and humoral immune reactions. Furthermore, they show that in principle several different antigens of the CNS

and PNS can mediate autoimmune inflammation or demyelination, respectively. The basic principle, however, consists of two mechanisms:

1) An inflammatory response (vasculitis) which in most (if not all) instances is T-cell mediated and directed against an autoantigen, which is liberated from myelin sheaths, and presented to T-cells by perivascular presenter cells (monocyte / microglia-like cells) and possibly by local cells of the CNS. This process leads to perivascular inflammation with disturbance of the blood-brain barrier and activation of effector cells. Clinical disease in this purely inflammatory disorder results from secondary vascular problems (edema, ischemia) rather than from direct damage to the nerve parenchyma.

2) A demyelinating response, which is specifically directed against antigens or epitopes, which are exposed on the extracellular surface of myelin sheaths. A role of antibodies against myelin surface determinants in the demyelinating process is well documented. There is at present little evidence for direct T-cell cytotoxicity in the pathogenesis of demyelination. Destruction of myelin, opsonized by antibodies is mainly accomplished by monocytes / macrophages, which had been activated previously in the course of the T-cell mediated unflammatory process.

Although at present likewise detailed data are not available for multiple sclerosis lesions, the striking similarities between EAE and human inflammatory demyelinating diseases suggest that comparable mechanisms may be responsible for the human disorders.

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