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RESEARCH ARTICLE

Autoimmunity to Myelin Oligodendrocyte Glycoprotein in Rats Mimics the Spectrum of Multiple Sclerosis Pathology

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Multiple sclerosis is a chronic inflammatory disease characterized by perivenous inflammation and focal destruction of myelin. Many attempts have been undertaken previously to create animal models of chronic inflammatory demyelinating diseases through autoimmunity or virus infection. Recently, however, a new model of myelin oligodendrocyte glycoprotein (MOG) induced autoimmune encephalomyelitis became available, which, in a very standardized and predictable way, leads to chronic (relapsing or progressive) disease and widespread CNS demyelination.

In the present study we actively induced MOG-experimental autoimmune encephalomyelitis (EAE) in different inbred rat strains using different immunization protocols. The pathology found in our models closely reflects the spectrum of multiple sclerosis (MS) pathology: Classical MS as well as variants such as optic neuritis, Devic´s disease and Marburg´s type of acute MS are mimicked in rats immunized with MOG antigen. Furthermore we demonstrate, that by using the proper strain/sensitization regime, subforms of MS such as for instance neuromyelitis optica can be reproducibly induced. Our study further supports the notion, that incidence and expression of the disease in this model, alike the situation in multiple sclerosis, is determined by genetic and environmental factors.

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Introduction

Multiple sclerosis is a chronic inflammatory disease, which is associated with widespread plaque like demyelination in the central nervous system (7). In its classic variant large demyelinated plaques may be found at any site of the brain and spinal cord, although some areas, such as the optic system, periventricular and subcortical white matter, cerebellum, brain stem and spinal cord are sites of predilection (28). In addition to classical form of MS, a number of variants are known, which exhibit specific pathological and/or clinical features. These include optic neuritis, neuromyelitis optica (11) and acute/subacute forms such as Marburg's type of acute MS (21) or Schilder's diffuse sclerosis (39) and may either represent entirely different disease entities or alternatively, different pathological manifestations of a single disease mechanism acting within different genetic environments. A key question for studies, dealing with the pathogenesis of this disease, is whether this wide spectrum of pathological entities can be reproduced in an animal model.

Many attempts have been undertaken to create animal models of chronic inflammatory demyelinating diseases through autoimmunity (23, 36, 40) or virus infection (4, 9, 10, 15, 21, 37). Although these models in essential aspects reflect MS, many differences still exist regarding the nature, size and distribution of lesions in the nervous system. Recently, however, a new model of myelin oligodendrocyte glycoprotein (MOG) induced autoimmune encephalomyelitis became available, which, in a very standardized and predictable way, is characterized by chronic (relapsing or progressive) disease and widespread CNS demyelination (1, 2, 17, 20)

The expression of multiple sclerosis depends upon the interplay of genes (13) with environmental factors (8). Furthermore, genomic screening suggests many different gene loci in association with disease incidence in MS (14, 18, 38). Thus, it can be expected that the whole spectrum of disease can only be mimicked in an animal model, when many different inbred strains and different

Animals			General Neuropathology					Patterns of Lesional Distribution						
strain	dosage	days	disease inc.	n: only I	n:DM	I.I.	DM score	ADLE	SC	CER	NM OPTICA	ON	PV	MIXED
DA	50-100	20-82	101/106 =95.28%	8/101 =7.92%	93/101 =92.08%	0.83	6.63	16/93 =17.21%	30/93 =32.26%	12/93 =12.9%	34/93 =36.56%	0/93 =0%	0/93 =0%	1/93 =1.08%
BN	100	34-70	35/45 =77.78%	0/35 =0%	35/35 =100%	0.31	9.66	1/35 =2.86%	5/35 (a) =14.3%	0/35 =0%	27/35 =77.14%	1/35 =2.86%	0 =0%	1/35 =2.86%
Lew.1AV1	50	40-48	13/14 =92.86%	2/13 =15.38%	11/13 =84.62%	0.1	3.9	2/11 =18.8%	8/11 =72.73%	0/11 =0%	0/11 =0%	0/11 =0%	1/11 (b) =9.09%	0/11 =0%
Lew.1A	50	40	11/11 =100%	0/11 =0%	11/11 =100%	0.14	3.27	4/11 =36.36%	5/11 =45.45%	0/11 =0%	0/11 =0%	0/11 =0%	0/11 =0%	2/11 18.18%
Lew.1N	50	40	6/6 =100%	0/6 =0%	6/6 =100%	.25	7.6	0/6 =0%	6/6 (c) =100%	0/6 =0%	0/6 =0%	0/6 =0%	0/6 =0%	0/6 =0%
all strains			166/182 =91.21%	10/166 =6.02%	156/166 =93.98%	0.6	6.92	23/156 =14.74%	54/156 =34.62%	12/156 =7.69%	61/156 =39.1%	1/156 =0.64%	1/156 =0.64%	4/156 =2.56

(a) including 2 animals with destructive transverse myelitis
(b) this lesion shows also Balo-like morphology
(c) including 3 animals with destructive transverse myelitis

disease inc. = disease incidence, I = inflammation, DM = demyelination, I.I. = inflammatory incidence (as described in methods), DM score = demyelination score (as described in methods), ADLE = acute disseminated leucoencephalomyelitis, SC = spinal type, CER = cerebellar type, NM OPTICA = neuromyelitis optica, ON = optic neuritis, PV = periventricular type, MIXED = mixed plaque distribution

Table 1. Summary of general neuropathology and lesional topography.

disease induction protocols are used. In the present study, by using this approach, we demonstrate that the spectrum of MS pathology is closely reflected in MOG induced experimental autoimmune encephalomyelitis (EAE) and that the genetic background and gender of the animals as well as the mode of sensitization has a highly significant impact on disease expression. Our study further underlines, that, by using the proper strain/sensitization regimes, subforms of multiple sclerosis, such as for instance neuromyelitis optica, can be reproducibly induced in this model.

Materials and Methods

Animals. BN and DA rats were obtained from Charles River (Sulzfeld, FRG), Lew.1A and Lew.1AV1 (19) animals were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, FRG) and Lewis.1N rats (19) were kindly provided by Prof. H. Hedrich (Medizinsche Hochschule Hannover, FRG). Homocygosity of congenic breeding pairs was routinely examined by a microsattelite marker located within the RT1 region. A total of 182 rats were analyzed in this study.

Sensitization procedure. Recombinant protein (rMOG) corresponding to the N-terminal sequence of rat MOG (amino acids 1-125) was expressed in E. coli and purified to homogeneity (1, 2). The purified protein dissolved in 6M urea was dialysed against PBS (100mM, pH7.4) to obtain a precipitated preparation that was stored frozen at -20°C. Alternatively MOG was dialysed against 20 mM sodium acetate buffer (pH 3.0) to obtain a soluble preparation. Complete and incomplete Freunds adjuvant (CFA, IFA) and heat killed Mycobacterium tuberculosis (H37Ra) were purchased from Difco (Detroit, USA). Purified protein derivative (PPD) was purchased from the State Serum Institute, Denmark. Rats were immunized under light ether anaesthesia sub-cutaneously (s.c.) at the base of the tail. 50-100 µg rMOG were emulsified in CFA containing 225 µg of heat killed Mycobacterium tuberculosis (H37Ra) or IFA in a total volume of 100µl, as indicated in the text. Sensitization with precipitated MOG in general leads to a protracted chronic form of the disease. Soluble MOG generally induces a fulminant acute fatal disease (data not shown, Stefferl and Brehm et al., manuscript in preparation). In some animal strains however a very reproducible chronic demyelinating EAE can be induced by sensitization with soluble MOG (Tab. 2), especially when IFA is used as adjuvant (Tab. 2).

Animals were weighed and examined daily for clinical signs of EAE that were scored on the following scale: 0.5, partial loss of tail tone; 1, complete tail atony; 2, hind limb weakness; 3, hind limb paralysis; 4, tetraplegy, moribund state; 5, death. Ataxia was routinely assessed.

Neuropathology. At various time points after sensitization (late stages of disease) animals were sacrificed and perfused via the aorta with 4% paraformaldehyde. Brains, spinal cords and peripheral nerves were dissected and routinely embedded in paraffin. Paraffin sections were stained with hematoxylin/eosin, luxol fast blue, and Bielschowsky silver impregnation to assess inflammation, demyelination, and axonal pathology, respectively.

In adjacent serial sections immunohistochemistry was performed with antibodies against following targets: macrophages/activated microglia (ED1; Serotec, Oxford, UK), T-cells (W3/13; Seralab, Sussex, UK), C9 (32), rat Ig (biotinylated a-rat, Amersham, Buckinghamshire, UK), glial fibrillary acidic protein (= GFAP; Boehringer, Mannheim, Germany), myelin oligodendroglia glycoprotein (= MOG; Dr. Sara Piddlesden, Cardiff, UK, (31)), 2´3´-cyclic nucleotide phosphodiesterase (= CNPase; Affinity Research Products, Ilkeston, UK), proteolipid protein (= PLP; Dept. of Biochemistry, Cardiff, UK). Bound primary antibody was detected with a biotin-avidin technique as previously described in detail (43). Control sections were incubated in the absence of primary antibody or with non-immune rabbit serum.

Quantitative evaluation of inflammation and demyelination and statistical analysis.

Inflammatory index (I.I.). the number of perivascular inflammatory infiltrates was determined for each animal on an average of 15 complete cross sections of spinal cord.

Demyelination score. = sum score of spinal cord + brain demyelination evaluated as follows:

		score
+/-	traces of perivascular or subpial demyelination	1
+	marked perivascular or subpial demyelination	2
++	confluent perivascular or subpial demyelination	4
+++	massive confluent demyelination (e.g. half spinal cord, one optic nerve complete)	6
++++	extensive demyelination (transverse myelitis, half cerebellar white matter or more, both optic nerves complete)	8

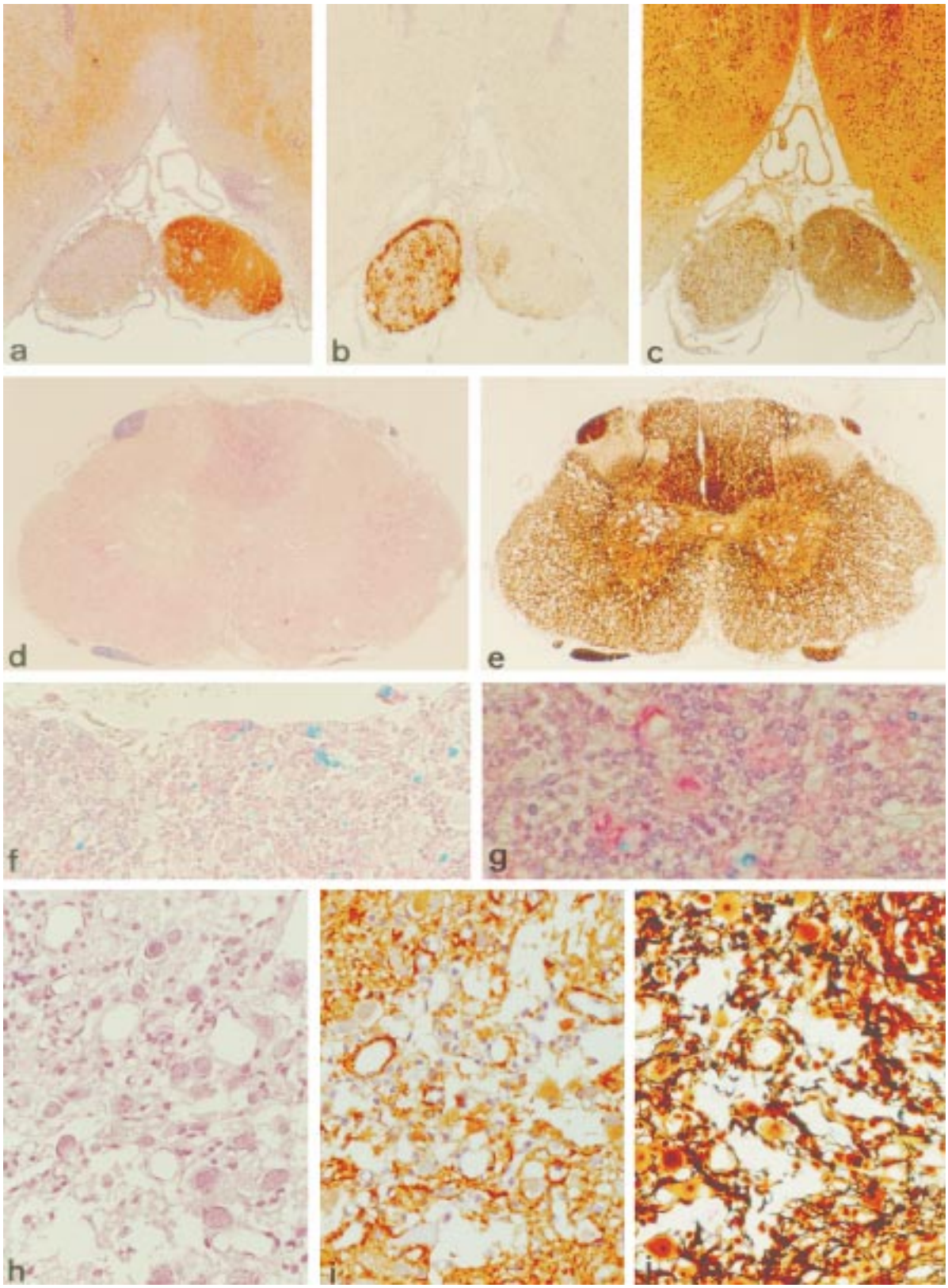
The scores were evaluated in brain and spinal cord separately and then a sum score of brain and spinal cord was obtained. As an example: spinal cord demyelination of 1 + brain demyelination of 4 = sum demyelination 5.

Animals	Immunization Protocol			General Neuropathology					Patterns of Lesional Distribution					
strain	MOG prep.	dosage	adjuvant	days	disease inc.	n: only I	n: DM	I.I.	Inflamm. cells	DM score	ADLE	SC	CER	NM OPTICA	ON	PV	MIXED
1. BN female	soluble	100	IFA	34-70	35/45 =77.78%	0/35 =0%	35/35 =100%	0.31	M/PMN, EOS (b)	9.66	1/35 =2.86%	5/35 14.3%	0/35 =0%	27/35 =77.14%	1/35 =2.86%	0/35 =0%	1/35 =2.86%
2. DA female	soluble	75	IFA	20-40	40/40 =100%	1/40 =2.5%	39/40 =97.5%	0.92	M/PMN, EOS (b)	6.28	2/39 =5.13%	15/39 =38.46%	4/39 =10.26%	17/39 =43.59%	0/39 =0%	0/39 =0%	1/39 =2.56%
3. DA male	soluble	75	IFA	23-40	16/18 =88.89%	5/16 =31.25%	11/16 =68.75%	0.22	M	4.45	3/11 =27.27%	5/11 =45.45%	3/11 =27.27%	0/11 =0%	0/11 =0%	0/11 =0%	0/11 =0%
											P=0.0294 (1 versus 2), P=0.0248 (2 versus 3) compared are patterns of lesional distribution (Chi-square test)						
4. DA female	precipitated	50	CFA++	23-82	16/16 =100%	1/16 =6.25%	15/16 =93.75%	0.75	M/PMN, EOS (b)	8.42	0/15 =0%	3/15 =20%	0/15 =0%	12/15 =80%	0/15 =0%	0/15 =0%	0/15 =0%
5.DA female	soluble	50-100	CFA++	20-60	6/6 =100%	1/6 =16.67%	5/6 =83.33%	1.77	PMN>>M	2.8	5/5 =100%	0/5 =0%	0/5 =0%	0/5 =0%	0/5 =0%	0/5 =0%	0/5 =0%
											P=0.0001 (4 versus 5 and 2 versus 5) compared are patterns of lesional distribution (Chi-square test)						

(a) physicochemical properties (soluble or precipitated antigen) are described in methods
(b) in active demyelinating areas polymorphonuclear cells (PMN), being to a high proportion eosinophils (EOS), may occur

Inflamm. cells = inflammatory cells, further abbreviations see table 1.

Table 2. Influence of strain, gender, physiochemical (a) properties of the antigen and adjuvant used on disease.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Statistical analysis. For statistical evaluation Bonfferoni Chi square test of contingency tables was performed to test whether or not there is a significant difference between different strains/immunization protocols or differences between male and female animals with respect to lesional distribution.

Results

1.Clinical course of disease. Most of the animals selected for this study developed a chronic relapsing disease course (111/156), while smaller numbers followed either a chronic progressive disease (16/156) or developed a stable course with neurological deficit (17/156). Only those animals with predominant or selective involvement of the optic nerve (optic neuritis) failed to develope clinical signs that could be scored. In some animals, with prominent lesions in the brain stem and cerebellum gait ataxia was sometimes the only clinical alteration.

2. General neuropathology. As described before in other models of MOG induced EAE (1, 2, 17, 20) the pathology of chronic MOG induced EAE was characterized by perivenous inflammation, the formation of confluent plaques of demyelination (Fig. 1a, d; 3, 4) with relative sparing of axons (Fig. 1c; 3c) and consecutive glial scar formation (Fig. 2b). This pattern was found in 133 of the 156 animals investigated. The other 23 animals showed pathological alterations of acute disseminated leucoencephalomyelitis characterized by severe perivenous inflammation with little or absent demyelination. Neuropathological changes were restricted to the central nervous system.

Inflammatory infiltrates were present around veins and venules and inflammation was also localized in the meninges with dispersion to the parenchyma adjacent to the pia mater. Inflammation was dominated by mononuclear cells, consisting of W3/13 positive T-cells and ED1 positive macrophages (Fig. 1b) , although in certain strains/protocols the presence of polymorphonuclear cells (PMN cells) or eosinophilic granulocytes was noted (see below; Tab. 2; Fig. 5b, d)(Fig. 6).

The extent of active or inactive demyelination varied. Frequently plaques containing actively demyelinating as well as inactive areas were detected. Sometimes ongoing lesional activity occured side by side with inactive or remyelinating areas.

In comparison to demyelination there was a relative preservation of axons, although axonal density was reduced in all lesions in comparison to the adjacent normal white matter (Fig. 1c; 3c). In addition, a small number of animals exhibited destructive lesions characterized by loss of myelin, axons and astrocytes (Fig. 1d-j). Active demyelination was associated with deposition of immunoglobulin (Ig) and complement component 9 (C9). At the plaque margin Ig and C9 were deposited along degenerating myelin sheaths (Fig. 2g, h) and macrophages in actively demyelinating areas (Fig. 2c) contained Ig (Fig. 2d), C9 (Fig. 2e) and myelin degradation products (Fig. 2f) in their cytoplasm.

Inactive plaques were mostly associated with a profound gliotic scaring (Fig. 2b). In the above mentioned destructive lesions a patchy loss of astrocytes occured besides demyelination and severe axonal pathology.

Remyelinating shadow plaques, characterized by very thin myelin sheaths, were frequently found (Fig. 2a). In the spinal cord at later stages of disease also Schwann cell remyelination was found, in particular in animals with destructive lesions (Fig. 1d, f, g).

3. Plaque growth and orientation of lesions. In contrast to most other models of EAE, where multiple small demyelinated areas are scattered over the entire brain and spinal cord, the pathology of MOG-EAE in rats was dominated by the presence of a restricted number, in some cases single, large focal demyelinated plaques in the CNS (Fig. 3, 4).

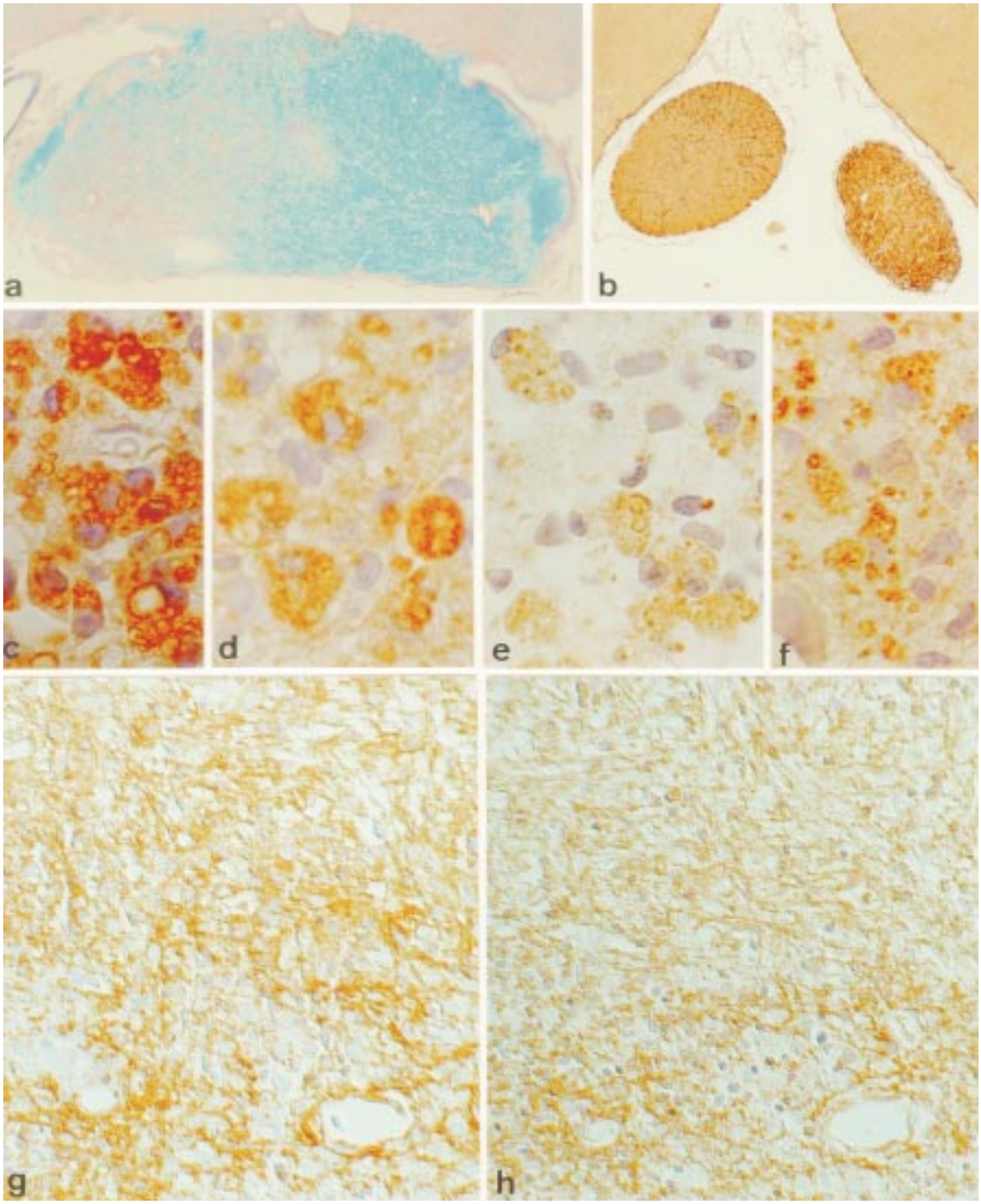
Figure 1. (Opposing page) General neuropathology of chronic myelin oligodendrocyte glycoprotein induced autoimmune encephalomyelitis (I)

a - c. Demyelination with relative sparing of axons.

Serial forebrain sections stained with different markers. One optic nerve is completely demyelinated (**a.** immunocytochemistry for myelin oligodendrocyte glycoprotein) with relative sparing of axons (**c.** Bielschowsky silver impregnation). **b.** Inflammation mainly consists of ED1 positive macrophages (immunocytochemistry for ED1). **a - c.** X 40.

d - j. Destructive plaque.

Serial spinal cord cross sections stained with different markers and stains. Destructive transverse myelitis with loss of myelin (**d.** luxol fast blue myelin stain; X 40), destruction of axons (**e, j.** Bielschowsky silver impregnation; **e.** X 40, **j.** X 300) and astrocytes (**i.** immunocytochemistry for glial fibrillary acidic protein; X 300). Cystic alterations are present in particular in the gray matter (**h.** hematoxylin/eosin; **i.** immunocytochemistry for glial fibrillary acidic protein, **j.** Bielschowsky silver impregnation; X 300) and extensive Schwann cell remyelination, recognizable by the violet myelin rings, is found (**f, g.** luxol fast blue, which stains myelin of the CNS turquoise blue and myelin of the PNS violet; **f.** X 260, **g.** X 860).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Most lesions were centered on small veins and frequently exhibited finger-like extensions at their periphery (Fig. 4f). However, other lesions were orientated towards the cerebrospinal fluid (CSF), including the inner and outer surfaces of brain and spinal cord.

4. Patterns of plaque distribution. The highest incidence of lesions was found in the spinal cord, the optic nerves/tracts and optic chiasms, the cerebellar white matter, the cerebellar peduncles and the brain stem, frequently involving the central portions of cranial nerves. Lesions were also found in the pons, the periventricular white matter, the cortex, fimbria hippocampi, habenulae, septum and thalamus, but these were smaller and occured at a low frequency.

The development of lesions at these predilection sites resulted in patterns of lesional distribution that closely resembled many pathological variants of MS (summary Tab. 1). An inverse relationship between involvement of brain and spinal cord was evident.

Neuromyelitis optica. The most frequent distribution pattern (39,1% overall) was characterized by the combined appearance of major lesions in the optic system (optic tracts, chiasms, nerves) and the spinal cord (Fig. 3d, e, f).

In 19 out of 35 BN rats and 16 out of 34 DA rats, classified as neuromyelitis optica, lesions were restricted to optic nerves and spinal cord. The other cases showed additional minor lesions in the cerebellar white matter and cerebellar peduncles, the medulla oblongata, the pons and in BN rats furthermore in the septum and fimbria hippocampi. Frequently the lesions were symmetric in the optic system. 9 out of 35 BN rats exhibited complete demyelination of both optic nerves and one out of these presented a complete atrophy of both optic nerves combined with severe spinal cord atrophy.

Optic neuritis. One animal showed exclusive affection of the optic nerves (Fig. 3a, b, c).

Spinal type. Besides neuromyelitis optica the next frequent distribution pattern observed was the predominant or exclusive precipitation of lesions in the spinal cord (overall 34,62%; Fig. 4c, d). Minor lesions at other predilection sites were extremely rare in this type. Overall one quarter of the spinal type animals presented a transverse myelitis, which was combined with severe atrophy in few animals.

Cerebellar type. The predominance of demyelination in the cerebellar white matter was found with an incidence of 7,69%. These lesions mostly included half or more than half of the cerebellar white matter and were frequently monofocal (Fig. 4a, b).

Periventricular type. Predominant involvement of the periventricular white matter (Fig. 4e, f) was only found in one animal. The lesion affected the entire periventricular white matter and extended to the adjacent cortex. This lesion was furthermore characterized by concentric layering of myelinated and demyelinated tissue, with similarities to the concentric sclerosis Baló (3) (Fig. 4f).

Acute disseminated leucoencephalomyelitis (ADLE) type. A pathology resembling ADLE was noted (14,74%), characterized by either small rims of perivenous demyelination throughout brain and spinal cord or by subpial demyelination evenly distributed on the surface of spinal cord and brain.

Destructive transverse myelitis. 5 rats developed a destructive transverse myelitis also affecting axons, neurons and astrocytes with cystic alterations throughout the gray and white matter of the spinal cord, combined with pronounced atrophy and extensive Schwann cell remyelination (Fig. 1d-j).

5. Influence of strain, gender, physicochemical properties of the antigen and adjuvant on general neuropathology and lesional topography. As predicted the individual sensitization procedures in different rat strains revealed highly significant differences, when an overall analysis of all groups was performed ($p = 0.0001$). These results clearly demonstrate that the genetic background and gender of the animals as well as environmental factors, determined by the sensitization protocol had a pronounced influence on the development of the disease.

Differences related to strain. Although all rat strains, included in this study, were susceptible for chronic EAE

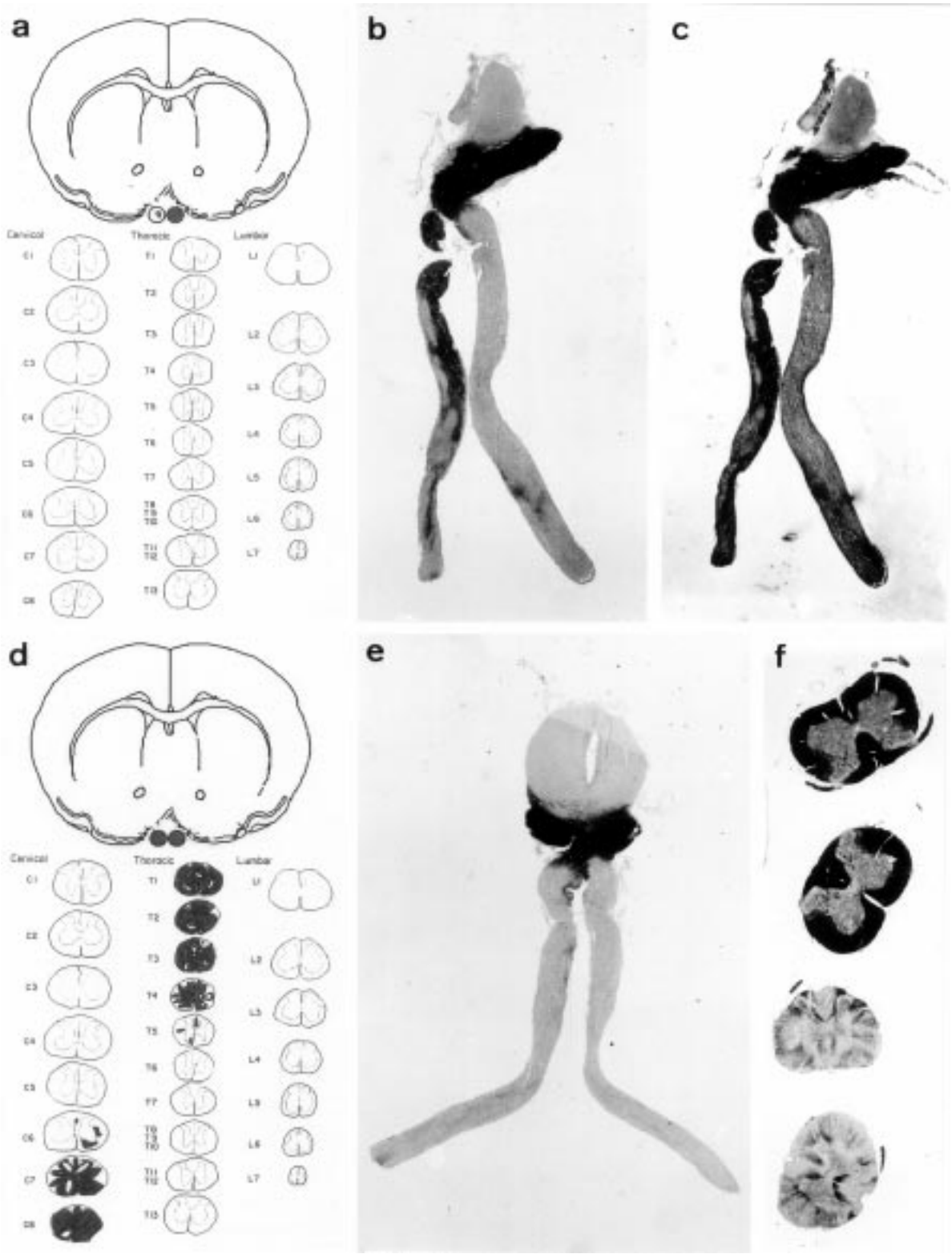
Figure 2. (Opposing page) General neuropathology of chronic myelin oligodendrocyte glycoprotein induced autoimmune encephalomyelitis (II)

a, b. Shadow plaque and glial scar formation.

a. Remyelinating shadow plaque located in the optic chiasm (luxol fast blue myelin stain; X 80). **b.** One optic nerve shows gliotic scaring (immunocytochemistry for glial fibrillary acidic protein; X 70).

c - h. Immunopathology.

Serial brain sections stained with different markers. Active demyelination is associated with deposition of immunoglobulin (Ig) and complement component 9 (C9). At the plaque margin Ig (**g**) and C9 (**h**) are deposited along degenerating myelin sheaths and macrophages (**c**) in actively demyelinating areas contain Ig (**d**), C9 (**e**) and myelin degradation products (**f**) in their cytoplasm. Immunocytochemistry for IgG (**d, g**), C9 (**e, h**), ED1 (**c**) and proteolipid protein (**f**). **g, h.** X 400; **c - f.** X 1000.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induction, profound strain dependent differences in the total extent as well as in the patterns of demyelination were observed (Tab. 1). The extent of demyelination was dependent upon major histocompatibility genes, since animals with the n-haplotype (Lew.1N) showed significantly more demyelination compared to those with the a-haplotype (Lew.1A and Lew.1AV1), conversely the incidence of optic nerve involvement, leading to neuromyelitis optica, was dependent on non-MHC genes, since Lew.1N rats never showed optical involvement in contrast to the MHC identical BN rats (Tab.1). Furthermore the incidence of neuromyelitis optica was much higher in BN compared to DA rats (Tab. 1). This was also evident, when BN and DA rats of the same gender and sensitized with identical protocols were compared with each other (Tab. 2). However, as shown in Table 2 this strain difference could be overcome by changing the sensitization protocol. DA rats, sensitized with a precipitated preparation of MOG showed a predominant expression of neuromyelitis optica comparable to BN rats immunized with a soluble preparation (Tab. 2).

Differences related to gender. Significant differences in optic nerve involvement were found also, when in DA rats male or female rats were immunized with identical protocols (Tab. 2). A high incidence of neuromyelitis optica was found in female rats, whereas non of the male animals showed optic nerve involvement. Furthermore in male rats infiltration by eosinophilic granulocytes never occured.

Differences related to sensitization protocols. Sensitization, using solubilized MOG with complete Freund's adjuvant induced a very aggressive disease that was often acutely lethal. Surviving animals showed massive inflammation with little demyelination. Demyelination scores were massively increased by either replacing CFA with IFA or by using a precipitated MOG preparation for sensitization (Tab. 2).

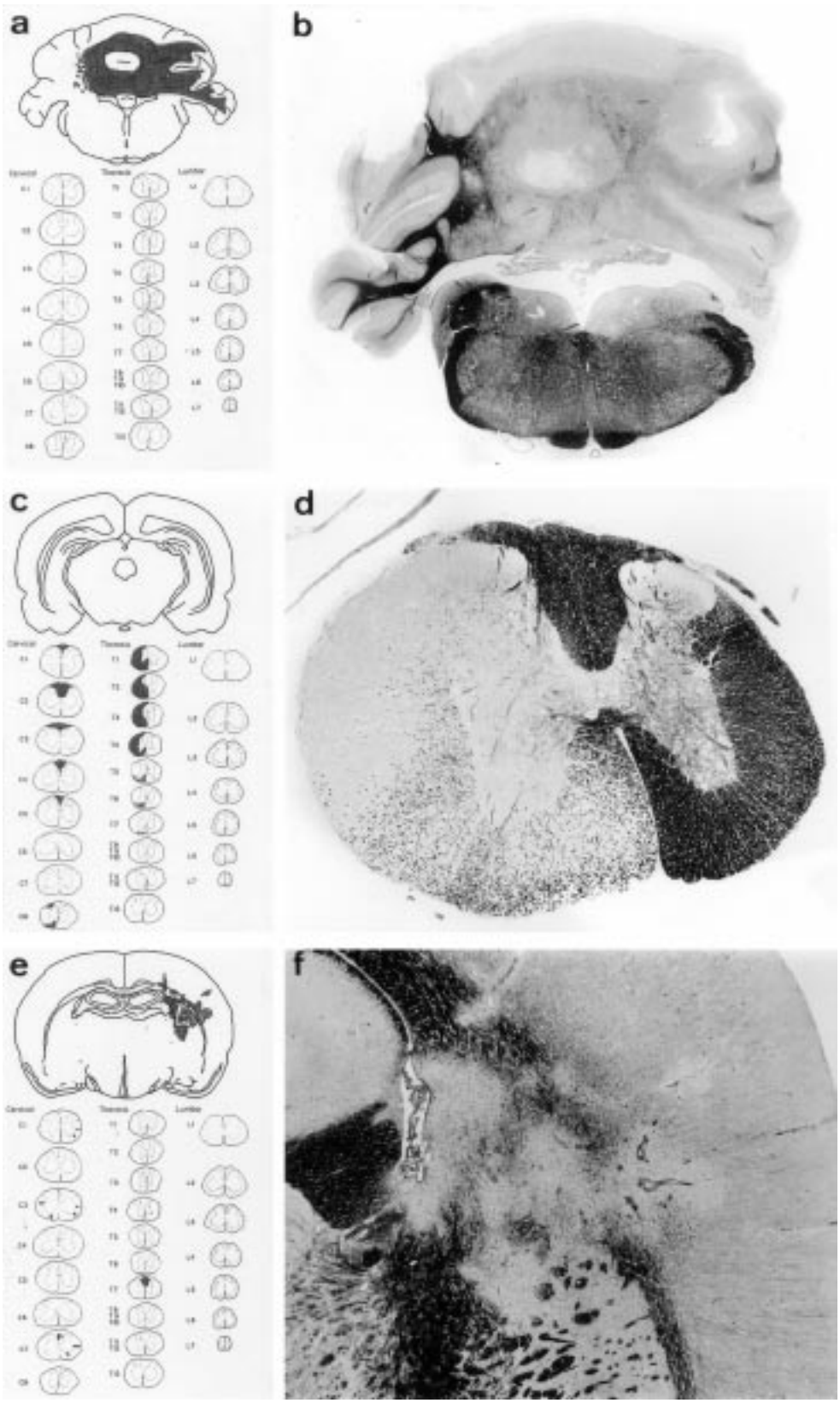
Optic neuritis in MOG-EAE is associated with acute inflammatory infiltrates containing neutrophilic and eosinophilic granulocytes. Most of the acut demyelinating lesions in MOG-induced EAE are characterized by an inflammatory infiltrate composed of mononuclear cells (T-cells and macrophages). However, in animals presenting with neuromyelitis optica and to some extent those animals developing a spinal cord dominated pathology, the inflammatory infiltrate in actively demyelinating lesions contained granulocytes (5,5 - 42% of inflammatory cells, MEAN = 18,18 % +/- SEM 3,24) and in particular eosinophils (37,5 -91,1% of granulocytes, MEAN = 68,42 % +/- SEM 4,4) (Fig. 5). 89,5% (17 / 19) of neuromyelitis optica cases with active areas showed eosinophilic infiltration (Fig. 5), whereas only 32% (8 / 25) of spinal type cases and 3,4% (1 / 27) of the other lesional distribution patterns presented eosinophils in actively demyelinating areas (Fig. 6).

Discussion

It is well established that essential features of multiple sclerosis, the chronic (relapsing) disease course, the inflammatory reaction, plaque like demyelination with axonal sparing and reactive gliosis are well reproduced in models of chronic relapsing autoimmune encephalomyelitis (23, 35). The present study, however, shows that the pathological characteristics of classical MS as well as those of its variants, such as optic neuritis, Devic's disease and Marburg's type of acute MS, are closely mimicked in rats, sensitized with a single CNS antigen such as MOG. This not only establishes the value of MOG-EAE for multiple sclerosis research in general, but also provides new and reproducible model systems, which allow the detailed study of lesional development in neuroanatomically defined areas such as for instance the optic system.

In contrast to most other EAE models which are induced by a single antigen such as myelin basic protein or proteolipid protein, the pathophysiology of MOG induced EAE is more complex. In this model the lesions are induced not only by T-cell mediated immune reactions, but are essentially dependent upon the presence, titers and properties of demyelinating anti-MOG anti-

Figure 3. (Opposing page) Patterns of plaque distribution in chronic myelin oligodendrocyte glycoprotein induced autoimmune encephalomyelitis (I)
Representative cases are shown.
a - c. Optic neuritis.
Serial longitudinal sections of optic nerves and chiasm (**b, c**). **b.** One optic nerve is completely and the other one partially demyelinated. **c.** Demyelination is associated with relative sparing of axons. **b.** luxol fast blue myelin stain, **c.** Bielschowsky silver impregnation; X 10.
d - f. Neuromyelitis optica.
Longitudinal section of optic nerves and chiasm (**e**) and spinal cord cross sections (**f**). Lesions are restricted to optic nerves and spinal cord (**d**). Complete demyelination of both optic nerves (**e**) and focal plaques of demyelination in the thoracic spinal cord (**f**). **e, f.** luxol fast blue myelin stain; X 10.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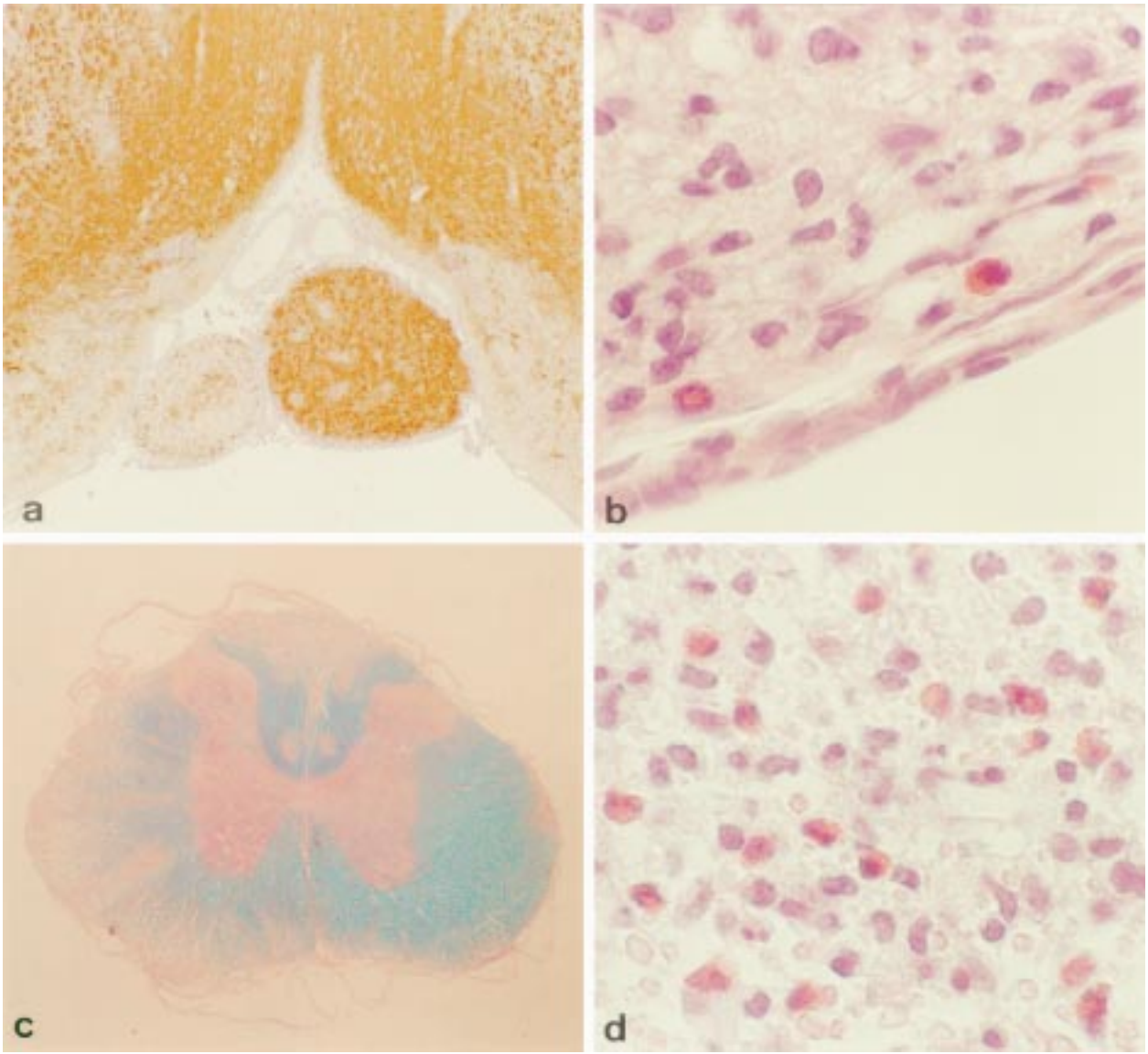

Figure 4. (Opposing page) Patterns of plaque distribution in chronic myelin oligodendrocyte glycoprotein induced autoimmune encephalomyelitis (II) Representative cases are shown.
a, b. Cerebellar type.
Monofocal (**a**) extensive demyelination in the cerebellar white matter (**b.** luxol fast blue myelin stain; X 12).
c, d. Spinal type.
Exclusive precipitation of lesions in the spinal cord (**c**). **d.** Focal plaque of demyelination in the thoracic portions of the spinal cord. Demyelination is associated with edema. Luxol fast blue myelin stain; X 30.
e, f. Periventricular type.
Predominant involvement of the periventricular white matter with additional minor lesions in the spinal cord (**e**). **f.** Large confluent plaque of demyelination in the periventricular white matter adjacent to the lateral ventricle wall with extension to the adjacent cortex. This lesion also shows concentric layering of myelinated and demyelinated tissue. Luxol fast blue myelin stain; X 65.

Figure 5. (Above) Granulocytes/eosinophils in actively demyelinating lesions in myelin oligodendrocyte glycoprotein induced neuromyelitis optica.
Animal presenting with neuromyelitis optica.In actively demyelinating areas of lesions in the optic nerves (**a.** immunocytochemistry for 2´3´-cyclic nucleotide phosphodiesterase; X 50) and spinal cord (**c.** luxol fast blue myelin stain; X 35) inflammatory infiltrates contain granulocytes and in particular eosinophils (**b, d.** hematoxylin/eosin; X 990).

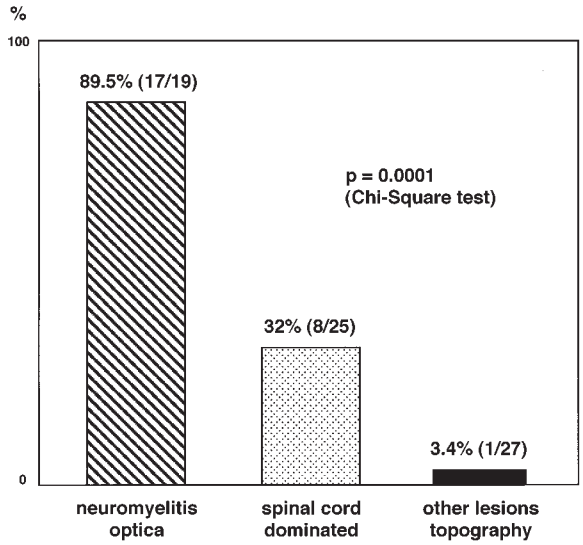

Figure 6. Occurence of eosinophils in cases of MOG-induced EAE with areas of active demyelination (DA and BN in rats).

bodies. While the T-cell mediated autoimmune reaction is responsible for induction of brain inflammation and the recruitment of activated effector cells (5, 26), the anti-MOG antibodies are instrumental in the induction of demyelination (22, 26, 44). It can thus be predicted, that the final outcome of the disease is controlled by genetic factors, regulating both, T- and B-cell responses, as well as by modes of sensitization, which differentially stimulate T- or B-cell responses. In such a situation, a broad spectrum of disease can be expected when animals of different genetic background are sensitized by different protocols. This in fact was observed in our present study.

Many of the differences in disease expression described here can basically be explained by differences in the balance between T-cell mediated and antibody mediated immune reactions. When inflammatory demyelinating lesions are induced in vivo by cotransfer of encephalitogenic T-lymphocytes and demyelinating antibodies, the balance between these two different pathogenetic factors to a large extent determines incidence and structure of the lesions (24). Similarly, in actively induced EAE an immunization procedure, which shifts the balance towards the T-cell arm of the immune systems, such as for instance a genetic background of DA animals, highly permissive for the induction of Th-1 reactions, and the usage of CFA in the sensitization medium will result in an acute/subacute disease, where inflammation is prominent, but demyelination is sparse. On the contrary, BN rats sensitized with MOG in IFA, which mount a very prominent antibody response but are relatively resistent to induction of classical T-cell mediated EAE, develope a chronic disease with very pronounced demyelination. Such a mechanism may also lead to additional recruitment of granulocytes into actively demyelinating lesions, as observed in animals with neuromyelitis optica. However, a chronic demyelinating disease can also be induced in DA rats sensitized with MOG in CFA, when the antigen is applied in a precipitated form, which results in very slow antigenic release from the sensitization site. In this situation it can be expected that the T-cell mediated immune response develops gradually, reaching significant intensities at times, when the antibody response is fully developed. In addition, however, we observed differences, which are difficult to explain purely on the basis of a balance between cellular and humoral immune reactions. The significant differences found between female and male rats of EAE induced in the same strain by identical sensitization procedures clearly demonstrate influences, which involve genes outside the major histocompatibility antigen region. This is further underlined by the differences observed between strains with identical MHC but different non-MHC background genes such as for instance BN and Lew.1N rats. A more detailed study on the effect of MHC and non-MHC genes on the clinical and neuropathological expression of the disease as well as on T-and B-cell mediated immune reactions is currently been undertaken in the model of MOG induced EAE and may provide interesting insights into the genetic control of multiple sclerosis.

The fine-characterization of multiple sclerosis lesions, which is currently performed in several different laboratories (6, 12, 27, 31, 34) suggests a profound heterogeneity of the lesions and indicates that different immunopathogenetic pathways may congrue on a common final mechanisms, which induces the inflammatory demyelinating lesions (25, 41). In one subgroup of multiple sclerosis patients the immunopathology of the lesions suggests that demyelinating antibodies act in concert with a T-cell mediated encephalitogenic T-cell reaction. This is reflected by deposition of immunoglobulin at the interface between degenerating myelin sheaths and macrophages (33), the uptake of myelin fragments into macrophages through coated pits and vesicles (34) and the precipitation of complement (16) and in particular of the complement C9neo antigen (42) on myelin at the site of active demyelination. This pattern of demyelination leads to inflammatory demyelinating plaques, which in all their essential criteria close-

ly mimick those, found in MOG induced EAE (42). Such a pattern of demyelination in multiple sclerosis may also be sometimes associated with granulocyte and in particular eosinophilic granulocyte deposition in active lesions, which can be encountered in cases with fulminant acute MS or neuromyelitis optica (Lassmann unpublished). Interestingly, in a subgroup of patients with fulminant Devic's disease, granulocytes have been described to occur in CSF samples taken during the stage of disease activity (30). This observation is closely reflected in the model of MOG-induced EAE, in which granulocyte and eosinophilic infiltrations in active lesions were mainly observed in animals with affection of optic nerve and spinal cord.

In summary our present study shows the close similarity of MOG-induced EAE in rats with multiple sclerosis. Our studies further support the notion that incidence and expression of the disease in this model, alike the situation in multiple sclerosis, is determined by genetic and environmental factors. MOG-induced EAE in rats therefore qualifies for an excellent model of multiple sclerosis, which will help to unravel genetic factors and immunological mechanisms involved in the pathogenesis of this disease.

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References

1. Adelmann M, Wood J, Benzel I, Fiori P, Lassmann H, Matthieu JM, Gardinier MV, Dornmair K, Linington C (1995) The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. *J Neuroimmunol* 63: 17-27
2. Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, Matthieu JM, Baker D (1994) Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol* 153: 4349-4356
3. Baló J (1928) Encephalitis periaxialis concentrica. *Arch Neurol* 19: 242-264
4. Barac-Latas V, Suchanek G, Breitschopf H., Stuehler A, Wege H, Lassmann H (1997) Patterns of oligodendroglia pathology in corona virus induced subacute demyelinating encephalomyelitis in the Lewis rat. *Glia* 19: 1-12
5. Ben-Nun A, Wekerle H, Cohen IR (1981) The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur J Immunol* 11: 195-199
6. Bonetti B, Raine CS (1997) Multiple sclerosis: oligodendrocytes display cell death-related molecules in situ but do not undergo apoptosis. *Ann Neurol* 42 (1): 74-84
7. Charcot JM (1868) *Histologie de la sclérose en plaque*. Gaz Hôpital (Paris) 41: 554-556
8. Compston A, Sadovnick D (1992) Epidemiology and genetics of multiple sclerosis. *Curr Opin Neurol Neurosurg* 5: 175-181
9. Dal Canto MC, Rabinowitz SG (1981) Experimental models of virus-induced demyelination of the central nervous system. *Ann Neurol* 11: 109-127
10. Dal Canto MC, Melvold RW, Kim BS, Miller SD (1995) Two models of multiple sclerosis: experimental allergic encephalomyelitis (EAE) and Theiler´s murine encephalomyelitis (TMEV) infection. A pathological and immunological comparison. *Microsc Res Tech* 32: 215-229
11. Devic C (1894) Myélite subaigúe compliquée de névrite optique. *Bull Med* 8: 1033
12. D´Souza SD, Bonetti B, Balasingam V, Cashham NR, Barker PA, Troutt AB, Raine CS, Antel JP (1996) Multiple sclerosis: Fas signaling in oligodendrocyte cell death. *J Exp Med* 184: 2361-2370
13. Ebers GC, Bulman DE, Sadovnick AD, Paty DW, Warren S, Hader W, Murray TJ, Seland TP, Duquette P, Grey T et al. (1986) A population-based study of multiple sclerosis in twins. *N Eng J Med* 315: 1638-1642
14. Ebers GC, Kukay K, Bulman DE, Sadovick AD, Rice G, Anderson C, Armstrong H, Cousin K, Ball RB; Hader W et al. (1996) A full genome search in multiple sclerosis. *Nat Genet* 13: 472-476
15. Fazarkerley JK, Buchmeier MJ (1993) Pathogenesis of virus-induced demyelination. *Adv Virus Res* 42: 249-324
16. Gay D, Esiri M (1991) Blood-brain-barrier damage in acute multiple sclerosis plaques. *Brain* 114: 557-572
17. Genain CP, Abel K, Belmar N, Villinger F, Rosenberg DP, Linington C, Raine CS, Hauser SL (1996) Late complications of immune deviation therapy in a nonhuman primate. *Science* 274: 2054-2075
18. Haines JL, Ter-Minassian M, Bazyk A, Gusella JF, Kim DJ, Pericak-Vance MA, Rimmler JB, Haynes CS, Roses AD, Lee A et al. (1996) A genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. The multiple sclerosis genetics group. *Nat Genet* 13: 469-471
19. Hedrich HJ (ed) (1990) *Genetic monitoring of inbred strains of rats.* Gustav Fischer: Stuttgart/New York
20. Johns TG, Kerlero de Rosbo N, Menon KK, Abo S, Gonzales MF, Bernard CCA (1995) Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. *J Immunol* 154: 5536-5541

21. Jordan CA, Friedrich VL Jr, Godfraind C, Cardellechio CB, Holmes KV, Dubois-Dalcqu M (1989) Expression of viral and myelin gene transcripts in a murine CNS demyelinating disease caused by a corona virus. *Glia* 2: 318-329
22. Kerlero de Rosbo N, Honegger P, Lassmann H, Matthieu JM (1990) Demyelination induced in aggregating brain cell cultures by a monoclonal antibody against myelin/oligodendroglia glycoprotein. *J Neurochem* 55: 583-587
23. Lassmann H (1983) Comparative neuropathology of chronic experimental allergic encephalomyelitis and multiple sclerosis. *Schriftenr Neurol* 25: 1-135
24. Lassmann H, Brunner C, Bradl M, Linington C (1988) Experimental allergic encephalomyelitis: The balance between encephalitogenic T lymphocytes and demyelinating antibodies determines size and structure of demyelinating lesions. *Acta Neuropathol* 75: 566-576
25. Lassmann H (1998) Immunopathology of multiple sclerosis. *J Neuroimmunol* in press
26. Linington C, Bradl M, Lassmann H, Brunner C, Vass K (1988) Augmentation of demyelination in rat acute encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am J Pathol* 130: 443-454
27. Lucchinetti CF, Brück W, Rodriguez M, Lassmann H (1996) Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. *Brain Pathol* 6: 259-274
28. Lumsden CE (1970) The neuropathology of multiple sclerosis. In *Handbook of Clinical Neurology*, Vinken PI, Bruyn GW (eds), vol 9, pp.217-309, Elsevier: New York
29. Marburg O (1906) Die sogenannte “akute Multiple Sklerose”. *Jahrb Psychiatrie* 27: 211-312
30. O´Riordan JI, Gallagher HL, Thompson AJ, Howard RS, Kingsley DP, Thompson EJ, McDonald WI, Miller DH (1996) Clinical, CSF, and MRI findings in Devic´s neuromyelitis optica. *J Neurol Neurosurg Psychiatry* 60 (4): 382-387
31. Ozawa K, Suchanek G, Breitschopf H, Brück W, Budka H, Jellinger K, Lassmann H (1994) Patterns of oligodendroglia pathology in multiple sclerosis. *Brain* 117: 1311-1322
32. Piddlesden SJ, Storch MK, Hibbs H, Freemann AM, Lassmann H, Morgan BP (1994) Soluble recombinant complement receptor 1 inhibits inflammation and demyelination in antibody-mediated allergic encephalomyelitis. *J Immunol* 152: 5477-5484
33. Prineas JW, Graham JS (1981) Multiple sclerosis: capping of surface immunoglobulin G on macrophages engaged in myelin break-down. *Ann Neurol* 10: 149-158
34. Prineas JW (1985) The neuropathology of multiple sclerosis. In *Handbook of Clinical Neurology*, JC Koetsier (ed), Vol 47, pp 337-395, Elsevier Science
35. Raine CS (1985) Experimental allergic encephalomyelitis and experimental neuritis. In *Handbook of Clinical Neurology*, JC Koetsier (ed), Vol 47, pp 429-466, Elsevier Science
36. Rivers TM, Sprunt DH, Berry GP (1933) Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J Exp Med* 58: 39-53
37. Rodriguez M, Prayoonwiwat N, Howe C, Sanborn K (1994) Proteolipid protein gene expression in demyelination and remyelination of the central nervous system: a model for multiple sclerosis. *J Neuropath Exp Neurol* 53: 136-143
38. Sawcer S, Jones HB, Feakes R, Gray J, Smaldon N, Chataway J, Robertson N, Clayton D, Goodfellow PN, Compston A (1996) A genomic screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat Genet* 13: 464-468
39. Schilder P (1912) Zur Kenntnis der sogenannten diffusen Sklerose (über Encephalitis periaxialis diffusa). *Z Gesamt Neurol Psychiatr* 10: 1-60
40. Stone SH, Lerner EM (1965) Chronic disseminated allergic encephalomyelitis in guinea pigs. *Ann NY Acad Sci* 122: 227-241
41. Storch M, Lassman H (1997) Pathology and pathogenesis of demyelinating diseases. *Curr Opin Neurol* 10: 186-192
42. Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H (1998) Multiple sclerosis: in situ evidence for antibody- and complement mediated demyelination. *Ann Neurol* 43: 465-471
43. Vass K, Lassmann H, Wekerle H, Wisniewski HM (1986) The distribution of Ia antigen in the lesions of rat acute experimental allergic encephalomyelitis. *Acta Neuropathol* 70: 149-160
44. Vass K, Heininger K, Schäfer B, Linington C, Lassmann H (1992) Interferon gamma potentiates antibody mediated demyelination in vivo. *Ann Neurol* 32: 198-206