Heterogeneity of Multiple Sclerosis Lesions: Implications for the Pathogenesis of Demyelination

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Multiple sclerosis (MS) is a disease with profound heterogeneity in clinical course, neuroradiological appearance of the lesions, involvement of susceptibility gene loci, and response to therapy. These features are supported by experimental evidence, which demonstrates that fundamentally different processes, such as autoimmunity or virus infection, may induce MS-like inflammatory demyelinating plaques and suggest that MS may be a disease with heterogeneous pathogenetic mechanisms. From a large pathology sample of MS, collected in three international centers, we selected 51 biopsies and 32 autopsies that contained actively demyelinating lesions defined by stringent criteria. The pathology of the lesions was analyzed using a broad spectrum of immunological and neurobiological markers. Four fundamentally different patterns of demyelination were found, defined on the basis of myelin protein loss, the geography and extension of plaques, the patterns of oligodendrocyte destruction, and the immunopathological evidence of complement activation. Two patterns (I and II) showed close similarities to T-cell–mediated or T-cell plus antibody–mediated autoimmune encephalomyelitis, respectively. The other patterns (III and IV) were highly suggestive of a primary oligodendrocyte dystrophy, reminiscent of virus- or toxin-induced demyelination rather than autoimmunity. At a given time point of the disease—as reflected in autopsy cases—the patterns of demyelination were heterogeneous between patients, but were homogenous within multiple active lesions from the same patient. This pathogenetic heterogeneity of plaques from different MS patients may have fundamental implications for the diagnosis and therapy of this disease.

Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707–717

Much emphasis has been placed on identifying the single pathogenetic mechanism in multiple sclerosis (MS) that would allow the development of therapeutic strategies applicable to all patients. However, recent genetic studies indicate multiple genetic factors with moderate individual effects may contribute to disease susceptibility, suggesting a potential multifactorial etiology.[1–3] In addition, the clinical course of MS both within and between patients is highly variable, including the variable response to immunomodulatory therapies.[4,5] Heterogeneity in lesional profiles has also been reported in magnetic resonance imaging and magnetic resonance spectroscopy studies of MS patients.[6]

Pathological analysis of actively demyelinating multiple sclerosis lesions—so far performed on very small case numbers in the individual reports—revealed many different structural and immunological features, suggesting multiple possible mechanisms in this process. These include involvement of activated macrophages or microglia,[7–9] cytotoxic cytokines,[10,11] reactive oxygen or nitrogen species,[12,13] or specific demyelinating antibodies and activated complement components.[14–17] In other cases, signs of oligodendrocyte dystrophy were noted, reflected by impaired expression of certain myelin proteins, such as myelin-associated glycoprotein (MAG) or dystrophic changes in most distal oligodendrocyte processes.[18–21] The pathological features of these lesions are so different from each other that a pathogenetic heterogeneity of demyelination in different MS patients has seriously to be considered.

In this study, we analyzed the patterns of demyelination in a large series of actively demyelinating lesions from MS patients. Our studies suggest that the target of injury (myelin or oligodendrocytes) and the mechanisms of demyelination are distinctly different in subgroups of the disease and at different stages of disease development.

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Received Nov 17, 1999, and in revised form Feb 21, 2000. Accepted for publication Feb 22, 2000.

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Materials and Methods

This study was performed on archival material of 51 biopsies and 32 autopsies with histologically proven active MS. Material was collected in the Department of Neuropathology at the Mayo Clinic, Rochester, MN (n = 32), the Neuropathological Institute at the University of Göttingen, Germany (n = 18), and the Institute of Brain Research at the University of Vienna, Austria (n = 33). The inclusion criteria for cases in this study were as follows: (1) tissue diagnosis of inflammatory demyelination confirmed by a neuropathologist (W.B., J.P., B.S., H.L.) to be consistent with MS, with the presence of confluent plaques in active stage of myelin destruction, relative sparing of axons and glial scaring; cases of acute disseminated (perivenous) leukoencephalomyelitis were excluded; (2) no clinical, radiological, serological, or pathological evidence of neoplasm, infection, vascular, or nondemyelinating inflammatory etiology; and (3) no structural or immunocytochemical evidence for an inflammatory demyelinating disease induced by known virus infections, such as subacute sclerosing panencephalitis or progressive multifocal leukoencephalopathy. Table 1 summarizes the mean age, sex ratio, and number of lesions analyzed.

Detailed clinical histories were available on 73 cases, with 32 cases clinically evaluated by a Mayo neurologist. The mean duration of clinical course prior to autopsy was 39 months (standard deviation [SD], 15 months; median, 3 months; range, 0.25–384 months), whereas the mean interval between first symptom and biopsy was 9 months (SD, 3 months; median, 1.75 months; range, 0.3–120 months). Total follow-up on biopsy cases was 37 months (SD, 79 months; median, 12 months; range, 1–424 months).

Neuropathological Techniques and Immunocytochemistry

All cases underwent detailed neuropathological examination, including assessment of 1 to 6 tissue blocks per biopsy case and up to 20 blocks per autopsy case. All tissue blocks were classified with regard to lesional activity.[9] Paraffin-embedded 5-μm sections were stained with hematoxylin-eosin, Luxol fast blue myelin stain, periodic acid–Schiff (PAS) reaction, and Bielschowsky's silver impregnation axonal stain.

Immunocytochemistry

Immunocytochemistry was performed without modification on paraffin sections using an avidin-biotin or an alkaline-phosphatase/anti–alkaline phosphatase technique as described in detail previously[22] with the antibodies listed in Table 2. The primary antibodies were omitted in controls. In situ hybridization was performed using digoxigenin-labeled riboprobes specific for proteolipoprotein (PLP). The source and specificity of the probes, the labeling techniques, and the methods of in situ hybridization have been described in detail previously.[23] To visualize degenerating cells in tissue sections, DNA fragmentation within cell nuclei was determined with the method of in situ tailing.[24] The sections were then processed for immunocytochemistry with antibodies against myelin oligodendrocyte glycoprotein (MOG), glial fibrillary acidic protein (GFAP), T cells, and macrophages as described above. Apoptotic oligodendrocytes were defined by nuclear condensation and fragmentation in cells stained by either MOG or cyclic nucleotide phosphodiesterase (CNPase) antibodies.

Quantitative Evaluation of Labeled Cells

The number of cells stained by immunocytochemistry, in situ hybridization, or in situ tailing per square unit of tissue was determined on serial sections. Appropriate areas of the sections were selected according to the demyelinating activity within the lesions. As a basis for quantitative evaluation, a topographical map was established for each lesion according to defined areas, including periplaque white matter, zone of active myelin destruction, inactive plaque center, and regions of remyelination. The number of cells were determined in each of these distinct plaque areas in 10 standardized microscopic fields of 25,000 μm^2, each defined by an ocular morphometric grid. Values in Table 3 represent the number of cells per square millimeter.

Statistical Analysis

Nonparametric group tests were used to compare groups. All values are expressed as means ± standard error of the mean. Differences in clinical expression of disease between patterns of demyelination were analyzed by χ^2 test.

Results

General Neuropathology

All biopsy and autopsy cases fulfilled the neuropathological diagnostic criteria of inflammatory demyelinating disease consistent with MS (Fig 1a, b). All cases contained at least one lesion in the active stage of demyelination. Lesions with active demyelination were characterized by reduced density of myelinated fibers and irregular ensheathment of axons. These lesions were infiltrated by macrophages and activated microglia (see Fig 1f, k; Fig 2b, d), containing intracytoplasmic granules of myelin debris that were reactive for MOG, myelin basic protein (MBP), and PLP, and expressed the early activation markers MRP 14 and

Table 1. Actively Demyelinating Multiple Sclerosis Cases and Lesions Incorporated in This Study

	No. of Cases	Mean Age (yr)	F/M Ratio	No. of Active Lesions	No. of Total Lesions
Autopsy	32	39.65 (range, 19–69)	22/10	173	325
Biopsy	51 (49)[a]	38.25 (range, 10–69)	29/22	62	71

[a]One patient underwent sequential brain biopsies, and another patient had an autopsy after the initial biopsy.

Table 2. Antibodies Used for Immunocytochemistry

Antigen	Antibody Type	Target	Source
CD3	mAb	T cells	Dako, Glostrup, Denmark
L-26	mAb	B cells	
human IgG	mAb	B cells, plasma cells	
KiM1P	mAb	Monocytes, microglia	Radzun et al[42]
CD68	mAb	Macrophages	Dako, Glostrup, Denmark
27E10	mAb	Activated macrophages	BMA Biomedicals, Augst, Switzerland
MRP 14	mAb	Activated macrophages	
C9neo	mAb	Activated terminal compl	Storch et al[15]
C9neo	polyAb	Activated terminal compl	
MBP	mAb	Myelin	Boehringer, Mannheim, Germany
GFAP	mAB	Astrocytes	
PLP	mAb	Myelin	Serotec, Oxford, UK
MAG B11F7	mAb	Myelin	Doberson et al[43]
MAG D7E10	mAb	Myelin	
MAG	polyAb	Myelin	Matthieu et al[44]
MOG 8-18C5	mAb	Myelin/oligodendrocytes	Piddlesden et al[45]
MOG Y10	mAb	Myelin/oligodendrocytes	
MOG Z12	mAb	Myelin/oligodendrocytes	
CNPase	mAB	Myelin/oligodendrocytes	Affinity Res Prod, UK

mAb = monoclonal antibody; polyAb = polyclonal antibody; compl = complement.

Table 3. Structural and Immunological Features of Different Patterns of Active Multiple Sclerosis Lesions

Feature	Pattern I	Pattern II	Pattern III	Pattern IV
Inflammation				
Composition of Infiltrates				
CD3 T cells	197 ± 68	133 ± 18	145 ± 23	134 ± 71
Plasma cells	5.9 ± 1.9	9.3 ± 2.1	5.4 ± 1.6	3.8
Macrophages	1,158 ± 105	931 ± 71	842 ± 91	1,650 ± 30
C9neo	−	++	−	−
Demyelination				
Perivenous pattern	+	+	−	±
Lesion edge	Sharp	Sharp	Ill-defined	Sharp
Concentric pattern	0/10	0/45	8/25	0/3
Oligodendrocytes				
#OG in DM	295 ± 73	249 ± 30	51 ± 24	55 ± 55
DNA frag in OG	±	±	++APO	++PPWM
OG apoptosis	−	−	14–37%	−
Myelin protein loss	Even	Even	MAG ≫ Others	Even
Remyelination				
Shadow plaques	++	++	−	−

Values given in the table represent cells per square millimeter.

#OG = density of oligodendrocytes in inactive demyelinated plaque center; DNA frag in OG = oligodendrocytes showing nuclear DNA fragmentation; OG apoptosis = apoptotic cell death of oligodendrocytes in active lesional areas; MAG = myelin-associated glycoprotein; PPWM = DNA fragmentation in oligodendrocytes in the periplaque white matter.

27E10.[9] The number of active lesions per case ranged from 1 (mainly in cases in the late chronic stage of the disease or from biopsy cases) to up to 35 (in some cases of Marburg's type of acute MS).

Different Pathological Features of Actively Demyelinating Lesions in MS

Despite the fact that lesions included in this study were defined by the same stringent criteria for demyelinating activity, we found a profound heterogeneity in their immunopathological appearance. Although all lesions had in common inflammatory infiltrates by T lymphocytes and macrophages, they segregated into four different patterns, based on the distribution of myelin protein loss, the plaque geography and extension, the pattern of oligodendrocyte destruction, and the immunopathological evidence of immunoglobulin (Ig) and activated complement deposits. Based on these essential

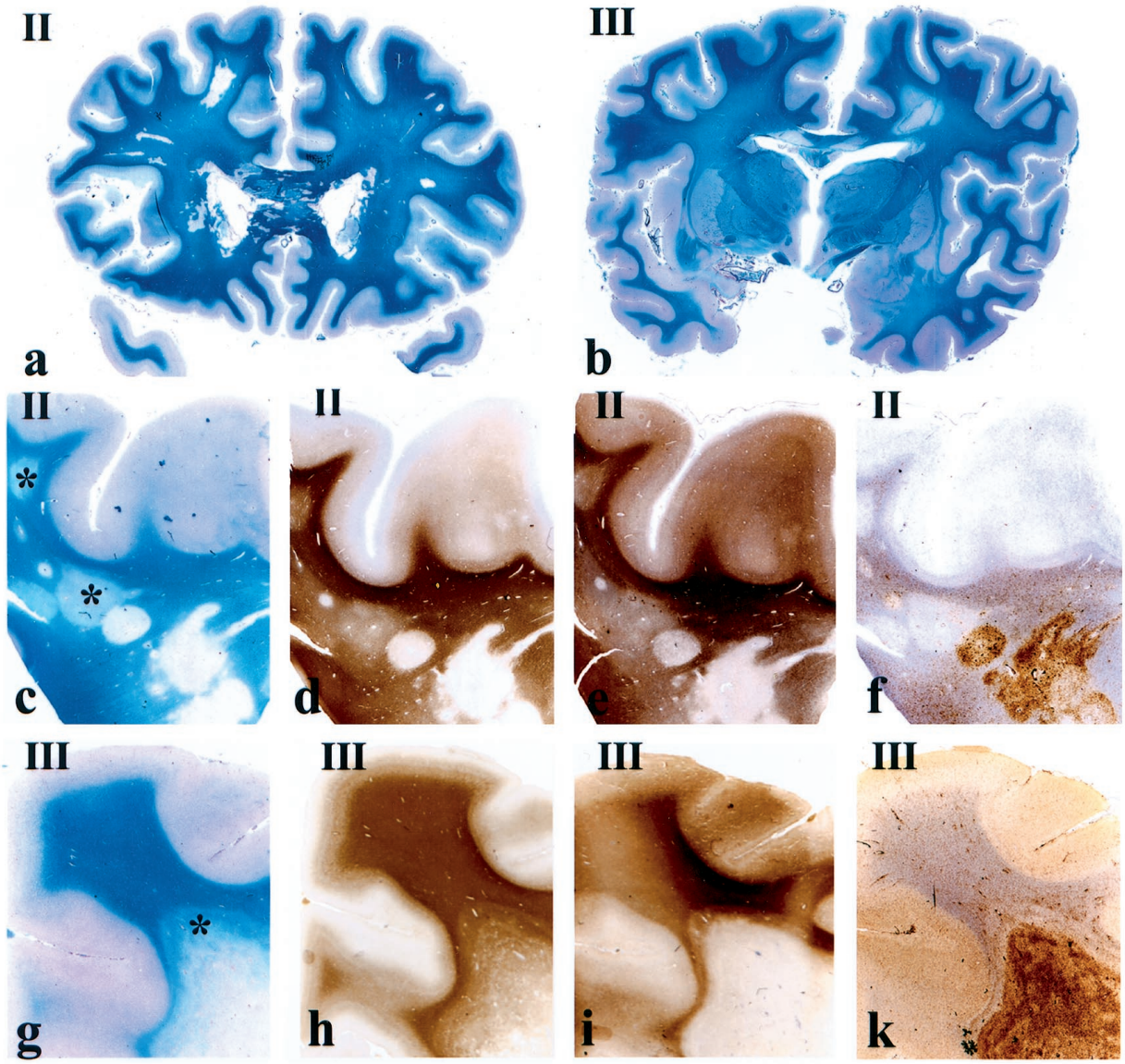

Fig 1. a. Acute multiple sclerosis (MS; autopsy, female, aged 51 years, 7 months' disease duration); pattern II; perivenous, confluent pattern of demyelination, accentuated in the periventricular regions (original magnification, ×2). b. Acute MS (autopsy, male, aged 35 years, 6 weeks' disease duration); pattern III; large demyelinated plaques in the deep white matter and the corpus callosum, showing concentric layering of demyelination (original magnification ×2). c–f. Chronic relapsing-remitting MS with actively demyelinating lesions (autopsy, female, aged 34 years, 156 months' disease duration); pattern II; multiple plaques in the subcortical white matter with perivenous extensions, some of them with pronounced macrophage infiltration, in particular in the perivenous extensions. Other plaques with pale blue staining in Luxol fast blue (LFB) presenting as classical remyelinated shadow plaques (asterisks). *The latter plaques are also free of macrophage infiltration. (c, LFB; d, MOG; e, MAG; f, CD68; original magnification, ×3). g–k. Acute MS (autopsy, male, aged 45 years, 3 weeks' disease duration); pattern III; subcortical plaque with ill-defined borders and some concentric layering of demyelinated tissue. In comparison with LFB and MOG, there is a significantly greater loss of MAG in the lesions* (asterisks). *(g, LFB; h, MOG; i, MAG; k, CD68; original magnification, ×3.)*

features, which are summarized in Table 3, the cases appeared to fall into one of the four patterns.

PATTERNS I AND II. These two patterns of demyelination shared several similar features. Active demyelination was associated with a T-lymphocyte– and macrophage-dominated inflammation. The major feature distinguishing pattern I from pattern II lesions was the prominent deposition of Igs (mainly IgG) and complement C9neo antigen at sites of active myelin destruction, found exclusively in pattern II lesions (see Fig 2e). A diffuse Ig reactivity in the tissue and astrocyte

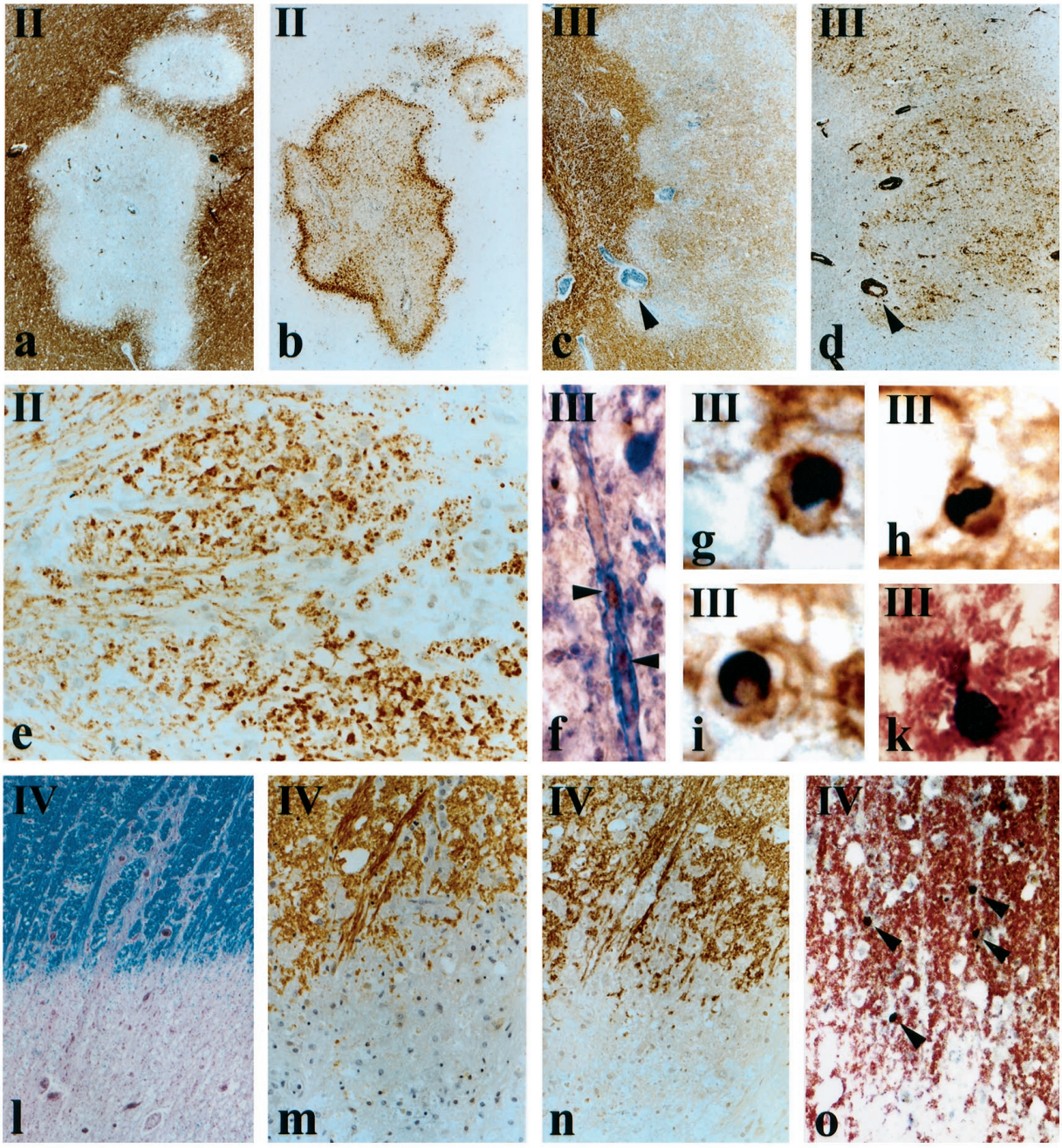

Fig 2. a and b. Acute multiple sclerosis (MS; autopsy, male, aged 52 years, 6 weeks' disease duration); pattern II; perivenous confluent pattern 2 lesion with macrophage rim at active border. (a, MOG; b, CD68; original magnification, ×4). c and d. Acute MS (autopsy, male, aged 45 years, 3 weeks' disease duration); pattern III; demyelinating lesion with ill-defined borders. The perivenous areas around inflamed vessels show lack of macrophage infiltration and demyelination (arrows). *(c, MOG; d, CD68; original magnification ×10.) e. Chronic relapsing-remitting MS (autopsy, female, aged 47 years, 384 months' disease duration); pattern II lesion with massive C9neo deposition at the actively demyelinating border. C9neo antigen (red) is present on myelinated fibers and in macrophages. (Original magnification ×700.) f. Acute MS (autopsy, male, aged 45 years, 3 weeks' disease duration); pattern III lesion; double staining for PLP (blue) and MAG (brown); actively demyelinating area. Partly demyelinated fiber still contains immunoreactivity for PLP; MAG staining is reduced to spot-like reactivity in periaxonal areas* (arrows). *(Original magnification, ×1,000.) g–k. Acute MS (g, i: biopsy, female, aged 22 years, 2 weeks' disease duration; h, k: autopsy, male, aged 35 years, 6 weeks' disease duration); apoptotic oligodendrocytes in pattern III lesions. (g–i, CNPase-positive oligodendrocytes with typical nuclear alterations of apoptosis; k, double staining for in situ tailing [DNA fragmentation; black] and CNPase [red]; oligodendrocyte with labeled nucleus; original magnification, ×2,000.) l–o. Primary progressive MS (autopsy, female, aged 37 years, 24 months' disease duration); pattern IV lesion. Myelin antigens are similarly distributed in the lesions, and DNA fragmentation of oligodendrocytes is seen in the periplaque white matter (o). (l, LFB; m, MOG; n, MAG; o, double staining of in situ tailing [DNA fragmentation] and CNPase [myelin and oligodendrocytes]; original magnification: l–n, ×200; o, ×400.)*

cytoplasm was found throughout the lesions regardless of the patterns of demyelination, reflecting blood-brain barrier damage. However, pattern II was distinguished from the other lesional patterns by a pronounced and accentuated Ig reactivity, associated with degenerating myelin at the active plaque edge and profound Ig reactivity of myelin degradation products within macrophages. Sections stained for C9neo antigen lacked this diffuse background staining, and the immunoreactivity was specifically associated with degenerating myelin and myelin degradation products (see Fig 2e). Pattern I and II demyelinated plaques were typically centered on small veins and venules and showed sharply demarcated edges with perivenous extensions (see Fig 1a, c–f and Fig 2a, b). Loss of all myelin proteins from damaged myelin sheaths appeared to occur simultaneously.

When active and inactive lesions were present side by side in autopsy cases of patients with these patterns of active myelin destruction, a variable loss of oligodendrocytes at the active lesional border with reappearance of high numbers of oligodendrocytes in the inactive plaque center was observed, as described in detail in a single case previously.[15] This was associated with a high incidence of remyelinated shadow plaques (see Table 3 and Fig 1c–f), defined as sharply demarcated plaques with uniformly thin myelin sheaths throughout the whole lesion.

PATTERN III. These lesions also contained an inflammatory infiltrate, composed mainly of T lymphocytes, with macrophages and activated microglia (see Fig 1g–k). Deposition of Ig and complement was absent. In contrast to pattern I and II lesions, demyelination in pattern III lesions was not centered by veins and venules. Instead, preservation of a rim of myelin was frequently observed around inflamed vessels within the demyelinated plaque (see Fig 2c, d). The borders of active lesions were ill defined, showing diffuse spread into the surrounding white matter. In addition, in 8 of the 22 cases containing this lesional pattern, concentric balo-like alternating rims of demyelinated and myelinated tissue were found at the periphery of the lesions (see Fig 1b, g–k). The striking feature in these cases was a preferential loss of MAG, while other myelin proteins (PLP, MBP, CNP) were still present within the partly damaged myelin sheaths (see Fig 1g–k). Loss of MAG was associated with alterations in the periaxonal MAG immunoreactivity, showing irregular punctuate staining between axons and myelin (see Fig 2f). At sites of preferential MAG loss, a range of 14 to 37% of MOG^+ or $CNPase^+$ oligodendrocytes revealed nuclear condensation and fragmentation, typical for apoptosis (see Fig 2g–i). These cells were also stained by in situ tailing for DNA fragmentation (see Fig 2k).

This pattern of demyelination typically demonstrated a pronounced loss of oligodendrocytes at the active plaque border, sometimes extending into the apparently normal periplaque white matter. The inactive center was almost completely devoid of oligodendrocytes, and remyelinated shadow plaques were absent (see Table 3).

PATTERN IV. The inflammatory infiltrates in these lesions were also dominated by T lymphocytes and macrophages. Deposition of Igs and complement C9neo antigen was absent. Demyelination was associated with oligodendrocyte death in a small rim of periplaque white matter, adjacent to the zone of active myelin destruction. Oligodendrocyte death was revealed by DNA fragmentation; however, the cells did not show the morphological features of apoptosis (see Fig 2o). This process generally was associated with a sharply demarcated plaque of demyelination with radial expansion of the lesion (see Fig 2l). The comparative immunocytochemistry for myelin proteins (MAG, MBP, PLP, CNP, and MOG) revealed no differences in staining patterns (see Fig 2m, n). A nearly complete loss of oligodendrocytes in active as well as inactive areas of these lesions was associated with lack of remyelinated shadow plaques.

Multiple Active Plaques in Individual Brain Autopsies Reveal Identical Morphological and Immunopathological Alterations

This was demonstrated in 27 cases in which multiple active plaques were identified in the autopsy tissue (Table 4). We found no evidence for intraindividual heterogeneity, since the morphology of the plaques remained the same within each patient. Therefore, further calculations on the frequency of the above-described patterns are based on cases rather than on lesions.

Overall Frequency of Different Patterns of Demyelination in Relation to Clinical Disease at Biopsy or Autopsy

The frequency of the above-described patterns of demyelination in our sample of MS cases is summarized in Figure 3. The pattern most frequently observed in the overall sample of MS cases was pattern II, followed

Table 4. Distribution of Demyelinating Patterns within Multiple Plaques of Autopsy Cases

No. Cases	No. Active Lesions	Pattern I	Pattern II	Pattern III	Pattern IV
1	3	3/3	0/0	0/0	0/0
16	115	0/0	115/115	0/0	0/0
7	43	0/0	0/0	43/43	0/0
3	9	0/0	0/0	0/0	9/9

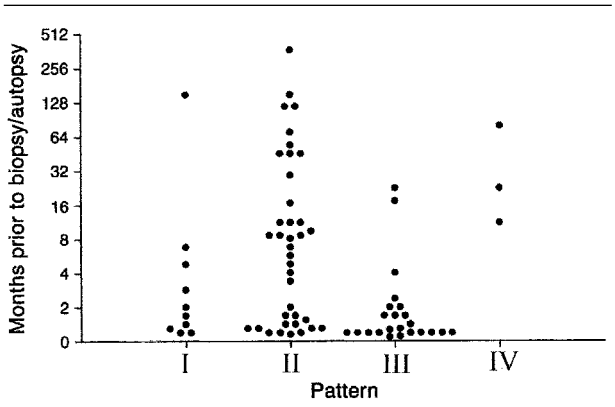

Fig 3. Incidence of different patterns of demyelination in relation to clinical duration of the disease at the time of biopsy or autopsy.

in order of magnitude by pattern III, pattern I, and pattern IV. Significant differences in the distribution of demyelinating patterns were found between acute MS (patients who die or are subjected to biopsy within the first year after disease onset) and chronic MS. While the incidence of pattern II and III lesions was similar in acute MS, the latter became rare in chronic MS ($p < 0.005$, χ^2 test).

Pattern I and II lesions were found in patients who presented with all different clinical subtypes of the disease before biopsy or death (Table 5). In contrast, pattern III lesions were found mainly in patients with a disease of less than 2 months' duration before biopsy or autopsy. Pattern IV lesions so far were found in only 3 patients, each suffering from a variant of primary progressive disease with prominent cognitive, cerebellar, and brainstem involvement.

Follow-Up of Biopsy Patients Reveals that Clinically Definite Multiple Sclerosis Develops Regardless of the Initial Pattern of Demyelination

Clinical follow-up data were available on 43 patients who underwent biopsy at disease onset (Table 6). From those patients, 33 developed clinically definite MS according to the Poser criteria. The other 10 patients either died in the acute phase of the disease (n = 4) or showed a monophasic disease with recovery and no further bout until the last follow-up (up to 36 months after disease onset). The relative incidence of acute or monophasic disease in relation to chronic disease was significantly higher in patients with pattern III lesions at onset in comparison with those with pattern I or II lesions ($p < 0.05$, χ^2 test). However, 5 patients with pattern III lesions experienced clinically definitive relapsing-remitting MS showing magnetic resonance imaging abnormalities, consistent with classical MS (Fig 4).

Table 5. Immunopathological Patterns of Demyelination in Relation to Clinical Disease Course before Biopsy or Autopsy (n = 73 cases)

Pattern	Acute (<1 yr)	RR (>1 yr)	SP (>1 yr)	PP (>1 yr)	Total
I	6	1	1	1	9
II[a]	20	9	6	4	39
III[a]	20	1	1	0	22
IV	0	0	0	3	3
Total	46	11	8	8	73

[a]Incidence of disease in acute versus chronic stage significantly different ($p < 0.005$; χ^2 test).

RR = relapsing-remitting disease; SP = secondary progressive disease; PP = primary progressive disease.

Discussion

This study demonstrates a pronounced heterogeneity in the immunopathological profiles of lesions between different MS patients. Although most cases share in common the T-cell– and macrophage-dominated inflammatory reaction, lesions segregated into those with close similarities to autoimmune encephalomyelitis (patterns I and II) and those with signs of oligodendrocyte dystrophy (patterns III and IV).

Pattern I and II lesions all show the typical perivenous distribution of lesions, which by confluence result in large demyelinated plaques and thus resemble the structural hallmarks of MS lesions, as defined by Rindfleisch[25] and Dawson.[26] Yet, even in these two types of lesions, the mechanisms of myelin injury are apparently different. Whereas in pattern II lesions the pronounced deposition of Igs and complement C9neo antigen at sites of active demyelination suggests an important role of antibodies,[14–16] this was not detectable in pattern I lesions. In these lesions, the destructive process may be induced mainly by products of activated macrophages, such as, for example, tumor necrosis factor-α.[10,11,27] The heterogeneity between pattern

Table 6. Follow-Up Data from 43 Patients with a Biopsy at Onset of Disease

Pattern	Acute	Mono	RR	SP	PP	Total
I	0	1	3	2	1	7
II[a]	3	0	8	9	5	25
III[a]	1	5	5	0	0	11
Total	4	6	16	11	6	43

No biopsies were available showing pattern IV lesions.

[a]Incidence of acute and monophasic disease in relation to chronic disease is significantly higher in pattern III compared with pattern II cases ($p < 0.05$; χ^2 test).

Acute = fatal disease during the first year after disease onset; Mono = monophasic disease without further relapse in follow-up; RR = relapsing-remitting disease in follow-up; SP = secondary progressive disease; PP = primary progressive disease.

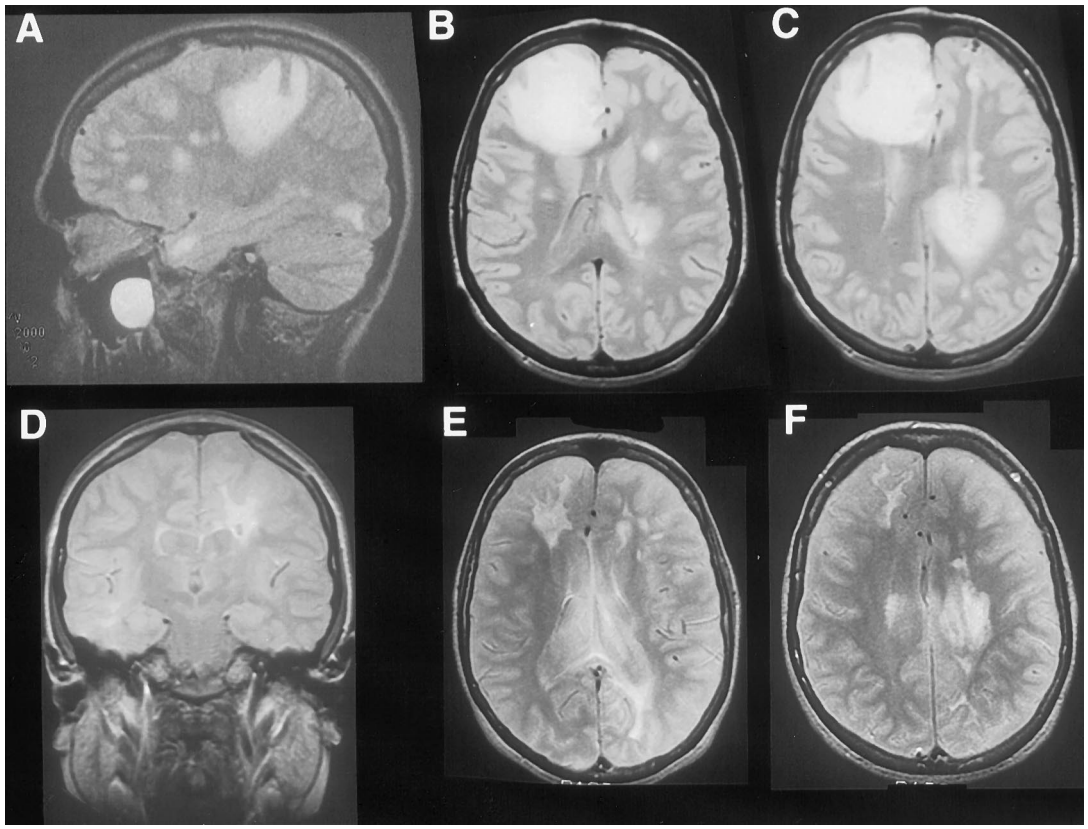

Fig 4. Magnetic resonance images (MRIs) and case report of a patient with biopsy-proven pattern III lesions at disease onset. Case report: This 22-year-old woman presented in May 1988 with a 2-week history of progressive right-sided weakness. MRI revealed multifocal lesions associated with mass effect, which were suspected for tumor (ie, lymphoma, multicentric glioma, metastasis). The patient underwent stereotactic brain biopsy of the left cerebral lesion, which revealed active demyelination. She dramatically improved after a course of steroids, and by August she was walking independently with minimal residual deficit. In October of that year, she had a second exacerbation characterized by increasing right-sided weakness, which improved after 2 weeks on another course of oral steroids. In January 1989, she experienced a third exacerbation associated with increasing incoordination and tremor of the right upper extremity, migratory paresthesias, and diurnal fatigue. She responded to another course of steroids. In December 1989, she developed a fourth exacerbation with increasing gait difficulties and falling. Examination revealed increase in right lower extremity weakness, which again markedly improved after a course of steroids. In February 1990, she experienced a 3-week episode of hemisensory loss on the left, which resolved spontaneously. In March 1990, she had her sixth exacerbation, which was characterized by profound right arm weakness associated with left-sided trigeminal neuralgia. She improved on a 5-day course of intravenous steroids. She was stable until September 1991, when she experienced another exacerbation characterized by vertigo, increased right arm weakness, and spasticity. Symptoms resolved over 4 days. In October 1991, she complained of a 2-week episode of a new visual scotoma on the right, which again resolved spontaneously. In 1992 she was enrolled in the sulfasalazine clinical trial for relapsing-remitting multiple sclerosis. She was stable with no further exacerbations until 1994, when she had a 3-week episode of right-sided painful tonic spasms. At last follow-up in 1996, her examination revealed minimal right hemiparesis and minimal right lower extremity spasticity. The remainder of the examination was unremarkable. MRI results: Initial MRI examination of the head, dated June 1988, demonstrates multiple large areas of T2 signal abnormality in the white matter of both cerebral hemispheres (A–C). The largest are located in the anterior right frontal region and extend into the genu of the corpus callosum and left parietal region. There is considerable mass effect associated with both lesions. There are several additional periventricular lesions oriented perpendicularly to the axis of the lateral ventricles. Follow-up MRI, dated November 1989, demonstrates residual T2 signal abnormality in the deep left posterior frontal anterior parietal region (D). Another follow-up MRI, dated September 1991, reveals almost complete resolution of the large lesion that was present in the right subfrontal white matter area and considerable decrease in size of the large left posterior frontal anterior parietal white matter lesion (E, F). There are numerous foci of increased T2 signal abnormalities in a pattern consistent with demyelinating disease.

I and pattern II lesions is reflected by the situation in autoimmune encephalomyelitis, where depending on the genetic background of the animals and the mode of immunization, demyelination either may be mediated by antibodies[28,29] or may ensue in the absence of a pathogenic B-cell response.[30]

Pattern III lesions are fundamentally different in several important aspects and have never been described

in any model of autoimmune encephalomyelitis. Demyelination is not centered on inflamed blood vessels, and the contours of the lesions are ill defined. Another unusual feature of pattern III lesions was the concentric layering of myelinated and demyelinated tissue in 8 of the 22 respective cases. Furthermore, signs of oligodendrocyte dystrophy, such as loss of MAG and oligodendrocyte apoptosis, were regularly present.

There is a body of literature that suggests that the relative distribution of MAG and MBP or PLP in diseased white matter may reflect primary oligodendrocyte injury in the pathogenesis of demyelination.[18,31–33] In adults, MAG is found exclusively in the periaxonal regions of myelinated fibers, where the oligodendroglial surface membranes, inner mesaxons, and the innermost layers of compact myelin are located.[34,35] This location is the most distal site from the oligodendrocyte cell body and, similar to the distal axon, represents the part of the oligodendrocyte most susceptible to injury. Loss of MAG is prominent in active lesions of progressive multifocal leukoencephalopathy (PML[31]), a known viral infection of the oligodendrocyte. Although several previous MS neuropathological studies had described a reduction in the periplaque expression of MAG compared with other myelin proteins,[18,36] these findings were not confirmed in a later series,[37] which were, however, based on a small sample of cases.

Ultrastructural evidence for oligodendrocyte-based demyelination in MS lesions was suggested by Rodriguez and Scheithauer,[19] who described early changes in the periaxonal oligodendrocyte processes, consistent with a "dying back oligodendrogliopathy." Experimentally, these peculiar alterations of oligodendrocyte processes have previously been described in conditions of toxic oligodendrocyte damage[38] and in the course of virus-induced inflammatory demyelinating diseases.[39] Similarly, the observation of dying-back oligodendrogliopathy in animals with a genetic deletion of MAG[40] indicates that selective loss of MAG and degeneration of distal oligodendrocyte processes are closely associated. All these data suggest that in MS patients with pattern III lesions, demyelination is induced by a functional disturbance of oligodendrocytes, possibly as a result of infection with a hitherto unknown virus or damage mediated by some unknown toxin.

Most patients showing pattern III lesions had a clinical course of less than 8 weeks before biopsy or autopsy. However, patients with pattern III lesions in a biopsy taken within the first weeks after disease onset later developed a disease clinically and radiologically consistent with clinically definite relapsing-remitting MS.[41] At present, it is unresolved whether these patients develop new lesions following the same pattern of demyelination as during the initial phase, thus retaining the phenotype of pattern III, or, alternatively, reflect an initial (possibly virus-induced) starter lesion, which may subsequently—later in the disease course—switch to the more classical autoimmune type of demyelination, such as that seen with patterns I or II. Only sequential biopsy and autopsy studies, which so far are not available, will ultimately clarify this point.

Pattern IV lesions, which were exclusively present in a subgroup of patients with primary progressive MS, showed similarities to the classical pattern I and II lesions, such as the perivenous and radial plaque growth and the simultaneous loss of all myelin proteins. The extensive loss of oligodendrocytes, the lack of remyelinated shadow plaques and the DNA fragmentation in oligodendrocytes in the periplaque white matter, however, suggest that in these cases, too, the oligodendrocytes are impaired.

The reason for the different patterns of demyelination in active MS lesions, as described in this study, is not clear, and there is no direct evidence for pathogenetic mechanisms. The differences could be due to the duration of the lesion, the severity of the offending agent (which may variably affect myelin sheaths or oligodendrocytes), the presence or type of previous lesions in the area of active demyelination, and varying patient susceptibility. However, in the present study we tried to exclude—as far as possible in human pathological tissue—effects of duration and severity of the lesions by exactly staging the demyelinating activity and by including in the analysis lesions of different size and destructiveness. Furthermore, the contribution of previous lesions may be rather small, since most cases included in this series presented with only a very short clinical course before biopsy or autopsy. Thus, when these patterns are compared with those seen in the experimental and clinical literature, as discussed earlier, it is tempting to speculate that they may reflect pathogenetic variability. Obviously, from the data presented here, no conclusions can be drawn regarding the question of whether these lesional patterns remain constant during the evolution of the disease or whether they may change in the course of the transition from the early acute stage of MS to chronicity of the disease. Detailed prospective follow-up of biopsied patients by clinical and neuroradiological means, as well as more data on autopsies of previously biopsied patients, is required to resolve this issue.

In conclusion, our data provide a first indication that the mechanisms and targets of demyelination in MS may be fundamentally different in distinct subgroups or stages of the disease. Thus, a therapy that may be useful in one group of patients or at one stage of the patient's disease may be deleterious in another. To tailor MS therapy or develop novel therapeutic strategies, future studies need to define specific clinical and paraclinical parameters that allow differentiating the described pathological patterns during the patient's life.

This study was funded by the Austrian Science Foundation, Project 12658 Med, the Gemeinnützige Hertie-Stiftung (GHS 2/439/97), and the Mayo Foundation Scholarship.

We also appreciate the contribution of Mr and Mrs Eugene Appelbaum for the financial support of this project. We thank Helene Breitschopf, Marianne Leisser, Petra Tassoti, Angela Kury, and Jutta Wakley-Neuninger for expert technical assistance.

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