doi:10.1093/brain/awl217 *Brain* (2006), **129**, 3165–3172

Remyelination is extensive in a subset of multiple sclerosis patients

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Although spontaneous remyelination does occur in multiple sclerosis lesions, its extent within the global population with this disease is presently unknown. We have systematically analysed the incidence and distribution of completely remyelinated lesions (so-called shadow plaques) or partially remyelinated lesions (shadow plaque areas) in 51 autopsies of patients with different clinical courses and disease durations. The extent of remyelination was variable between cases. In 20% of the patients, the extent of remyelination was extensive with 60–96% of the global lesion area remyelinated. Extensive remyelination was found not only in patients with relapsing multiple sclerosis, but also in a subset of patients with progressive disease. Older age at death and longer disease duration were associated with significantly more remyelinated lesions or lesion areas. No correlation was found between the extent of remyelination and either gender or age at disease onset. These results suggest that the variable and patient-dependent extent of remyelination must be considered in the design of future clinical trials aimed at promoting CNS repair.

Keywords: multiple sclerosis; remyelination; shadow plaques

Abbreviations: MBP = myelin basic protein; PLP = proteolipid protein

Received March 31, 2006. Revised July 17, 2006. Accepted July 18, 2006. Advance Access publication August 18, 2006.

Introduction

Multiple sclerosis is an inflammatory CNS disease associated with focal white matter plaques of demyelination (Charcot, 1868; Lumsden, 1970). Plaques can in part become repaired by remyelination. Reappearance of oligodendrocytes within active lesions associated with early stages of remyelination are frequently seen in patients with acute or early multiple sclerosis (Lassmann, 1983; Prineas *et al.* 1984; Prineas, 1985; Raine and Wu, 1993; Prineas *et al.* 1993*a*; Bruck *et al.*, 1994; Lucchinetti *et al.*, 1999). This may give rise to completely remyelinated plaques, the so called shadow plaques (Schlesinger, 1909; Lassmann, 1983; Prineas, 1985). It has, however, been suggested that remyelination is a transient phenomenon (Frohman *et al.,* 2006) and that remyelinated shadow plaques may become affected by new bouts of demyelination (Prineas *et al.,* 1993*b*). In patients with late stage progressive disease, remyelination is thought to be sparse and largely restricted to a small zone at the borders of inactive plaques (Suzuki, 1969). Yet, in a correlative radiological-pathological study in autopsy brains, remyelinated areas were found in 42% of the lesions studied. Partial remyelination was observed in 19% of the lesions and 23% of the lesions were completely remyelinated shadow plaques (Barkhof *et al.*, 2003). Due to these divergent results a systematic study of remyelination at different stages and in different forms of multiple sclerosis seems to be necessary.

Since there is no established and reliable surrogate MRI marker of remyelination, it is still necessary to rely on post-mortem histopathological analysis. In this study we observed that in some patients remyelination is extensive, whereas in others it largely fails. Patients who had died at an older age

Table 1 Clinical data of all cases

Clinical subtypes	Age onset (years)	Age at death (years)	Disease duration (months)	Female:male
Acute	45 (35–51)	45 (35–51)	3.4 (0.6–7)	0.67:1
RRMS	31 (16–48)	49 (20–67)	136 (48–234)	0.50:1
SPMS	31 (19–50)	48 (34–71)	204 (72–408)	1.70:1
PPMS	39 (25–49)	55 (28–75)	188 (30–411)	1.75:1
Unknown	14, 47	51 (42–73)	108, 708	3.50:1

Averages shown with range in parentheses. Disease duration in months. Only two disease durations known in the unknown clinical course group.
RR = relapsing-remitting; SP = secondary progressive; PP = primary progressive; MS = multiple sclerosis.

and after a longer disease duration showed more extensive remyelination when compared to those who died earlier in the disease course.

Patients and methods

Brain tissue from 51 multiple sclerosis cases, comprising 5 acute, 7 relapsing–remitting, 18 secondary progressive and 11 primary progressive cases, and 10 cases with an unknown clinical course, was analysed for evidence and extent of remyelination. The average age at death was 49.96 years (range 20–75), the female to male ratio 1.5:1, and the average disease duration was 175.21 months (0.6–708). Clinical records, which allowed for an unequivocal identification of disease course, were available from 41 of the total 51 patients and for all patients with large hemispheric or double hemispheric brain sections. The other 10 patients died in the progressive phase of the disease but could not be classified as primary or secondary progressive. Patient demographics are given in Table 1. Clinical courses were determined by retrospective chart reviews performed either by a neurologist (H.R., P.S.S. and C.L.) or a neuropathologist (C.S.), blinded to the outcome of our neuropathological analysis.

Our study was restricted to the analysis of the forebrain. A total of 1026 lesions were analysed. Whole hemispheric or double hemispheric brain sections were performed for 23 cases. Double hemispheric sections were obtained in a standardized fashion, including one section at the level of the anterior part of the corpus callosum and one at the level of the mid thalamus. Single hemispheric brain slices were available from multiple brain regions, including the frontal, parietal and occipital lobe. Depending on the macroscopic identification of demyelinated lesions additional small tissue blocks were taken from various regions of the forebrain (see also Table 2). For the remaining cases multiple regular tissue blocks (average number of small sections per case = 3.97, approximate average size = 2×3 cm^2) formed the basis of the analysis. Brains were fixed in formaldehyde and embedded in paraffin. In three of the cases, tissue blocks, which were fixed in glutaraldehyde and embedded in epoxy resin, were available for analysis in addition to the paraffin embedded material. Paraffin sections from all cases were stained with haematoxylin and eosin, Luxol fast blue, Bielschowsky's silver stain and by immunocytochemistry for proteolipid protein (PLP) or myelin basic protein (MBP). The epoxy-embedded sections were stained with Toluidine blue.

Immunocytochemistry

Immunocytochemistry was performed using an avidin/biotin method. Sections were de-paraffinized, and antigen retrieval was performed in a steamer for 90 min with EDTA buffer at pH 8.5. Primary antibodies against the following targets were used: PLP (polyclonal, Serotec, Oxford, UK; AHP 261; monoclonal, Serotec, Oxford, UK; MCA839G), MBP (polyclonal, DAKO, Glostrup, Denmark A0623), neurofilament (polyclonal; Chemicon, Temecula, USA; AB1983; monoclonal; Affinity, Mamhead, UK; SMI 31 and SMI 33) and the macrophage antigen CD68 (monoclonal, DAKO, M0814). Incubation of the sections with primary antibodies overnight at 4°C was followed by application of biotinylated secondary antibodies (sheep anti-mouse or donkey anti-rabbit, Amersham, Buckinghamshire, UK; RPN 1001 and 1004) and peroxidase labelled avidin (Sigma, St Louis, USA; A3151). Bound antibodies were visualized by developing the sections with diaminobenzidine and nickel.

Immunocytochemical double staining was performed for conventional light microscopy. In a first step, myelin antigens were labelled by polyclonal antibodies against PLP or MBP. Then, neurofilament epitopes were stained with a mixture of the monoclonal antibodies SMI 31 and 33. Rabbit polyclonal antibodies were detected with an alkaline phosphatase labelled donkey anti-rabbit antibody (Jackson ImmunoResearch, West Grove, USA; 59678) using fast red BB salt as chromogen. Finally, the sections were incubated with biotinylated sheep anti-mouse antibody and avidin peroxidase. The reaction product was visualized with diaminobenzidine.

In addition confocal laser microscopy was performed for double and triple staining. Myelin was stained with polyclonal primary antibodies against PLP or MBP and bound antibodies were visualized with biotinylated donkey anti-rabbit (Amersham, Oxford, UK; RPN1004) and Cy^{TM}2 labelled streptavidin (Jackson ImmunoResearch, West Grove, USA; 59678). Neurofilaments were stained with a mixture of SMI 31 and 33 mouse monoclonal antibodies and a Cy^{TM}3 donkey anti-mouse antibody (Jackson ImmunoResearch, Dianova, Hamburg; 715-165-151). For active lesions we additionally used the monoclonal antibody against CD68, which was then visualized with an Alexa Fluor 660 goat anti-mouse secondary antibody (Molecular Probes, Eugene, OR, USA; A21055).

Table 2 Sub-study showing the percentage of remyelination in large versus small sections

Case no.	Large sections	Small sections
1	84 (2)	71 (5)
2	78 (1)	71 (6)
3	85 (1)	80 (5)
4	8 (2)	2 (6)
5	9 (2)	15 (13)
6	5 (1)	0 (3)

Number of sections in parentheses.

For control, sections were reacted in the absence of primary antibodies or by using irrelevant primary antibodies of the same immunoglobulin subclass.

Strategy for quantitative analysis of remyelination

Shadow plaques and shadow plaque areas (incomplete shadow plaques), defined according to the criteria described in the Results section, were first identified on consecutive sections stained with Luxol fast blue (LFB) and by immunocytochemistry for PLP. When such lesions were present, sections from two representative tissue blocks per case were double stained for confocal laser microscopy and by conventional immunocytochemistry to confirm the presence of remyelination. In the three cases where additional epoxy resin embedded material was available, remyelination was further confirmed in plastic sections of osmicated material. Sections were digitally scanned, printed, and the areas of demyelination and remyelination were labelled. The images were overlaid by a morphometric grid and the number of grid-points located over the total white matter area, as well as the area of demyelinated or remyelinated plaques, was determined. To analyse the relation between total plaque size and remyelination, correction was made for the enlargement of the images and all data were standardized to the actual mm^2 of tissue area. The extent of remyelination was then expressed as the percentage of the total plaque area.

Determination of the extent of remyelination may be biased when the analysis is performed on small tissue blocks, which were macroscopically selected for the presence of demyelination. In order to assess the reliability and consistency of our quantitative methods, we also compared in a sub-study on six patients the extent of remyelination in hemispheric or bi-hemispheric sections with the extent of remyelination in randomly sampled small MS blocks of the same case. The percentage of remyelination within the total lesions was overall comparable in small sections to that seen in the large sections when $\geqslant$3 small sections were used (Table 1). It was noted, however, that the extent of remyelination in most small sections is slightly underestimated compared to that seen in the large sections, likely reflecting a tissue sampling bias.

Statistical analysis

Statistical analysis was performed using Statgraphics Plus 4.0. Spearman's rank correlations were used to identify interdependence of variables, comparing remyelination, age at disease onset, disease duration and age at death. Group differences were analysed using non-parametric group tests (Kruskal–Wallis) to compare genders, high and low remyelination cases, and the different clinical subtypes. *P*-values of $\leqslant$0.05 were considered significant.

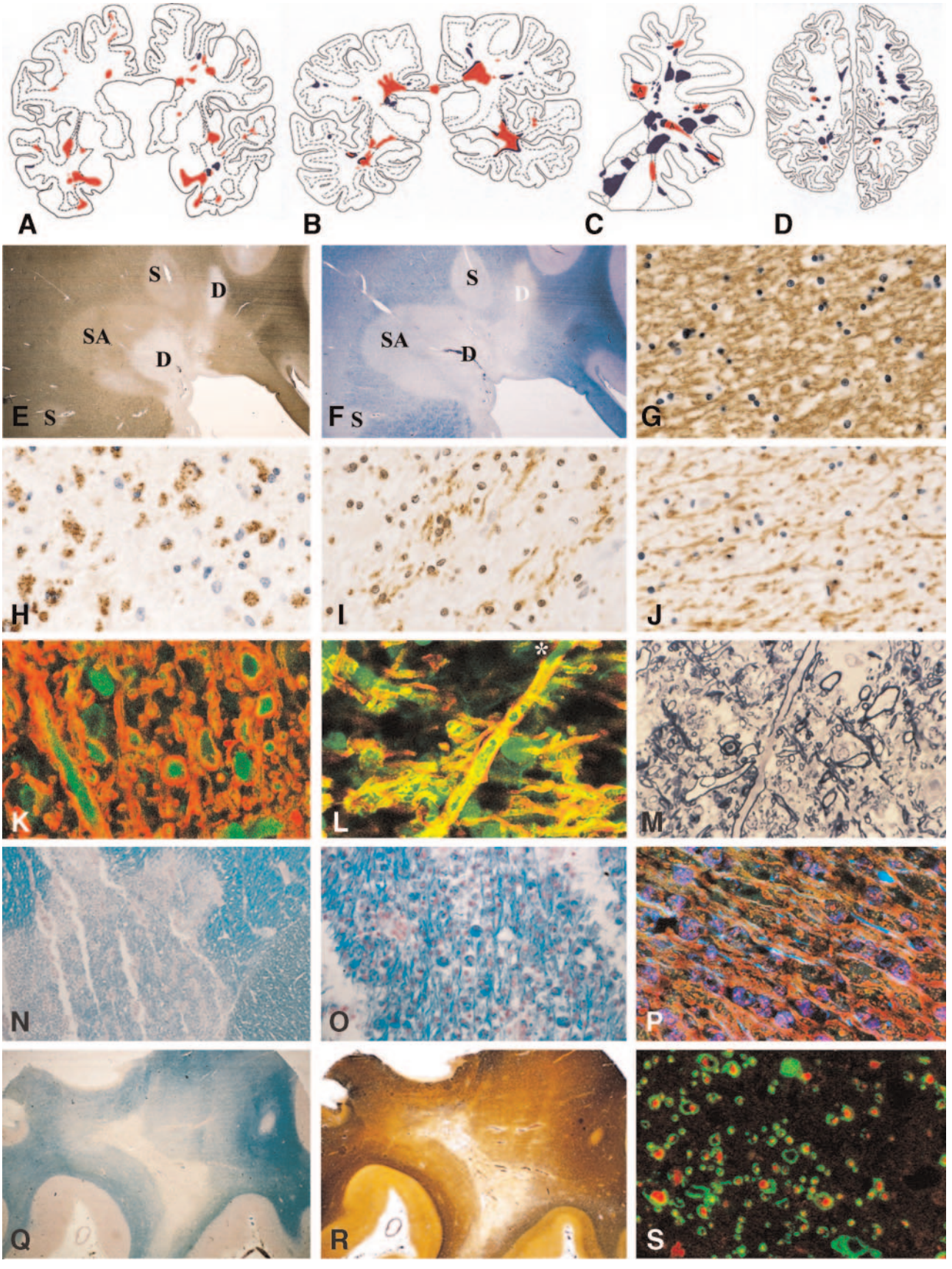
Results

Definition of remyelinated areas in the brains of multiple sclerosis patients

Previous studies on the fine structure of multiple sclerosis lesions have defined areas of remyelination based on the abundance of thinly myelinated fibres with shortened internodes and widened nodes of Ranvier (Prineas, 1985). In conventional myelin stains of such lesions, remyelination appears as focal areas with reduced myelin density when compared to the surrounding normal appearing white matter (shadow plaques; Fig. 1E and F). These lesions are sharply demarcated from the surrounding normal appearing white matter and generally centred on a small or medium sized vein. The pale myelin stain is due to a uniform reduction of myelin sheath thickness throughout the whole lesion and both shortened internodes and widened nodes of Ranvier can be detected by inspection at high magnification (Fig. 1I, J, L and M). Other characteristic features of shadow plaques were a moderate reduction of axonal density (Kornek *et al.*, 2000), the absence of macrophages with early myelin degradation products (Fig. 1I and J) and a low number of perivascular inflammatory infiltrates. In addition, in cases with profound remyelination, which still contained actively demyelinating lesions (Fig. 1C and D), the whole spectrum of remyelination was seen. Complete demyelination was present in classical active lesions (Fig. 1H), which contained abundant macrophages with early myelin degradation products. In lesions, which were still hypercellular and infiltrated by macrophages with empty vacuoles, small clusters of thin myelin sheaths were found which were only detectable by immunocytochemistry for PLP (Fig. 1I). Other plaques with only minimal residual macrophage infiltration contained abundant axons with very thin PLP reactive myelin sheaths with only faint staining for LFB (Fig. 1J). These lesions were present together with classical shadow plaques within the same case or section.

However, focally reduced myelin density within multiple sclerosis brains can also occur for reasons other than remyelination. Actively demyelinating lesions are also sharply demarcated areas of reduced myelin density; however, these lesions are infiltrated by macrophages with early myelin degradation products and the remaining myelin sheaths within the lesions are of comparable thickness to those in the surrounding white matter and may show signs of dissolution (Fig. 1N–P). Another possible reason for reduced myelin density around an established plaque may reflect Wallerian degeneration, due to axonal destruction and loss within the lesion. Such areas of secondary degeneration are poorly demarcated areas of reduced myelin density, surrounding the plaques in ill-defined contours (Fig. 1Q–S). At higher magnification, a profound reduction of axonal density is seen and the remaining fibres are enveloped by myelin sheaths, which are similar in thickness when compared to those in the surrounding normal appearing white matter. Sometimes they may reveal an unusually high density of thick myelinated fibres, since thin axons are preferentially destroyed in multiple sclerosis lesions (Evangelou *et al.*, 2001).

In our further quantitative studies, only those lesions with reduced myelin density were included which fulfilled the criteria for remyelinated shadow plaques or shadow plaque areas as defined above. Shadow plaques contained uniformly thin myelin sheaths throughout the whole lesion, while shadow plaque areas (incomplete shadow plaques) only affected part of the lesions.



Distribution of shadow plaques and extent of remyelination

Major differences in the incidence of shadow plaques and in the extent of remyelination became apparent between individual patients (Fig. 1A–D). In 10 patients most focal plaques within the forebrain were shadow plaques, resulting in 60–96% of total plaque area being remyelinated (Fig. 1C and D; Table 3). In 34 other patients, remyelination was sparse and restricted to small zones at the edge of demyelinated lesions leading to a total percentage of remyelinated areas between 0 and 25% (Fig. 1A and B; Table 3). The remaining seven patients had an intermediate range with a percentage of remyelination between 25 and 60% of the plaque area (Table 3). Similar results were also obtained when the analysis was restricted to cases where large hemispheric or double hemispheric sections were available (Table 4), however, most of these large-section cases segregated into two distinct groups of either high or low remyelination (Fig. 2B). There was no significant difference between the total plaque area analysed in the high versus low remyelinating cases either when all cases were analysed ($P = 0.41$) or when the cases of acute disease were excluded from the analysis ($P = 0.14$).

The extent of remyelination was in part dependent upon the location of the lesion. Although complete remyelination was present in some periventricular plaques, the global extent of remyelination was lower in these lesions compared to those located in the deep white matter or subcortically (Fig. 1E and F; Tables 4 and 5). This was seen not only in the global multiple sclerosis sample, but also in cases with a high extent of remyelination, in whom large (hemispheric or double hemispheric) sections were available.

Relation between extent of shadow plaque formation and clinical course of the disease

Shadow plaque areas were rare in acute cases, accounting for only 0–13% of total plaque area. This may have been due to the fact that most lesions examined were in a stage of early active demyelination (Kutzelnigg *et al.*, 2005). However, as described previously (Lucchinetti *et al.*, 1999), in many of these lesions activated oligodendrocytes with signs of early myelin formation were seen.

The frequency and size of shadow plaques or shadow areas were highly variable between different patients with chronic illness. They were more frequent in patients with relapsing–remitting and primary progressive disease compared to those with secondary progressive illness, but these differences did not reach statistical significance (Tables 3 and 4). We did not find a significant correlation between the extent of shadow plaque formation with either the age of disease onset or gender in the global sample or when acute cases were excluded from the analysis. However, there was a significant correlation ($P = 0.017$, $r = 0.34$) between the extent of remyelinated plaque area and the age of death of the patients. Those patients who died at an older age showed significantly more shadow plaques or shadow plaque areas compared to those who died at a younger age (Fig. 2A). This was also seen when acute patients were excluded from the analysis ($P = 0.03$; $r = 0.32$). A similar correlation was found when only the large hemispheric or double hemispheric sections were analysed ($P = 0.026$, $r = 0.48$; Fig. 2B). A significant correlation was also found between remyelination and disease duration ($P = 0.021$, $r = 0.38$), as shown in Fig. 3.

Fig. 1 (**A–D**) Schematic diagrams showing the proportion of remyelination (blue) and demyelination (red) in different MS patients. (**A**) Case 12; SPMS with 8% RM, (**B**) Case 11; SPMS with 24% RM, (**C**) Case 5; RRMS with overall 78% RM, and (**D**) Case 19; PPMS with overall 84% RM. The rows below the schematic diagrams show the features differentiating remyelination from active MS plaques and axonal loss secondary to Wallerian degeneration. (**E**) Case 21; PPMS; PLP stained section (low power view) showing sharply demarcated demyelinated and shadow plaques; the periventricular lesion is completely demyelinated in the periventricular area (**D**), but contains a large shadow plaque area in the rest of the plaque (SA); most of the lesions in the deep white matter are sharply demarcated shadow plaques (S); ×3. (**F**) In the consecutive sections stained with LFB pale myelin stain is seen in the shadow plaques and plaque areas; ×3. (**G**) Case 19; PPMS; high power view of PLP staining in the normal appearing white matter of the same case, shown in **H–L**; ×100. (**H**) PLP stained section of an active plaque showing macrophages containing myelin degradation products; there is no remyelination in this lesion; ×200. (**I**) PLP stain showing early remyelination with clusters of very thin sheaths in a lesion, which is still hypercellular; ×200. (**J**) later stage of remyelination showing abundant thin myelin sheaths; ×150. (**K**) Confocal fluorescence image of PLP (red) and axonal neurofilaments (SMI 31 and 33 stained green) showing thick myelin sheaths in normal appearing white matter adjacent to the lesion, depicted in **L**; ×800. (**L**) same section as in (**K**) showing thin myelin sheaths enveloping axons in a shadow plaque; note the widened node of Ranvier (asterisks); ×800. (**M**) Case 5; RRMS; Toluidine blue stain showing thin myelin sheaths and a widened node of Ranvier; ×800. Further features of acute plaques (Case 3; acute): (**N**) LFB low power view showing reduced myelin staining and a sharply demarcated border; ×15; (**O**) higher power view showing massive macrophage infiltration; ×150; (**P**) confocal fluorescence image of PLP (red), CD68 stained macrophages (blue), and SMI 31 and 33 neurofilament stainings (aqua) showing abundant macrophages with myelin degradation as well as myelinated and demyelinated axons; ×300. Features of Wallerian degeneration (WD) secondary to axonal injury: (**Q**) Case 4; RRMS; LFB low power view of a plaque (PL) in a patient with acute MS and extremely destructive white matter lesions, which is surrounded by Wallerian degeneration showing the reduced myelin staining and an indistinct border; ×3; (**R**) Bielschowsky's silver stain showing reduced axonal densities surrounding the plaque; ×3; (**S**) Case 7; RRMS; confocal fluorescence image with PLP (green) and neurofilaments (SMI 31 and 33 stained red) showing reduced axonal density and axolysis, namely hollow myelin sheaths and alterations in axonal diameters; ×700. RR = relapsing-remitting; SP = secondary progressive; PP = primary progressive; MS = multiple sclerosis.

Discussion

Three key observations are reported in our study. Remyelination, defined by the presence of shadow plaques, is extensive in a considerable proportion of multiple sclerosis patients. It is not restricted to early stages of the disease and it occurs in all manifestations of the disease, including primary progressive multiple sclerosis.

It was first suggested by Marburg (1906) that spontaneous remyelination may occur in multiple sclerosis. This was later confirmed when electron microscopy was introduced into multiple sclerosis research (Perier and Gregoire, 1965; Suzuki *et al.*, 1969). In the following years it became clear that shadow plaques, originally identified by Schlesinger in 1909 as areas of incomplete demyelination, represent fully remyelinated plaques (Lassmann, 1983; Prineas, 1985).

Table 3 Distribution of numbers of cases ($n = 51$) from all clinical subtypes into the different remyelination groups

Clinical subtypes	0–25%	26–59%	60–100%
Acute	5	0	0
RRMS	4	0	3
SPMS	14	3	1
PPMS	7	1	3
Unknown	4	3	3
Total	34	7	10

RR = relapsing-remitting; SP = secondary progressive; PP = primary progressive; MS = multiple sclerosis.

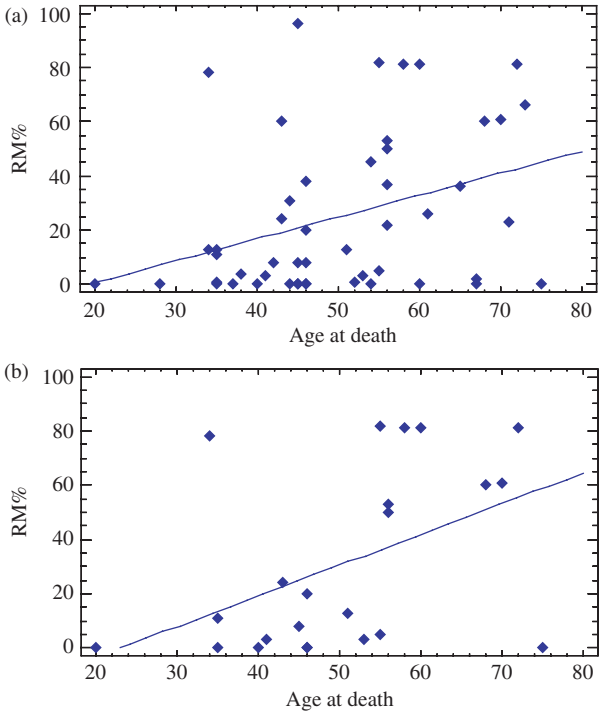


Fig. 2 Graph showing the statistically significant correlation of increased remyelination with increased age at death in (**A**) the global sample ($n = 50$; $P = 0.017$; $r = 0.34$) and (**B**) in patients where large hemispheric or bi-hemispheric sections were available ($n = 23$; $P = 0.026$; $r = 0.48$).

Table 4 Lesion distribution in large tissue sections

Case	Type	Age	Disease duration (months)	Periventricular			Deep WM			Subcortical			Total	RM%
				SP	ISP	DM	SP	ISP	DM	SP	ISP	DM		
1	Ac	35	1.5			5			15				20	0
2	Ac	46	3			1							1	0
3	Ac	51	5			19	17	7	92				135	13
4	RR	20	48			13			28				41	0
5	RR	34	156	2		1	47	11	11	3		1	76	78
6	RR	40	120			2			1			2	5	0
7	RR	58	120	2	1			1					4	81
8	RR	60	234	6	1		8		1			1	17	85
9	SP	35	n.a.		4	4	13	12	15	20	3	2	73	9
10	SP	41	156		1	5	5	1	3			3	18	5
11	SP	43	192		3	1	10	4	9				27	24
12	SP	45	240			1	1	1	25	1		9	38	8
13	SP	46	n.a.	1		2	2		5				10	20
14	SP	46	264			6			11			6	23	0
15	SP	53	241		2		5	4	6	1			18	3
16	SP	55	366		4	5	7	1	7	3		6	33	8
17	SP	56	408				4	4	1				9	50
18	SP	70	120		1	1	2			2		1	7	61
19	PP	55	150	5		2	84	12	16	12		3	134	84
20	PP	56	132	1		5			2	1			9	53
21	PP	68	336	2	5	6	7	1	6			4	31	60
22	PP	72	411		2	1	4			19	1	5	32	81
23	PP	75	314						2			1	3	0

SP = shadow plaques; ISP = incomplete shadow plaques (demyelinated plaques with shadow plaque areas); DM = demyelinated plaques; Ac = acute multiple sclerosis (MS); RR = relapsing/remitting MS; SP = secondary progressive MS; PP = primary progressive MS; RM% = percentage of remyelinated area in all lesions; WM = white matter; n.a.: not available.

Table 5 Percentage of plaque types and total number of plaques in different brain regions in all cases versus high remyelinators with large sections (>50% global remyelination)

All cases	SP%	ISP%	DM%	Total plaques
Periventricular	10.8	30.6	58.6	196
Other plaques	38.2	12.0	49.8	830
Cases with extensive remyelination				
Periventricular	40.9	22.7	36.4	44
Other plaques	70.2	10.9	18.9	275

SP = shadow plaque; ISP = incomplete shadow plaque; DM = demyelinated plaque.

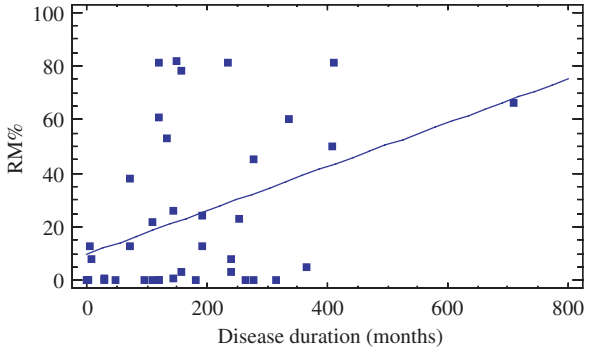


Fig. 3 Graph showing the statistically significant correlation between remyelination and disease duration (global sample; $n = 39$; $P = 0.021$; $r = 0.374$).

Remyelination starts early after onset of demyelination and, indeed, many active lesions show signs of oligodendrocyte activation and initial stages of remyelination whilst still containing macrophages loaded with myelin degradation products (Prineas *et al.*, 1993*a*; Raine and Wu, 1993; Lucchinetti *et al.*, 1999; Wolswijk, 2000). From these studies it was concluded that remyelination transiently occurs during the acute and early relapsing stage of the disease, being sparse or absent in patients with late chronic disease (Prineas *et al.*, 1993*a*; Ozawa *et al.*, 1994; Frohman *et al.*, 2006).

Contrary to these earlier studies we found that extensive remyelination is present in a subset of patients with chronic multiple sclerosis and that in such patients virtually all inactive white matter plaques are remyelinated shadow plaques. In our global sample we found a profound diversity in the amount of remyelination between cases. However, when the analysis was restricted to patients where large hemispheric or bi-hemispheric sections were available, they clearly segregated into two groups with either extensive or low remyelinating capacity. Why this dichotomy was only visible in the substudy based on large sections is not clear so far. Although we were able to show that the extent of remyelination overall was comparable in case samples with large and small sections, we can not exclude that the extent of remyelination is at least in part underestimated when only small tissue blocks are used, which are selected on the basis of macroscopically visible lesions. Alternatively, the smaller number of cases in whom large sections were available may confer a sampling bias. In addition, we used a stringent classification for remyelinated shadow plaques. Thus we excluded all lesions or lesion areas which were suggestive of secondary Wallerian tract degeneration as well as actively demyelinating plaques, which also contain some remyelination. Thus, the global extent of remyelination in our study is rather underestimated than exaggerated.

It is unresolved as to why in some patients remyelination is widespread whilst in others it is sparse (Franklin, 2002). Several mechanisms have been suggested that may result in blockade of remyelination in multiple sclerosis. These include the loss and destruction of oligodendrocyte progenitor cells within the lesions in the course of repeated de- and remyelination (Prineas *et al.*, 1993*b*). In some lesions, however, progenitor cells are abundantly present (Chang *et al.*, 2000; Chang *et al.*, 2002), but they may fail either because the axons are not permissive for remyelination (Charles *et al.*, 2002) or the maturation of progenitor cells is inhibited (John *et al.*, 2002). Whether the extent of remyelination depends upon the genetic background of the patients has yet to be determined.

According to our results shadow plaques were not restricted to patients with early and relapsing disease, but were prominent in patients with long-standing chronic disease, who died at old age. However, this diverse capacity to form shadow plaques did not correlate with clinical subtype, age of disease onset or gender. The incidence of active demyelination in focal white matter plaques in general decreases with chronicity of the disease (Kutzelnigg *et al.*, 2005). In early stages the illness, remyelination occurs in lesions, which are either still active or may be subjected to repeated de- and remyelination (Prineas *et al.*, 1993*b*). Such remyelination may be unstable and the formation of persistent shadow plaques may be sparse in contrast to that in inactive lesions at the late stage of the disease. Another factor, which seems to determine remyelination in a given lesion, is its location. We found more remyelination in lesions located subcortically or in the deep white matter than in periventricular plaques. The reason for this difference is currently not clear. Finally, we have shown before that remyelination protects against progressive axonal injury (Kornek *et al.*, 2000). Thus, completely demyelinated in contrast to remyelinated plaques may progressively shrink, thus increasing the relative percentage of remyelinated areas in patients with long-standing multiple sclerosis. Although this factor may contribute to our findings in some patients, it is unlikely to be a major factor in patients with a very high extent of remyelination. In these patients we found that complete demyelination is only present in lesions with recent demyelinating activity (see Fig. 1C and D).

Our study shows that remyelination in focal white matter plaques is extensive in a subset of multiple sclerosis lesions. Although functional restoration of conduction has been shown by electrophysiology in some lesions (Kriss *et al.*, 1988), it is currently undetermined as to what extent this

occurs in the global multiple sclerosis population. In this context it is important to note that our study was restricted to the analysis of forebrain lesions. Whether the remyelinating capacity of lesions is regionally different in the human brain and spinal cord awaits further investigation. Despite this caveat, new paraclinical tools have to be developed to assess the extent of spontaneous remyelination in the context of therapeutic trials aimed to promote remyelination in multiple sclerosis.

Acknowledgements

This study was funded by the European Union (Project LSHM-CT-2005-018637) and the NIH (Grant Number: R01-NS049577-01A2). C.S. is supported by the Gemeinnützige Hertie Stiftung and the Medical Faculty of Goettingen (junior research group). We thank Ulrike Köck, Angela Kury and Marianne Leisser for expert technical assistance.

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