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A quantitative analysis of oligodendrocytes in multiple sclerosis lesions
A study of 113 cases

Claudia Lucchinetti,[1,*] Wolfgang Brück,[4,*] Joseph Parisi,[2] Bernd Scheithauer,[2] Moses Rodriguez[1,3] and Hans Lassmann[5]

Departments of [1]Neurology, [2]Neuropathology and [3]Immunology, Mayo Clinic, Rochester, USA, [4]Institute of Neuropathology, Göttingen, Germany and [5]Institute of Neurology, Vienna, Austria

Correspondence to: Dr Claudia Lucchinetti, Department of Neurology, Mayo Clinic, 200 First Street, SW, Rochester, Minnesota, USA
E-mail: lucchinetti.claudia@mayo.edu

**These authors contributed equally to the contents of this paper*

Summary
We studied quantitatively the fate of oligodendrocytes (OGs) during lesion formation in 395 lesion areas from biopsy and autopsy tissue of 113 multiple sclerosis cases. The density of OGs in multiple sclerosis lesions was variable at all stages of demyelinating activity, ranging from nearly complete loss to values exceeding those in the periplaque white matter (range 0–970 OGs/mm^2). To determine whether there were distinct patterns of OG pathology in different patients, we restricted our analysis to the 56 cases in which the longitudinal extent of the lesion extended from periplaque white matter into the active demyelinating edge and inactive plaque centre. Two major groups of OG pathology were defined by the presence or absence of increased OGs within the lesion. In 70% (39 out of 56) of the cases, OGs were variably reduced during active stages of myelin destruction, but reappeared within inactive or remyelinating areas. In inactive areas, an increased number of OGs expressing proteolipid protein (PLP) mRNA compared with those expressing myelin oligodendrocyte glycoprotein (MOG) suggested these cells may have been derived from the progenitor pool. In the remaining 30% (17 out of 56) of the cases, extensive destruction of myelinating cells at active sites of demyelination was observed, but OGs were absent in inactive plaque areas without remyelination. In all lesions from a given patient the pattern of OG pathology remained consistent. A highly significant negative correlation was observed between number of macrophages in lesions and number of MOG- and PLP mRNA-labelled OGs (MOG: $r = -0.32$, $P < 0.0000118$; PLP mRNA: $r = -0.23$, $P < 0.00238$). OG density did not correlate with T-cell and plasma cell inflammation, or axonal loss. The profound heterogeneity in extent and topography of OG destruction in active demyelinating lesions suggests that in subsets of multiple sclerosis patients, myelin, mature OGs and possibly OG progenitors are differentially affected.

Keywords: multiple sclerosis; oligodendrocytes; demyelination; pathology

Abbreviations: CNPase = 2′3′-cyclic nucleotide 3′-phosphodiesterase; GFAP = glial fibrillary acidic protein; LFB = luxol fast blue myelin stain; MBP = myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; OG = oligodendrocyte; PAS = periodic acid–Schiff reaction; PLP = proteolipid protein

Introduction
Multiple sclerosis is an inflammatory demyelinating disease of the CNS associated with focal myelin sheath destruction and astrocytic scar formation (Lumsden, 1970). The sequence of morphological and immunological events leading to myelin breakdown as well as the ultimate fate of oligodendrocytes (OGs) in the evolution and repair of the demyelinating lesion remains uncertain. *In vitro* studies demonstrate that OGs are highly susceptible to damage by a variety of immune effector mechanisms present within an inflammatory response. These include direct T-cell mediated cytotoxicity through the Fas-Fas ligand pathway (D'Souza *et al*., 1996; Dowling *et al*., 1996), products from T cells (perforin and lymphotoxin) (Scolding *et al*., 1990; Selmaj *et al.*, 1991*a*, *b*), activated macrophages (cytokines or reactive oxygen radicals) (Selmaj

Table 1 *Summary of 148 multiple sclerosis lesions analysed*

	No. cases	Mean age (years ± SEM)	F : M ratio	No. blocks	No. lesions	No. lesional areas	Early active	Late active	RM	DM	WM
Autopsy	29	37 ± 7	21 : 7	46	61	131	37	19	10	28	37
Biopsy	87*	36 ± 4	51 : 32	187	87	330	61	35	82	61	91

The data are reported with respect to the number of total lesions examined, as well as the number of topographical lesional areas within a single lesion including the periplaque white matter, areas of active demyelination and the plaque centre. In two cases, the sex of the patient was unknown. F = female; M = male; RM = remyelination; DM = inactive demyelination; WM = periplaque white matter. *Three patients underwent sequential brain biopsies.

and Raine, 1988; Griot *et al*., 1990) and serum components (antibodies and complement) (Bornstein and Appel, 1961; Brosnan *et al*., 1977). Immunocytochemical studies on a limited number of multiple sclerosis cases and lesions suggest that some of these mechanisms are also relevant *in vivo* (Prineas *et al*., 1993; Dowling *et al*., 1996; Storch *et al*., 1998*a*).

Previous neuropathological studies examining the fate of OGs in multiple sclerosis lesions have been limited by inadequate tissue preservation, technical difficulties in identifying OGs in archival paraffin material, the lack of stringent criteria to define lesional activity and, most importantly, the relatively small number of cases examined (from 1 to 28) (Raine *et al*., 1981; Prineas *et al*., 1989; Brück *et al*., 1994; Ozawa *et al*., 1994). Most investigators agree that in long-standing sclerotic multiple sclerosis plaques, OGs are largely absent from the lesions (Lumsden 1970; Lassmann 1983; Prineas 1985; Mews *et al*., 1998). However, there is disagreement over the fate of OGs within actively demyelinating multiple sclerosis lesions and during early stages of the disease. Immunocytochemical, light microscopic and electron microscopic studies have reported a variable degree of OG survival in actively demyelinating lesions (Prineas 1975; Raine *et al*., 1981; Lassmann 1983; Prineas *et al*., 1989; Rodriguez *et al*., 1993; Brück *et al*., 1994; Ozawa *et al*., 1994; Rodriguez and Scheithauer, 1994; Lucchinetti *et al*., 1996; Storch *et al*., 1998*a*). Using new OG markers on brain biopsy and autopsy specimens from multiple sclerosis patients, we previously postulated that the degree of OG pathology may be different between early and late stages of the disease, as well as between different patients (Brück *et al*., 1994; Ozawa *et al*., 1994; Lucchinetti *et al*., 1996). When multiple lesions from a single patient were analysed, very uniform patterns of inflammation, demyelination, OG pathology, axonal loss and remyelination were found. However, between different multiple sclerosis patients, a high variability of lesional structure was observed. This variation may be due to dynamic changes during the evolution of lesions or over the course of disease. Alternatively, the broad spectrum of structural changes observed in demyelinating lesions may reflect distinct pathogenetic mechanisms operating in subsets of multiple sclerosis patients. In order to establish a dynamic pathology of multiple sclerosis lesion formation and repair, we examined the fate of OGs in brain biopsy and autopsy specimens from 113 multiple sclerosis patients and correlated OG numbers with stages of demyelination and lesion topography, as well as the extent of inflammation and axonal destruction.

Material and methods

Clinical history of patients

This study was performed on archival material of 113 cases with histologically proven inflammatory demyelination consistent with multiple sclerosis. Material was collected in the Department of Neuropathology at the Mayo Clinic (n = 48), the Neuropathological Institute at the University of Göttingen (n = 33) and the Institute of Neurology at the University of Vienna (n = 32). The inclusion criteria for cases in this study were: (i) tissue diagnosis of inflammatory demyelination confirmed by a neuropathologist (W.B., J.P., B.S. or H.L.); (ii) no clinical, radiological, serological or pathological evidence of neoplasm, infection, vascular or non-demyelinating inflammatory aetiology; (iii) no evidence of acute disseminated or haemorrhagic encephalomyelitis, defined pathologically as demyelination limited to perivenular areas (Hart and Earle, 1975); (iv) formalin-fixed paraffin embedded material; (v) sufficient tissue sample per paraffin block available for further study. The data are reported with respect to lesion areas. An individual case may have had a single lesion biopsied, although several different lesion areas may have been sampled including the periplaque white matter, areas of active demyelination or the plaque centre.

Table 1 summarizes the mean age (years), sex ratio, number of lesions and lesioned areas analysed and the distribution of lesional staging in the biopsy and autopsy cases. Detailed clinical histories were available on 95 cases, with 48 cases clinically evaluated by a Mayo neurologist. According to both neuropathological and Poser's criteria (Poser *et al*., 1983), all subtypes of multiple sclerosis were included in this study and are summarized for both autopsy and biopsy cases in Table 2. Only 10 cases had an isolated demyelinating event at last follow-up. The mean (± standard error of the mean) duration of clinical course prior to autopsy was 38 ± 8

Table 2 *Distribution of 95 inflammatory demyelinating cases (autopsies and biopsies with clinical follow-up)*

Multiple sclerosis type	Autopsy (n = 29)	Biopsy (n = 66)	Total
Acute (Marburg)	15	2	17
Balo	3	2	5
Devic's disease	1	1	2
Relapsing–remitting	3	23	26
Secondary progressive	4	14	18
Primary progressive	3	13	16
Progressive–relapsing	0	1	1
Isolated event	0	10	10
Disease duration (months; mean ± SEM)	38 ± 8 (range 0.5–384)	29 ± 3 (range 0.3–460)	–
Interval first symptom to biopsy (months)	–	20 ± 2 (range 0.3–424)	–

Balo = Balo's concentric sclerosis; Devic's disease = neuromyelitis optica.

months (range 0.5 ± 384 months), whereas the mean interval between first symptom and biopsy was 20 ± 2 months (range 0.3–424 months). Total follow-up on biopsy cases was 29 ± 3 months (range 0.3–460 months).

Neuropathological techniques and immunocytochemistry

All cases underwent detailed neuropathological examination including assessment of one to six tissue blocks per biopsy case and up to four blocks per autopsy case (Table 1).

Although up to 20 blocks per autopsy case were classified with regard to lesional activity, detailed investigations of OG pathology were restricted to one to four representative lesions per case. Paraffin-embedded 5-μm sections were stained with haematoxylin–eosin, luxol fast blue (LFB) myelin stain, periodic acid–Schiff (PAS) reaction and Bielschowsky's silver impregnation axonal stain.

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 (Vass *et al*., 1986). The primary antibody was omitted in controls. The following primary antibodies were used. *Myelin/OGs*: anti-myelin basic protein (anti-MBP; Boehringer–Mannheim, Mannheim, Germany), anti-proteolipid protein (anti-PLP; Serotec, Oxford, UK), anti-2′3′-cyclic nucleotide 3′-phosphodiesterase (anti-CNPase; Affinity Research Products, UK) and anti-myelin oligodendrocyte glycoprotein (anti-MOG) provided by Dr S. Piddlesden (University of Cardiff, UK); *inflammatory cells*: T and B cells (anti-CD3, anti-L26 and anti-human IgG; Dako, Glostrup, Denmark); *monocytes/macrophages*: anti-KiM1P (provided by Dr Radzun, University of Göttingen, Göttingen, Germany), anti-27E10, anti-MRP14, anti-25F9 and anti-MRP8 (BMA Biomedicals, Augst, Switzerland); *astrocytes*: anti-glial fibrillary acidic protein (anti-GFAP; Dako).

In situ *hybridization*

In situ hybridization was performed using digoxigenin-labelled riboprobes specific for PLP. The source and specificity of the probes, the labelling techniques and the methods of *in situ* hybridization have been described in detail previously (Breitschopf *et al*., 1992). The specificity of the reaction was controlled by comparing hybridization with antisense and sense riboprobes. Positive controls included normal white matter from rat and human brain. Following *in situ* hybridization, the sections were either counterstained with haematoxylin or processed for immunocytochemistry with anti-PLP antibodies as described above.

Lesional staging

A stringent classification scheme to determine lesional activity was applied to all lesioned areas available for study. This was performed independently by at least two of the authors (C.L., W.B. and H.L.) in each lesion with 100% inter-observer reliability. Lesional activity was determined within a plaque by studying the structural profile and chemical composition of minor (MOG) and major (PLP) myelin degradation products within macrophages, and examining the expression of inflammatory macrophage activation antigens in the lesions (MRP14, 27E10, 25F9, MRP8). According to previously published criteria (Brück *et al*., 1994; Ozawa *et al*., 1994; Brück *et al*., 1995; Lucchinetti *et al*., 1996) the following stages of demyelinating activity were defined.

Early active. These lesions were located at the plaque border between demyelinated plaques and periplaque white matter. Macrophages contained myelin-degradation products which stained with LFB and were immunoreactive for all myelin proteins including MOG. These macrophages expressed the acute-stage inflammatory macrophage markers MRP14 and 27E10.

Late active. In these lesions, myelin degradation was more advanced with macrophages containing myelin degradation products immunoreactive for the major myelin proteins MBP and PLP, but not for MOG. Macrophages still expressed the acute-stage inflammatory marker 27E10.

Inactive demyelinated. These areas showed complete demyelination. Macrophages, when present, contained either empty vacuoles or PAS-positive degradation products, and expressed the chronic-stage inflammatory macrophage marker 25F9.

Early remyelinating. These lesions were similar to inactive, demyelinated plaques, but contained small clusters of axons surrounded by very thin myelin sheaths.

Late remyelinating (shadow plaques). These lesions represented focal plaques of astrocytic gliosis and reduced myelin density. Axons were surrounded by thin myelin sheaths. Few macrophages expressing late activation markers were occasionally present.

Quantitative morphometry of labelled cells

The number of cells stained by immunocytochemistry or *in situ* hybridization per square unit of tissue was determined on serial sections. For the purposes of blinding, the pathological specimens had no identifier except their biopsy or autopsy number. In initial phases of this project, quantification of cells was performed on every tissue sample by three authors independently (C.L., W.B. and H.L.), with an inter-observer reliability of ~95%. At later stages, the reliability of quantitative results was verified by reviewing all the sections qualitatively at each of the three centres. A topographical map was established for each lesion outlining the periplaque white matter, zone of active myelin destruction, inactive plaque centre and region 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.

Determination of axonal density

In sections stained with Bielschowsky's silver impregnation, axonal density was determined by point sampling, using a 24-point Zeiss eyepiece. Measurements of all lesions were performed at an identical final magnification of $\times 800$ to guarantee comparability. Random points were superimposed on the plaques and the periplaque white matter. The number of points crossing axons was determined as a fraction of the total number of points of the stereological grid. Axonal density was compared with the periplaque white matter.

Statistical analysis

Non-parametric group tests were used to compare groups. All values are expressed as mean $\pm$ standard error of the mean.

Results

General neuropathology

Inflammatory infiltrates, mainly composed of macrophages and lymphocytes, were present to a variable degree in all tissue samples. T cells and plasma cells were present in highest numbers within perivascular inflammatory cuffs, with additional dispersion into the CNS parenchyma in most active lesions. The dominant inflammatory cells within the parenchyma of demyelinated plaques were macrophages, which, dependent upon the stage of demyelinating activity, contained different myelin degradation products and expressed different activation antigens. In addition, a variable extent of microglia activation was observed along the active plaque edge, extending into the surrounding periplaque white matter. Astrocytic gliosis was observed in most lesions and consisted of predominantly large protoplasmic astrocytes occasionally containing cytoplasmic inclusions or undergoing mitosis. Axonal density within lesions was reduced from 0 to 90% compared with the periplaque white matter. Lesions from acute Marburg cases were characterized by particularly pronounced axonal injury with multiple spheroids and necrotic areas.

Lesional activity

To draw meaningful conclusions regarding patterns of OG pathology in relation to myelin destruction, it was necessary to examine a large sample of lesions from different patients as well as multiple lesional areas in different stages of demyelination within the same multiple sclerosis plaque. The criteria for identification of active plaques remains controversial (Traugott *et al*., 1983; Traugott 1987; Sobel *et al*., 1990; Sanders *et al*., 1993; Woodroofe and Cuzner, 1993; Washington *et al*., 1994; Brück *et al*., 1995; Gay *et al*., 1997; Lassmann *et al*., 1998). In the present study we relied upon a uniform and stringent classification scheme to define lesional activity based upon the presence of myelin degradation products within macrophages and the expression of macrophage activation markers (Lassmann and Wisniewski, 1979; Brück *et al*., 1994, 1995; Lassmann *et al*., 1998). Table 1 summarizes the distribution of demyelinating activity within the 461 lesional areas. Early and late active lesional areas were found in only 40% of biopsy plaques investigated in this study, despite a preference in our material towards biopsies typically obtained within days to weeks of initial clinical presentation. These data suggest that active destruction of myelin may represent only a short phase in plaque formation and underscore the importance of stringent criteria for lesional activity in studies on disease immunopathogenesis.

Morphometric assessment of OGs

OGs were identified in both the normal appearing white matter and the lesions using two principal markers (PLP

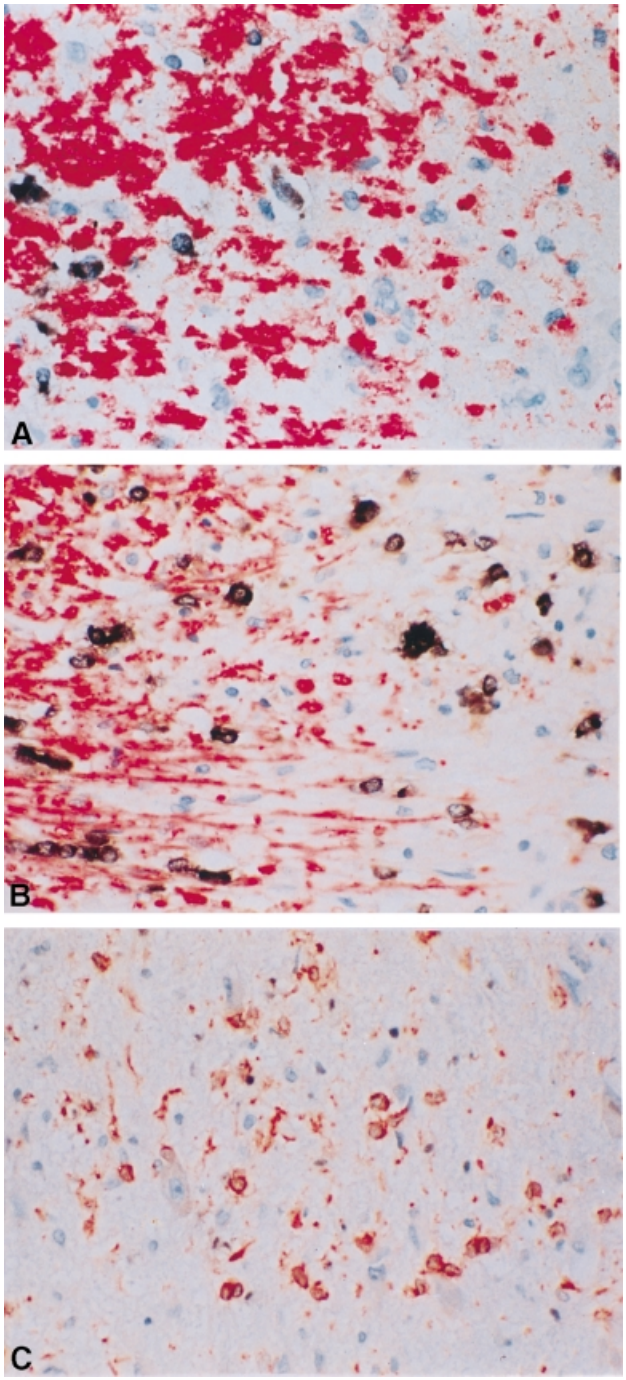

Fig. 1 Detection of OGs in multiple sclerosis plaques. (**A**) Edge of an actively demyelinating lesion stained for PLP mRNA cells (black cells) and PLP protein (red); cell nuclei are counterstained with haematoxylin (blue); OGs are present in the periplaque white matter; at the active edge there is loss of PLP mRNA-expressing cells (×400). (**B**) Edge of an actively demyelinating plaque; same staining as in **A**; note many PLP mRNA-positive OGs in periplaque white matter as well as in the plaque (×400). (**C**) MOG protein positive cells in inactive demyelinated plaque centre visualized by immunocytochemistry; many MOG-positive cells are present, some with fine cell processes (×300).

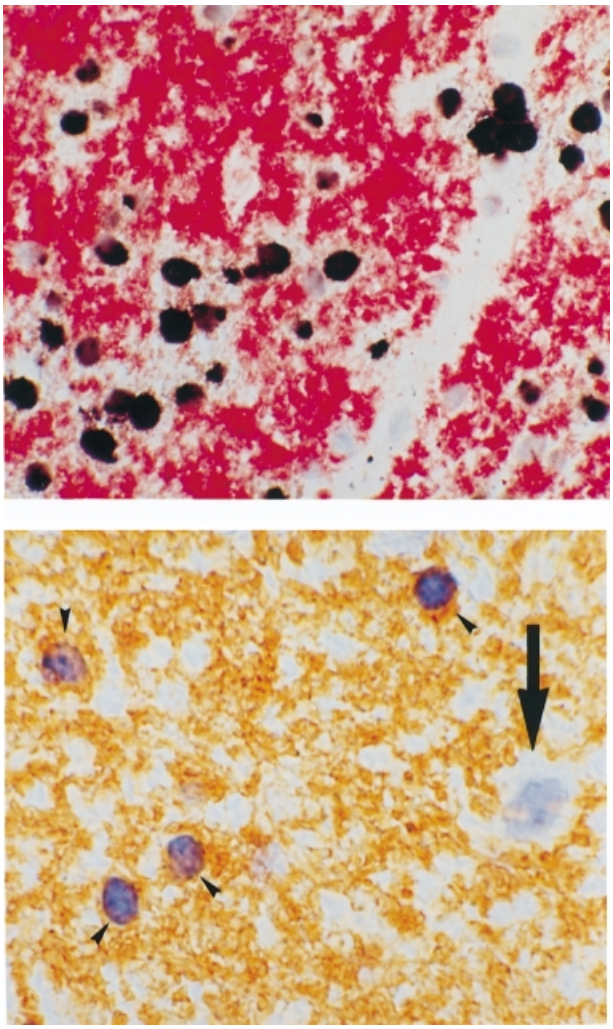

Fig. 2 Detection of OGs in normal-appearing white matter. OGs in the periplaque white matter stained for PLP mRNA (top panel, black cells; ×600) and immunocytochemistry for MOG protein (bottom panel, brown cells, indicated by arrowheads; ×800). The large arrow shows an unlabelled astrocyte for control of staining specificity.

mRNA within the cells and MOG protein on their surface; Figs 1 and 2) (Puckett *et al*., 1987; Matthieu and Amiguet, 1990). OG quantification was performed on a total of 395 lesional areas (Table 3). In order to be included in the quantitative analysis, lesional areas needed to demonstrate uniform staining with routine immunohistochemical stains (GFAP, LFB/PAS and Bielschowsky). The remaining 66 of the total 461 lesion areas were excluded for technical reasons, either because of insufficient material and/or fixation quality. This was mainly the case for blocks where *in situ* hybridization for PLP mRNA gave a weak and inconsistent staining in the normal periplaque white matter. Figure 3 illustrates the density of OGs in individual lesions at different stages of demyelinating activity. The average number of OGs in the periplaque white matter was 434 MOG-positive cells/mm^2 and 464 PLP mRNA-positive cells/mm^2. The density of OGs in multiple sclerosis lesions was variable at all stages

Table 3 *Distribution of material available for analyses*

Lesional stage	OG quantification (n = 113 cases)	OG patterns of pathology (n = 56 cases)
Early active	89	67
Late active	54	52
Remyelination	67	41
Inactive demyelination	81	63
Periplaque white matter	104	79
Total lesional areas	395	302

OG quantification was performed on a total of 395 lesional areas from 113 cases. To determine whether there were distinct patterns of OG pathology between different patients, we restricted our analysis to the 56 cases in which the longitudinal extent of the lesion was present, extending from the periplaque white matter into the active demyelinating edge and towards the plaque centre. A total of 302 lesional areas were examined from these cases. The distribution of lesional activity for these two groups is indicated.

of demyelinating activity, ranging from nearly complete loss to values exceeding those in the periplaque white matter (range 0–970 OGs/mm^2). Despite our material preferentially representing patients with early multiple sclerosis (biopsies obtained generally within weeks of clinical presentation and fulminant autopsies), some of these cases nevertheless showed pronounced OG loss. This challenges previous observations that OG loss is restricted to chronic established multiple sclerosis plaques.

Patterns of OG loss relative to lesional activity

To determine whether there are distinct patterns of OG pathology in individual patients, we examined 56 of the original 113 cases (24 autopsies and 32 biopsies), where the longitudinal extent of a lesion extending from the periplaque white matter into the active demyelinating edge and towards the inactive or remyelinating plaque centre was available. The distribution of lesional stages in these 56 cases is summarized in Table 3. Quantitative assessment of OGs across a single lesion was examined by determining the percentage increase or decrease in OG numbers relative to the periplaque white matter from that particular case. This ensured that each case acted as its own internal control, thereby limiting potential variability in OG counts which could result by comparing and combining absolute OG numbers between lesioned areas from different cases. Two major groups of OG pathology were defined by the presence (group I) or absence (group II) of OG recruitment within the lesion. In 70% (39 out of 56) of the cases, we observed OG recruitment as evidenced by increased numbers of OGs in demyelinated and/or remyelinated areas compared with the active plaque edge. However, in the remaining 30% (17 out of 56) of the cases, OG numbers in inactive or remyelinating areas were similar or even lower compared with those in their active counterparts. Within these two broad groups, the following subtypes were differentiated.

IA. OG loss in active lesions with pronounced recruitment of OGs in the plaque centre

In 23 out of 56 cases (41%), the density of MOG-labelled OGs was decreased by an average of 65% in the active region; however, there was a pronounced increase in OG-density in demyelinated or remyelinated areas (Fig. 4A). Even in some of the most destructive acute lesions characterized by widespread macrophage infiltration and demyelination paralleled with profound loss of axons, astrocytes and OGs at the active edge, a relative increase in OGs was observed in the demyelinated or remyelinated plaque centre compared with the regions of active demyelination. In these lesions, remyelination of preserved axons was regularly observed.

IB. Minimal OG loss throughout all lesional stages

In seven out of 56 cases (13%), we observed a minor reduction of MOG-labelled OGs at the active edge (average 15%) with an increase of OG densities in demyelinated or remyelinated areas in comparison with early active areas (Fig. 4B). In some cases this resulted in a higher OG density in the demyelinated plaque compared with that in the periplaque white matter.

IC. Recruitment of OGs in remyelinated areas, but absent in demyelinated regions

In nine out of 56 cases (16%) we observed an intermediate subtype where the total number of MOG-labelled OGs was decreased by an average of 60% in early active areas with comparably low OG densities in inactive demyelinated areas. However, increased densities of OGs were present in remyelinated areas (Fig. 4C).

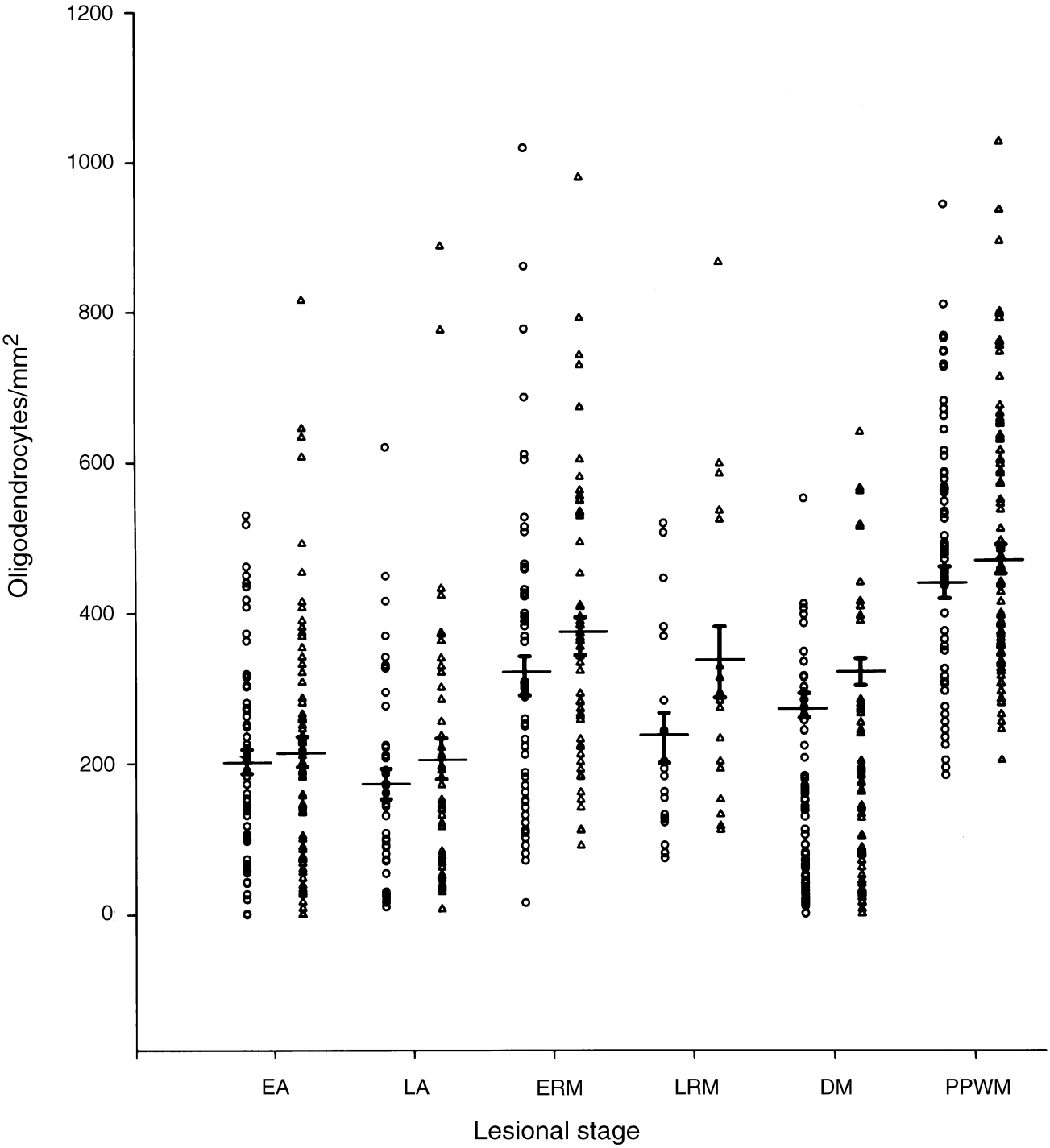

Fig. 3 OG densities in multiple sclerosis plaques. Density of MOG-labelled (circles) and PLP mRNA-labelled (triangles) OGs/mm^2 at different stages of demyelinating activity. Data expressed per group with bar showing mean ± standard error of the mean. EA = early active demyelination; LA = late active demyelination; ERM = early remyelination; LRM = late remyelination; DM = inactive; PPWM = periplaque white matter.

IIA. Absent OG recruitment with impaired remyelination

In nine out of 56 cases (16%), there was a variable, but significant reduction of MOG-labelled OGs in active lesions (average reduction 75%, range 40–100%) with no increase of OG density in the plaque centre (Fig. 4D). Regardless of the extent of OG loss in active areas, the density of OGs in inactive areas remained the same or lower compared with active regions. We found no evidence of remyelination in these plaques.

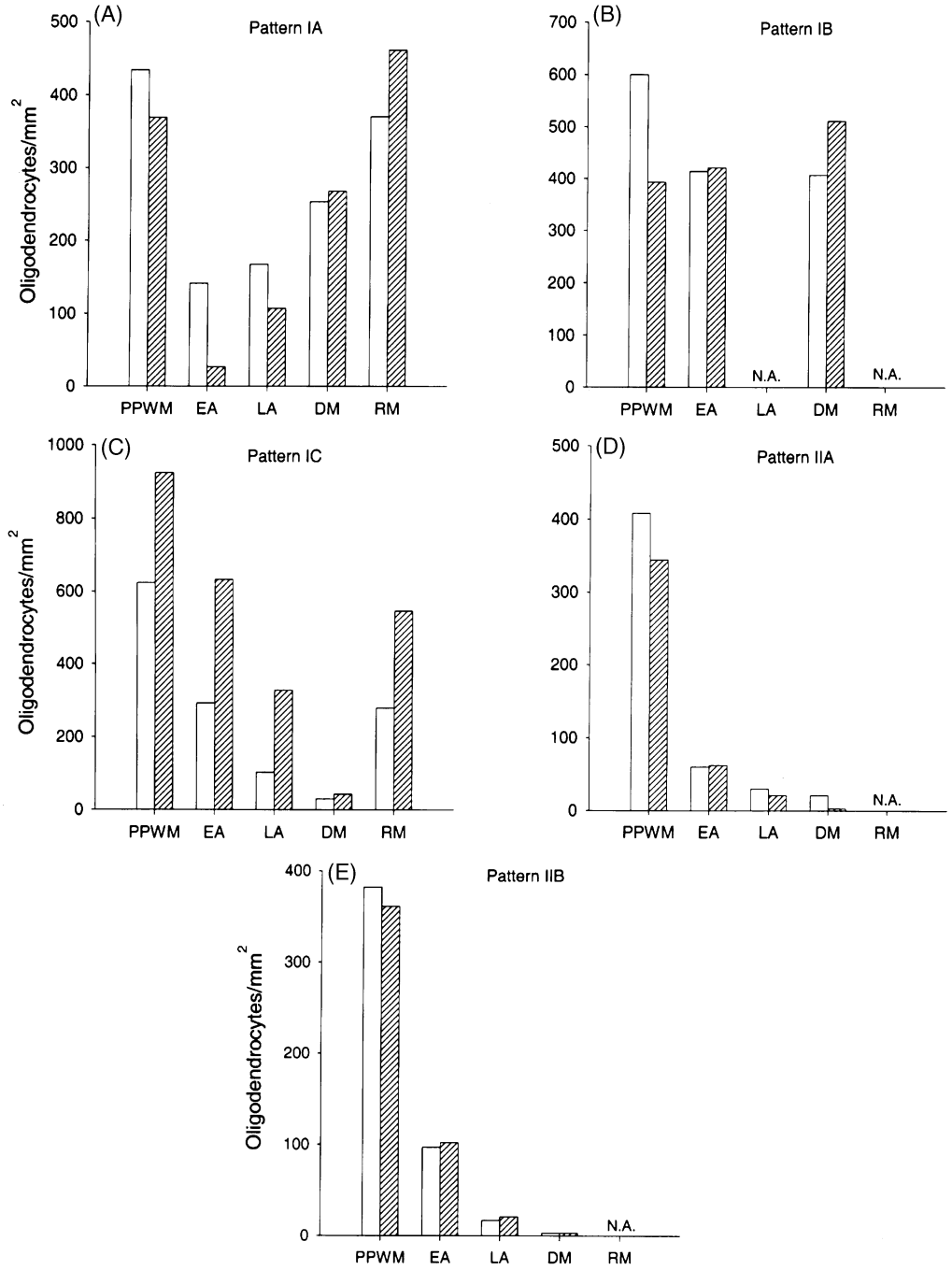

Fig. 4 Patterns of OG pathology. Density of MOG-labelled (open columns) and PLP mRNA-labelled (cross-hatched columns) OGs/mm^2 in different plaque regions from five different multiple sclerosis cases. **A** illustrates a case (pattern IA, 41% of cases) with loss of MOG- and PLP mRNA-labelled OGs in early active (EA) and late active (LA) regions compared with the periplaque white matter (PPWM). However, there is a pronounced increase in OG density in demyelinated and remyelinated regions within the plaque centre. **B** illustrates a case (pattern IB, 13% of cases) in which minimal reduction of MOG or PLP mRNA-labelled OGs is observed at different stages of demyelinating activity across a single lesion. **C** illustrates a case (pattern IC, 16% of cases) with evidence of recruitment of OGs in remyelinated areas but absent in demyelinated regions. **D** illustrates a case (pattern IIA, 16% of cases) with profound loss of both MOG- and PLP mRNA-labelled OGs throughout the lesion. **E** illustrates a case (pattern IIB, 14% of cases) with a gradient of MOG- and PLP mRNA-labelled OG loss extending from the active plaque edge towards the inactive plaque centre. NA = not available in these cases; DM = inactive demyelination; RM = remyelination.

Table 4 *Percentage of MOG and PLPmRNA-labelled OGs in lesions compared with the periplaque white matter*

Pattern OG pathology	% of MOG-labelled OG density relative to PPWM			% of PLP-labelled OG density relative to PPWM		
	Active	DM	RM	Active	DM	RM
IA	35 ± 4**	35 ± 6**	74 ± 12**	24 ± 4*	41 ± 6*	85 ± 11*
IB	85 ± 9	79 ± 23	NA	82 ± 12	110 ± 12	NA
IC	4 ± 4**	15 ± 5**	64 ± 8**	50 ± 5**	15 ± 5**	87 ± 9**
IIA	25 ± 6	20 ± 7	NA	23 ± 7	23 ± 9	NA
IIB	35 ± 3**	8 ± 3**	NA	35 ± 7**	9 ± 3**	NA

Data expressed as mean ± standard error of the mean; NA = not available; PPWM = periplaque white matter; ANOVA was performed and pair-wise comparisons between active, demyelinated (DM) or remyelinated (RM) plaque regions within each pattern were made and found to be statistically significant by Dunn's method (*) or Student–Newman–Keuls method (**). The *P*-values for these comparisons are as follows: pattern IA: EA-MOG versus RM-MOG, $P = 0.002$; RM-MOG versus DM-MOG, $P < 0.001$; EA-PLP versus RM-PLP, $P < 0.05$; RM-PLP versus DM-PLP, $P < 0.05$; pattern IC: EA-MOG versus RM-MOG, $P = 0.01$; EA-MOG versus DM-MOG, $P = 0.004$; RM-MOG versus DM-MOG, $P < 0.001$; EA-PLP versus RM-PLP, $P < 0.001$; EA-PLP versus DM-PLP, $P = 0.001$; RM-PLP versus DM-PLP, $P < 0.001$; pattern IIB: EA-MOG versus DM-MOG, $P < 0.001$; EA-PLP versus DM-PLP, $P = 0.005$.

IIB. Gradient of OG loss

In eight out of 56 cases (14%) we observed a gradual loss of OGs extending from the active plaque edge into the plaque centre, with evidence of ongoing OG death towards the plaque centre (Fig. 4E). There was no evidence of OG recruitment or remyelination.

Table 4 illustrates the density of both MOG- and PLP mRNA-labelled OGs in active, demyelinated and remyelinated regions for each pattern observed, expressed as a mean percentage (± standard error of the mean) of OGs relative to the periplaque white matter. A comparison between the patterns revealed highly significant differences. The most important differences are between groups I and II. Group I lesions demonstrate evidence of OG recruitment and remyelination, whereas group II lesions do not. Figure 5 illustrates representative topographical maps for each of these five patterns indicating the specific areas of the plaque chosen for quantification.

Subtypes of lesional pathology are uniform for individual patients

In vivo and *in vitro* experimental data suggests multiple effector mechanisms are capable of producing an inflammatory demyelinating lesion (Storch and Lassmann, 1997). Multiple effector mechanisms could occur in parallel within a single patient and/or lesion. Alternatively, a dominant immune effector mechanism could operate in different patients or subgroups of the disease. To address this possibility, we examined the pattern of OG pathology in multiple lesions from individual patients. This was possible only in autopsy cases that contained multiple active lesions or in rare cases that underwent sequential brain biopsies. In all lesions studied in a given patient, the pattern of OG pathology remained consistent. We examined two to five active lesions from each of 18 autopsies and two active lesions from a sequential biopsy obtained after a 9-month interval. Within these cases the distribution of lesional pathology was as follows: IA ($n = 7$ autopsies; 2 lesions were examined in each of four cases, 3 lesions in each of two cases and 5 lesions in one case), IB ($n = 0$), IC ($n = 3$ autopsies,1 sequential biopsy; 2 lesions examined in each of the four cases), IIA ($n = 3$ autopsies; 2 lesions examined in each case) and IIB ($n = 5$ autopsies; 2 lesions examined in one case, 3 lesions in each of three cases and 5 lesions in one case). Figure 6 illustrates the consistency of these patterns when examining different lesions from the same patient. No example was observed in which more than one pattern of pathology was present in the CNS of an individual case. However, since there were no autopsy or sequential cases demonstrating lesional pattern IB, it is impossible to determine whether a IA lesion potentially develops into a IB lesion. These findings suggest the mechanisms of OG injury in multiple sclerosis may be uniform for the individual patient, but heterogeneous between different patients.

Relationship of MOG protein versus PLP mRNA expression

To address the issue of progenitor recruitment we compared the absolute number of MOG-labelled OGs with the number of OGs expressing PLP mRNA in individual cases. MOG is a marker of terminally differentiated mature OGs (Matthieu and Amiguet, 1990) and PLP mRNA is expressed in mature as well as immature OGs engaged in myelin synthesis or maintenance (Puckett *et al*., 1987). Total OG counts were compared between the early active edge (37 cases) and the plaque centre consisting of late active, inactive and, when present, remyelinated areas (43 cases). Six of the early active cases were excluded for technical reasons (lacked either PLP mRNA or MOG staining). In 15 out of 37 (41%) cases with early active lesion areas, there was a greater reduction in PLP mRNA-positive cells compared with MOG protein expressing cells (Table 5). This relationship was reversed in 29 out of 43 (67%) inactive demyelinated or remyelinated lesions where PLP mRNA-positive cells outnumbered MOG-

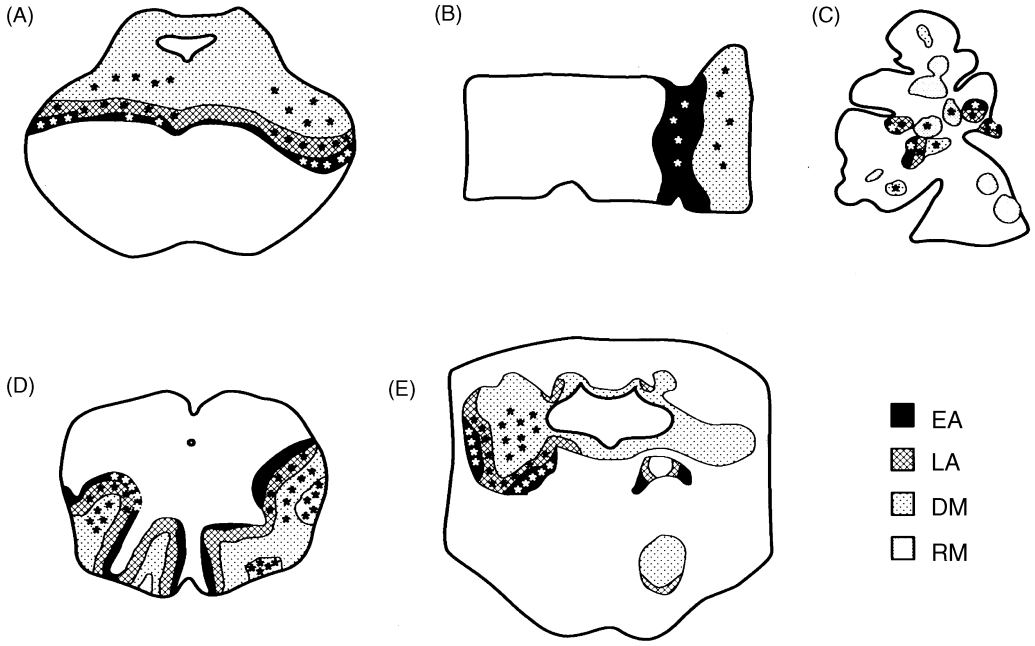

Fig. 5 Lesional maps of the five representative cases presented in Fig. 4, illustrating the topography of the lesion, areas of defined lesional activity and the specific areas chosen for quantification. The asterisks represent the areas of quantification. (**A**) An autopsy section of medulla; (**B**) a biopsy from the right parietal lobe; (**C**) an autopsy hemispheric section through frontal brain; (**D**) an autopsy section of caudal pons; (**E**) an autopsy pontine section. EA = early active demyelination; LA = late active demyelination; DM = inactive demyelination; RM = remyelination.

positive cells ($P = 0.0004$). This observation suggested that during active demyelination myelin protein synthesis in preserved OGs is impaired. In contrast, the high number of PLP mRNA-expressing cells devoid of MOG protein expression suggests that these cells may be immature, possibly being recruited from the progenitor pool.

OG loss correlates with macrophage infiltration

OGs are highly susceptible to damage by a variety of immune effector mechanisms present within an inflammatory response (Bornstein *et al*., 1961; Brosnan *et al*., 1977; Selmaj and Raine, 1988; Selmaj *et al*., 1991*a*, *b*; Griot *et al*., 1990). We examined whether the quantity and nature of the inflammatory response (T cells, plasma cells and macrophages) correlated with either the overall degree of OG survival in the lesion or the pattern of OG pathology observed (group I or II). No correlation was observed between the total number of MOG-labelled or PLP mRNA-labelled OGs in multiple sclerosis lesions with the inflammatory response consisting of T lymphocytes or plasma cells (Fig. 7A–D). No differences in mean number of T cells was observed between lesions which demonstrated OG recruitment and remyelination (groups IA–C, 147 ± 33 T cells/mm^2) and those that did not (groups IIA and B, 160 ± 43 cells/mm^2). The mean number of plasma cells/mm^2 was greater in lesions demonstrating remyelination (16 ± 5 cells/mm^2) compared with those lesions which did not (7 ± 3 cells/mm^2), but this was not statistically significant because of the small numbers. A highly significant negative correlation was observed between the mean number of macrophages/mm^2 in the lesion and the number of MOG- and PLP mRNA-labelled OGs/mm^2 (MOG: $r = –0.32$, $P < 0.0000118$; PLP mRNA: $r = –0.23$, $P < 0.00238$; Fig. 7E and F). As the number of macrophages increased within the lesion, the number of OGs decreased. Since both OG markers (MOG protein and PLP mRNA) were affected in a similar way, it is unlikely these results merely reflected functional OG impairment during the stage of active demyelination. The mean number of macrophages/mm^2 was also greater in those lesions, demonstrating a pattern of OG pathology in which remyelination and recruitment were limited (groups IIA and B, 1230 ± 148 cells/ mm^2) compared with lesions demonstrating effective recruitment and repair (groups IA–C, 987 ± 81 cells/mm^2; $P = 0.125$). The relationship between greater numbers of macrophages in lesions associated with OG loss and impaired remyelination suggests that macrophage toxins may play a major pathogenic role in OG injury (Sriram and Rodriguez, 1997).

Correlation of OG density with axonal injury

Although multiple sclerosis is considered a primary demyelinating disease, it is well recognized that a variable degree of axonal injury occurs in multiple sclerosis lesions (Ferguson *et al*., 1997; Trapp *et al*., 1998). We examined axonal density in 142 lesion areas at different stages of demyelinating activity. The overall mean reduction in axonal density in all lesion areas examined relative to the periplaque

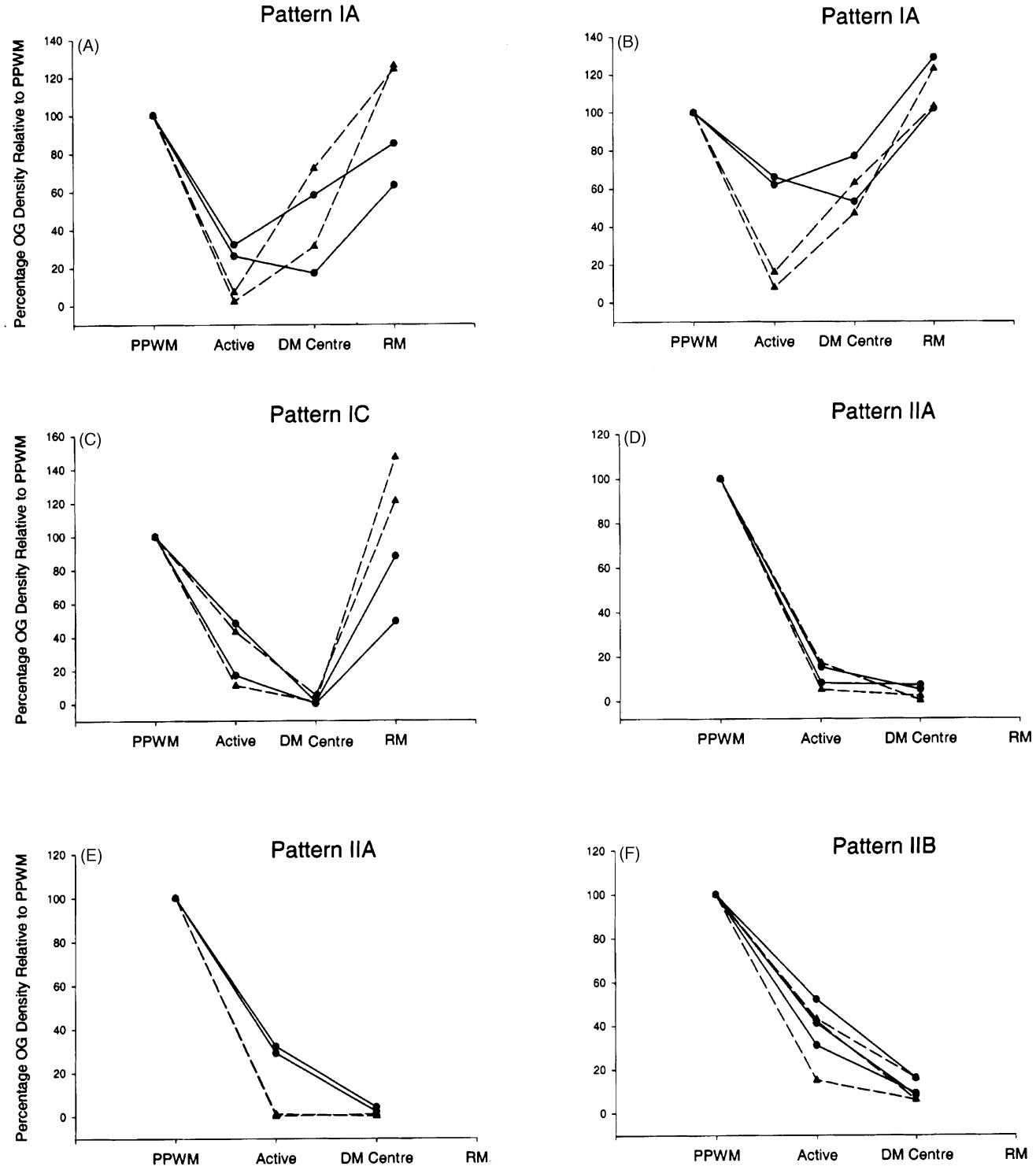

Fig. 6 Homogeneity of OG pattern. Six representative multiple sclerosis cases illustrating the density of MOG-labelled (circles) and PLP mRNA-labelled (triangles) OGs (expressed as a percentage relative to the periplaque white matter) at different stages of demyelinating activity within multiple lesions from a given case. The corresponding OG pattern of pathology is indicated for each case as defined in the manuscript. PPWM = periplaque white matter; Active = refers to actively demyelinating edge; DM centre = demyelinated inactive centre; RM = remyelinated region.

white matter was 57 ± 5% (range 0–90%). The greatest mean reduction in axonal density was observed in completely demyelinated inactive plaque regions (66 ± 2%, n = 41). Remyelinated plaque regions (n = 37) demonstrated a 49 ± 3% mean reduction in axonal density, whereas actively demyelinating regions (n = 64) demonstrated a 56 ± 2%

Table 5 *Comparison of MOG- and PLP mRNA-positive OGs at active edge and plaque centre*

Relationship of PLP mRNA to MOG	Early active edge	Plaque centre (late active + inactive + remyelinated regions)
PLP mRNA > MOG	14	29
PLP mRNA = MOG	8	12
PLP mRNA < MOG	15	2
Total cases	37	43

The comparison of OG reactivity between the early active edge and the plaque centre (consisting of late active + inactive + remyelinated regions) is highly significant by χ^2 Test (P = 0.0004).

mean reduction in axonal density. The differences in the mean values among each of these groups was statistically significant (P = 0.000123). Because we preferentially analysed lesions at the early stages of multiple sclerosis, our observations support previously published results (Ferguson *et al*., 1997; Trapp *et al*., 1998) that axonal injury may be an early event in disease pathogenesis and is not restricted to long-standing chronic multiple sclerosis lesions. Furthermore, axonal injury occurs at all stages of demyelinating activity.

Since OGs rely, in part, upon axonal contact for survival, we were also interested in determining whether OG survival versus loss was merely a function of axonal preservation or injury. No correlation was found between the extent of axonal injury and OG density (Fig. 7G and H). Even in some cases with greater than 80% reduction in axonal density compared with the periplaque white matter, OGs were present to a variable degree within the lesion. In addition, there were no statistical group differences in mean reduction in axonal density between cases that appeared effectively to recruit OGs (57 ± 2%, groups IA–C) versus those demonstrating impaired recruitment and remyelination (49 ± 5%, groups IIA and B).

Correlation of OG densities with disease duration and variants of multiple sclerosis

It is generally accepted that OGs are largely absent in multiple sclerosis lesions of long duration (Lumsden, 1970; Prineas, 1985). Although no correlation was observed between disease duration and OG survival, we were unable to address this point adequately in this study due to a bias in our material towards early multiple sclerosis cases. The low number of cases in our series with disease duration greater than 5 years precludes any definitive conclusions regarding OG loss at late stages of the disease. However, there was no difference in the duration of clinical illness before sampling in the two main pathological groups (groups I and II). The average disease duration (± standard error of the mean) in subtypes IA–C was 33 ± 6 months, compared with 25 ± 6 months in subtypes IIA and B. Table 6 illustrates the correlation of groups I and II with the clinical course in 47 of the 56 cases in which clinical follow-up was available. In cases of relapsing–remitting or secondary progressive multiple sclerosis there is a major dominance of group I lesions, whereas group II lesions are mainly found in acute or primary progressive (PP) multiple sclerosis cases. Table 7 compares the densities of OGs in inactive plaques from different variants of multiple sclerosis. The mean number of OGs was lowest in acute multiple sclerosis compared with the other variants, although this was not statistically significant.

Discussion

Our present study revealed three major results. First, OG loss within different multiple sclerosis plaques was highly variable, resulting in a spectrum of lesions ranging from complete absence of these cells to normal or even increased OG density. Secondly, the patterns of OG loss were highly heterogeneous between different multiple sclerosis patients, while being homogeneous in different active plaques of the same patient. Thirdly, the intensity of macrophage infiltration of the plaque tissue was the major correlate of OG loss within the lesions.

Pathogenetic heterogeneity

Extensive *in vitro* and *in vivo* data suggest that different toxic and immunological mechanisms can lead to myelin and/or OG injury in multiple sclerosis lesions. Consistent with these experimental observations, this study demonstrates a profound heterogeneity in the structural patterns of demyelination, remyelination, inflammation, axonal injury and OG pathology between different multiple sclerosis patients. This involves, in particular, the extent and topography of OG destruction in active lesional areas, as well as the extent of reappearance of OGs and remyelination in inactive lesions. Due to the limited number of cases it is difficult to make definitive conclusions regarding the relationship between OG pathology and clinical disease. However, group I lesions in which there was evidence of OG recruitment and extensive remyelination were typically associated with relapsing–remitting or secondary progressive multiple sclerosis, whereas group II lesions characterized by the relative loss of OGs and lack of remyelination were observed predominantly in cases of acute or PP multiple sclerosis.

A comparison of the patterns described here with those found in experimental models of inflammatory demyelination may help to unravel the underlying pathogenetic mechanisms. The most common pattern described in our study—demyelination with variable loss of OGs at active sites with increased numbers of OGs in the inactive plaque centre—is closely reflected in MOG-induced autoimmune encephalomyelitis in rodents and primates (Linington *et al*., 1992; Storch *et al*., 1998). In this model demyelination is accomplished by specific antibodies on the background of a T-cell mediated encephalitis. Since MOG is highly expressed

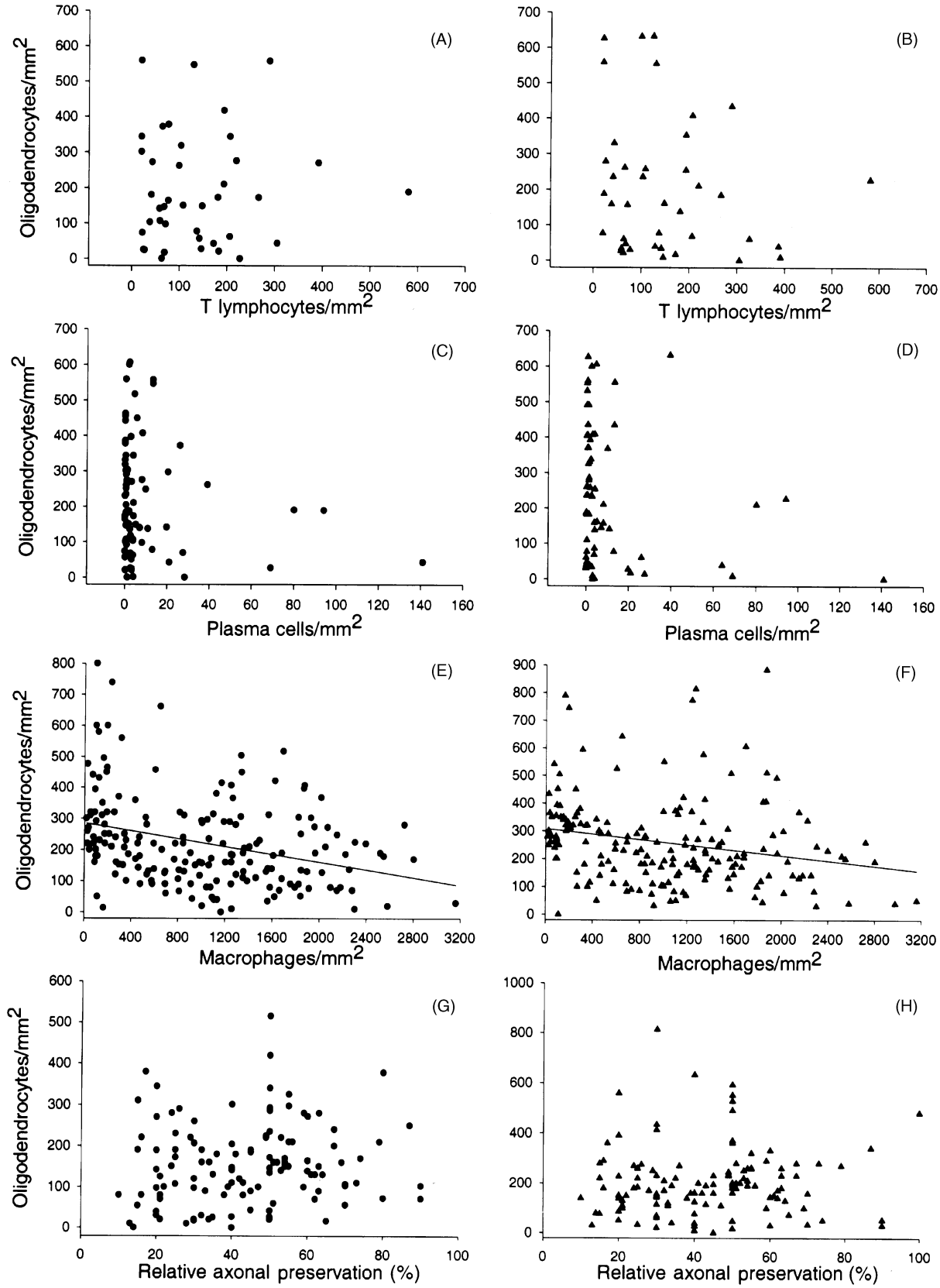

Fig. 7 Correlation of OG density with inflammation and axonal density. Correlation of OG densities/mm^2 with numbers of T lymphocytes (**A** and **B**), plasma cells (**C** and **D**), macrophages (**E** and **F**) and axonal density (**G** and **H**). A strong negative correlation was found between the density of macrophages/mm^2 and the number of MOG-labelled (filled circles; $r = -0.32$, $P < 0.0000118$) and PLP mRNA-labelled (filled triangles; $r = -0.23$; $P < 0.00238$) OGs/mm^2 within a lesion. No correlation was found between OG density and T-cell/plasma cell infiltration or axonal injury.

Table 6 *Correlation of pathological pattern with clinical course*

Multiple sclerosis variant	Group I*	Group II†
Acute multiple sclerosis	10	7
Relapsing–remitting multiple sclerosis	9	2
Secondary progressive multiple sclerosis	10	0
Primary progressive multiple sclerosis	4	4
Progressive–relapsing multiple sclerosis	1	0

*OG recruitment and remyelination; †no OG recruitment or remyelination.

Table 7 *Correlation of OG density with clinical classification of demyelinating disease*

Multiple sclerosis variant	MOG OGs	*n*	PLP mRNA	*n*
Acute	63 ± 21	9	95 ± 30	10
Isolated event	129 ± 53	6	153 ± 89	3
Relapsing–remitting	154 ± 39	16	215 ± 52	17
Secondary progressive	192 ± 48	16	190 ± 53	13
Progressive–relapsing	64 ± 45	2	14	1
Primary progressive	178 ± 59	9	183 ± 61	9
Balo	141 ± 81	4	114 ± 40	8

Numbers represent cells/mm^2 ± SEM; Balo = Balo's concentric sclerosis.

on the surface of myelin sheaths, but to a much lower extent on the surface of OGs (Brunner *et al*., 1989), the myelin sheath appears to be the primary target for destruction and OGs are lost to a variable degree, dependent upon the local concentration of anti-MOG antibodies. Furthermore, since MOG is not expressed on OG progenitor cells, this cell population is presumably unharmed during active myelin destruction. In keeping with this pathogenetic interpretation, massive deposition of immunoglobulin and complement C9 neo-antigen has been observed in a single multiple sclerosis case with a similar pattern of myelin and OG pathology (Storch *et al*., 1998). Multiple sclerosis lesions with virtual preservation of OGs may just reflect a milder form of the same pathogenetic principle. As shown in the model of MOG-EAE (experimental allergic encephalitis induced by active immunization with MOG), repeated episodes of demyelination may result in permanent sclerotic plaques with loss of OGs and limited remyelination (Linington *et al*., 1992). A similar mechanism has been suggested previously in multiple sclerosis cases. In these studies, dynamic changes in plaque pathology were related to clinical duration and revealed that in multiple sclerosis patients who died in the early stages of their disease, there was an initial loss of OGs in the most recent lesions, whereas in slightly older lesions there was a reappearance of increased OG densities associated with pronounced remyelination (Prineas, 1985; Prineas *et al*., 1989, 1993). However, low OG densities were present in inactive demyelinated plaques. These previous studies support our observations in a subgroup of multiple sclerosis lesions and may indicate that the initial insult in such lesions occurs without major OG injury; however, these cells are gradually lost at later stages of plaque development.

The situation appears to be fundamentally different in those cases where extensive or even complete loss of OGs occurs during active demyelination and no myelinating cells or remyelination are found in the inactive plaque centre (patterns IIA and B). In this case it seems likely that the OG or possibly the progenitor cells are primary targets of the disease process. Direct OG injury may occur through several different mechanisms, including activation of cytotoxic cytokines, such as tumour necrosis factor-α or lymphotoxin (Selmaj and Raine, 1988; Selmaj *et al*., 1991; Probert *et al*., 1995), through Fas-Fas-L interactions (D'Souza *et al*., 1996) or through virus (Rodriguez *et al*., 1983). Such a process may lead to OG death followed by secondary destruction of myelin sheaths. Furthermore, immature OG progenitor cells may become a target through cellular cytotoxicity (Freedman *et al*., 1991; Selmaj *et al*., 1991;) or specific antibodies (Rodriguez *et al*., 1983).

Consequences for remyelination

Our studies clearly show that remyelination during the early stages of formation of some multiple sclerosis lesions can be extensive. Furthermore, the extent of remyelination largely correlates with the presence of OGs within the lesions. This finding, at first glance, casts doubts on the practical usefulness of therapeutic strategies in multiple sclerosis aimed to stimulate remyelination in this disease. As long as cells capable of remyelination are present in the plaques, repair of myelin appears to occur spontaneously. When they are no longer present, no cell will be available that can be stimulated for remyelination. However, there may be situations in which stimulation of remyelination might be feasible and useful.

First, the presence of cells in very early stages of OG development have been identified in completely demyelinated plaques devoid of mature OGs (Wolswijk, 1997; Archelos *et al*., 1998). To what extent these cells can be stimulated to divide, repopulate the lesions and initiate remyelination must still be demonstrated. Secondly, little is known about the fate and function of preserved mature OGs in multiple sclerosis lesions. Current concepts obtained from *in vitro* studies suggest that remyelination can only be accomplished by cells which originate from an undifferentiated pool, still capable of going through one or more cycles of cell division (Targett *et al*., 1996). There is, however, limited experimental evidence to suggest that mature terminally differentiated OGs which survive demyelination are capable of dedifferentiating into proliferating remyelinating progenitor cells (Ludwin, 1984; Ludwin and Bakker, 1988). It might prove useful to determine to what extent terminally differentiated OGs that have survived acute demyelination may be stimulated by cytokines or trophic factors to become remyelinating cells. This strategy could massively expand the pool of cells available for tissue repair. Although the specific cytokines and trophic factors present within active multiple sclerosis plaques may create

an environment that facilitates dedifferentiation of mature OGs, it must also be considered that these mature cells remain useless and eventually die. Finally, in some lesions many cells express low levels of PLP mRNA in the absence of remyelination. These cells may represent dormant OG progenitors, which may require additional trophic factors to become efficient remyelinating cells. Whether this situation is comparable to the experimental lesions of Theiler's virus induced demyelination, where effective remyelination can be stimulated by immunoglobulins, remains to be determined (Miller and Rodriguez, 1995).

Correlation of OG survival with inflammation and axonal injury

An important question is to determine the mechanism of OG loss observed in multiple sclerosis lesions. Three possibilities have been addressed in this study: (i) T-cell mediated OG cytotoxicity; (ii) macrophage dependent injury; (iii) loss of axon contact or neuronal derived growth factors. We correlated the extent of OG injury with the number of T cells, plasma cells, macrophages and axons in the lesion. A correlation was only found between increased numbers of macrophages and reduced OG numbers. No correlation was observed between OGs and T cells, plasma cells or axonal density. These studies suggest that a principal effector for OG loss in some lesions may be the macrophage/microglial arm of the inflammatory response (Sriram and Rodriguez, 1997). However, in addition to secreting soluble factors which may be cytotoxic to myelin and OGs, it is increasingly recognized that microglia and macrophages produce a range of growth factors and cytokines which may promote OG proliferation, differentiation and survival (Diemel *et al*., 1998). Therefore, the complex role of the macrophage in promoting both demyelination and perhaps remyelination needs to be better defined.

In conclusion, our data support the concept of an inter-individual pathogenetic heterogeneity of OG responses in multiple sclerosis lesions. The variable extent of OG survival and degree of remyelination observed in active demyelinating lesions suggests that in subsets of multiple sclerosis patients, myelin, mature OGs and possibly OG progenitors are differentially affected. Multiple sclerosis may therefore be a syndrome with multiple immunopathological mechanisms which may merge into a final common pathway of CNS injury. If this hypothesis is proven true, than it raises major challenges for the development and evaluation of future therapeutic strategies targeted toward specific subgroups of multiple sclerosis patients. Future studies need to define the specific immune effector mechanisms operating in these lesions and determine whether they are related to the specific patterns of OG pathology we have identified. In addition, a defined clinical–radiological–pathological correlation of these different patterns is necessary, since unique subtypes of the disease may ultimately require different therapeutic regimens.

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