Relapsing and Remitting Multiple Sclerosis: Pathology of the Newly Forming Lesion

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The study describes the clinical and pathological findings in 12 patients with relapsing and remitting multiple sclerosis, who died during or shortly after the onset of a relapse. Pathological changes not previously associated with the formation of new symptomatic lesions were observed in seven cases, namely, extensive oligodendrocyte apoptosis and microglial activation in myelinated tissue containing few or no lymphocytes or myelin phagocytes. No current laboratory model of multiple sclerosis, in particular, experimental allergic encephalomyelitis, is known with these features, which raises the possibility of some novel process underlying new lesion formation in multiple sclerosis.

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The pathological changes associated with the formation of new symptomatic lesions in patients with relapsing and remitting multiple sclerosis (MS) have remained largely unknown, because such lesions are rarely fatal. Among previous reports of newly forming lesions of known clinical duration, the earliest appear to be two "7-day" brainstem lesions reported by Adams and colleagues in 1989.[1] Such "acute" lesions generally are described as actively demyelinating lesions in which myelin phagocytes directly engage normal-appearing myelin sheaths in the presence of infiltrating T cells. Because similar changes are observed in some forms of experimental allergic encephalomyelitis (EAE), this has encouraged the view that tissue destruction in MS is the result of a T-cell–mediated cellular immune response directed against myelin. This study describes the clinical and pathological findings in a young patient with relapsing and remitting MS who died within 24 hours of the onset of a new symptomatic brainstem lesion. In view of the unusual findings, we reviewed tissue obtained at autopsy from other patients with rapidly deteriorating MS. Nine lesions were found that were essentially identical to the fatal brainstem lesion in the first case. The earliest structural change shared by all 10 lesions was extensive oligodendrocyte apoptosis in tissue exhibiting early microglial activation but few or no infiltrating lymphocytes or myelin phagocytes. This is unlike any current laboratory model of the disease, in particular, experimental allergic encephalomyelitis, which raises the possibility of some novel disease process underlying lesion formation in relapsing and remitting MS.

Patients and Methods

Patients

The clinical and pathological findings in the most acute case (Case 1) are described below. Tissue from 11 other patients with early MS (6 with new lesions similar to the fatal lesion in Case 1 [Table 1] and 5 without such lesions) and 6 patients with long-standing MS selected from an archival bank of 307 MS cases also was examined. Death in the early MS group was caused by rapidly worsening disease or by isolated brainstem or upper spinal cord lesions.

No biopsy cases or cases of Devic's disease or acute disseminated encephalomyelitis were included in the study.

Histology

Six-micrometer-thick paraffin sections were stained using hematoxylin and eosin, or for myelin using Luxol fast blue (LFB) periodic acid–Schiff. Bodian's silver stain or a silver stain[2] combined with Luxol fast blue were used to stain axons. To determine cell densities in a particular section, we examined 10 fields, each 0.1mm^2, using a calibrated eyepiece reticule and a $\times 40$ objective. Results are shown as a mean $\pm$ SEM.

Immunocytochemistry

Deparaffinized 3 to 6μm-thick sections on poly-L-lysine–coated slides were immunostained using primary antibodies listed in Table 2. Binding of biotinylated second antibody was visualized using avidin-biotin horseradish peroxidase complex (Vector ABC elite kit; Vector, Burlingame, CA) or a labeled streptavidin biotin system (LSAB+ kit; Dako Corporation, Carpinteria, CA) and 3,3′-diaminobenzidine as chromogen. Nickel sulphate was used to intensify staining on occasion.[3] Hematoxylin or nuclear-fast red was used as a nu-

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Table 1. Clinical and Pathological Characteristics: Cases with "Apoptotic" Lesions

Case	Sex	Age (yr)	Disease Duration	Duration of Fatal Episode	Discrete Attacks	No. of Apoptotic Lesions	Remyelinating Shadow Plaques
1	F	14	9 mo	<24 hr	4	1	+
2	M	14	18 days	18 days	1	1	+
3	F	31	3 mo[a]	7 days	2	1	+
4	M	25	29 days	29 days	1	2	+
5	F	23	30 days	60 hr	3	2	+
6	F	20	28 days	28 days	1	1	+
7	F	40	12 yr[b]	42 days	2	2	?[c]

Several of the cases have been the subject of previous reports (Cases 3 and 6[1]; Case 4[64]; and Case 7[65]).

[a]Optic neuritis 3 months before death.
[b]Optic neuritis at age 26 years.
[c]Cerebral hemispheres and brainstem only available for study.

clear counterstain. Sections were reacted with primary antisera for periods ranging from 30 minutes at 37°C to 3 days at 4°C, according to the detection kit and antibodies used. Negative controls included substitution of primary antibodies of other specificities, the use of nonimmune immunoglobulin (Ig) of the same isotype applied at approximately the same concentration, and omission of the primary antibody. Renal biopsies from patients with glomerulonephritis

Table 2. Primary Antibodies Used for Immunocytochemistry

Antigen	Clone	Dilution	Source
Macrophage/microglia			
RCA-1 lectin		1:4,000	Vector, Burlingame, CA
LCA (CD45)	PD7–26, 2B11	1:200[a]	Dako, Carpinteria, CA
MRP-14	BMA-S36,48	1:25[a]	Accurate Chemical and Scientific Corp, Westbury, NY
MAC-1 (macrophages, monocytes, endothelial cells)	HAM-56	1:1,000[b]	Dako, Carpinteria, CA
Complement			
C3	Polyclonal	1:15[a]	Biogenex, San Ramon, CA
C3d (opsonin)[c]	Polyclonal	1:2,000[a]	Dako, Carpinteria, CA
C9neo (MAC)[c]	B7	1:20[b]	B.P. Morgan, Cardiff, UK
Serum proteins			
IgG	Polyclonal	1:3,200[a]	Organon Teknika, West Chester, PA
		1:8,000[b]	Dako, Carpinteria, CA
IgM	Polyclonal	1:8,000[b]	Dako, Carpinteria, CA
κ light chains	A8B5	1:200[b]	Dako, Carpinteria, CA
λ light chains	N10/2	1:200[b]	Dako, Carpinteria, CA
Myelin/oligodendrocytes			
MBP (myelin)	Polyclonal	1:500[a]	Dako, Carpinteria, CA
CNP (oligodendrocytes, myelin)[c]	Polyclonal	1:6,400[a]	Dako, Carpinteria, CA
MOG (oligodendrocytes, myelin)	Y10	1:50[a]	S. Piddlesen, Cardiff, UK
	Z12	1:100[a]	S. Piddlesen, Cardiff, UK
HNK-1(CD57) (oligodendrocytes, myelin)	Leu-7	1:8[a]	Becton Dickinson, San Jose, CA
Other cell markers			
GFAP (astrocytes)	Polyclonal	1:2,500[a]	Dako, Carpinteria, CA
CD3 (T lymphocytes)	CD3–12	1:100[a]	Serotec, Oxford, UK
CD45RO (activated T cells)	UCHL1	1:150[a]	Novocastra, Newcastle-Upon-Tyne, UK
	OPD4	1:50[a]	Dako, Carpinteria, CA
PCNA	PC10	1:80	Dako, Carpinteria, CA
Activated caspase-3	Polyclonal	1:200[a]	R&D Systems, Minneapolis, MN

[a]Microwave antigen retrieval in a citric acid–based retrieval solution.
[b]Proteinase K predigestion (Dako, Carpinteria, CA) for 5–20 minutes at 37°C.
[c]Procedure done twice.

RCA -1 = ricinus communis agglutinin-1; LCA = leukocyte common antigen; MRP-14 = 14kDa subunit of human macrophage migration inhibitory factor; MAC = membrane attack complex; MBP = myelin basic protein; CNP = 2′, 3′-cyclic nucleotide 3′-phosphodiesterase; MOG = myelin oligodendrocyte glycoprotein; HNK = human natural killer cell antigen NK1; GFAP = glial fibrillary acidic protein; PCNA = proliferating cell nuclear antigen.

and autopsy brain tissue from patients with Alzheimer's disease were used as positive staining controls for C3, C3d and C5b-9.[4] Sections of breast adenocarcinoma were used as a positive staining control for activated caspase 3.[5]

Case History

A 14-year-old white female presented 9 months before death with paraesthesiae affecting the left leg and trunk to the level of the breast. Symptoms resolved without sequaelae over the course of 3 weeks. A second attack occurred 5 months before death and consisted of numbness of the right face and tip of tongue, and an unsteady gait. The symptoms again resolved over 3 weeks. It was noted in both of these attacks that symptoms peaked within 48 hours of onset. Sixteen and a half weeks before death, a third discrete episode, consisting of paraesthesiae of the right neck and upper limb, occurred.

The fourth and final attack began 1 week after a mild upper respiratory tract infection. The patient awoke with severe vertigo and nausea. Later the same day, she began coughing pink frothy sputum and was admitted to hospital with signs of acute pulmonary edema. She was alert and oriented, with no abnormality detected on limited neurological examination. Despite treatment, including hydrocortisone 100mg intravenously, the patient suffered a cardiac arrest and was pronounced dead 17 hours after onset of this exacerbation.

Autopsy examination 13 hours after death confirmed the presence of pulmonary edema. Gross inspection of the fixed brain, brainstem, and spinal cord, sectioned serially in a coronal plane, showed four discrete lesions noted as patches of gray discoloration, 6 to 16mm in diameter, in the cerebral hemispheres, but none elsewhere.

Results

Case 1

Myelin-stained sections from 21 blocks of tissue sampled (cerebrum 9, brainstem and cerebellum 5, spinal cord 7) showed a total of 12 lesions: 6 remyelinating shadow plaques, 1 remyelinating shadow plaque with edge activity, and 5 actively demyelinating lesions. The latter were defined as demyelinated or partially demyelinated lesions infiltrated by macrophages containing LFB-positive particles of myelin. Seven of the 12 lesions were located in the cerebrum and five in the brainstem, with none in the optic nerves or chiasm, cerebellum, or spinal cord below C1 (Fig 1).

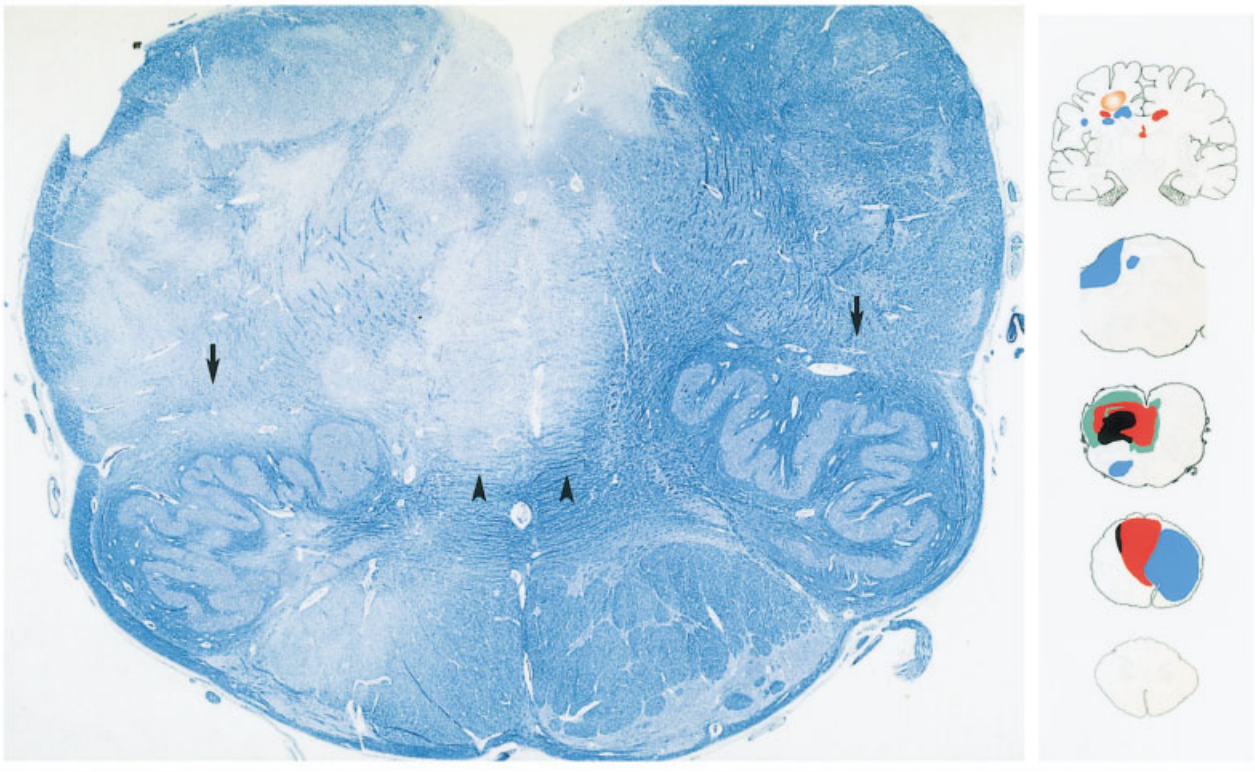

Fig 1. The 17-hour lesion at the level of the lower medulla. A patchy reduction in myelin density involves midline structures and most of the section above the left inferior olivary nucleus. In no area is myelin loss complete. The pale area on the left toward the bottom of the section is an old remyelinated shadow plaque. The areas indicated by arrowheads and arrows are shown at higher magnification in Figures 2 and 3. The diagram shows the location of this and the 11 other lesions found at autopsy in Case 1. (left) Luxol fast blue periodic acid–Schiff, magnification ×7. (right) The colors indicate the predominant tissue alteration. Red = active myelin phagocytosis; green = prephagocytic myelin vacuolation and/or dissolution; black = oligodendrocyte apoptosis; blue = remyelination.

The only abnormality detected in tissue not at the site of a plaque was the presence of occasional perivascular cuffs of mononuclear cells scattered in hemispheric white matter, especially close to the lateral ventricles and at the corticomedullary junction, with rare cuffs also present in the subarachnoid space. No hint of myelin breakdown was discernible in adjoining tissues, and the vascular endothelium appeared normal.

The 17-Hour Lesion

As in previous reports of patients with MS and neurogenic pulmonary edema,[6–9] the lesion, which was more than 14mm in length, extended from the dorsomedial region of the lower medulla to involve the first cervical segment of the spinal cord.

The most striking feature apparent in myelin-stained sections was that there was relatively little loss of myelin throughout the lesion (see Fig 1). In broad areas within and extending from the lesion, myelin sheaths showed only a slight reduction in staining intensity, whereas all normal appearing oligodendrocytes were replaced by apoptotic oligodendrocytes, that is, oligodendrocytes with shrunken nuclei and annular or compact condensation of nuclear chromatin. Thirty percent of the affected cells showed commencing nuclear fragmentation ("nuclear body" formation) with approximately 10% showing condensed cytoplasm and rounding up of the cell body, both features typically associated with apoptotic cell death. Only 2% of the apoptotic oligodendrocytes appeared to be immunoreactive for activated caspase 3. Ramified HAM-56–negative microglia showing signs of very early activation (thickened processes) were present in increased numbers, but macrophages, T cells, MRP-14–positive mononuclear cells and enlarged astrocytes were absent in such areas (Figs 2–4). Using differential interference contrast microscopy to optically section cells, we occasionally observed microglia embracing or apparently endocytosing apoptotic oligodendrocyte nuclei and nuclear fragments, that is, appearances strongly suggestive of phagocytosis (Fig 5). No similar engagement of apoptotic nuclei by any other cell type, including astrocytes, was observed.

The remainder of the lesion consisted of adjoining patches of vacuolated tissue lightly infiltrated by large amoeboid prephagocytic HAM-56–positive cells (Fig 6) or areas, some perivascular, of commencing phagocytosis of fragmenting vacuolated myelin sheaths by HAM-56–positive macrophages containing LFB-positive particles of myelin (Fig 7). No oligodendrocytes were visible in these areas, and cells of any description with apoptotic nuclei were rare or absent. Low numbers of large, strongly HNK-1–positive and faintly 2′3′-cyclic nucleotide 3′-phosphodiesterase (CNP)-positive cells resembling oligodendrocyte precursor cells[10,11] were, however, present together with myelin phagocytes in areas of active demyelination (Fig 8), and enlarged oligodendrocytes with enhanced CNP immunoreactivity were present in narrow bands of normally myelinated tissue lying between some vacuolated areas and areas of commencing phagocytosis. Several such "lines" of intact myelin, identical to those seen in Baló's concentric sclerosis,[12] can be seen in Figure 1 toward the top left of the lesion.

In contrast with areas exhibiting conspicuous oligodendrocyte apoptosis, CD4, CD8, and particularly CD45RO-positive T cells were observed among macrophages in areas of commencing phagocytosis and in perivascular cuffs within and close to such areas.

No extensive zones of oligodendrocyte apoptosis were observed in any of the other actively demyelinating lesions in this patient, although scattered apoptotic oligodendrocytes were sometimes present in white matter immediately bordering such lesions. Regenerative activity also was more advanced, with often numerous oligodendrocytes present in deeper parts of such lesions.

"Apoptotic" Lesions in Other Patients

Nine additional lesions similar to the 17-hour lesion in Case 1 were identified in six other cases with clinically active disease who died shortly after onset of the disease (Cases 2–7, see Table 1). These lesions formed a very small fraction of the total number of actively demyelinating lesions examined. No "apoptotic" lesions were found in the six patients with chronic MS included in the study, although rare apoptotic nuclei were observed in bordering white matter (counts based on one to four plaques in each case of chronic MS showed a density of apoptotic nuclei of $6.5 \pm 3.3/mm^2$ within 0.5mm of the plaque margin, and $3.5 \pm 1.7/mm^2$ 2mm away from the plaque margin).

Except for a very large lesion in one cerebral hemisphere in Case 7, the lesions were all relatively small (2–10mm in diameter), with curved or scalloped borders and usually no obvious perivascular relationship. Broad areas of tissue were present in which most or all oligodendrocytes appeared apoptotic in the presence of microglia with activated morphology (Figs 9 and 10). Cells containing LFB-positive debris were rare or absent, astrocytes were inconspicuous, myelin sheaths stained palely but were otherwise largely intact, and T cells and MRP-14 monocytes were rare or absent. As an example, in one of the two apoptotic lesions present in Case 4, T cells numbered $3.6 \pm 4.7/mm^2$ in the apoptotic lesion and $71 \pm 28/mm^2$ in an adjacent zone of active demyelination. As in Case 1, few apoptotic cells reacted positively for activated caspase 3.

Where apoptotic areas formed part of more complex lesions, adjacent areas of vacuolated tissue devoid of oligodendrocytes were present, as well as areas of active myelin phagocytosis and oligodendrocyte regeneration identical to those observed in Case 1. Other common features included the presence of 'lines' of preserved my-

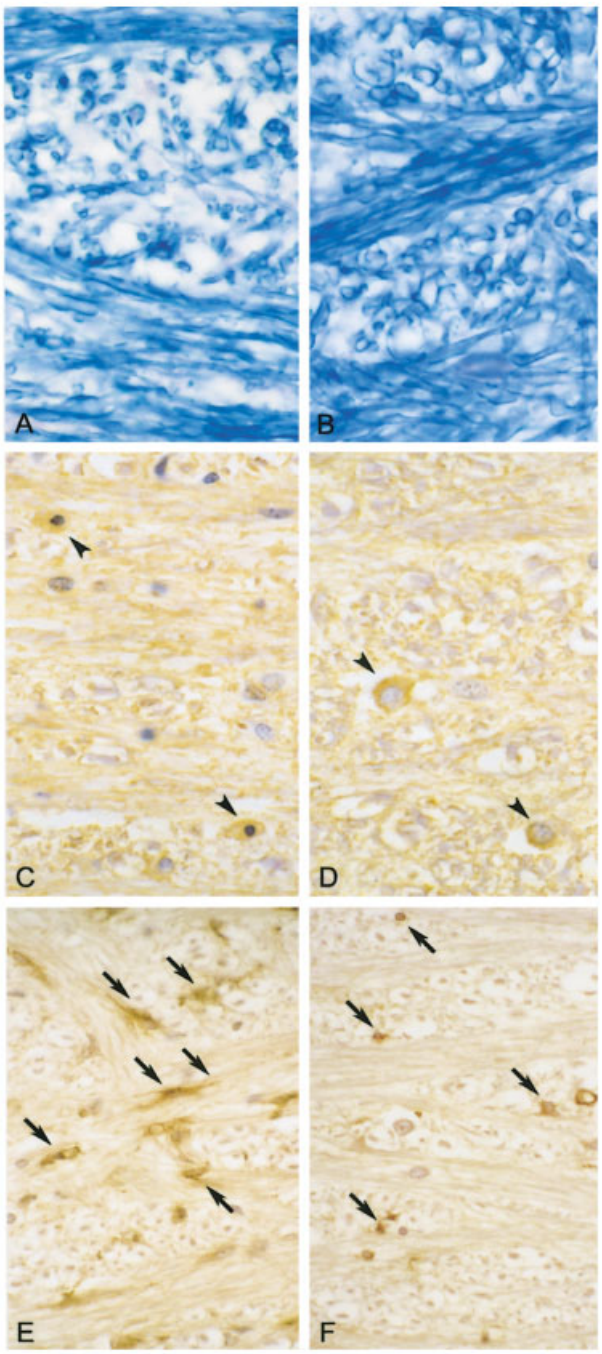

Fig 2. Typical early "prephagocytic" white matter changes in the 17-hour lesion. (A, C, E) Area within the lesion indicated by left arrowhead in Figure 1. (B, D, F) Corresponding unaffected area indicated by right arrowhead in Figure 1. (A, B) Affected tissue within the lesion appears vacuolated and paler than normal when stained for myelin. (C, D) Oligodendrocytes (arrowheads) *in unaffected tissue appear normal, with typical open nuclei. In contrast, the nuclei of oligodendrocytes within the lesion are markedly reduced in volume and show extreme compaction of nuclear chromatin. (E, F) Ramified microglia* (arrows) *are enlarged and increased in number within the lesion. (A, B) Luxol fast blue periodic acid–Schiff. Magnification ×400. (C, D) Immunostained for HNK-1. Magnification ×400. (E, F) RCA-1 lectin. Magnification ×330.*

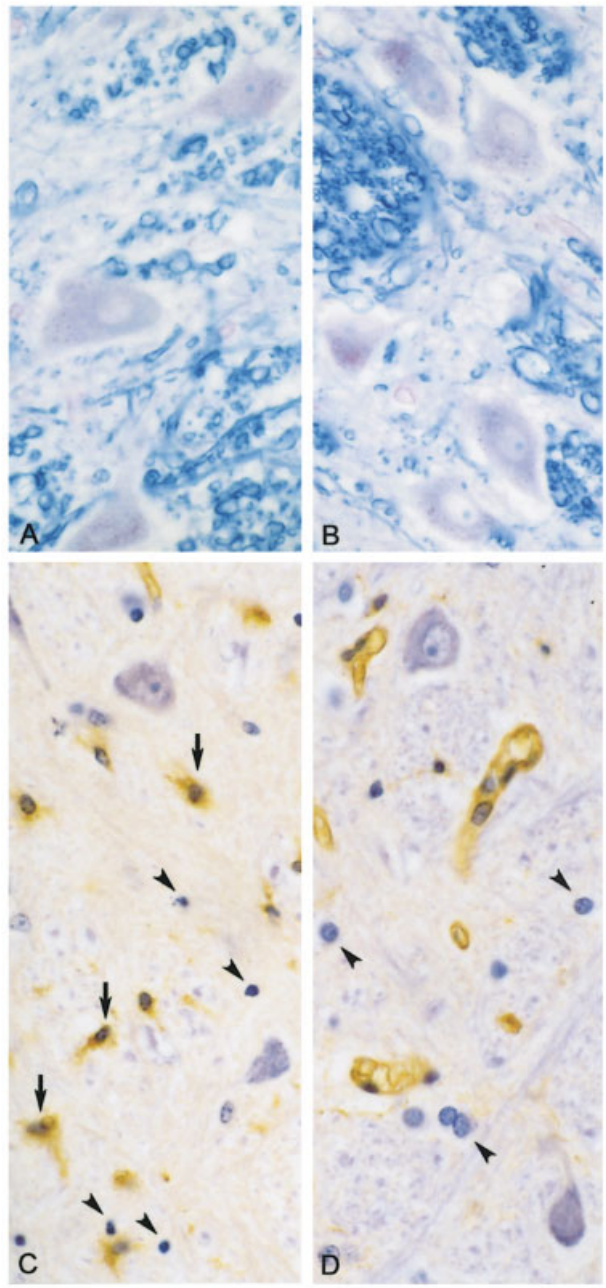

*Fig 3. Typical early "prephagocytic" changes in gray matter located within the 17-hour lesion. (A, C) A nucleus located within the lesion approximately 1.5mm above the middle of the left inferior olivary nucleus (*left arrow, *Fig 1). (B, D) The corresponding nucleus on the unaffected side (*right arrow, *Fig 1). The changes present are the same as those seen in affected white matter, namely, myelin pallor (A, B) together with oligodendrocyte apoptosis and microglial activation in the absence of myelin phagocytes (C, D). Arrowheads indicate oligodendrocytes; arrows indicate RCA-1 lectin-positive enlarged microglia. Neurons are unaffected. (A, B) Luxol fast blue periodic acid–Schiff. Magnification ×400. (C, D) RCA-1 lectin. Magnification ×400.*

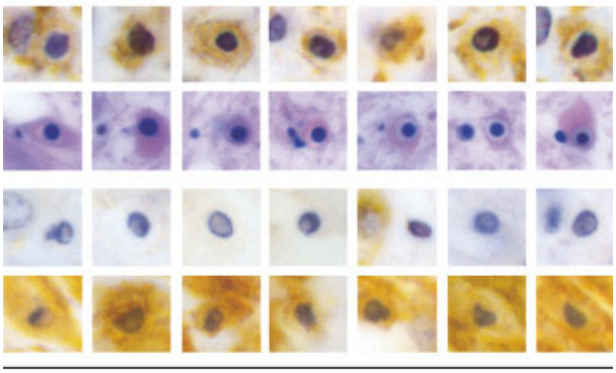

Fig 4. Morphology of apoptotic oligodendrocyte nuclei in prephagocytic areas. All show a reduction in nuclear volume together with condensed chromatin. Annular chromatin condensation is best seen in cells with unstained cytoplasm (third row from top). Fragmenting nuclei with extruded "nuclear bodies" in cells with condensed cytoplasm are shown in second row from top. (first row) Case 1 immunostained for the oligodendrocyte marker CNP. (second row) Case 1 stained with hematoxylin and eosin. (third row) Case 4 immunostained from the leucocyte and microglial marker CD45. (fourth row) Case 4 immunostained for CNP. Magnification ×756.

elin and oligodendrocytes in three of the nine apoptotic lesions, and the presence of remyelinating shadow plaques in all but one case (see Table 1). In Case 5, relapses followed by improvement occurring 4 weeks and 2 weeks before death could be linked to two small, discrete pontine lesions exhibiting extensive new myelin formation.

In the five acute MS cases without apoptotic lesions, occasional apoptotic oligodendrocytes were identified in tissue bordering some fresh lesions. Several typical

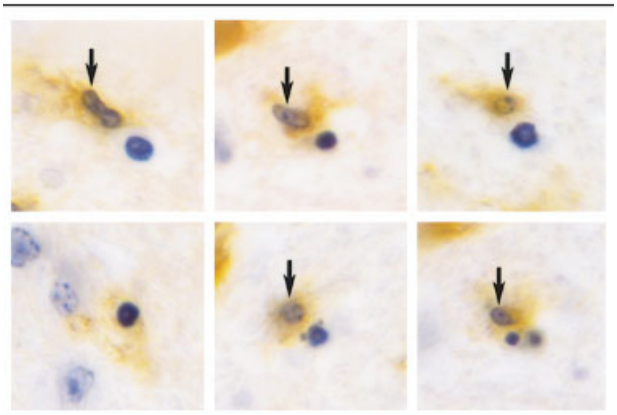

Fig 5. Phagocytosis of apoptotic oligodendrocyte nuclei and nuclear bodies by activated microglia in sections immunostained for the microglial marker RCA-1 lectin. Microglial nuclei (arrows) *appear small because of their orientation and the use of differential interference contrast microscopy to optically section cells located in the same optical plane. RCA-1 lectin. Magnification ×756.*

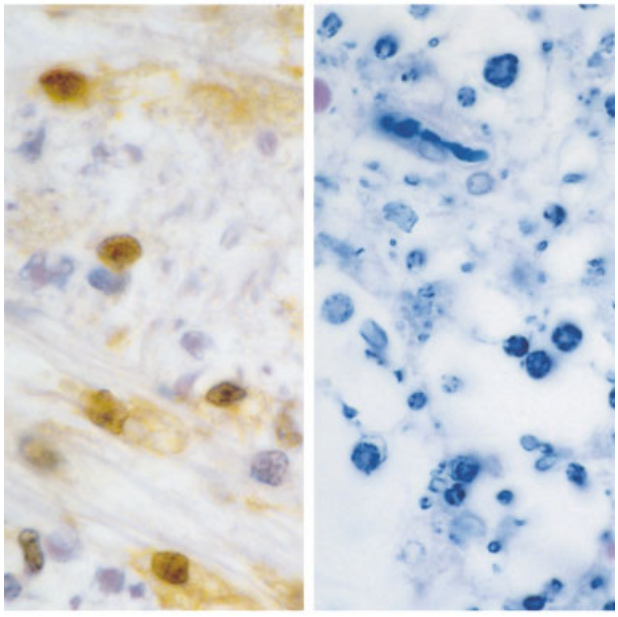

Fig 6. The second type of prephagocytic change identified in the 17-hour lesion and in other newly forming lesions, namely, vacuolation and/or smudging of myelin sheaths in tissue lightly infiltrated by proliferating microglia. Oligodendrocytes and apoptotic nuclei were rare or absent in such areas. (left) Case 1, showing nuclei of microglia staining positively for PCNA (brown); myelin sheaths stain faintly with hematoxylin (blue). (right) vacuolated tissue in a newly forming spinal cord lesion stained for myelin using Luxol fast blue periodic acid–Schiff. Magnification left ×630, right ×400.

vacuolated zones lacking oligodendrocytes and with the same myelin sheath changes as those observed in Case 1 were identified, however, bordering what appeared to be typical actively demyelinating lesions. Remyelinating shadow plaques were present in three of the five cases.

Although axonal interruption was observed and swollen axons were common in subacute lesions, axonal changes in general were relatively inconspicuous in newly forming lesions in the 12 acute MS cases studied.

Immunoglobulins and Complement

IgG was detected in macrophages, axons, astrocytes, some normal oligodendrocytes and neurons within, and in tissue bordering, all apoptotic and actively demyelinating lesions, that is, a distribution consistent with simple diffusion of a serum protein from an open blood–brain barrier. The only exceptions, possibly indicative of antigen–antibody complex, were unidentified periodic myelin-associated structures of stereotyped appearance which disappeared together with myelin loss at plaque margins (Fig 11). Although normal myelin sheaths, apoptotic oligodendrocytes, and altered myelin usually showed no unequivocal enhanced immunoreactivity for IgG, all forms of altered myelin within lesions, including palely staining myelin sheaths in apoptotic lesions,

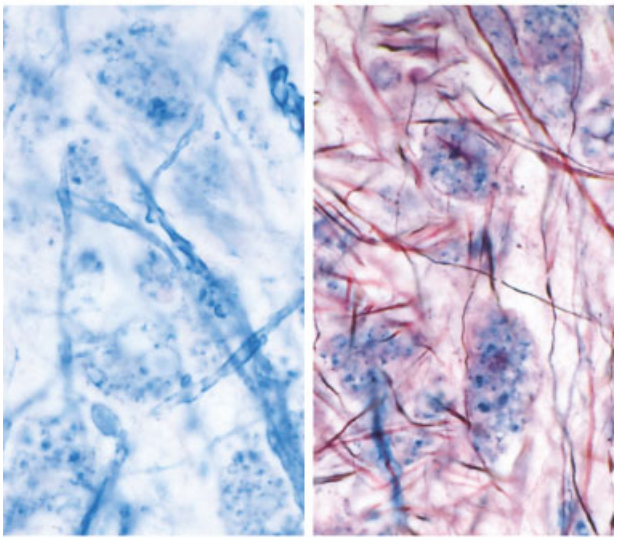

Fig 7. Myelin phagocytosis in an area adjacent to that illustrated on the left in Figure 6. (left) Luxol fast blue periodic acid–Schiff. The linear profiles are remnants of sheaths still attached to axons, the granules particles of myelin located within macrophages. (right) Luxol fast blue periodic acid–Schiff and a silver stain for axons showing numerous normal appearing axons located among macrophages containing particles of myelin. Magnification ×400.

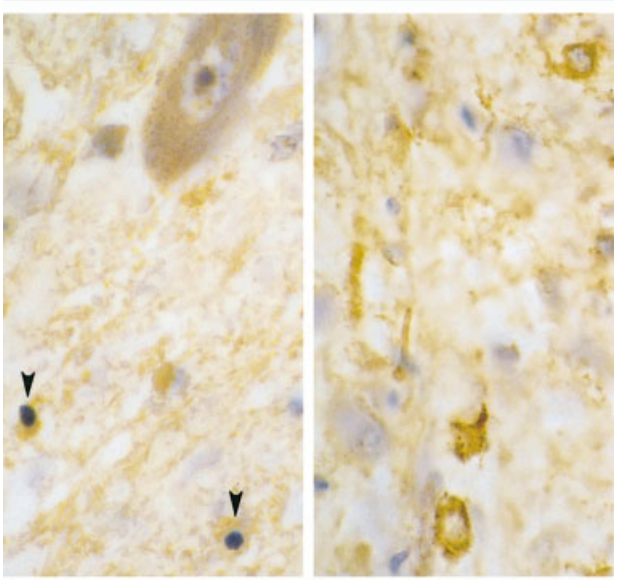

Fig 8. Oligodendrocyte degeneration and regeneration within the 17-hour lesion. These two fields lay approximately 3mm apart. (left) Apoptotic oligodendrocytes (arrowheads) *within the zone of apoptotic oligodendrocytes. (right) Large strongly HNK-1–positive cells that resemble oligodendrocyte precursors are present in a zone of advancing phagocytosis. These cells, unlike normal or newly generated oligodendrocytes, stain only faintly for CNP and have been shown to express markers specific for oligodendrocyte precursors.[10] Immunostained for the HNK-1 epitope. Magnification ×400.*

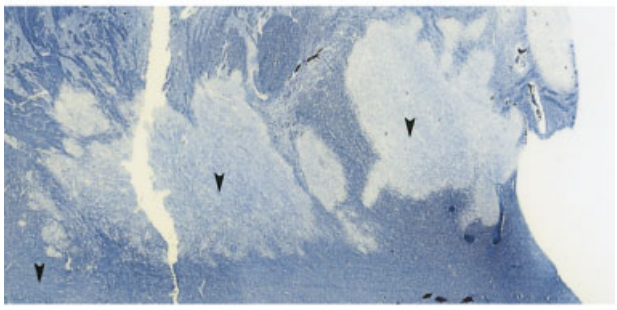

Fig 9. Case 3. A newly forming apoptotic lesion in the pons in which a broad zone of apoptotic oligodendrocytes (middle arrowhead) *lies adjacent to a vacuolated zone of commencing myelin phagocytosis in tissue largely devoid of oligodendrocytes* (right arrowhead). *Areas indicated by the arrowheads are shown at higher magnification in Figure 10. Luxol fast blue period acid–Schiff. Magnification ×5.4.*

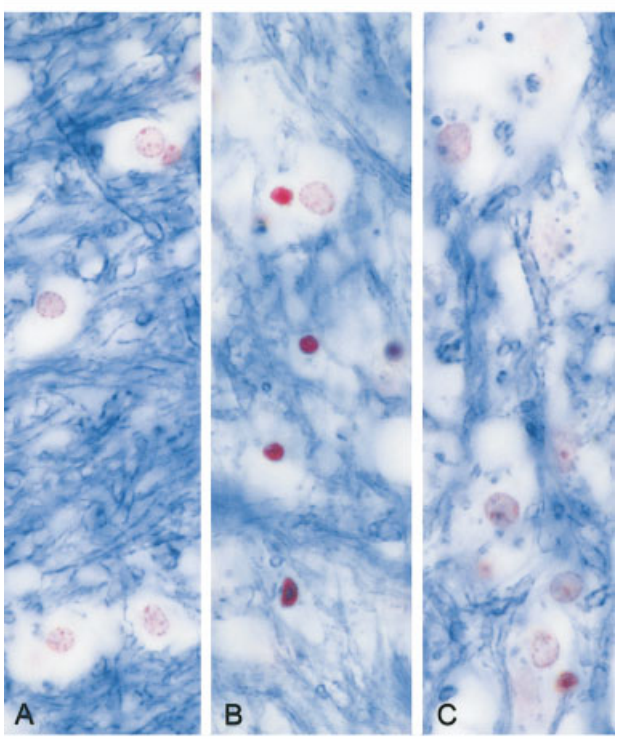

*Fig 10. (A) Unaffected white matter (*left arrowhead, *Fig 9). A stain that colors nuclear chromatin red has been used to identify nuclei in tissue stained blue for myelin. Glial nuclei in this field appear normal. (B) Apoptotic oligodendrocytes with shrunken nuclei and densely compacted nuclear chromatin in area indicated by middle arrowhead in Figure 9. (C) Commencing phagocytosis of vacuolated myelin in area indicated by the right arrowhead in Figure 9. Apoptotic nuclei and normal appearing oligodendrocytes were almost entirely absent in this region of the plaque. Luxol fast blue periodic acid–Schiff with nuclear fast red counterstain. Magnification ×630.*

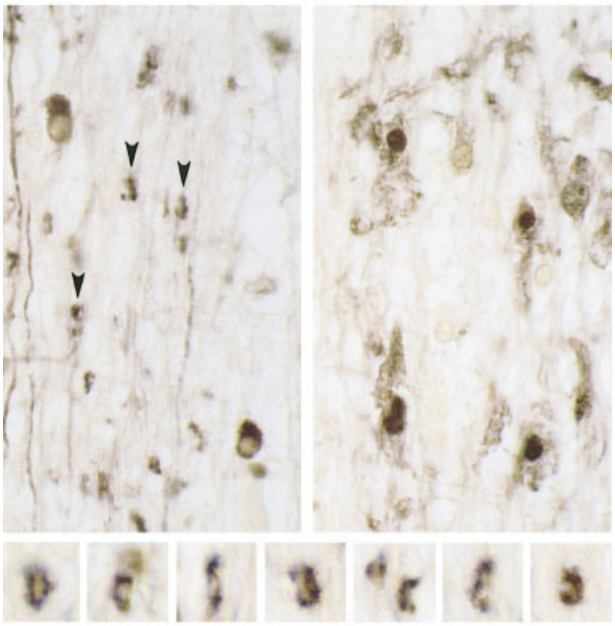

Fig 11. A bundle of normally myelinated fibers approaching (left) and entering (right) an actively demyelinating pontine plaque immunostained for λ light chains. (left) Normal white matter into which serum proteins have seeped. Some normal appearing oligodendrocytes, some axons, and a small unidentified component of intact myelin sheaths (arrowheads) *stain positively for Ig light chains. (right) Within the plaque only macrophages react positively. (bottom) Enlarged view of the myelin-associated structures reactive for IgG, λ, and κ light chains and activated complement located in normal white matter bordering plaques in several cases of acute MS. Immunostained for λ light chains. Magnification top left and right ×400; bottom ×630.*

sheaths distended by intramyelinic edema or those with a smudged outline in vacuolated tissue, and fragmenting sheaths contacted by phagocytes, stained positively for both C9neo and C3d (Fig 12 and 13). Macrophage contents and intracellular membranes were also consistently positive for C9neo and C3d (Fig 14). C3d was also detected in some astrocytes, neurons, axons, and rare apoptotic oligodendrocytes (Fig 15).

Discussion

The clinical, electrophysiological, and magnetic resonance imaging (MRI) features that accompany the formation of a new lesion in relapsing and remitting MS are sufficiently stereotyped and unusual to suggest that the underlying pathological changes are equally unusual and stereotyped.[13–15] The fact that symptoms usually peak within 2 to 3 days, plateau, and begin disappearing within a few weeks indicates that the size of the lesion responsible for conduction block is fully established within a few days of onset of symptoms in many cases. In this study, we have identified a series of changes likely to be related to, if not the cause of, the initial 2 to 3-day progressive phase of a typical relapse, namely, an abrupt loss of oligodendrocytes throughout a circumscribed and relatively small volume of tissue. Degenerate oligodendrocytes have been observed before in evolving MS lesions[16–21] but this report is the first to our knowledge to link oligodendrocyte destruction with the formation of a new lesion in relapsing and remitting MS.

Formation of a New Lesion

This study and other studies of MS plaques sampled before much myelin loss[21–27] provide the following description of how new lesions form.

At an early stage in their evolution, new lesions may not be visible in slices of fixed brain tissue owing to the presence throughout the lesion of still largely intact myelin sheaths. Within hours, oligodendrocytes throughout the affected tissue appear apoptotic, myelin sheaths stain positively for activated complement while immunoreactivity for CNP and MAG[24,26] is diminished, and rami-

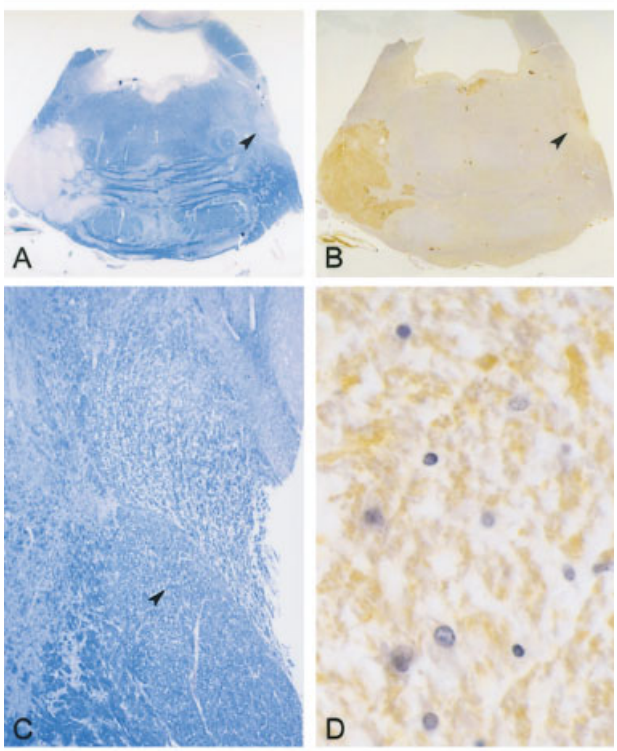

Fig 12. Case 6. (A, B) A newly forming apoptotic lesion in the pons (arrowheads). *Also visible are two subacute lesions, one subependymal and a larger lesion on the left. (C) An enlarged view of the new lesion. The arrowhead points to the zone of apoptotic oligodendrocytes. The spongy tissue above this is a zone of vacuolated myelin and commencing phagocytosis. (D) Some of the myelin within the zone of apoptotic oligodendrocytes stains positively for activated complement. (A, C) Luxol fast blue periodic acid–Schiff. Magnification: (A) ×1.5; (C) ×16; (B): immunostained for microglia/macrophages (CD45), ×1.5; (D) immunostained for C9neo, magnification ×400.*

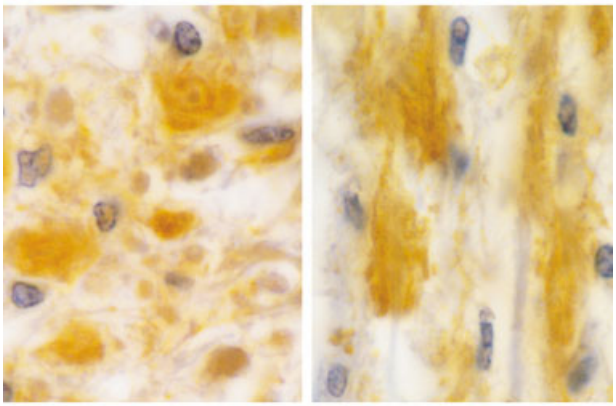

Fig 13. Fragmenting swollen myelin sheaths in one of the subacute lesions illustrated in Figure 12 immunostained for activated complement (C9neo). (left) Transversely sectioned disrupted sheaths. (right) Longitudinally sectioned swollen sheaths. Magnification ×630.

fied microglia with thickened processes appear in increased numbers.[23] T cells,[23] early activated macrophages,[22] (MRP-14–positive cells) and myelin phagocytes are rare or absent in the apoptotic zone but are present elsewhere in the lesion.

After 1 or 2 days, oligodendrocytes disappear, most presumably phagocytosed by the now amoeboid microglia present in the tissue. The tissue appears vacuolated because of the presence of widespread intramyelinic edema, which is the usual accompaniment of oligodendrocyte loss.[28]

The third and most protracted stage involves fragmentation and uptake of vacuolated and smudged (vesiculated[29,30]) myelin sheaths by macrophages in the presence of infiltrating T cells and MRP-14–positive cells.[31]

Regenerative activity begins with the appearance of large cells closely resembling HNK-1–positive, faintly CNP-positive oligodendrocyte precursor cells[10,32] among myelin phagocytes within 2 days of the onset of symptoms. The same time course has been observed in experimental demyelination[11,33–35] and is in keeping with other evidence that macrophages provide trophic factors that drive remyelination.[36,37] Within a week or two, numerous differentiated oligodendrocytes (somewhat smaller cells strongly reactive for CNP) appear within the lesion, including lesions in which apoptotic oligodendrocytes continue to appear at the margin.[17]

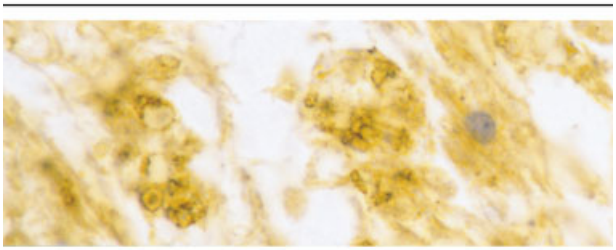

Fig 14. Case 4. Myelin phagocytes stain positively for activated complement (C3d). Magnification ×630.

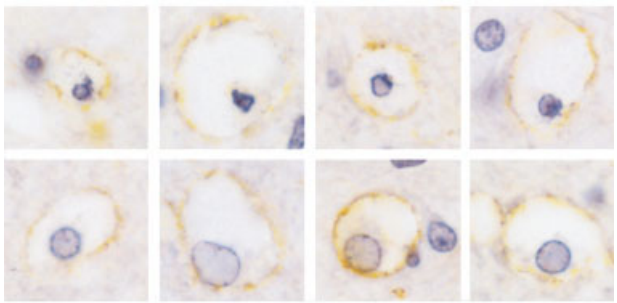

Fig 15. Case 6. Apoptotic oligodendrocytes (top row) and oligodendrocytes with enlarged nuclei (bottom row) in white matter bordering a subacute lesion stain positively for activated complement (C3d). Immunoreactivity appears to be restricted to the surface of each swollen oligodendrocyte. Magnification ×630.

The observation that oligodendrocytes disappear before commencing phagocytosis of myelin by macrophages is contrary to the long-standing view that macrophages are the primary mediators of myelin destruction in MS.[23,38–43] Our findings suggest that macrophage–myelin engagement in new lesions involves the same mechanism that normally initiates the removal of apoptotic plasma membranes. During programmed cell death, including caspase-independent apoptosis,[44] phosphatidylserine, a component of the inner leaflet of the plasma membrane, is externalized to the surface of the cell. Here, it is engaged by phosphatidylserine receptor–bearing constitutive phagocytes, resulting in macropinocytosis of the affected membrane.[45] Apoptosis also causes activated complement to be deposited on the cell surface, further promoting phagocytosis, via macrophage complement receptors,[46] of the damaged plasma membrane.

Although apoptosis typically allows dead cells to be removed without inciting an inflammatory reaction,[47–49] the multiple sclerosis lesion might be an exception in view of the relatively huge quantity of apoptotic plasma membrane, in this case altered myelin, generated by each dying oligodendrocyte. Inflammation is associated with postapoptotic necrosis, which occurs when normal clearance mechanisms of apoptotic cells are overwhelmed,[47]and it is possible that this mechanism contributes to the inflammatory response in MS.

Oligodendrocyte Apoptosis

The earliest change observed in the lesions examined in this study was widespread oligodendrocyte apoptosis in tissue in which T cells, macrophages, activated microglia, reactive astrocytes, and neurons appeared normal. This observation points to some change in the local environment to which oligodendrocytes are especially

susceptible and which triggers what appears on present evidence to be an activated caspase 3 independent form of apoptosis.[50,51] Poor preservation of caspase 3 immunoreactivity in central nervous system tissue obtained at autopsy and subjected to prolonged formalin fixation also could plausibly explain the latter point.

In previous studies in which partly myelinated lesions resembling those described here have been examined, it has been noted that no similar lesions are known to occur in any experimental model involving T cell– or antibody-mediated immunity directed against an oligodendrocyte or myelin antigen.[23,24,26,52,53] It has been suggested that some novel process might be involved, perhaps a virus infection of oligodendrocytes[24,26]; hypoxic stress secondary to ischemia or an immune-mediated vasculitis[22,52]; or diffusion from Virchow Robin spaces,[23] or secretion by activated microglia, of cytokines that have been shown to selectively damage oligodendrocytes in vitro[54–58] and that have been detected in some MS lesions.[59–61]

It is difficult to ascribe oligodendrocyte destruction in MS to the action of proinflammatory cytokines alone, however, in view of the fact that extensive demyelination and oligodendrocyte loss is not usual in T-cell–mediated EAE,[30,62] acute disseminated encephalomyelitis, or paraneoplastic encephalomyelitis.[63] Although this study provides no direct evidence of what might be the cause of oligodendrocyte apoptosis, one unexplained finding that may relate to the genesis of a new lesion was the occasional occurrence of perivascular cuffs of mononuclear cells adjacent to periventricular white matter, at the corticomedullary junction and close to the pia, all areas where new MS lesions tend to form.

Heterogeneity

This study confirms a recent report by Lucchinetti and colleagues[26] that new lesions in some MS cases exhibit extensive zones of apoptotic oligodendrocytes. In contrast, however, this study shows that such lesions, referred to as "type 3" lesions,[26] are not exclusively limited to a subset of MS patients in whom remyelinating shadow plaques are absent and actively demyelinating lesions show no evidence of complement activation. Rather, type 3 lesions and the lesions described in this study probably represent a very early stage in the formation of most if not all lesions responsible for typical acute exacerbations of the disease.

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