J Clin Pathol 1986;39:615–621

Monoclonal antibody to macrophages (EMB/11) labels macrophages and microglial cells in human brain

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SUMMARY Normal and diseased human central nervous system (CNS) tissues were studied immunohistochemically by a monoclonal antibody to human macrophages (EBM/11), antisera to glial fibrillary acidic protein (anti-GFAP), and α-1-antichymotrypsin (α1-ACT). EBM/11 reacted with brain macrophages located mainly around blood vessels in normal brain; it also reacted with resting microglia in normal brain and with numerous reactive microglia and macrophages in brain tumours and inflammatory lesions. Microglia did not react with anti-GFAP or α1-ACT. An EBM/11 positive phenotype, therefore, is shared by microglia and macrophages and suggests that microglial cells form a specialised part of the mononuclear phagocyte system.

The nature and origin of microglial cells in the central nervous system (CNS) has been a subject of debate for many decades. del Rio Hortega[1 2] was the first to identify cells termed "resting" microglia in normal undamaged CNS and "reactive" or "activated" microglia in traumatic and inflammatory CNS lesions. Intermediate forms also existed, and del Rio Hortega considered that the two forms were interchangeable and were of mesodermal origin. This view has been supported by light and ultrastructural histochemistry.[3] Experimental autoradiographic studies have indicated that at least some of the cells with morphological features of activated microglia and macrophages infiltrating CNS lesions are derived from circulating blood mononuclear cells.[4 5] Perivascular adventitial cells also provide a source of phagocytic cells in injured brain parenchyma.[6] Some authors have recently questioned the view that resting and activated microglia are derived from blood monocytes; this has arisen because antigens normally expressed by macrophages and blood monocytes are not detectable in resting microglia.[4 5 7–10] Macrophages, however, are a heterogeneous population, both functionally and in their antigenic properties. The fact that certain macrophage markers have not been detected on resting microglia does not, therefore, conclusively refute their originating from blood monocytes. Furthermore, recent studies on the localisation of the mouse macrophage marker F4/80 in developing and adult mouse brain and retina, which show that F4/80 positive cells migrate into the mouse CNS during development and subsequently differentiate into cells with appearances of resting microglia,[11 12] suggest that these cells may have a macrophage function. The monoclonal antibody EBM/11, raised against lung macrophages, has been shown to react with a cytoplasmic determinant in human macrophages from various organs[13] and other cells of presumptive macrophage origin (E Bliss, unpublished observations).[14 15] In a preliminary survey of undiseased necropsy material EBM/11 reacted with microglial cells in human brain (E Bliss, unpublished observations).[13 16]

This report shows that EBM/11 reacts not only with macrophages but also with microglia in normal and diseased human CNS tissues, indicating that microglial cells and macrophages are phenotypically (and presumably) functionally similar.

Accepted for publication 23 January 1986

Material and methods

Fresh frozen cryostat sections were prepared from surgical and necropsy specimens from the following: normal cerebral cortex and white matter (five cases), brain stem (three cases), and spinal cord (three cases); cerebral gliomas (six cases), meningiomas (two cases), pituitary adenoma (one case), multiple sclerosis plaques and surrounding white matter (five cases), progressive multifocal leucoencephalopathy (one case), acute perivenous leucoencephalitis (one case),

cerebral infarction (three cases), cerebral granuloma (two cases), acute necrotising encephalitis (one case), and head injury (two cases).

Sections 10 μm thick were mounted on glass slides, fixed in acetone for 10 minutes, washed in 0·15M/l sodium chloride buffered with Tris-hydrochloric acid, pH 7·4 (Tris saline), and covered by the EBM/11 antibody, applied as neat ascitic fluid for 30 minutes at 22°C. After washing with Tris saline peroxidase conjugated rabbit antimouse IgG (Dako) (diluted 1/50 with 1/20 normal human serum) was applied to the slides for 30 minutes, washed in Tris saline incubated with diaminobenzidine and H_2O_2 for five minutes. Slides were counterstained with haematoxylin. Sections adjacent to those treated with EBM/11 antibody were incubated with antibody to glial fibrillary acidic protein anti-GFAP (Monosan). In selected cases double staining was done with EBM/11 antibody, using peroxidase labelled second antibody for detection of this antibody and alkaline phosphatase labelled second antibody for detection of anti-GFAP. Immunoreactivity of CNS tissues with EBM/11 antibody was also compared with reactivity to α1-antichymotrypsin antiserum (α1-ACT) (Dako) (1/1000 dilution), which was detected by using the PAP immunoperoxidase technique on adjacent sections.

Results

NORMAL CENTRAL NERVOUS SYSTEM

A regular array of small, bipolar, or multipolar cells reactive for EBM/11 was shown in cerebral white matter (Fig. 1). These cells, therefore, had the typical appearances of microglial cells. Nuclei of reactive cells were spaced roughly 30–70 μm apart, and their processes in places lay within smaller distances of each other but did not appear to overlap. Similar relatively regular spacing of EBM/11 reactive microglial cells was seen in the white matter of the spinal cord and brain stem and in grey matter of the cerebral cortex. Some of these cells lay close to capillaries and others, in grey matter, close to neurone cell bodies. EBM/11 reacted with a cytoplasmic component of the positively stained cells, and the reaction product was distinctly granular in appearance. The nuclei of reactive cells both in grey and white matter were small, moderately stained with haematoxylin, oval, or comma shaped, and had stippled chromatin. These small EBM/11-reactive cells in CNS parenchymal tissues did not react with either the antiserum to α1-ACT or that to GFAP (Figs. 1 and 2). The latter antiserum labels astrocytes, which are larger and have longer smoother processes than microglia (Fig. 2).

In addition to reacting with the microglial cells described above, EBM/11 reacted with larger cells of polygonal or spindle shape, with abundant cytoplasm found immediately adjacent to parenchymal blood vessels of various types (Fig. 1). Similar cells were detected in the leptomeninges where they were also often, but not exclusively, associated with blood vessels (Fig. 3). The distribution and appearance of these cells suggested that they were macrophages and peri-

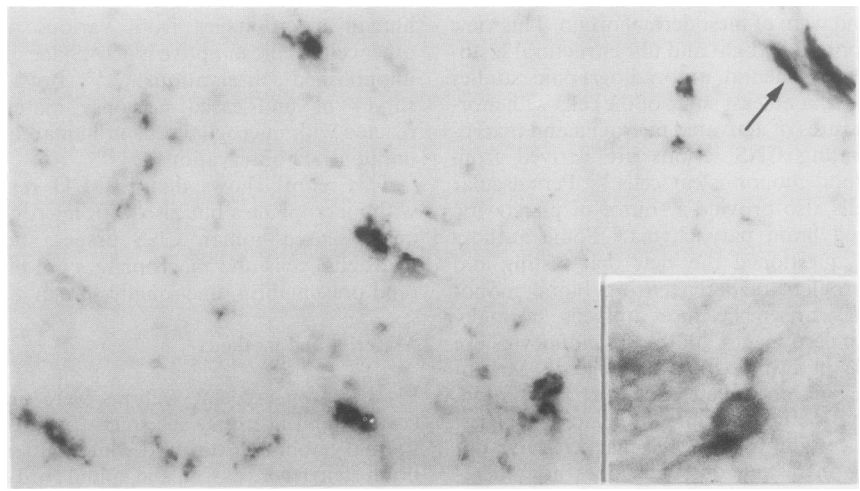

Fig. 1 *Cryostat section of cerebral white matter treated with monoclonal antibody EBM/11 followed by peroxidase reaction with diaminobenzidine showing reaction product in scattered microglial cells. Top right shows small venule (arrow) flanked by two reactive perivascular cells, which are larger than parenchymal microglial cells. (Counterstained with haematoxylin.)* × *350. Inset: bottom right, single bipolar microglial cell at higher power.* × *700.*

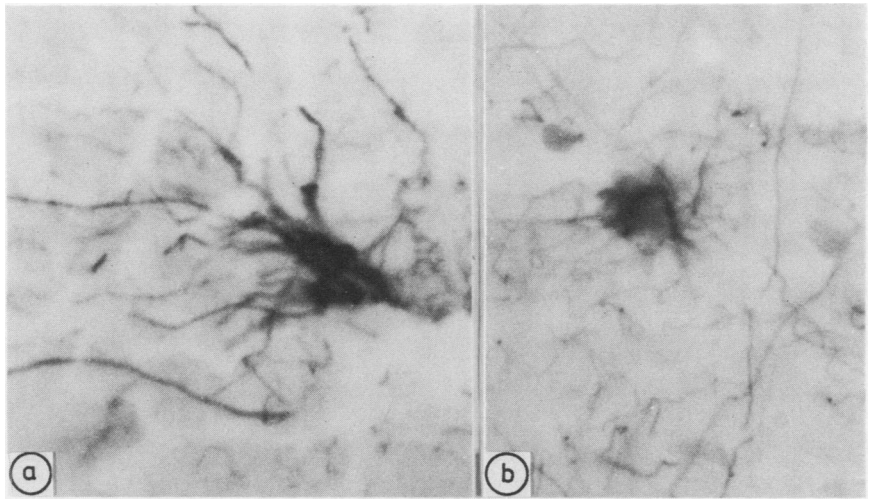

Fig. 2 *Examples of white matter astrocytes from same block as Fig. 1 shown with anti-GFAP antibody and peroxidase reaction with diaminobenzidine. These cells are larger and have more numerous and longer processes than cells shown by monoclonal antibody EBM/11 seen in Fig. 1.* ×*950.*

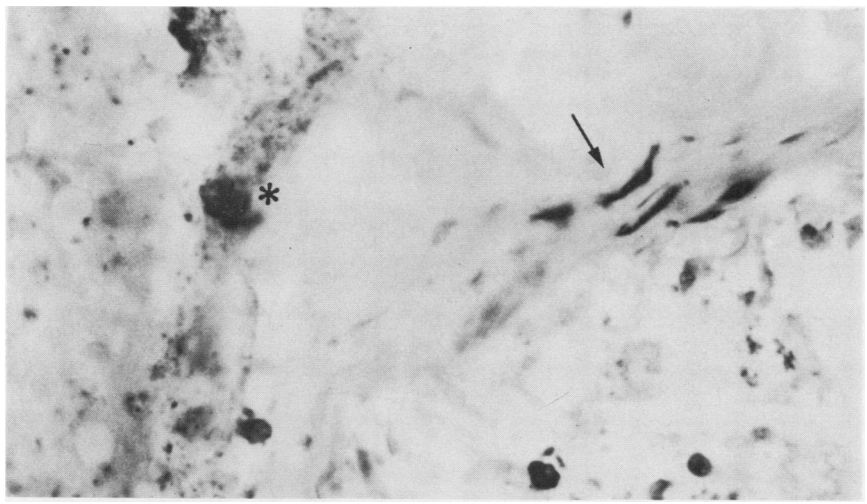

Fig. 3 *Cryostat section of spinal cord leptomeninges containing plump macrophages (example asterisked) and perivascular spindle shaped cells (arrow) reacting with monoclonal antibody EBM/11. (Peroxidase reaction with diaminobenzidine counterstained with haematoxylin.)* ×*450.*

cytes. Some, but not all of the cells of this type, reacted weakly with the antiserum to α-ACT.

TUMOURS

Cerebral gliomas contained scattered EBM/11 positive cells that were larger than those seen in normal brain and had a more rounded outline with fewer processes (Fig. 4). Their density varied from one part of a tumour to another and from tumour to tumour. There was an accumulation of EBM/11 positive cells at the edge of necrotic areas in glioblastomas (Fig. 5). The pattern of reaction with EBM/11 was quite different from that with the GFAP antiserum, which reacted with many of the tumour cells in astrocytomas and glioblastomas (Fig. 6). In general, the EBM/11 reactive cells were ubiquitous and quite numerous in gliomas. In one glial tumour from a child with tuberous sclerosis almost half the cells within the

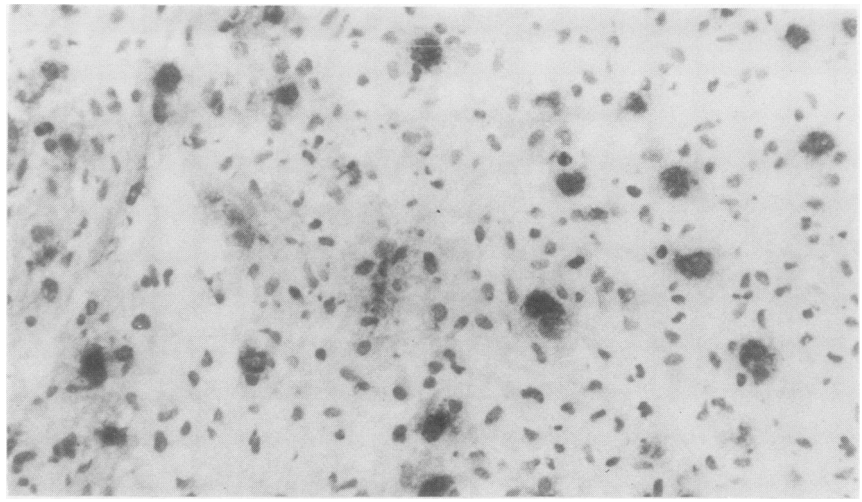

Fig. 4 *Cryostat section of infiltrating cerebral astrocytoma treated with monoclonal antibody EBM/11 followed by peroxidase reaction with diaminobenzidine. Positively reacting plump cells are scattered throughout tumour. Nuclei of unreactive astrocytes are interspersed between EBM/11 reactive cells. (Counterstained with haematoxylin.)* ×300.

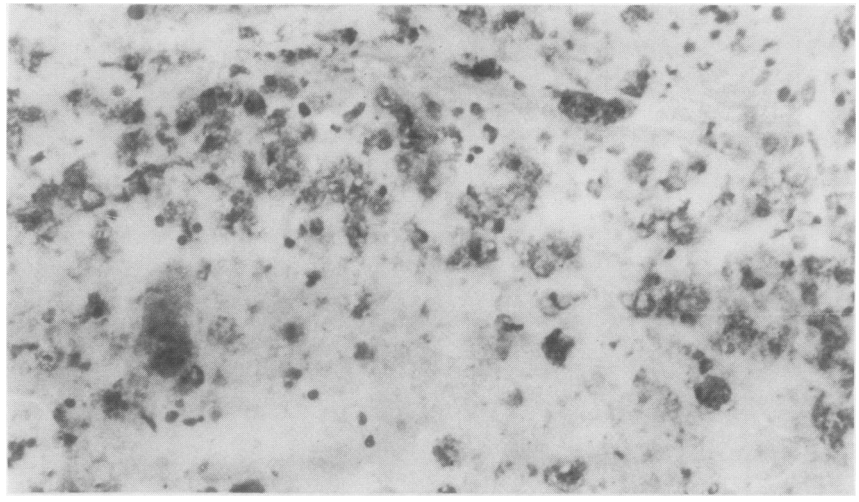

Fig. 5 *Cryostat sections of cerebral glioblastoma treated with monoclonal antibody EBM/11 followed by peroxidase reaction with diaminobenzidine. Zone containing reactive macrophages runs fom left to right, bordering an area of necrosis below. (Counterstained with haematoxylin.)* ×250.

tumour section reacted strongly with EBM/11, but notably, these cells were smaller and had smaller less pleomorphic nuclei than those of the unreactive cells (Fig. 7).

The meningiomas studied showed some foci of positive reactivity with EBM/11. In these tumours the reactive cells were spindle shaped and appeared to be part of the tumour itself.

The pituitary adenoma tumour cells showed no reactivity with EBM/11, but there were occasional positively reacting large cells adjacent to blood vessels in this tumour.

The gliomas and other tumours studied were not convincingly reactive with the α1-ACT antibody.

INFLAMMATORY AND OTHER NON-NEOPLASTIC LESIONS

Large macrophages or Gitter cells from the case of

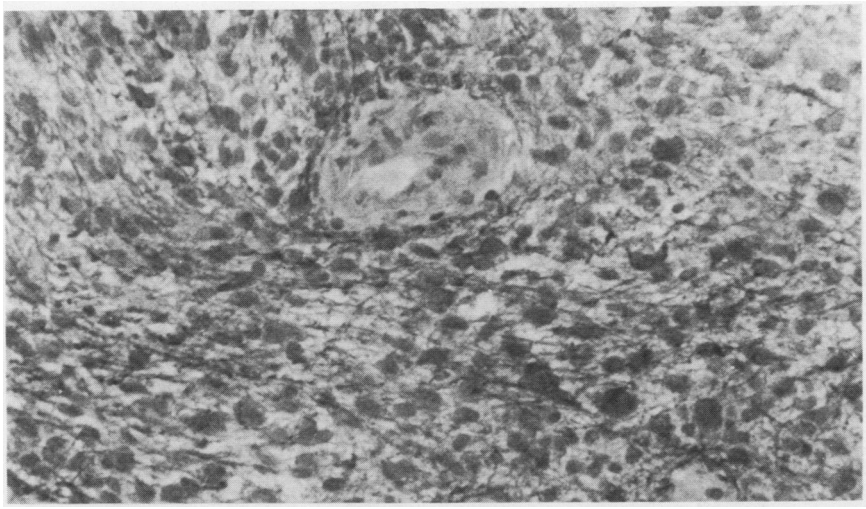

Fig. 6 *Cryostat section of astrocytoma illustrated in Fig. 4 treated with anti-GFAP antiserum followed by peroxidase reaction with diaminobenzidine. Numerous astrocyte cell bodies and their processes are stained. Compare appearances with those of Fig. 4. (Counterstained with haematoxylin.)* ×*300.*

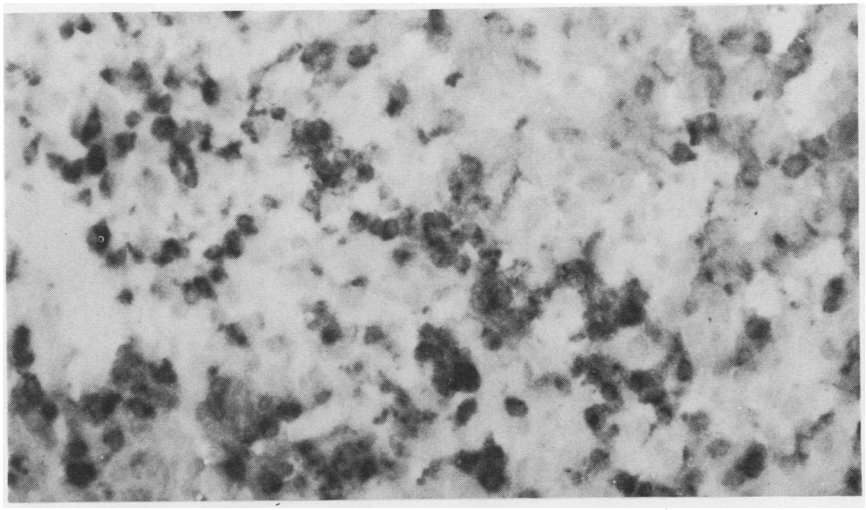

Fig. 7 *Cryostat section of large celled astrocytoma from subependymal region from a case of tuberous sclerosis treated with monoclonal antibody EBM/11 following by the diaminobenzidine reaction. Numerous EBM/11 positive cells are present interspersed among the unreactive tumour cells. (Counterstained with haematoxylin.)* ×*300.*

necrotising encephalitis, the cases of cerebral infarction, and the margins of multiple sclerosis plaques reacted strongly with EBM/11 (Fig. 8). The cerebral granulomas also contained numerous intensely reactive spindle and epithelioid type cells. Clusters of reactive microglial cells in the cases of head injury were also easily identified and reacted intensely with EBM/11 (Fig. 9). Some of the Gitter cells reacted weakly with α1-ACT antiserum; the EBM/11 antibody showed far more reactive cells that stained more intensely. In lesions of progressive multifocal leucoencephalopathy and acute perivenous encephalitis numerous large EBM/11 reactive cells were present, whereas few cells reacted for α1-ACT. All of these lesions also contained numerous large reactive astrocytes. For the most part, astrocytes were clearly unreactive with EBM/11, but the astrocytes in the progressive multifocal leucoencephalopathy

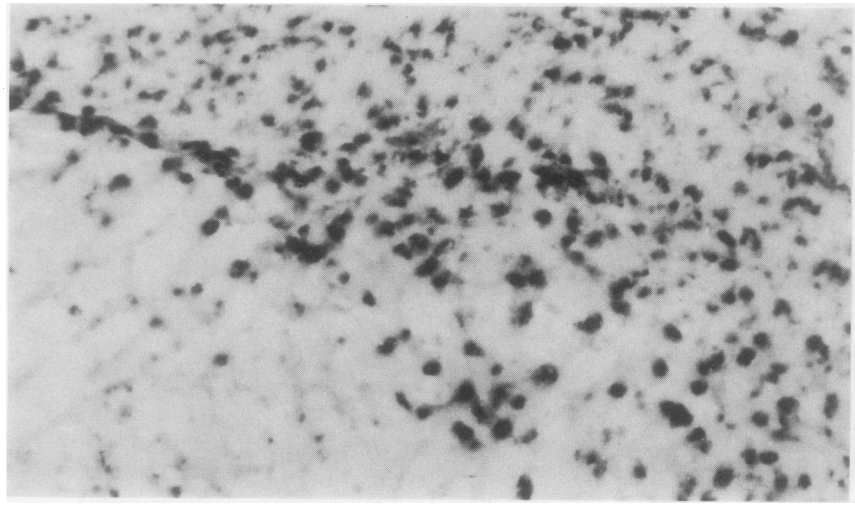

Fig. 8 *Cryostat section from margin of multiple sclerosis plaque (bottom left) treated with monoclonal antibody EBM/11 followed by diaminobenzidine reaction. Large numbers of EBM/11 reactive cells occupy marginal zone. (Counterstained with haematoxylin.)* × *120.*

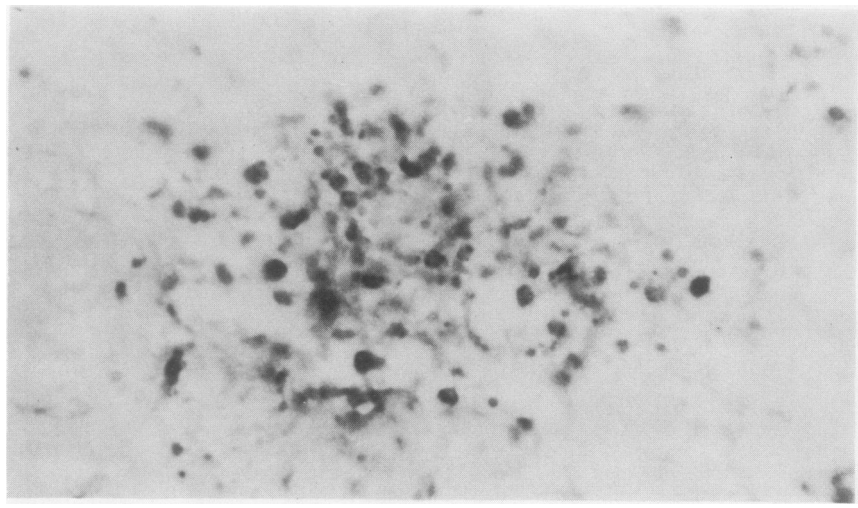

Fig. 9 *Cryostat section from cerebrum from a case of severe head injury with eight days' survival treated with monoclonal antibody EBM/11 followed by the diaminobenzidine reaction. Cluster of reactive microglial cells is strongly reactive. (Counterstained with haematoxylin.)* × *350.*

lesions and at the margins of some multiple sclerosis plaques showed a weak diffuse reaction with EBM/11, though this was much less intense and lacked the granular quality of the staining seen in macrophages and microglial cells.

Discussion

We found the EBM/11 antibody to be an excellent reagent for detecting microglial cells in undiseased human brain. The arrays of small cells with multiple processes shown in this study closely resembled those identified as reactive with the macrophage specific antibody F4/80 in mouse brain. (E Bliss, unpublished observations). These cells correspond morphologically to the classically described microglial cells of del Rio Hortega.[1 2] In addition to these parenchymal microglial cells, EBM/11 also reacted with macro-

phage like perivascular and leptomeningeal cells in normal brain.

In cerebral glial tumours we detected a high number of large reactive microglial cells and macrophages. Heavy macrophage infiltration of human gliomas was also found by Morantz *et al.*[17] EBM/11 positive cells tended to form an array in tumours lacking necrosis, rather like that of microglial cells in normal brain. These cells may represent a modified population of microglial cells in tissue that has been diffusely infiltrated rather than destroyed by the tumour. In tumours with extensive necrosis large numbers of additional macrophages were also present, particularly around the necrotic zones. In non-neoplastic cerebral disease strongly positive EBM/11 cells were present in large numbers. Results of comparison of these cells with those positive for GFAP generally showed no overlap, but in some cases in which numerous EBM/11 positive cells were present GFAP positive astrocytes also showed a weak diffuse reaction for EBM/11. This may result from release of the EBM/11 antigen from macrophages and uptake into reactive astrocytes. In general, EBM/11 is a sensitive and specific marker for macrophages and microglial cells in the CNS. It reacts with many more cells than does antiserum to α1-ACT and it shows the normal microglial population more reliably than silver stains. It does, however, require the use of frozen as opposed to formalin fixed material. It is likely to prove a valuable tool in differentiating macrophages and microglial cells from other CNS components and for studying entry of macrophages into the human CNS during development. Our findings using EBM/11 support del Rio Hortega's original view that microglia form a part, albeit a specialised part, of the mononuclear phagocyte system.

"Microglioma" and "microgliomatosis" are now presumed to be lymphoreticular malignancies. Fresh tissues from these conditions were not available when this study was done. The reaction of EBM/11 in these disorders and cerebral lymphomas will form part of a future study to determine whether any or all of these malignancies express this macrophage marker.

This work was supported by grants from the Multiple Sclerosis Society of Great Britain and Northern Ireland (MME) and the Cancer Research Campaign (JO'DMcG). Miss M Reading gave excellent technical help. Miss L Watts typed the manuscript.

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