Resident Macrophages (Ramified Microglia) of the Adult Brown Norway Rat Central Nervous System Are Constitutively Major Histocompatibility Complex Class II Positive

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Summary

A flow cytometric phenotype for isolated adult central nervous system (CNS) ramified microglia was previously defined ($CD45^{low}$ $CD11b/c^+$) in the Lewis strain rat, that clearly distinguished these cells from all blood-derived leucocytes, the latter being $CD45^{high}$. Consistent with the reported lack of major histocompatibility complex (MHC) expression in the CNS, isolated microglia were mostly MHC class II^-. Employing these phenotypic criteria, we now show that a proportion of microglia in Brown Norway (BN) strain rats are constitutively MHC class II^+. In spinal cord, up to 25% of microglia are distinctly positive and most have some level of expression. In situ staining of MHC class II^+ microglial cells in BN rats indicates that positive cells are typical of ramified microglia on the grounds of both morphological appearance and anatomical location. In Lewis (LEW) rats, the few MHC class II–expressing cells isolated from the normal CNS are $CD45^{high}$ blood-derived cells and not resident microglia. After infection of both LEW and BN rats with a neurotropic murine hepatitis virus (MHV-JHM), MHC class II was rapidly upregulated on microglia in the BN but not in the LEW strain. In the latter, inflammatory cells were the predominant MHC class II–expressing population. Nevertheless, most microglia in the LEW strain could, after some delay, be induced to express MHC class II after transfer of an experimental autoimmune encephalomyelitis (EAE)–inducing encephalitogenic T cell line. Paradoxically, strains resistant to EAE (exemplified by the BN) contained more constitutive MHC class II–expressing microglia than susceptible ones, when a variety of strains were examined. The results clearly establish that the normal CNS may contain MHC class II–expressing cells that are a resident rather than a transient blood-derived population. It is significant that this expression is strain related, but there is no evidence that microglial cell constitutive MHC class II expression predisposes to EAE susceptibility.

Until relatively recently, the prevailing view of the central nervous system (CNS)[1] from an immunological perspective, was dominated by the apparent deficiencies of this tissue, which include the lack of real lymphatic drainage, the exclusion of most lymphoid cells because of the presence of the blood-brain barrier, and the lack of MHC expression on most CNS parenchymal cells (for reviews see references 1 and 2). However, a number of lines of experimental (3) and circumstantial evidence (2) now indicate that despite these conditions, the CNS is effectively patrolled by the immune system. Exactly how this is achieved given the conditions under which the immune system must operate is not absolutely clear. Nevertheless, the demonstration of MHC class II induction on glial cells by T cell factors such as IFN-γ (4), the presence in the CNS of macrophage-like perivascular cells with in vivo APC ability (5), and the observation that activated T cells may nonspecifically cross the blood-brain barrier (1, 6), alleviate to some extent the mystique surrounding likely processes of immune system intervention within the CNS.

There are still many facets of immune–central nervous system interactions that require clarification. In particular, it is still not known exactly what role glial cells such as as-

[1] *Abbreviations used in this paper:* BN, Brown Norway; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; F_1, (LEW × BN)F_1; LEW, Lewis; MBP, myelin basic protein; MHV, murine hepatitis virus.

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trocytes and microglia may play in the development of immune responses. Astrocytes are effective APC in vitro, at least for secondary CD4+ T cell responses (1, 7) whereas after the development of inflammation in the human and animal CNS, microglia (together with inflammatory monocytes) are the most abundant MHC class II–expressing population (8, 9). MHC expression on either cell type after inflammation is always taken to indicate that T cell–glial cell interactions can then proceed, leading to further T cell activation. There is no evidence to suggest that this actually occurs in vivo, despite the substantial body of in vitro data including our own studies with astrocytes (7) supporting the role of glial cells as effective APC for proliferative T cell responses. Indeed, a recent study indicates that the environment of the CNS is not particularly conducive to T cell activation (10).

Employing new methodology for the isolation and characterization of ramified (resident) microglia from the adult rat CNS (11), we now report that in some strains of rats, the Brown Norway (BN) in particular, a high proportion of microglia are constitutively MHC class II+. The BN strain is one of the most resistant to the induction of the CD4+ T cell–mediated disease, experimental autoimmune encephalomyelitis (EAE). In highly EAE-susceptible strains such as the Lewis (LEW), few microglia express MHC class II, and in this case, nonresident blood–derived cells are the predominant MHC class II–expressing population isolated from the normal CNS. The result stress the point that resident cells of the CNS can express MHC class II under normal, noninflammatory conditions, but that this expression is very strain dependent. It is significant that mere expression of MHC class II on microglia is not indicative of heightened susceptibility to inflammatory T cell responses in the CNS.

Materials and Methods

Animals and Irradiation Chimeras. 8–10-wk-old female rats were obtained from Zentralinstitut für Versuchstierzucht (Hannover, Germany); Moellegard Ltd. (Skensved, Denmark); Olac Ltd. (Bicester, UK); or were bred in our own facilities (Blackburn, University of Sydney). Strains used were Lewis (LEW, $RT1^l$), Brown Norway (BN, $RT1^n$), (LEW × BN)F_1 (F_1), PVG ($RT1^c$), F344 ($RT1^{lv1}$), and Wistar Furth ($RT1^u$). Athymic PVG ($RT1^c$, rnu/rnu) were from Olac Ltd. (LEW × BN)F_1 → LEW and (LEW × BN)F_1 → BN irradiation bone marrow chimeras were prepared and assessed as described (11).

Induction of CNS Inflammation. Viral encephalomyelitis was induced by intracerebral injection of rats with the neurotropic JHM strain of murine hepatitis virus (MHV-JHM), as described (11). EAE was induced by subcutaneous injection of rats with myelin basic protein (MBP)/CFA (12) or by transfer of the CD4+ MBP-specific encephalitogenic T cell line, 266/87, to naive LEW rats (13).

Isolation of Microglia and CNS-associated Leucocytes. The general procedures for vascular perfusion, collagenase/DNase digestion of CNS material, and Percoll gradient separation of CNS cells are detailed elsewhere (11), but note should be made of some changes and corrections to this original method: (*a*) 10^3 U of DNase should be used per rat CNS, not 10^4 U; and (*b*) other types of collagenase may be employed in addition to Serva type II (used at ~ 10 U/CNS). Sigma Immunochemicals (St. Louis, MO) type I (260 U/mg) gives high recoveries when used at up to 15 U/CNS.

Monoclonal and Polyclonal Antibodies. Biotinylated rat mAb that distinguish between MHC class I of LEW and BN rats (mAb R2/15S, $RT1.A^{l+}$ and $RT1.A^{n-}$; mAb YR5/12, $RT1.A^{n+}$ and $RT1.A^{l-}$) were those used in a previous study (11). Mouse mAb specific for rat cell surface antigens were MRC OX1 (anti-rat CD45), MRC OX6 (anti-rat monomorphic MHC class II, *RT1.B* [I-A]), MRC OX18 (anti-rat monomorphic MHC class I, *RT1.A*), MRC OX26 (anti-rat transferrin receptor), and MRC OX42 (anti-rat CD11b/c). (See 11, 14, 15 for cross-reference details). Control mouse IgG1 mAb were MRC OX21 (anti-human C3bi; [16]), L180/1 (anti-sheep LFA-3; [17], provided by Thomas Hünig, Institüte für Virologie und Immunbiologie, Würzburg, FRG), and K-1-21 (anti-human free κ light chains, noncrossreactive on rat Ig). Rat Ig-absorbed FITC-conjugated donkey anti-mouse Ig and PE-streptavidin were from The Jackson Laboratory (Bar Harbor, ME). FITC-conjugated goat (Fab) anti-mouse Ig was from Cappell Laboratories (Cochranville, PA) and rabbit anti-mouse Ig coupled to peroxidase or alkaline phosphatase were from Dako (Glostrup, Denmark).

Cell Labeling and Flow Cytometry. Double labeling of cells for flow cytometry (FACScan®; Becton Dickinson & Co., Mountain View, CA), with biotinylated rat mAb and mouse mAb tissue culture supernatants, was performed as detailed (11). Double labeling of cells with two mouse mAb involved the sequential addition of the first mAb as tissue culture supernatant, FITC-conjugated goat (Fab) anti-mouse Ig in the presence of 20% normal rat serum, biotinylated second mAb in the presence of 20% normal mouse serum and streptavidin-PE.

Immunohistology. Rats were injected intravenously with 2 ml MRC OX26 ascites (anti-rat transferrin receptor) plus 5 mg MRC OX18 IgG (anti-rat MHC class I) and 30 min later, the vascular bed perfused with 250 ml PBS. Spinal cord and brain were removed, snap frozen, and 6-μm cryostat sections cut and fixed in 100% acetone. Anti-transferrin receptor (14) and MHC class I mAb binding to brain endothelial cells was revealed by addition of peroxidase-conjugated anti-mouse IgG followed by diaminobenzidine (DAB) substrate (brown product). MRC OX6 (anti-MHC class II), MRC OX42 (anti-CD11b/c), or control mAb were then added to the sections. The heavy deposition of DAB substrate virtually prevented binding of these mouse mAb by the peroxidase-conjugated anti-mouse IgG already on the sections. MHC class II or CD11b/c staining was revealed by addition of alkaline phosphatase-conjugated anti-mouse IgG and the (blue product) substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (ratio of 1:1.5 by weight).

Results

Irradiation Chimeras Establish Microglial Phenotype in BN and LEW Rats. Ramified microglia are irradiation resistant and do not swap to the donor phenotype in irradiation bone marrow chimeras (18). In a previous study, we utilized (LEW × BN)F_1 → LEW irradiation bone marrow chimeras (11) to establish a definitive phenotype for freshly isolated microglia in LEW rats. The most important phenotypic feature of these cells was their distinctly low expression of the CD45 molecule as compared to blood-derived leucocytes which were $CD45^{high}$. "Perivascular cells," a distinct population to microglia, are situated around vessels outside the parenchymal basement membrane (19). They are not only irradiation sensitive (20), but are strongly CD45+ by immunohistochemical staining, unlike microglia, so they can probably be classified as $CD45^{high}$. Microglia are also stained

with the mAb MRC OX42 (11, 21) which recognizes a determinant on both the rat CD11b (Mac-1) and CD11c (p150/95) molecules (21, 22). However, monocytes, granulocytes, and perivascular cells (20) are also positive for this mAb, so it is not a particularly discriminating marker. The results in Fig. 1 demonstrate that microglia isolated from BN rats have a similar phenotype to those found in the LEW strain. In these experiments, we have utilized the ability of a neurotropic murine hepatitis virus (MHV-JHM) to elicit a CNS inflammation in both LEW and BN rats. This response is generally of a lower magnitude in the latter strain. Inflammatory cells of donor phenotype can be distinguished easily from irradiation-resistant host cells in these chimeras by virtue of the former expressing LEW MHC class I in the case of $F_1 \rightarrow$ BN chimeras (Fig. 1, *d–f*) or BN MHC class I in the case of $F_1 \rightarrow$ LEW chimeras (Fig. 1, *j–l*). It is notable that the cells (microglia) that are negative for these MHC class I markers (that is of host and not donor type), are virtually all $CD45^{low}$ (Fig. 1, *e* and *k*) and $CD11b/c^+$ (Fig. 1, *f* and *l*). Microglia in both rat strains express some MHC class I even after isolation from the noninflamed CNS (11 and data not shown), and this is greatly increased after viral infection. In BN rats in particular, MHC class I expression of the $CD45^{low}$ population (microglia, Fig. 1 *b*) is at least as high as the inflammatory cells.

If progenitor cells within the bone marrow inoculum entered the adult CNS and differentiated into ramified microglia, one might expect these cells to express low levels of the CD45 molecule. However, in noninfected $F_1 \rightarrow$ parent chimeras up to 1 yr of age, we have no evidence to date that ramified microglia can develop from blood leucocytes. That is, all $CD45^{low}$ cells remain of host phenotype only and do not express MHC class I of donor type. In addition, relatively soon after viral infection (7–14 d) in both LEW and BN rats, no such transition has been observed (11). In Fig. 1, where the CNS was obtained 10 wk after infection, there is now some evidence that this process may occur, but only significantly in BN rats. The indicated population in Fig. 1 *e* is atypical in being of donor type but $CD45^{low}$. Triple staining is required to ascertain whether this population is also $CD11b/c^+$.

Constitutive MHC Class II Expression on BN Microglia. In $F_1 \rightarrow$ LEW chimeras, little evidence was found for constitutive MHC class II expression on microglia in the normal CNS (Fig. 2 *a*, *bottom right*). Most MHC class II^+ cells recovered from the perfused CNS, were of donor phenotype (Fig. 2 *a*, *top right*). At the peak of the inflammatory episode 7 d after viral infection, most MHC class II–expressing cells recovered from the CNS, were of donor type and therefore, inflammatory cells derived from the blood (Fig. 2 *b*, *top right*). MHC class II expression by LEW microglia was not increased at later time points (data not shown). On analysis of normal $F_1 \rightarrow$ BN chimeras, a very different situation was observed where up to 15% of BN microglial cells were MHC class II^+ (Fig. 2 *c*, *bottom right*, LEW MHC class I^-). After viral infection, a distinct MHC class II^+ microglial population emerged (Fig. 2 *d*, *bottom right*). Levels of expression were less than that on inflammatory cells (Fig. 2 *d*, *top right*), although there were relatively few of the latter.

To simplify the analysis of microglia in other strains and to establish that MHC class II expression on BN rat microglia

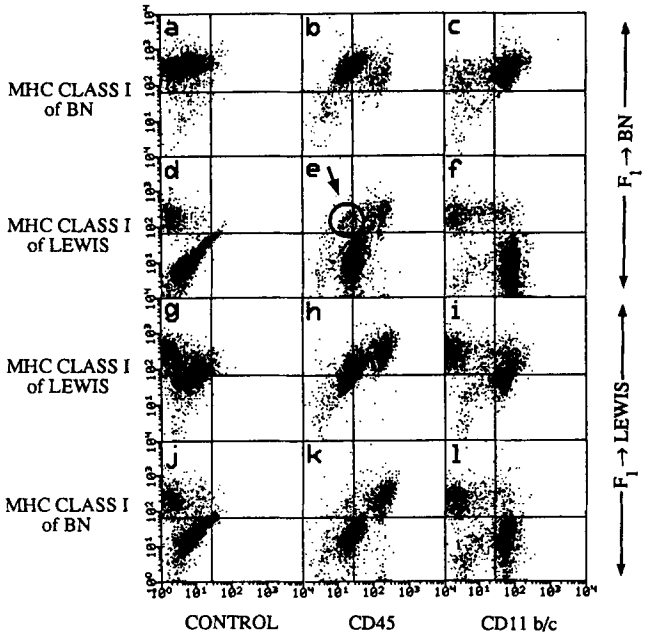

Figure 1. Microglial cell phenotype in BN and LEW rats. (LEW × BN)$F_1 \rightarrow$ LEW and (LEW × BN)$F_1 \rightarrow$ BN irradiation bone marrow chimeras were prepared at 4 wk of age and injected intracerebrally with the neurotropic virus, MHV-JHM 5 wk later. 10 wk after infection, cells were isolated from the CNS and double stained for flow cytometric analysis. The control mAb is MRC OX21 (mouse IgG1). The indicated population (*e*) are of donor cell type (LEW class I^+ in the BN host) but are $CD45^{low}$ (see text for details).

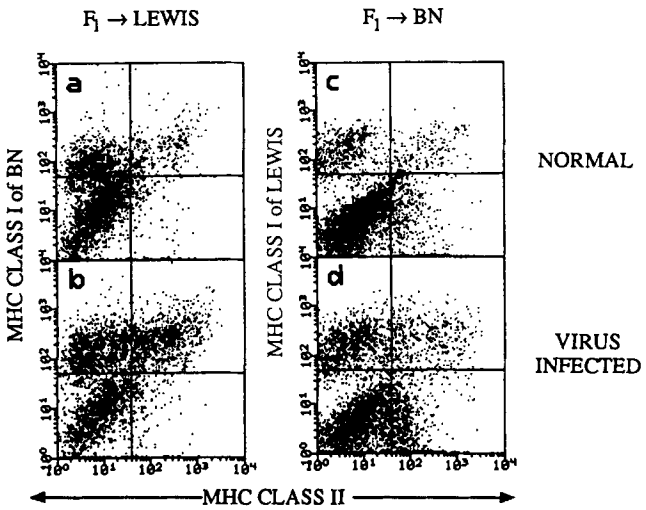

Figure 2. Constitutive and inducible MHC class II expression on microglia in chimeric BN rats. Chimeras were prepared as described in Fig. 1. Cells were isolated from the CNS of rats infected 7 d previously with MHV-JHM or from noninfected controls and double stained for flow cytometric analysis. Shown here are only those stainings which allow discrimination between donor and host cells. That is, BN MHC class I in $F_1 \rightarrow$ LEW and LEW MHC class I in $F_1 \rightarrow$ BN.

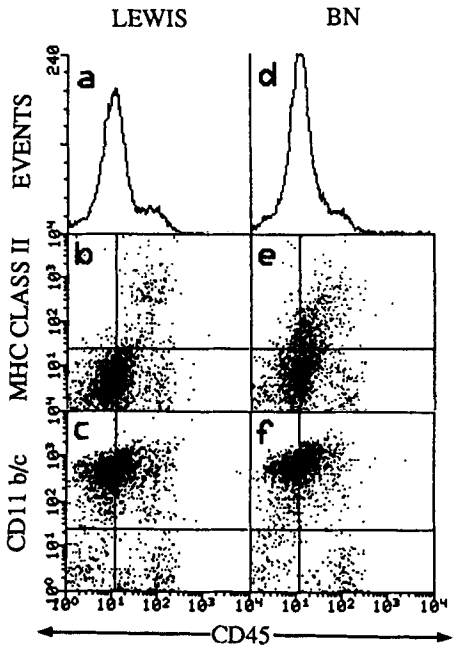

Figure 3. Constitutive MHC class II expression on CD45^{low} (microglial) cells in nonchimeric BN rats. CNS cells were isolated from normal LEW and BN rats and double stained for flow cytometric analysis.

was not an artefact of the chimeras, cells isolated from the CNS of normal rats were analyzed for CD45 and MHC class II expression by 2-color flow cytometry. Fig. 3, *a* and *d* illustrate CD45 histograms and show typical three-peak profiles of CD45^{-}, CD45^{low} (microglia), and CD45^{high} (blood-derived leucocytes). The results for MHC class II expression are entirely consistent with those in the chimeras (Fig. 2). MHC class II expression in LEW rats (Fig. 3 *b*) is virtually confined to the CD45^{high} population, whereas many CD45^{low} cells in BN rats (Fig. 3 *e*) are class II^{+}. In many cases using this staining protocol where MHC class II is detected with highly sensitive PE rather than FITC (Fig. 2), the entire BN CD45^{low} population shifts in the direction of MHC class II^{+}. Consistent with the fact that the CD45^{low} population are microglia, virtually all of them are CD11b/c^{+} (Fig. 3, *c* and *f*), whereas only around a half of the CD45^{high} cells are CD11b/c^{+}. These latter cells are either monocytes or granulocytes, whereas the CD45^{high} CD11b/c^{-} cells (Fig. 3, *c* and *f*, *bottom right panels*) are almost exclusively α/β^{+} T cells (data not shown).

The existence of MHC class II^{+} cells in the CNS of normal BN rats was established finally, by staining of cells in situ (Fig. 4). Vessels were highlighted by staining with mAb recognizing the transferrin receptor and MHC class I

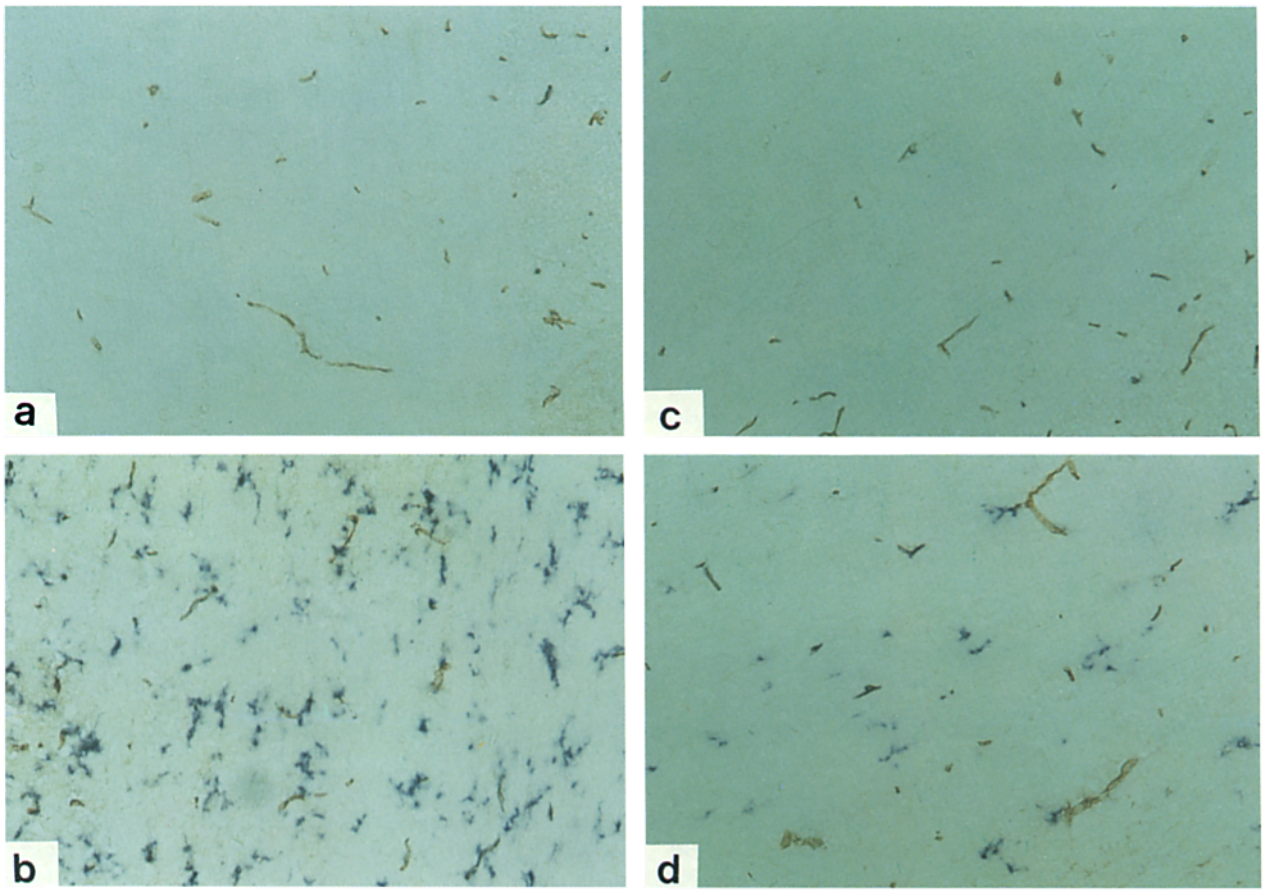

Figure 4. Constitutive MHC class II expression on BN microglia revealed in situ. Horizontal cryostat sections of thoracic spinal cord from normal LEW (*a* and *c*) and BN (*b* and *d*) rats. Brown staining in all cases reveals vessels. Blue staining shows cells positive for: (*a*) MRC OX21 (negative control); (*b*) CD11b/c; (*c* and *d*) MHC class II. In each case, the vessels are in focus. Some of the microglia are out of plane. ×130.

(brown staining), and other markers are revealed with a blue substrate. Addition of a control mAb to LEW spinal cord (Fig. 4 *a*) shows no staining other than the vessels. Numerous CD11b/c$^+$ cells (microglia) can be seen in BN tissue (Fig. 4 *b*). These pictures are the same for both LEW and BN rats. There are no MHC class II$^+$ cells seen in LEW spinal cord (*c*), whereas many positive cells are evident in tissue from BN rats (Fig. 4 *d*). Sections at low magnification are shown to illustrate the fact that positive cells are not infrequent in BN tissue. Most of the positive cells do not lie around vessels, and so are not perivascular cells (5, 23). Microglial MHC class II expression is not uniform throughout the normal CNS. It tends to be patchy, the highest levels are found in the lumbar cord, and this reduces as the cord is ascended. Lowest levels are found in the cerebral lobes. In the BN rat, if spinal cord only is used as a source of tissue, up to 25% of isolated microglia are truly MHC class II$^+$ and most have some expression, albeit weak.

EAE Susceptibility and MHC Class II Expression. Having established microglial phenotype without having to resort to chimeras for analysis, a variety of rat strains were tested both for microglial MHC class II expression and susceptibility to EAE as induced by standard procedures of MBP/CFA injection (Table 1). The clearest point to emerge from this study was that high constitutive MHC class II expression on microglia (in normal rats) is not associated with increased susceptibility to EAE. BN rat microglia (obtained from two sources) expressed the most MHC class II. Wistar Furth and PVG rats, both strains resistant or relatively resistant to EAE, were variable although clearly lower than the BN. LEW and (LEW × BN)F_1 rats are susceptible to EAE but consistently both were low expressors, F344 rats were included in the study as it is a strain which, under some circumstances (24), is susceptible to EAE. With the immunization protocol used here, minimal disease was observed, although MHC class II expression on microglia was similar to LEW and (LEW × BN)F_1 rats. Why there is some variability in levels of expression, particularly in the PVG and Wistar Furth strains is unclear, given the inbred nature of these animals. Environmental factors may be involved but, where we have had the opportunity to compare SPF and conventional rats, the trends in levels of expression remain the same — BN high, LEW low, and so on.

After viral infection of LEW rats, most of the MHC class II–expressing cells are inflammatory rather than resident microglia (11 and Fig. 2 *b*), so it is possible that microglial cells from this strain are relatively resistant to MHC class II enhancement, unlike BN rat microglia. However, in the face of a massive CD4$^+$ T cell inflammatory response as occurs in EAE, most microglia do express MHC class II (Fig. 5). 3 d after the transfer of an EAE-inducing T cell line, a proportion of blood-derived cells (CD45^{high}) are MHC class II$^+$ as seen in the normal CNS. Compared with control rats (not shown), slightly more CD45^{high} cells are detectable, indicating an early influx of inflammatory cells and/or the appearance of the injected T cell line in the CNS. There is no evidence at this stage of MHC class II expression by the majority CD45^{low} microglial population. By day 4, there is a further increase in the number of CD45^{high} cells recovered. Most microglia are still MHC class II$^-$. A caveat to the observations to day 4 is that our analysis of the whole CNS rather than the lower segments of spinal cord where the first infiltration is generally observed in EAE, may mean that localized induction of MHC class II on microglia is missed, because of a simple dilution effect. Early induction of MHC class II on cells in the vicinity of infiltrating T lymphocytes in LEW rats has been reported, although it is unclear whether the MHC class II$^+$ cells were resident microglia or inflammatory cells (8). By day 5, there is a massive inflammatory response and clear movement of the CD45^{low} cells to MHC class II expression. At day 6, most CD45^{low} cells are MHC class II$^+$. It should be noted that once there is a large influx of inflammatory cells, the analysis of CD45^{low} cells is more difficult in nonchimeric rats as, relative to the infiltrating cells, the numbers of microglia isolated are very low. Moreover, the demarcation between microglia and infiltrating cells becomes less distinct as the former upregulate their CD45 expression levels, presumably as a result of inflammatory cytokines (Fig. 5). However, the irradiation chimera studies

Table 1. *Strain Dependence of Microglial Cell Constitutive MHC Class II Expression*

Rat strain	Percent MHC class II$^+$ microglia*	EAE susceptibility	
		Clinical disease	Severity‖
Lewis	5.0	8/8	5
BN (A)	17.6‡	0/8	–
BN (B)	11.9‡	ND	ND
(Lewis × BN)F_1	5.2	2/4	3.5
F344	5.3	1/3	0.5
PVG	5.6§	1/4	1
Wistar Furth	6.8§	0/4	–

Microglia were isolated from the brain and spinal cord of groups of two or three noninfected, nonimmunized rats and double stained for FACS® analysis. Other groups of rats were injected with MBP/CFA to elicit EAE. BN rats were from Germany (A) or the UK (B). Data are from three experiments using groups of two Lewis and two BN (A) rats as internal controls for each experiment.

* Average scores are shown. Cells were stained for CD45 (MRC OX1 supernatant) and MHC class II (biotinylated MRC OX6; IgG1) or control mAb (biotinylated mouse IgG1 anti-sheep LFA-3). Figures are the percentage of CD45^{low} cells (microglia) staining greater than the negative control marker (as illustrated in Fig. 3). The small percentage of cells staining positive with the biotinylated control mAb (i.e., greater than the set marker) have been subtracted.

‡ The entire CD45^{low} population shifted towards MHC class II positivity.

§ Average of three rats. Range, 4–8.3% (PVG) and 4.5–10.6% (WF). Most cells were negative (like LEW) but in some rats, a distinct population was positive.

‖ Animals that were clinically ill.

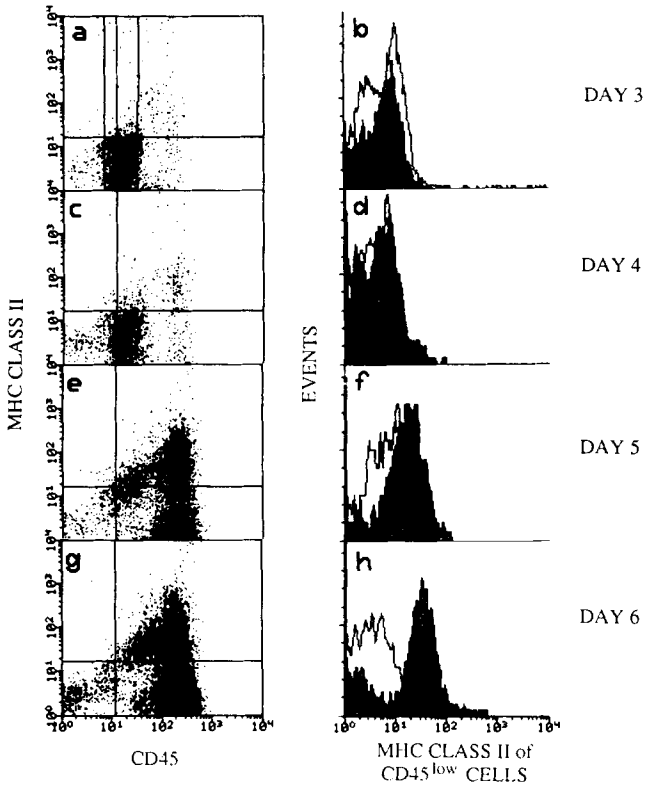

Figure 5. Upregulation of LEW microglial cell MHC class II expression after induction of EAE. LEW rats were injected intravenously with 1.5 × 10^6 MBP-specific 266/87 line T cells, CNS cells isolated 3–6 d later, and the recovered cells double stained for flow cytometry. Analysis quadrants (*a*, *c*, *e*, *g*) are based on control stainings using the mAbs MRC OX21 (FITC) and biotinylated K-1-21 (PE). Gain settings for Fl-1 and Fl-2 were not changed across the time course. MHC class II expression of microglia ($CD45^{low}$) cells is illustrated (*b*, *d*, *f*, and *h*; filled histograms). These histograms were generated by examining only those cells within an analysis gate placed around the $CD45^{low}$ population as illustrated in *a*. The position of this gate was changed for each day as the CD45 expression levels increased. (*Unfilled line*) Control mAb staining (CD45 and biotinylated K-1-21 with analysis only of $CD45^{low}$ cells) for each day.

(11 and Fig. 1) indicate that microglial CD45 expression rarely reaches that of PBL or inflammatory cells, so the use of CD45 levels to distinguish between microglia and inflammatory cells is still valid. Consistent with this is the fact that the majority of $CD45^{low}$ cells in Fig. 5 (days 3–6) are $CD11b/c^+$ (data not shown). Disease severity in the Fig. 5 rats was: day 3, none; day 4, loss of tone in tail only; day 5, hind limb weakness; and day 6, bilateral hind limb paralysis.

Discussion

These studies demonstrate three points. First, cells constitutively expressing MHC class II molecules are present in the normal CNS. These MHC class II^+ cells are not necessarily a transient population derived from the blood, but can be resident (ramified) microglial cells that are an integral part of the structure of normal CNS tissue. Second, levels of constitutive expression vary between strains, and some strains, the BN in particular, have considerable numbers of their microglia expressing MHC class II antigens. In other strains, such as the Lewis, the main MHC class II–expressing cells isolated from the normal CNS are a transient population derived from the blood, whereas microglial MHC class II expression is minimal. Third, susceptibility to CNS autoimmunity (EAE) is, if anything, inversely related to constitutive MHC class II expression. Certainly, the most EAE-susceptible strains do not constitutively express MHC class II on their microglia. It is probably reasonable in the light of this data to bury the concept that heightened susceptibility to CNS inflammation and constitutive MHC class II expression, at least on microglia within the CNS, are linked. In EAE-susceptible strains like LEW, MHC class II can be induced on microglia (Fig. 5), but it can also be further upregulated in the BN strain given the appropriate stimulus (Fig. 2).

The irradiation chimera experiments provide strong evidence that the MHC class II–expressing population in BN rats isolated in these studies, are a resident, irradiation-resistant population. The early conclusions by Ting et al. (25) using irradiation bone marrow chimeras, that brain Ia antigens have a bone marrow origin are, therefore, probably only correct when the normal constitutive levels in the host strain are very low. In $F_1 \rightarrow$ LEW rats for example, most of the MHC class II expression in the normal CNS, are derived from the donor cells and not the host (Fig. 2 *a*). CNS perivascular cells which are irradiation sensitive (5, 20) and may constitutively express MHC class II molecules (23), are one of the populations likely to be responsible for donor cell MHC class II expression in chimeras. The staining of MHC class II^+ cells in situ in BN rats confirms the predominantly parenchymal as opposed to perivascular location of the positive cells. True perivascular cells lie outside the parenchymal basement membrane in the perivascular space (19). Most of the positive cells in Fig. 4 are not of this type. Some of the MHC class II^+ cells were relatively close to vessels, but this is consistent with the recent observation that microglial processes can, like astrocyte endfeet, form part of the glia limitans (26).

Is constitutive MHC class II expression T cell dependent? It is now clear that activated T cells traffic through the normal CNS (6) and it is possible that these cells could be responsible for the constitutive MHC class II expression described here. Preliminary studies suggest however, that most of it is not dependent on T lymphocytes. First, at least 5–6% of microglia are MHC class II^+ in athymic PVG rats. Athymic BN rats are not available. Second, in BN rats infected with MHV-JHM we have found recently that increased MHC class II expression on microglia such as that illustrated in Fig. 2 *d*, can be prevented completely by irradiation of the rats with 600 R γ, which temporarily depletes most T cells. Applying this same procedure to normal (noninfected) BN rats, MHC class II–expressing microglia are mostly retained, although there is some loss of the highest expressing population (Schwender, S., and R. Dörries, unpublished observations).

Does constitutive MHC class II expression on microglia have any functional relevance for development of T cell responses in the CNS? It has been known for some time that

after development of inflammation in the human and animal CNS, microglia (together with inflammatory monocytes) are the most abundant MHC class II–expressing population (8, 9). On the basis of these observations, microglia are increasingly being viewed as the most likely candidates to fulfill the role of APC in the CNS. APC in this context is taken to mean a cell that will present antigen to T cells (unprimed or primed), as does a leucocyte dendritic cell for example, leading to further T cell activation and proliferation. There is evidence that microglia purified from the neonatal mouse CNS can act as APC for T cell lines (27). Perivascular cells which are distinct from microglia have a demonstrable APC function in vivo (5), but it is not known whether fully differentiated, ramified microglia from adult animals like those isolated in the present studies, are similarly effective in this regard. In essence, therefore, the appearance of MHC class II on microglia after the influx of inflammatory cells is not proof that these cells are effective APC in situ. Rather, it only means that they have responded to the presence of infiltrating T cells. The description here of constitutive MHC class II expression on microglia from (some) rat strains resistant to EAE and the low levels of expression on susceptible strains, has led us to consider whether microglial MHC expression imparts on these cells, the ability to interact with and downregulate T cell responses rather than amplify them. In support of this general concept is the demonstration that administration of IFN-γ intraventricularly just before the normal time of EAE onset (resulting in high MHC class II expression on CNS glial cells), effectively inhibits clinical disease (28).

Of course, it should be stressed that the data in the present study do not at this stage, establish that MHC-expressing microglia are the only or even a major contributory factor in strain resistance. A complex assortment of background genes are likely to be involved in susceptibility to EAE (29). This is exemplified by the PVG-RT1^c strain used here, in which inducible corticosterone levels are probably the dominant regulatory mechanism (30). Nevertheless, in other strains such as the PVG-RT1^u strain (same background, different MHC), steroid influences are less important (31). It remains to be tested whether in strains like the PVG-RT1^u and in the BN, glial cell–T cell interactions may contribute to strain resistance. To this end, we are currently preparing modified chimeras between the LEW and BN rat strains which will allow us to establish unequivocally the influence of the host target organ on susceptibility to EAE, and examining whether microglia freshly isolated from the BN vs LEW CNS have negative rather than positive regulatory influences on T cell function in vitro.

Finally, it is important to note the substantial differences between the results presented here and some in vitro studies on rat and mouse astrocytes (32) and rat brain vascular endothelial cells (33) where IFN-γ treatment has been shown to induce higher levels of MHC class II expression on cells isolated from EAE-susceptible strains. Notably, another in vitro study (34) demonstrated substantial IFN-γ–induced MHC class II expression on astrocytes from an EAE-resistant mouse strain after the astrocytes were cultured for an extended period of time. Thus, the contradictory findings may be attributed to a variety of factors, including the developmental stage of the cells (newborn vs adult-derived cells, length of culture time), the fundamental differences between the cell types examined, or the fact that the present study addresses in vivo rather than in vitro phenomena.

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