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HLA class II molecules (HLA-DR, -DP, -DQ) on cells in the human CNS studied *in situ* and *in vitro*

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SUMMARY

Human leucocyte antigen (HLA) class II molecules expressed by antigen-presenting cells (APC) are major restriction elements in the interaction between APC and T cells of the $CD4^+$ subtype. To explore the immune accessory function of cells in the central nervous system (CNS), we studied the expression of HLA-DR, -DP, and -DQ molecules on CNS cells *in situ* and *in vitro*. Reactive microglia and perivascular cells in multiple sclerosis lesions expressed all three HLA class II molecules, whereas microglia in the normal CNS expressed HLA-DR only. All three HLA class II molecules were up-regulated on cultured microglia after stimulation with interferon-γ (IFN-γ). Microglial stimulation of allogeneic $CD4^+$ T cells in a mixed lymphocyte reaction (MLR) was effectively blocked using anti-HLA-DR monoclonal antibodies (mAb) but not using anti-HLA-DQ mAb. HLA class II-positive astrocytes and endothelial cells were not identified in normal or diseased CNS. Cultured astrocytes stimulated with IFN-γ could, however, be induced to express HLA class II antigens of all subtypes, although great variability was observed between different donors. Our results indicate that although both microglia and astrocytes are capable of expressing all HLA class II subtypes *in vitro*, subtype expression differs between normal and pathological states *in situ*. Such selective expression may be associated with functional properties.

INTRODUCTION

Human leucocyte antigen (HLA) class II genes are composed of three closely linked subregions encoding the polymorphic HLA class II molecules HLA-DR, -DP and -DQ. HLA class II molecules are transmembranous glycoproteins primarily expressed on cells involved in immune functions, such as B cells, monocytes, macrophages, dendritic cells and Langerhans' cells. The membrane-distal domains of these molecules form a groove which binds processed peptide fragments of antigenic molecules. The molecular complex of peptide and HLA may thereafter be recognized by antigen-restricted $CD4^+$ T cells. Allelic variants of HLA class II molecules vary in ability to bind antigenic peptides, making the antigen-specific T-cell response of an individual restricted by the HLA haplotype.[1–3]

Multiple sclerosis (MS), a chronic disease of the human central nervous system (CNS) white matter, and experimental allergic encephalomyelitis (EAE), a putative animal model of MS, are both characterized pathologically by multiple foci of perivascular inflammation with varying degrees of demyelination. Myelin basic protein (MBP) reactive T cells of the CD4 subtype are of major pathogenetic importance in EAE.[4] The genetic linkage of MS to specific HLA class II alleles,[5] the identification of $CD4^+$ T cells recognizing MBP in the context of MS-associated HLA class II molecules,[6] and the identification in MS lesions of a restricted MBP-specific T-cell receptor repertoire,[7] suggest that T cells may also have a pathogenetic role in the development of MS.

We have recently shown that microglia isolated from adult human CNS can induce both a mixed lymphocyte reaction (MLR) and a recall antigen T-cell response. Preincubation of microglia with interferon-γ (IFN-γ) up-regulated HLA class II expression and antigen-presenting capability of microglia.[8,9] A similar *in vivo* function of microglia would permit T-cell responses to be initiated intrathecally, further strengthening a role for autoreactive T cells in MS.

Most autoreactive MBP-specific T cells are restricted by HLA-DR, but HLA-DP and HLA-DQ molecules have also been identified as restriction elements for MBP-reactive T cells.[6,10] The expression of HLA-DR on CNS cells has been extensively studied both *in vitro* and *in situ*, but little is known about HLA-DP and HLA-DQ expression in the normal and diseased CNS. To explore further the antigen-presenting

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capacity of CNS cells, we have studied the expression of HLA-DR, -DP and -DQ in normal and diseased CNS, and on microglia and astrocytes cultivated *in vitro* under basal and activating conditions.

MATERIALS AND METHODS

Patients

Ten patients with clinically definitive MS were studied. The diagnosis was confirmed by post-mortem examination of the CNS. Six patients with non-cerebral diseases were included as controls.

Tissue

Brain and spinal cord from MS patients and controls were obtained at various time intervals after death. Blocks of tissue from MS lesions and surrounding white matter as well as white matter from the controls were frozen in liquid nitrogen and sectioned (4–6 μm) on a cryostat. Histological staining of sections with Luxol fast blue was performed to delineate demyelinated areas.

For double-labelling studies by confocal microscopy, 10% formalin or 4% paraformaldehyde fixed CNS tissue was cryoprotected in 20% glycerol, embedded in 30% sucrose on a dry ice platform and sectioned (30 μm) on a sliding microtome.

Glial cell cultures

Isolated glial cells were obtained from patients undergoing surgical procedures for intractable epilepsy by a previously described protocol.[11] Histological examination of tissue revealed mild to severe gliosis. After 1 week in culture, culture flasks with microglia/astrocyte cultures were trypsinized. Mixed microglia/astrocyte cultures were seeded at 10^4 cells/9 mm Aclar fluorocarbon plastic coverslip (S. Kim, Vancouver, Canada) previously coated with 10 μg/ml poly-L-lysine (Sigma, St Louis, MO). Other culture flasks were subjected to 5 hr of 150 rpm shaking on an orbital shaker. The less adherent astrocytes floated off, and the remaining cells (>95% Leu-M5$^+$ cells) were then trypsinized and seeded onto coverslips or into microplate wells. In some experiments glial cells were stimulated with 100 U IFN-γ (UBI, Lake Placid, NY) for 24 hr before analysis. Culture medium was Eagle's minimum essential medium supplemented with 5% fetal calf serum (FCS), gentamicin 20 μg/ml, glucose 1 mg/ml, and 2 mM glutamine (Gibco, Burlington, Canada).

Lymphocytes

Peripheral blood mononuclear cells were obtained from young adult donors. Total T cells were isolated by rosetting with aminoethyl-isothiouroniumbromide hydrobromide (AET)-treated sheep erythrocytes, and CD4$^+$ cells prepared by complement-mediated lysis of CD8$^+$ cells.[9] Purity of CD4$^+$ T cells was assessed by FACScan analysis of T cells immunostained with fluorescein isothiocyanate (FITC)-conjugated anti-CD8 monoclonal antibody (mAb) (Leu-2a) and phycoerythrin-conjugated anti-CD4 mAb (Leu-3a; Becton Dickinson, Mountain View, CA). Purity of CD4$^+$ T cells was >96%.

Antibodies

The mAb used in this study are summarized in Table 1. Rabbit antibodies to glial fibrillary acidic protein (GFAP) and biotinylated rabbit anti-mouse immunoglobulins were purchased from Dakopatts (Glostrup, Denmark). Rabbit anti-glucose transporter antibodies (Glut-1) were obtained from Calbiochem (San Diego, CA). Rhodamine-conjugated goat anti-mouse immunoglobulins and fluorescein-conjugated goat anti-rabbit immunoglobulins were obtained from Cappel (West Chester, PA) and from Jackson Laboratories (West Grove, PA). Rhodamine-conjugated Ricinus Communis Agglutinin-1 (RCA-1), a lectin which labels macrophages, microglia and endothelial cells, was obtained from Vector Laboratories (Burlingame, CA).

Immunofluorescence analysis

Mixed astrocyte–microglia cultures were analysed by indirect double immunofluorescence labelling techniques in order to permit discrimination between astrocytes (GFAP$^+$) and microglia. Viable unfixed cells were incubated with murine mAb for 2 hr at 4°, and thereafter with rhodamine-conjugated goat anti-mouse antibodies diluted 1:100 for 45 min at 4°. Following fixation with 95% ethanol–5% glacial acetic acid mixture at −20° for 15 min, antibodies to GFAP were introduced for 45 min at room temperature. Fluorescein-conjugated

Table 1. Characteristics of mAb used

Antigen	mAb	Isotype	Reactivity pattern	Manufacturer*	Dilution
CD4	Leu-3a	IgG1	T helper/inducer; monocytes	B/D	5 μg/ml
CD8	OKT8	IgG2a	T suppressor/cytotoxic	Ortho	5 μg/ml
CD8	Leu-2a	IgG1	T suppressor/cytotoxic	B/D	5 μg/ml
CD11c	Leu-M5	IgG2b	Monocytes/macrophages	B/D	1 μg/ml
CD14	Leu-M3	IgG2b	Monocytes/macrophages	B/D	2·5 μg/ml
CD45	2B11 and PD7/26	IgG1	Leucocytes	Dako	1:20
HLA class II	CR3/43	IgG1	Leucocytes	Dako	1:100
HLA-DR	L243	IgG2a	Leucocytes	B/D	0·5 μg/ml
HLA-DP	B7/21	IgG1	Leucocytes	B/D	0·5 μg/ml
HLA-DQ	Leu-10	IgG1	Leucocytes	B/D	0·5 μg/ml
HLA-DQ	FN 81–1-3	IgG1	Leucocytes	S. Funderud	1 μg/ml

*B/D, Becton Dickinson, Mountain View, CA; Ortho, Ortho Diagnostic Systems, Raritan, NJ; Dako, Dakopatts, Glostrup, Denmark. The generous gift of mAb FN 81–1-3 by S. Funderud, The Norwegian Radiumhospital, Oslo, Norway, is appreciated.

goat anti-rabbit immunoglobulins diluted 1:100 were then applied for 45 min at room temperature. All incubations were followed by washings in phosphate-buffered saline, pH 7·2 (PBS). Coverslips were finally mounted on glass slides with Gelvatol (Air Products and Chemicals Inc, Allentown, PA).

For double labelling of cells *in situ*, free floating sections were permeabilized with 10% Triton X-100 for 30 min, incubated in 3% normal goat serum for 30 min, and then with primary antibodies or RCA-1 for 14 hr. Appropriate fluorescein- and rhodamine-conjugated secondary antibodies (1:100) were then applied for 1 hr. Sections were mounted in a Mowiol-based (Calbiochem) mounting medium containing paraphenylenediamine hydrochloride. Confocal microscopy was performed using a Leica Aristoplan confocal laser scanning microscope (Leitz Wetzlar, Heidelberg, Germany). Confocal images representing optical sections of approximately 0·35 μm axial resolution were collected. Final images represented the average of 32 line scans of each line of the chosen fields (line averaging). In some cases, eight to 10 consecutive optical sections were combined to form a 'through focus image'. Omission of primary antibody in parallel sections served as a control for non-specific background staining.

Immunohistochemistry

Sections were fixed in cold acetone for 5 min. They were thereafter incubated with 10% rabbit serum diluted in PBS for 30 min at room temperature, followed by overnight incubation at 4° in a humid chamber with mAb diluted in 10% rabbit serum. Biotinylated rabbit anti-mouse immunoglobulins diluted 1:300 were then applied for 30 min.

Avidin–biotin–peroxidase complex (ABComplex/HRP, Dakopatts) was prepared as recommended by the manufacturer, and allowed to react with sections for 30 min. Sections were finally treated with a 3-amino-9-ethyl-carbazole-containing buffer for the development of a coloured reaction product.

Purified microglia were stained with mAb for 60 min at 4°. The cells were then fixed with 95% ethanol–5% glacial acetic acid at −20° for 15 min. Microglia were thereafter immunostained as described above. All incubations were followed by washings in PBS. Controls consisted of similarly treated sections in which primary mAb were omitted. Sections and cells were finally counterstained with haematoxylin, mounted in Glycergel (Dakopatts), and analysed in a light microscope.

Mixed microglia–lymphocyte reactions

These experiments were performed as previously described.[9] Briefly, 1×10^5 allogeneic $CD4^+$ T cells were placed into individual wells of 96-well flat-bottom microtitre plates into which radiated microglia had been seeded at a concentration of 2×10^4 cells/well. Wells were rinsed twice before addition of anti-HLA mAb L243 [American Type Culture Collection (ATCC), Rockville, MD] or FN 81–1-3 (S. Funderud, The Norwegian Radium Hospital, Oslo, Norway) and T cells. Monoclonal antibodies were added at 20 μg/ml final dilution 1 hr prior to the addition of T cells.

Reactions were carried out for 5 days, and proliferative responses determined by pulsing cultures for 5 hr with [^{3}H]thymidine (1 μCi/well) (ICN Flow Laboratories, Mississagua, Canada), harvesting the wells, and counting radioactivity using a beta liquid scintillation counter (LKB, Fisher, Canada). Results were calculated as mean counts per minute (c.p.m.) of triplicate wells.

RESULTS

Normal white matter

$HLA\text{-}DR^+$ ramified cells were observed in both autopsy and biopsy brains (Fig. 1A). The $HLA\text{-}DR^+$ cells were $RCA\text{-}1^+$/$GFAP^-$ as shown by confocal microscopy. In two autopsy brains occasional $HLA\text{-}DP^+$ cells were observed in the parenchyma. HLA-DQ was not observed. Vessel-associated HLA class II expression was found on perivascular macrophages, but not on $Glut\text{-}1^+$ endothelium. Parenchymal ramified cells were $CD11c^+$ and $CD45^+$, but $CD14^-$. Occasional perivascular cells were stained by Leu-M3 against CD14. $CD4^+$ and $CD8^+$ T cells were not found.

MS lesions

HLA-DR was expressed on cells throughout the parenchyma and on perivascular cells in all lesions (Fig. 1B). $HLA\text{-}DR^+$ cells had an oval morphology within the lesions and at the borders, whereas cells just outside the borders and in the normal appearing parenchyma had a more ramified morphology. The intensity of staining was strongly enhanced within lesions and at the active borders.

HLA-DP and HLA-DQ antigens were identified on perivascular cells and on rounded cells within MS lesions and at the borders. $HLA\text{-}DP^+$ and $HLA\text{-}DQ^+$ cells with a ramified morphology were identified in the surrounding normal appearing white matter just outside the active borders (Fig. 1C, D), although less frequently than $HLA\text{-}DR^+$ cells. HLA-DP and HLA-DQ reactivity was weak or absent on cells farther away from the lesions. More cells were stained by mAb reactive with HLA-DP than with HLA-DQ.

HLA class II^+ parenchymal and perivascular cells were $RCA\text{-}1^+$ as seen by confocal microscopy (Fig. 2A). $RCA\text{-}1^+$ and $Glut\text{-}1^+$ endothelium did not co-localize with HLA class II immunopositivity as shown by confocal microscopy (Fig. 2A, B). Astrocytes were consistently negative for HLA class II molecules in all lesions studied, as verified by double immunofluorescence analysis with GFAP (Fig. 2C, D).

Cells reactive with mAb to CD11c and CD45 were prominent throughout the lesions, and had a morphology similar to the previously defined $HLA\text{-}DR^+$ cells. Cells in the perivascular inflammatory infiltrates were also $CD45^+$. Leu-M3 stained intravascular and perivascular cells, but also some round cells in the parenchyma. $CD4^+$ and $CD8^+$ cells were observed mainly in the perivascular inflammatory cuffs. Some $CD4^+$ and $CD8^+$ T cells were also present within the parenchyma.

Cultured glia

As shown in Fig. 3, 83% of unstimulated microglia were $HLA\text{-}DR^+$, whereas 96% of IFN-γ-stimulated microglia were $HLA\text{-}DR^+$. Thirteen per cent of unstimulated microglia were $HLA\text{-}DP^+$. The number of $HLA\text{-}DP^+$ cells increased to 63% after IFN-γ stimulation. Similar numbers for HLA-DQ were 10% positive before stimulation, 60% positive after stimulation. Of the four patients studied, only one patient had $HLA\text{-}DP^+$ and $HLA\text{-}DQ^+$ astrocytes. The number of astrocytes

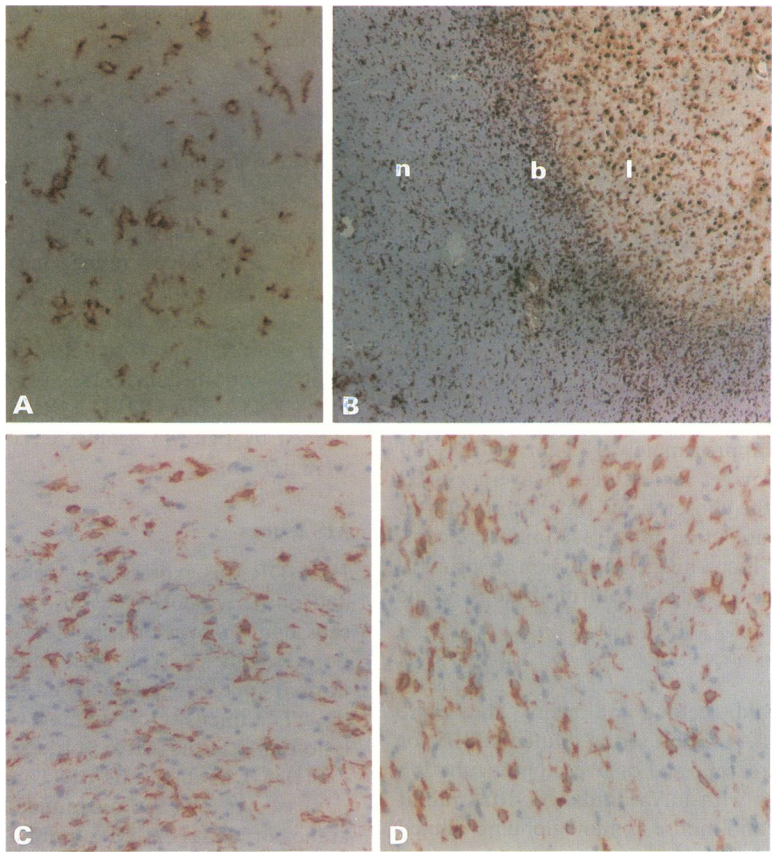

Figure 1

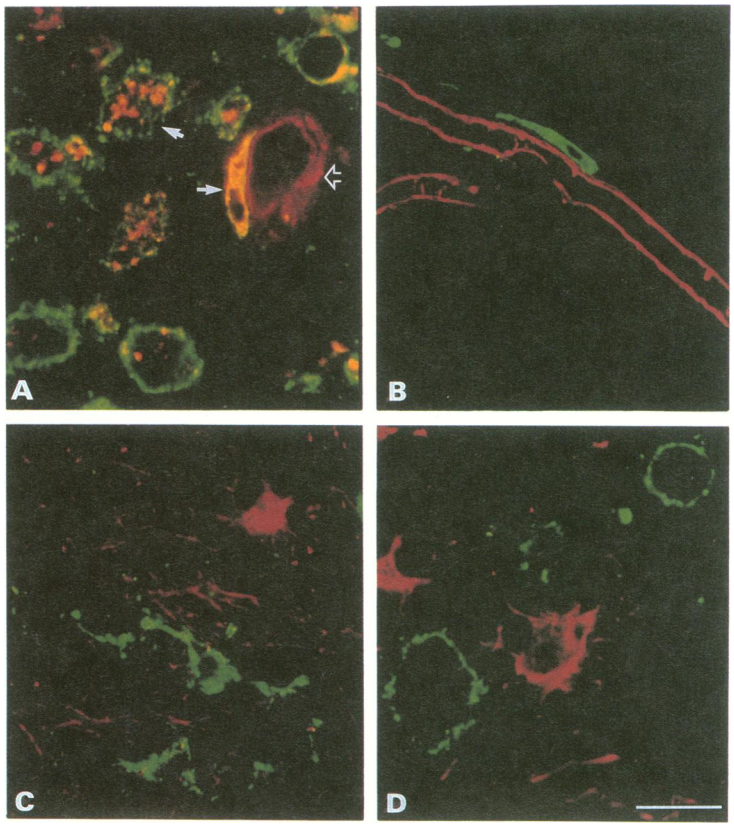

Figure 2

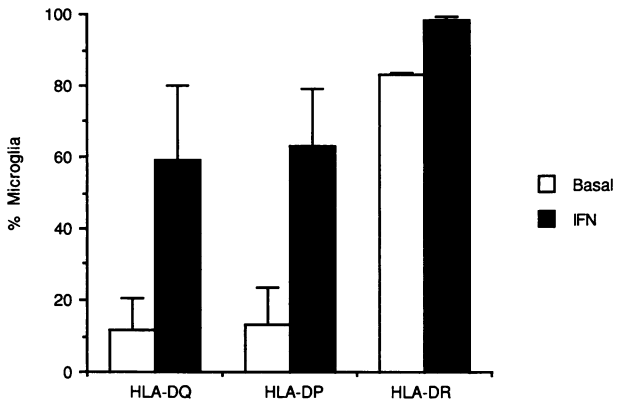

Figure 3. Comparison between basal and IFN-γ (100 U/ml) stimulated HLA-DQ (Leu-10), HLA-DP (B7/21), and HLA-DR (L243) expression on cultured human microglia. Bars represent per cent ± SD of microglia immunostained with anti-HLA class II mAb ($n = 4$ patients). One hundred microglia were counted blind on each of three different coverslips from each patient.

from this patient positive for HLA-DR was 20% and 57%, for HLA-DP 1% and 22%, and for HLA-DQ 0% and 10%, before and after IFN-γ stimulation respectively. Mean values of HLA-DR expressing astrocytes from the four patients were 7·6% before IFN-γ stimulation and 25·6% after stimulation.

Cultured human microglia were stained by mAb to CD11c and CD45, but not by mAb to CD4, CD8, or CD14.

Effect of anti-HLA class II mAb on microglia-induced T-cell proliferation

Microglia induced a significant proliferation of $CD4^+$ T cells prepared from allogeneic donors (13,988 ± 3882 c.p.m.). Previous incubation of microglia with anti-HLA-DR mAb reduced T-cell proliferation by more than 80% in all experiments ($n = 5$). Previous incubation of microglia with anti-HLA-DQ mAb had an inhibitory effect on T-cell proliferation (40–47% reduction) in four experiments whereas anti-HLA-DQ mAb enhanced the T-cell proliferative response in one experiment (49% increase).

DISCUSSION

Absence of HLA class II-expressing cells in the normal human CNS was previously interpreted as a manifestation of the immunologically privileged status of the brain.[12] However, using more sensitive immunohistochemical techniques several groups have in recent years reported HLA-DR expression on microglia in both normal and diseased CNS.[13–16] We found in the present study distinct HLA-DR expression on ramified microglia within the normal CNS, with a strongly increased expression on reactive microglia ($CD11c^+$, $CD45^+$, $CD14^-$) in MS lesions. HLA-DP and HLA-DQ molecules were detected on reactive microglia within MS lesions and at the active borders, but scarcely in normal CNS. HLA-DP and HLA-DQ molecules have previously been identified in the CNS from patients with Alzheimer's disease[17] and amyotrophic lateral sclerosis,[18] and recently HLA-DQ has been described on microglia in active MS lesions.[19] The expression of HLA-DP and HLA-DQ on reactive microglia may be used as activation markers of these cells, because resting microglia are largely negative for both molecules.

The expression of HLA-DQ on reactive microglia within MS lesions may be of particular significance as MS has a strong association to specific HLA-DQ alleles.[5] The effect of HLA-DQ on disease susceptibility is however largely unknown, although some data indicate that suppressor T cells are primarily HLA-DQ restricted.[20] Our functional data using mAb to block HLA class II molecules in the mixed microglia–T cell reaction indicate HLA-DR as the main restriction determinant for antigen-restricted T-cell stimulation, with HLA-DQ having a minor role. However, in one experiment an enhancing effect of anti-HLA-DQ mAb was observed, indicating a suppressive effect of this specific HLA-DQ allele on T-cell activation. Similar results have previously been obtained using stimulator cells obtained from peripheral blood in both allogeneic and MBP-specific T-cell activation.[21,22]

The expression of HLA-DR on astrocytes *in situ* is controversial.[14–16,23–25] Expression of HLA-DR on cultured adult human astrocytes has however been conclusively demonstrated, with an increased expression after treatment with IFN-γ.[26] In the present study HLA class II molecules were not detected on astrocytes *in situ*, although such expression was observed on astrocytes cultured *in vitro* under both basal and IFN-γ-stimulated conditions. Expression of HLA class II antigens on cultured astrocytes was extremely variable between donors, as previously shown by Yong *et al.*[26] These data may indicate a differential genetic capacity of patients to express HLA class II molecules on astrocytes. If these *in vitro* data are applicable to the *in vivo* situation, such data may explain some of the controversies in the literature regarding HLA-DR staining of astrocytes in MS lesions.

Although both astrocytes and microglia are able to express HLA class II molecules *in vitro*, microglia are most important as CNS antigen-presenting cells (APC) in the rodent system.[27] We have previously shown that human microglia, but not

Figure 1. (*opposite*) Immunohistochemical staining of cells in normal (A) and MS (B–D) CNS. Counterstaining with haematoxylin. (A) HLA-DR identified on ramified microglial cells in a CNS biopsy with mild gliosis (magnification × 181·5). (B) HLA-DR on microglial cells within an active MS lesion (l), at the lesion border (b), and in the normal appearing surrounding parenchyma (n) (× 38·5). (C) HLA-DP expression on microglia at the border of an active MS lesion (× 93·5). (D) HLA-DQ expression on microglia at the border of an active MS lesion (× 93·5).

Figure 2. (*opposite*) Confocal images of free-floating sections from MS brains double labelled for HLA class II (green) (A–D) and RCA-1 (A), Glut-1 (B), and GFAP (C,D). Inside the lesions, HLA class II was detected on $RCA\text{-}1^+$ cells (A, arrow) but not $RCA\text{-}1^+$ vascular endothelial cells (A, open arrow). Outside MS lesions, vessel-associated HLA class II is located on thin cells (B, green) that line Glut-1 transporter positive endothelial cells (B, red). Outside (C) and inside (D) the demyelinating region, the astrocyte cell body and processes are $GFAP^+$ (red), but not HLA class II^+ (green). Outside the lesion the HLA class II antibody stains cells with the morphology of microglia (C, green). Inside the lesion HLA class II is found on the surface of phagocytic cells (D, green). Scale bar = 30 μm.

astrocytes, can initiate a primary T-cell response *in vitro*,[9] thus implicating microglia as the main APC in the human CNS parenchyma.

The perivascular location of macrophages expressing HLA class II molecules in both normal and diseased CNS suggests an important role for these cells in antigen presentation.[16,28] Perivascular macrophages are the first HLA class II^+ cells within the CNS to encounter invading T cells. Such an encounter may induce activation and proliferation of antigen-restricted T cells, probably explaining the vasoconcentricity of T cells observed in MS and EAE lesions. The relative importance of CNS APC in EAE induction was recently demonstrated using mouse bone marrow radiation chimeras.[29] Although both resident CNS parenchymal cells and perivascular bone marrow-derived cells could present autoantigen and induce disease *in vivo*, perivascular bone marrow-derived APC were most efficient.

Occasional HLA-DR^+ endothelial cells have previously been identified in the CNS by some investigators[24,30] but not by others.[25] A recent report has shown that cultured human CNS endothelial cells are negative for HLA-DR, but become positive for HLA-DR after IFN-γ treatment.[31] In addition, rat endothelial cells express major histocompatibility complex (MHC) class II antigens after intravenous injections of IFN-γ.[32] Such data indicate that HLA-DR will be expressed on endothelial cells only in inflamed areas, where T cells may be a rich source of IFN-γ. We were not able however to identify HLA class II^+ endothelial cells in the present study.

Our *in vitro* studies indicate that IFN-γ is a potent inducer of HLA class II molecules on glial cells. IFN-γ is produced during immune responses by antigen-specific T cells, and induces a rapid increase in mRNA for HLA class II molecules, thus enhancing the antigen-presenting capability of inflammatory cells.[33] MS patients treated with IFN-γ experience exacerbations of the disease, and this has been attributed to an up-regulation of HLA class II molecules.[34] Because the blood–brain barrier of MS patients is permeable, intravenous injections of IFN-γ may cross the blood–brain barrier and stimulate expression of IFN-γ-responsive molecules on glial cells, thereby enhancing the CNS immune response.[32]

IFN-γ-producing T cells have been identified in MS lesions,[35] probably explaining the increased expression of HLA class II molecules on microglia within MS lesions. In the present study we found few T cells in the parenchyma of active MS lesions; the majority of T cells had a perivascular location. Similar results have previously been described by Boyle and McGeer[15] and Hofman *et al.*,[23] whereas Estes *et al.*[36] found T cells of both the CD4 and CD8 phenotypes throughout the lesions. These differences are probably related to the age and intensity of inflammation in the lesions.

Although major histocompatibility complex (MHC) class II expression by glial cells is thought to play an important role in the pathogenesis of demyelinating diseases, recent experimental data indicate that under certain conditions expression of MHC class II molecules on rat microglia may have a suppressive rather than enhancing effect on the CNS immune response.[37,38] Presence rather than absence of specific MHC class II molecules may consequently be important in the immunological privilege of the CNS. Our findings of aberrant HLA class II expression by microglia in MS lesions may accordingly be of importance in the local regulation of the CNS immune response.

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REFERENCES

1. Thorsby E. (1987) Structure and function of HLA molecules. *Transplant. Proc.* **19,** 29.
2. Fairchild P.J. & Wraith D.C. (1992) Peptide–MHC interaction in autoimmunity. *Curr. Opin. Immunol.* **4,** 748.
3. Brown J.H., Jardetzky T.S., Gorga J.C., Stern L.J., Urban R.G., Strominger J.L. & Wiley, D.C. (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*, **364,** 33.
4. Zamvil S.S. & Steinman L. (1990) The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* **8,** 579.
5. Spurkland A., Rønningen K.S., Vandvik B., Thorsby E. & Vartdal F. (1991) HLA-DQA1 and HLA-DQB1 genes may jointly determine susceptibility to develop multiple sclerosis. *Hum. Immunol.* **30,** 69.
6. Chou Y.K., Vainiene M., Whitham R., Bourdette D., Chou C.H.-J., Hashim G., Offner, H. & Vandenbark A.A. (1989) Response of human T lymphocyte lines to myelin basic protein: association of dominant epitopes with HLA class II restriction molecules. *J. Neurosci. Res.* **23,** 207.
7. Oksenberg J.R., Panzara M.A., Begovich A.B., Mitchell D., Erlich H.A., Murray R.S. *et al.* (1993) Selection of T-cell receptor Vβ-Dβ-Jβ gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature*, **362,** 68.
8. Williams K., Bar-Or A., Ulvestad E., Olivier A., Antel J.P. & Yong V.W. (1992) Biology of adult human microglia in culture: comparisons with peripheral blood monocytes and astrocytes. *J. Neuropathol. exp. Neurol.* **51,** 538.
9. Williams K., Ulvestad E., Cragg L., Blain M. & Antel J.P. (1993) Induction of primary T cell responses by human glial cells. *J. Neurosci. Res.* **36,** 382.
10. Joshi N., Usuku K. & Hauser S.L. (1993) The T-cell response to myelin basic protein in familial multiple sclerosis: diversity of fine specificity, restricting elements, and T-cell receptor usage. *Ann. Neurol.* **34,** 385.
11. Yong V.W. & Antel J.P. (1992) Culture of glial cells from human brain biopsies. In: *Protocols for Neural Cell Culture* (eds A. Richardson and S. Fedoroff), p. 81. The Humana Press Inc., St Louis.
12. Daar A.S., Fuggle S.V., Fabre J.W., Ting A. & Morris P.J. (1984) The detailed distribution of MHC class II antigens in normal human organs. *Transplantation*, **38,** 293.
13. Gehrmann J., Banati R.B. & Kreutzberg G.W. (1993) Microglia in the immune surveillance of the brain: human microglia constitutively express HLA-DR molecules. *J. Neuroimmunol.* **48,** 189.
14. Hayes G.M., Woodroofe M.N. & Cuzner M.L. (1987) Microglia are the major cell type expressing MHC class II antigens in human white matter. *J. Neurol. Sci.* **80,** 25.
15. Boyle E.A. & McGeer, P.L. (1990) Cellular immune response in multiple sclerosis plaques. *Am. J. Pathol.* **137,** 575.
16. Graeber M.B., Streit W.J., Büringer D., Sparks L. & Kreutzberg G.W. (1992) Ultrastructural location of major histocompatibility complex (MHC) class II positive perivascular cells in histologically normal human brain. *J. Neuropathol. exp. Neurol.* **51,** 303.
17. Luber-Narod J. & Rogers J. (1988) Immune system associated antigens expressed by cells of the human central nervous system. *Neurosci. Lett.* **94,** 17.

18. Kawamata T., Akiyama H., Yamada T. & McGeer, P.L. (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am. J. Pathol.* **140,** 691.
19. Li H., Newcombe J., Groome N.P. & Cuzner M.L. (1993) Characterization and distribution of phagocytic macrophages in multiple sclerosis plaques. *Neuropathol. appl. Neurobiol.* **19,** 214.
20. Altman D.M., Sansoom D. & Marsh S.G.E. (1991) What is the basis for HLA-DQ associations with autoimmune disease? *Immunol. Today*, **12,** 267.
21. Festenstein H., Fainboim L., Jaraquemada D. & Navarrete C. (1986) Changing antigenic profiles of HLA class II antigens on activated T cells and their biological effects. In: *HLA Class II Antigens. A Comprehensive Review of Structure and Function* (eds B. G. Solheim, E. Møller and S. Ferrone), p. 314. Springer-Verlag, Berlin.
22. Liblau R., Tournier-Lasserve E., Maciazek J., Dumas G., Siffert O., Hashim G. & Bach M.A. (1991) T cell response to myelin basic protein epitopes in multiple sclerosis patients and healthy subjects. *Eur. J. Immunol.* **21,** 1391.
23. Hofman F.M., von Hanwehr R.I., Dinarello C.A., Mizel S.B., Hinton D. & Merrill J.E. (1986) Immunoregulatory molecules and IL 2 receptors identified in multiple sclerosis brain. *J. Immunol.* **136,** 3239.
24. Traugott U., Scheinberg L.C. & Raine C.S. (1985) On the prescence of Ia-positive endothelial cells and astrocytes in multiple sclerosis lesions and its relevance to antigen presentation. *J. Neuroimmunol.* **8,** 1.
25. Lee S.C., Moore G.R.W., Golenwsky, G. & Raine, C.S. (1990) Multiple sclerosis: a role for astroglia in active demyelination suggested by class II MHC expression and ultrastructural study. *J. Neuropathol. exp. Neurol.* **49,** 122.
26. Yong V.W., Yong F.P., Ruijs T.C.G., Antel J.P. & Kim S.U. (1991) Expression and modulation of HLA-DR on cultured human astrocytes. *J. Neuropathol. exp. Neurol.* **50,** 16.
27. Matsumoto Y., Ohmori K. & Fujiwara M. (1992) Immune regulation by brain cells in the central nervous system: microglia but not astrocytes present myelin basic protein to encephalitogenic T cells under *in vivo*-mimicking conditions. *Immunology*, **76,** 209.
28. Hickey W.F. & Kimura H. (1988) Perivascular microglial cells of the CNS are bone marrow-derived and present antigen *in vivo*. *Science*, **239,** 290.
29. Myers K.J., Dougherty J.P. & Ron Y. (1993) *In vivo* antigen presentation by both brain parenchymal cells and hematopoietically derived cells during the induction of experimental autoimmune encephalomyelitis. *J. Immunol.* **151,** 2252.
30. Styren S.D., Civin W.H. & Rogers J. (1990) Molecular, cellular, and pathologic characterization of HLA-DR immunoreactivity in normal elderly and Alzheimer's disease brain. *Exp. Neurol.* **110,** 93.
31. Huynh H.K. & Dorovini-Zis K. (1993) Effects of interferon-γ on primary cultures of human brain microvessel endothelial cells. *Am. J. Pathol.* **142,** 1265.
32. Steiniger B. & van der Meide P.H. (1988) Rat ependyma and microglia express class II MHC antigens after intravenous infusion of recombinant gamma interferon. *J. Neuroimmunol.* **19,** 111.
33. Rosa F., Hatat D., Abadie A., Wallach D., Revel M. & Fellous M. (1983) Differential regulation of HLA-DR mRNAs and cell surface antigens by interferon. *EMBO J.* **2,** 1585.
34. Panitch H.S., Hirsch R.L., Schindler J. & Johnson K.P. (1987) Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology*, **37,** 1097.
35. Traugott U. & Lebon P. (1988) Multiple sclerosis: involvement of interferons in lesion pathogenesis. *Ann. Neurol.* **24,** 243.
36. Estes M.L., Rudick R.A., Barnett G.H. & Ransohoff R.M. (1990) Stereotactic biopsy of an active multiple sclerosis lesion. Immunocytochemical analysis and neuropathologic correlation with magnetic resonance imaging. *Arch. Neurol.* **47,** 1299.
37. Sedgwick J.D., Schwender S., Gregersen R., Dörries R. & ter Meulen V. (1993) Resident macrophages (ramified microglia) of the adult brown Norway rat central nervous system are constitutively major histocompatibility complex class II positive. *J. exp. Med.* **177,** 1145.
38. Uitdehaag B.M.J., de Groot C.J.A., Kreike A., van der Meide P.H., Polman, C.H. & Dijkstra, C.D. (1993) The significance of in-situ Ia antigen expression in the pathogenesis of autoimmune central nervous system disease. *J. Autoimmun.* **6,** 323.