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Immunohistochemical studies of adult human glial cells

Yannick Grenier, Theodora C.G. Ruijs, Yves Robitaille, André Olivier and Jack P. Antel

Department of Neurology and Neurosurgery, McGill University, Montreal, Que., Canada

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Summary

Using immunohistochemical techniques, we examined major histocompatibility complex (MHC) antigen expression on astrocytes, oligodendrocytes, and macrophages–microglia derived from surgically resected tissue from young adults and maintained in dissociated cell cultures supplemented with either fetal calf or human AB serum. The majority of these cells in culture expressed class I MHC antigens. MHC class II expression was observed on only a restricted proportion of astrocytes either under basal or induction conditions (γ-interferon, activated lymphocyte supernatants), on the majority of macrophages–microglia under inducing conditions, and not on oligodendrocytes. MHC class II expression on astrocytes in culture did not correlate with the extent of in situ gliosis or with in vitro cell morphology. MHC antigen expression was not detected in situ immunohistochemically. These data extend observations on the dissociation of in vivo and in vitro expression of MHC antigens on glial cells. The apparent greater expression of MHC class II antigens on macrophages–microglia compared to astrocytes raises the issue of the relative roles of each of these cell types in promoting immune reactivity under pathologic conditions.

Introduction

The central nervous system (CNS), although often referred to as an immunologically privileged site, can be the target of immune-mediated tissue injury. Systemic immune sensitization with myelin antigens in humans can result in the uniphasic disorder, acute disseminated encephalomyelitis; in animals, one can induce either an acute or chronic relapsing form of experimental allergic encephalomyelitis (EAE). Cellular immune-mediated mechanisms contribute to lesion formation in some chronic demyelinating diseases of the CNS induced by viral infection in animals, and are postulated to participate in the pathogenesis of the human chronic demyelinating disease, multiple sclerosis (MS). The above pathologic conditions are characterized by accumulation of lymphocytes, macrophages–microglia, and reactive astrocytes in the region of demyelination. The contribution of each of the cell types present in the region of these lesions to promoting ongoing immune reactivity remains under active study.

A major consideration with regard to specific cell types either promoting antigen-specific immune reactivity or becoming targets of antigen-

Address for correspondence: Dr. Jack P. Antel, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, Canada.

specific cell-mediated immune effector responses within the CNS, relates to the capacity of these cells to express major histocompatibility complex (MHC) antigens. MHC antigen expression, either class I or II, is usually undetectable within the CNS under non-pathologic conditions, using in situ immunohistochemical techniques. De Tribolet et al. (1984) did observe MHC class II-positive cells, some of which were tentatively identified as astrocytes, in apparently normal brain tissue. Lampson and Hickey (1986), in an in situ study of adult human brain tissue which surrounded tumor infiltrates, did not detect either class I or class II MHC antigen-positive astrocytes even among those histologically considered reactive. In active MS lesions, class I, and possibly class II, MHC antigens are reported to be present on astrocytes as well as on endothelial cells and macrophages present within the active lesions (Traugott et al., 1985; Hauser et al., 1986; Hofman et al., 1986). In EAE, several groups have detected class I but not class II MHC antigens on astrocytes in situ (Lassman et al., 1986; Sakai et al., 1986), whereas endothelial cells and macrophages expressed both classes. In the Theiler' murine encephalomyelitis virus (TMEV) model, both MHC antigen classes are detected on astrocytes, endothelial cells and macrophages, and seemingly also on a small proportion of oligodendrocytes (OGCs) (Rodriguez et al., 1987).

MHC antigen expression by astrocytes, OGCs and macrophages–microglia has been studied in vitro, largely using fetal or newborn neural tissue (Wong et al., 1985; Fontana et al., 1987). Under basal culture conditions, expression of class I MHC antigens on fetal murine astrocytes is undetectable or low by immunohistochemical criteria, although recognition of these cells by class I-restricted cytotoxic T cells may be a more sensitive means of assay (Skias et al., 1987); class II MHC antigen expression is usually non-detectable. Gamma-interferon, a potent inducer of MHC antigens on non-neural cells, and stimulated lymphocyte supernatants, markedly augment both class I (H-2) and II MHC expression on these cells. Massa et al. (1987) found a hyperinducibility of class II MHC antigens on astrocytes but not peritoneal macrophages of newborn rats from strains susceptible to EAE. Human fetal astrocytes maintained in culture are variably reported to spontaneously express DR antigens but can be induced to express them with γ-interferon (Pulver et al., 1987). Fetal murine oligodendrocytes do not express immunohistochemically detectable MHC antigens under basal conditions; class I, but not class II, MHC expression can be induced with γ-interferon, activated lymphocyte supernatants, and by viral infection (Wong et al., 1984; Suzumura et al., 1986; Takiguchi and Frelinger, 1986). Takiguchi and Frelinger (1986) demonstrated that, unlike astrocytes, OGCs cannot present exogenous antigen to T-cell hybrids, a capacity dependent on class II MHC antigen expression. Macrophages–microglia isolated from newborn mouse brain express class I MHC antigens under basal culture conditions, and both class I and II MHC antigens after exposure to γ-interferon (Frei et al., 1987; Suzumura et al., 1987).

The biologic properties of astrocytes, oligodendrocytes and macrophages–microglia of adult humans, and more specifically young adults — i.e., of an age most at risk for development of suspected autoimmune disease, namely multiple sclerosis, have been examined by in vitro tissue culture techniques to only a limited extent. Kim et al. (1985), using autopsy material of individuals of widely variable age (27–93 years), found that in tissue cultures which utilized minimum essential medium (MEM) and 5% fetal calf serum (FCS), 9–24% of glial fibrillary acidic protein (GFAP)-positive cells expressed DR (MHC class II) antigens; 0–16% of GalC-positive cells also expressed these antigens with cultures from 50% of donors being negative. All fibronectin-positive cells (fibroblasts) were HLA DR-negative. Lisak et al. (1983) did not detect DR antigens on human or rat OGCs. Hirayama et al. (1986), using autopsy material from apparently a single donor, found in cultures containing FCS that neither astrocytes nor OGCs expressed class II MHC antigens whereas 7% of OGCs, but none of the GFAP-positive cells expressed class I MHC antigens. Following addition of γ-interferon (1–20 μl per ml for 28–44 h), 80–98% of astrocytes and OGCs expressed class I MHC antigens; > 50% of astrocytes but none of the OGCs expressed MHC class II antigens. Induction was blocked by actinomycin-D and cycloheximide, indicating that synthesis

of new mRNA and protein was needed for MHC antigen induction. Barna et al. (1987) reported in a culture of GFAP$^+$ cells established from surgically derived human tissue that DR expression could be observed on 33% of cells after addition of γ-interferon or lymphokine-containing medium, although not on untreated cells. These data do suggest that MHC expression variably occurs on glial cells, but that conditions determining expression or non-expression require further definition.

The aim of the present study was to evaluate expression of MHC antigens on astrocytes, oligodendrocytes and macrophages–microglia contained within dissociated cell cultures established from human CNS tissue, surgically resected from young epileptic adults. The tissue was derived either from resections of temporal lobe tissue (combined grey and white matter) or from resections from the corpus callosum (i.e., pure white matter). The immunohistochemical results were correlated with the well-documented clinical-pathologic features of the individual cases, with in situ immunohistochemical findings and, in the case of astrocytes, with their morphologic features. We also compared MHC antigen expression by glial cells in the presence of human and calf serum, as well as the relative inducibility of MHC antigens on astrocytes compared to macrophages–microglia by γ-interferon and lymphocyte supernatants.

Materials and methods

Sources of material

CNS tissue was obtained from patients undergoing surgical procedures for treatment of epilepsy. The procedures were either local resection of tissue adjacent to the epileptic focus in the temporal lobe, or resection of corpus callosum. In the temporal lobe resection procedure, tissue was removed either on block or by Cavitron ultrasonic aspiration, which dissected the tissue into multiple pieces about 2–3 mm^3 in size. The corpus callosum tissue was removed by ultrasonic aspiration.

Cell isolation procedure

Meninges and blood vessels were removed from the tissue. Minced tissue was washed extensively in phosphate-buffered saline (PBS) to remove contaminating blood and endogenous proteases, and then incubated for 1 h with 0.25% trypsin in calcium-free PBS at 37°C in a 5% CO_2 humidified incubator. Serum was then added to inactivate the trypsin, along with DNAase (25 μg/ml) to dissolve DNA streaks liberated from broken nuclei. Tissue was further dissociated by passage through two nylon meshes (pore size 210 and 132 μm). A 30% Percoll gradient centrifugation was then performed as described by Kim et al. (1983). Glial cells were recovered from the layer of floating cells in between the top layer of myelin and the bottom layer of red blood cells. Cells were extensively washed in PBS, counted and plated on plastic flasks in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Grand Island Biologic Co.) or human AB serum (obtained from healthy volunteers). The sera were inactivated at 56°C for 30 min and filtered prior to use. Cells obtained from callosotomies were plated in DMEM with 5% serum. The average cell yields were approximately 1×10^6 cells per gram of tissue. All culture media contained penicillin (50 units per ml), streptomycin (50 μg per ml — Gibco), and tylosine (1.0 μg per ml).

Tissue culture

After 24–48 h, cells that had not attached to the tissue culture plate were removed, resuspended in DMEM with 5% serum, either FCS or human AB, at 5×10^5 cells per ml, and replated either on poly-L-lysine-coated plastic flasks or Aclar coverslips. The cultures established from the secondary plating were enriched in oligodendrocytes. The cultures containing residual adherent cells were enriched for astrocytes and macrophages–microglia. Adherent cells could be detached and transferred to coverslips after 1–2 weeks of culture by trypsinization.

Immunohistochemical and histologic studies in tissue culture

Cells maintained in culture for 1–8 weeks were stained on coverslips by double-immunofluorescence labelling techniques in order to permit evaluation both of cell type and expression of MHC antigen. Non-specific labelling was blocked by a pre-incubation of the cells in PBS + 10% normal goat serum (NGS) for 30 min.

Astrocytes. Cells were initially incubated with anti-MHC antibodies, either class I (W6/32 used at a 1:50 dilution) (Parham et al., 1979) or class II (SG465, a mouse IgG_{2A} recognizing DR, DP, and DQ epitopes, used at a 1:64 dilution for 30 min) (gift from Dr. Rafick Sekaly, Institut de Recherches Cliniques de Montréal). The specificity of the MHC class II expression was further evaluated using anti-DP (B7-21 mouse IgG_1 supernatant) (Watson et al., 1983); anti-DQ (TU22A mouse IgG ascites), and anti-DR (D1-12 mouse IgG_2 ascites) (Carrel et al., 1981) monoclonal antibodies. The second antibody used was a fluorescein-labelled $F(ab)'_2$ fragment of a rat monoclonal antibody recognizing mouse IgG_K (gift from Dr. Michael Julius, McGill University, Montreal) used at a 1:1000 dilution and applied for 30 min. After each incubation, the cells were washed 5 times in PBS plus 2% horse serum plus sodium azide (0.01%). After staining and washing, the cells were fixed with cold 5% acetic acid in ethanol for 2–5 min. Fixed cells were stained with a 1:300 dilution of polyclonal rabbit anti-GFAP antibody (Dako, lot No. 15, code Z334) for 30 min. To verify the specificity and staining pattern of the above reagent, a second polyclonal rabbit anti-GFAP antibody was also used (gift from Dr. S. David, McGill University, Montreal). The cells were then incubated for 30 min with a goat anti-rabbit IgG rhodamine-labelled antibody used at a dilution of 1:100 (Jackson Immunoresearch Laboratory). All incubations were performed at room temperature. As controls, some cultures were incubated with 10% NGS followed by rat anti-mouse IgG_K $F(ab')_2$ fragment, then fixed in 5% acetic acid and exposed to goat anti-rabbit IgG conjugated to rhodamine; other control cultures were incubated with NGS followed by mouse MHC class I or II antisera, then fixed, and exposed to goat anti-rabbit IgG-conjugated rhodamine. Neither displayed positive staining of cells.

As an attempt to correlate MHC immunohistochemical results with morphology of astrocytes, the $GFAP^+$ cells were subtyped based on their morphologic appearance and on their size, as described in the legend to Fig. 4.

Oligodendrocytes. Cells were initially exposed to the same anti-MHC antibodies and FITC-labelled second antibody as for astrocytes. After fixation in 4% paraformaldehyde and permeabilization with Nonidet, cells were stained with rabbit anti-CNPase (1:100, a gift from Dr. Peter Braun, McGill University, Montreal) followed by goat anti-rabbit rhodamine-conjugated IgG (1:100, Jackson Laboratories, West Grove, PA). Oligodendrocyte-enriched cultures were also double-labelled with a monoclonal mouse anti-galC (gift from Dr. S. Kim, University of British Columbia, Vancouver) and anti-GFAP antibody to determine the proportions of different cell types in the culture.

Macrophages–microglia. These were assessed using anti-LeuM5 antibody (1:20) (Beckton-Dickinson). In some studies this cell population was further identified by adding 1 μm diameter latex beads to the culture (1:100, Dow Diagnostics) for 3 h prior to staining cells with either anti-MHC, or anti-GFAP, anti-galC, or anti-LeuM5 antibodies.

Fibroblast-like cells were observed in some of our cultures. Immunostaining of > 2-week-old cultures with a polyclonal rabbit anti-fibronectin antibody (used at 1:100, gift from Dr. S. Carbonetto, Montreal General Hospital, Montreal) showed a fibronectin matrix, but only rare fibronectin-positive cells. The cell type(s) (fibroblasts, macrophages or astrocytes) were synthesizing the fibronectin is unknown.

Induction of MHC antigens on glial cells in vitro

In these studies, recombinant γ-interferon (Enzo Biochem., New York) 1–1000 units per ml was added to cultures 1–6 days prior to immunohistochemical analysis.

For the study of activated lymphocyte effect on MHC antigen expression by glial cells, mononuclear cells (MNCs) were isolated using a Ficoll-Hypaque gradient from the peripheral blood from either a normal control donor or from the patient whose tissue was used to establish the cell culture. The cells were allowed to adhere overnight. The non-adherent cells were then treated with 5 mM leucine methyl ester for 40 min at 37°C. These macrophage-depleted lymphocytes were added to adherent glial culture on coverslips at a ratio of lymphocyte to glia of 10:1 in duplicate 24-microwell culture plates. After 7 days, 100 μl aliquots of cultures (i.e. non-adherent cells) were collected

and pulsed with 1 μC of [^{3}H]thymidine for 5 h, then harvested with a MASH. Dried filters were counted in a scintillation counter.

In situ evaluation of MHC antigen expression by glial cells

Brain tissue block specimens to be stained were frozen in liquid nitrogen and stored at −70°C. 6 μm-thick sections were cut on a cryostat at −70°C. The sections were thawed and stained using the same MHC and GFA antisera as those mentioned above, and similar control stainings were carried out.

Results

Morphologic features of CNS cultures

Immunohistochemical studies of dissociated CNS cell cultures

(a) Astrocytes. In the presence of either fetal calf serum (FCS) or human AB serum, the majority of astrocytes cultured expressed class I MHC antigens (Table 1, Fig. 1). In contrast, only a minority of GFAP$^+$ cells expressed class II MHC antigens, as determined by immunostaining detectable by fluorescence microscopy (Table 1, Fig. 2). In the presence of FCS, 20–36% of cells

TABLE 1

EXPRESSION OF MHC CLASS I AND II ANTIGENS ON HUMAN ADULT ASTROCYTES AND OLIGODENDROCYTES IN VITRO

Data indicate observations on MHC antigen expression by astrocytes or oligodendrocytes derived from surgically resected tissue of young adults and maintained in dissociated cell culture. For the oligodendrocyte-enriched cultures, percentage of oligodendrocytes within the culture is given. The source of the serum supplement in all cultures is also stated. Gamma-interferon (γ-IFN) data are the highest percentage of positive cells observed over a dose range of 1–1000 units of γ-IFN. The letters in parentheses in Table 1 and Table 3 indicate when cells from the same donor were used.

Donor		Source of cells	Percent of total cells	Serum supplement	Percent of cells expressing MHC antigens			
Age	Sex				Class I		Class II	
					− γ-IFN	+ γ-IFN	− γ-IFN	+ γ-IFN
Astrocytes (GFAP$^+$ cells)								
18 (A)	M	Temporal lobe (TL)		FBS [a]			36	
48 (B)	M	Temporal lobe		FBS	96		20	
				Human AB	87		3	
32 (C)	F	Temporal lobe		FBS			24	
25 (D)	M	TL – culture 1		FBS	35		27	25
		TL – culture 2			> 80			
37 (E)	F	Temporal lobe		FBS	> 80			
				Human AB			7	2
15 (F)	M	Temporal lobe		Human AB	75		10	
Oligodendrocytes								
48 (B)	M	Temporal lobe	67	Human AB			Neg.	
32 (C)	F	Temporal lobe		FBS	> 80			
25 (D)	M	Temporal lobe	50	FBS	0		Neg.	
37 (E)	F	Temporal lobe	98	FBS	> 80		Neg.	
19	F	Temporal lobe	45	FBS			< 1	
17	F	Temporal lobe	68	FBS			Neg.	Rare
39	F	Temporal lobe		FBS	> 80	> 80		
24	F	C. callosum	50	FBS	> 80			
26	M	C. callosum	26	FBS	80–90			
19	F	C. callosum	50	Human AB	10		Neg.	
28	F	Temporal lobe	70	Human AB	80	80	Neg.	
32	M	Temporal lobe	75	Human AB	> 90			

[a] FBS = fetal bovine serum.

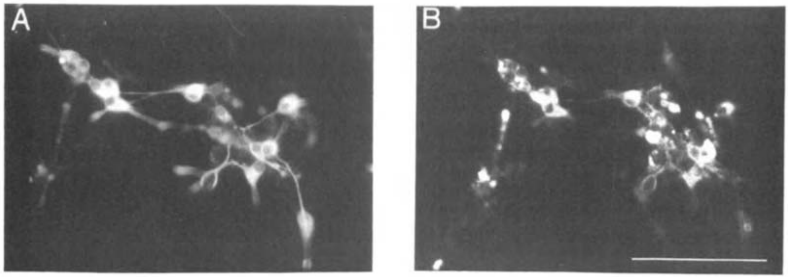

Fig. 1. Double immunostaining of cells within dissociated cell cultures derived from temporal lobes of young adult humans with polyclonal rabbit anti-GFAP antibody, followed by rhodamine-conjugated goat anti-rabbit Ig (*A*) and monoclonal mouse anti-human MHC class I antigen antibody (W6/32), followed by fluorescein-conjugated rat anti-mouse IgG_K antibody (*B*). Majority of $GFAP^+$ cells are immunostained with anti-MHC class I antibody. Scale bar = 100 μM.

had detectable fluorescence; this percentage was reduced in the presence of human AB serum (≤ 10%). The addition of γ-interferon (1–100 units) did not substantially increase the proportion of MHC class II-expressing astrocytes above a pre-existent baseline expression. As shown in Table 2, the proportion of $GFAP^+$ cells expressing class II MHC antigens was increased following 7 days of co-culture with allogeneic peripheral blood-derived lymphocytes compared to co-cultures con-

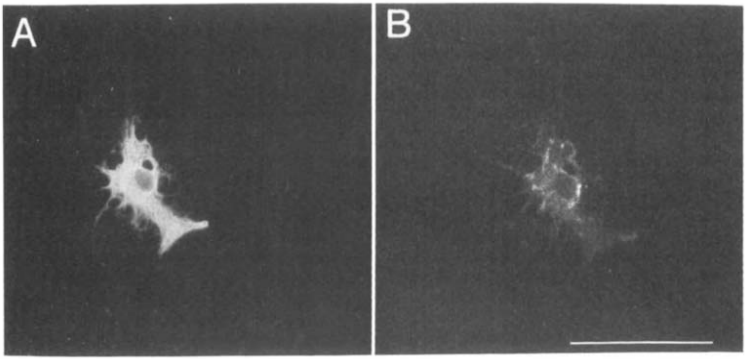

Fig. 2. Double immunostaining of a cell within a dissociated cell culture derived from the temporal lobe of a young adult human with polyclonal rabbit anti-GFAP antibody, followed by rhodamine-conjugated goat anti-rabbit IgG (*A*) and monoclonal mouse anti-human MHC class II antigen antibody (SG465), followed by fluorescein-conjugated rat anti-mouse IgG_K antibody (*B*). Scale bar = 50 μM.

TABLE 2

IN VITRO INTERACTION OF AUTOLOGOUS AND ALLOGENEIC LYMPHOCYTES AND DISSOCIATED CNS CELL CULTURES

4×10^5 T-cells (peripheral blood MNCs depleted of adherent cells) were co-cultured with 4×10^4 glial cells. After 7 days, non-adherent cells (MNCs) were removed and pulsed with [^{3}H]thymidine; adherent cells (astrocytes, macrophages–microglia) were then double-immunostained with anti-GFAP and anti-MHC class II antibodies. Cultures used in this study contained 10% human AB serum.

Culture	[^{3}H]Thymidine uptake by peripheral blood MNCs following 7-day co-culture with glial cell-enriched culture	Proportion of GFAP$^+$ cells expressing MHC class II antigens after 7-day culture
Glial cells +		
autologous T cells	349 ± 35 [a]	16%
allogeneic T cells	10247 ± 404 [a]	39%
no added T cells	46 ± 10 [a]	10%

[a] Standard error of mean calculated from six replicate cultures.

taining autologous lymphocytes. Allogeneic but not autologous T-lymphocytes were induced to proliferate markedly within these cultures.

The proportion of MHC class I or II antigen-expressing astrocytes did not correlate with the proportion of non-glial cells within the cultures, nor did the addition of indomethacin, up to 3 mg per ml, alter the number of GFAP$^+$ cells expressing MHC class II expression (data not shown).

The GFAP$^+$ cells in the cultures displayed heterogeneous morphologic features both with respect to cell shape and size (Fig. 3). For each donor, the relative distribution of GFAP$^+$ cells into the four morphologic subtypes was rather constant amongst replicate cultures. As shown in Table 3, fibrous-type cells as compared to flat cells comprised the majority of cells. We attempted to correlate this in vitro morphology with the extent of gliosis in the surgically resected tissue as independently rated by a neuropathologist (Dr. Yves Robitaille) based on sections prepared from tissue adjacent to that used to establish the cultures. The proportion of flat cells in vitro was suggestively increased in donors whose tissue showed severe gliosis. The proportion of such cells was also increased in a culture derived from tissue subsequently interpreted histologically as a low-grade ganglioglioma. We did not find a correlation between either the extent of gliosis, as

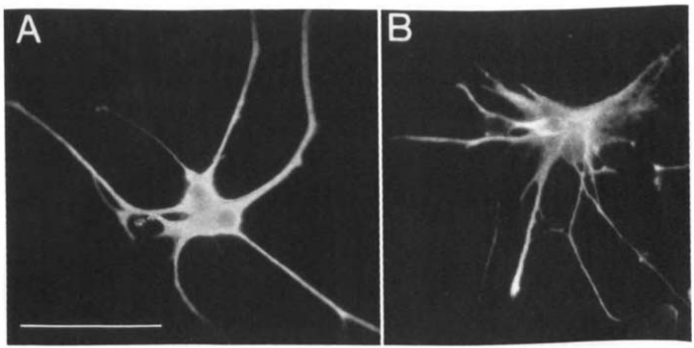

Fig. 3. Morphologic features of GFAP$^+$ cells present in dissociated cell cultures derived from temporal lobes of young adults. The GFAP$^+$ cells were subgrouped on the basis of their morphologic features. The two major subgroups were uni- or multipolar cells with well-developed processes, as illustrated in (*A*), and cells with a relatively larger somal area (flat cells), as illustrated in (*B*). Subdivisions within each group could be made on the basis of size; however, no correlation was found between cell size and MHC class II antigen expression. Scale bar = 50 μM.

TABLE 3

CORRELATION OF IN VITRO MORPHOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF ADULT HUMAN ASTROCYTES WITH CLINICAL AND HISTOLOGIC PARAMETERS OF INDIVIDUAL CASES FROM WHICH THE CELLS WERE DERIVED

The data presented in this table include the pre-operative clinical diagnosis, histopathologic assessment of surgically resected tissue, and morphologic features of astrocytes in vitro. All cases presented with intractable seizures, but only in a minority were specific etiologies identified. Case B had a low-grade glioma resected 5 years previously; no tumor was found at the current surgical exploration. The qualitative histopathologic grading scale for severity of gliosis in situ utilized a range of $0–4^+$, using as criteria densities of hypertrophic astrocytes in cortex, cell size, and extent of processes. The in vitro morphologic data is presented as proportion of $GFAP^+$ cells falling into either of two major categories (fibrous or flat). Within each category are combined cell types of different sizes. The serum supplement in cultures consisted of 10% fetal bovine serum (FBS) or human AB serum. The numbers in parentheses for cases F and G indicate proportion of $GFAP^+$ cells in each morphologic category expressing MHC class II antigens.

Donor		Clinical diagnosis of seizures	Histology Severity of gliosis		Serum supplement	Morphology of $GFAP^+$ cells in vitro	
Age	Sex		Hippocampus	Lateral temporal lobe		Fibrous	Flat
18 (A)	M		1^+	2^+	FBS	100	0
48 (B)	M	Status post glioma	0	1^+	FBS	98	2
32	F	Post-trauma	0	$1–2^+$	FBS	97	3
36	F		1–2	1	FBS	96	4
16	M		0	3^+	FBS	93	7
26	F		4^+	1^+	FBS	94	6
24	F		2^+	3–4	FBS	88	12
25 (G)	M	Post-trauma	0	$2–3^+$	FBS	68	32
		Re-operation × 3				(27)	(26)
15 (F)	M	Ganglioglioma	4^+		Human AB	65	35
						(14)	(0)

judged histologically, and the proportion of $GFAP^+$ cells in vitro expressing MHC class II antigens or the in vitro cell morphology and MHC class II antigen expression.

An immunostained culture for individual MHC class II epitopes showed that only 2% of $GFAP^+$ cells were labelled with the anti-DQ-specific monoclonal antibody, while 9% and 10% of $GFAP^+$ cells were labelled with anti-DP- and anti-DR-directed monoclonal antibodies.

Using temporal lobe tissue from a case with severe gliosis (Fig. 4*A*), we did not detect MHC

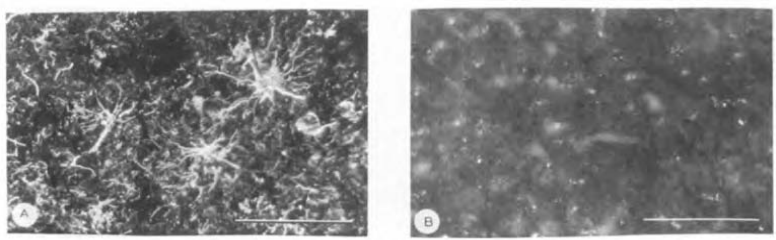

Fig. 4. Single immunostaining of 6 μm-thick frozen sections in situ, with anti-GFAP and anti-MHC antibodies. (*A*) $GFAP^+$ cells from the temporal lobe of a case with severe gliosis; no cells immunoreactive with anti-MHC antibodies were noted. (*B*) Anti-MHC (TU22A, 1 : 1000) class II immunostaining of frontal lobe tissue resected from a case of chronic encephalitis. Scale bars = 100 μM.

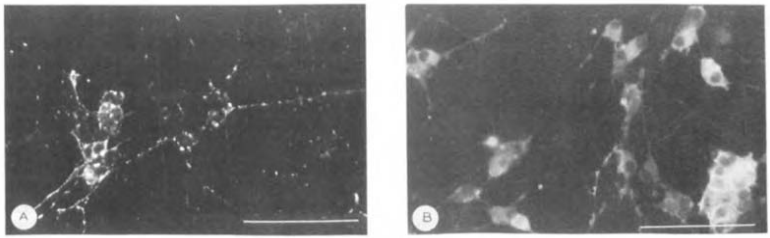

Fig. 5. Single immunostaining of cells in an oligodendrocyte-enriched culture derived from the temporale lobe of young adults, with monoclonal mouse anti-galactocerebroside C followed by rat anti-mouse IgG_K (187.1) (*A*), and with polyclonal rabbit anti-CNPase antibody followed by a rhodamine-conjugated goat anti-rabbit Ig (*B*). Scale bars = 100 μM.

antigen expression, either class I or II, in situ. Although astrocyte cultures were not available in this case, oligodendrocytes derived from this case did express class I MHC antigens in vitro. As a control for the in situ studies, we analyzed frontal lobe tissue from a case of chronic encephalitis and were able to detect MHC class I and II immunostaining. This staining was predominantly associated with non-GFAP$^+$ cells, likely macrophages (Fig. 4*B*).

(b) Oligodendrocytes. These cultures were established either from callosotomy-derived tissue or from temporal lobe tissue. Successful cultures were obtained from 6 of 6 callosotomy cases and 65% (22 of 32) temporal lobe resections. We have not consistently derived cultures from autopsy material. GalC or CNPase$^+$ cells began to extend processes within 3 days of initial culture (Fig. 5). The cells tended to grow best when in contact with other cells, including other galC$^+$ cells. Rarely in the longer-term cultures did we detect individual cells immunostaining with both anti-galC and anti-GFAP antibodies.

The majority of galC$^+$ cells, with the exception of one donor, expressed MHC class I antigen (Fig. 6). We did not detect consistent MHC class II antigen on the oligodendrocytes in our cultures, even with γ-interferon supplementation (Table 1).

(c) Macrophages–microglia. Within all cultures, one could detect cells with macrophage–microglia properties. These cells avidly ingested latex particles (Fig. 7) and were immunostained with

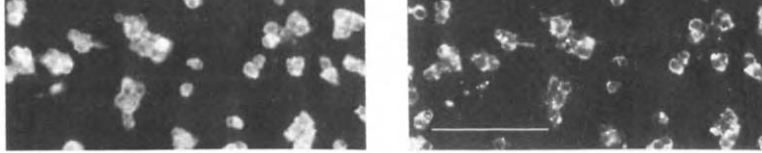

Fig. 6. Double immunostaining of cells in an oligodendrocyte-enriched dissociated cell culture derived from the temporal lobe of a young adult human, with polyclonal rabbit anti-CNPase antibody, followed by rhodamine-conjugated goat anti-rabbit Ig (*A*) and monoclonal mouse anti-human MHC class I antigen antibody (W6/32), followed by fluorescein-conjugated rat anti-mouse IgG_K antibody (*B*). Almost all CNPase$^+$ cells express MHC class I antigens. Scale bar = 100 μM.

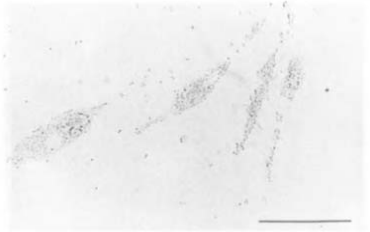

Fig. 7. Non-GFAP$^+$ cells contained within a dissociated culture derived from temporal lobe of young adults. These cells have ingested latex particles added to the culture 3 h prior to immunostaining the cells. Scale bar = 50 μM.

anti-LeuM5 antibody. These cells could be highly enriched by repeated trypsinization of cultures. We attempted to quantify MHC class II antigen expression on such cells using a culture enriched for macrophages–microglia. 15% of cells expressed MHC class II antigens under basal conditions; addition of γ-interferon, over the same dosage range used on astrocyte-containing cultures, induced marked MHC class II antigen expression (> 75% of the cells were positive). The level of MHC class II antigen expression was qualitatively greater on macrophages–microglia than on astrocytes under basal and γ-interferon induction conditions (Fig. 8).

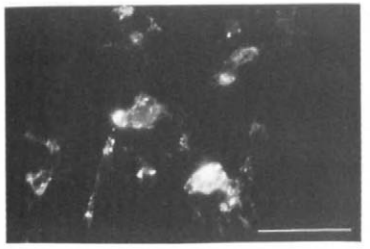

Fig. 8. MHC-class II antigen expression by LeuM15$^+$, non-GFAP$^+$ cells in a dissociated cell culture derived from the temporal lobe of a young adult. Scale bar = 50 μM.

Discussion

In this study, we have immunohistochemically evaluated in vitro MHC antigen expression by astrocytes, oligodendrocytes, and macrophages–microglia derived from surgically resected tissue from young adult humans. This material was obtained under rather constant conditions, avoiding the pre-mortem, particularly septicemia (Traugott and Lebon, 1988), and post-mortem variables introduced by use of autopsy material. The age of our tissue donors largely overlaps with the age of peak incidence of multiple sclerosis. Unlike studies of fetal murine or occasional human CNS tissue cultures, the cultures derived from adult tissue contain mixed cell types, although one could enrich for specific cell types.

With regard to astrocytes, we found that the majority of these cells in vitro, whether maintained in fetal calf or human AB serum-supplemented media and whether derived from cases with or without severe gliosis, expressed class I MHC antigens. We could not detect class I MHC expression on glial cells in situ from tissue with severe gliosis unassociated with inflammation, although we could detect MHC antigen in situ on non-GFAP$^+$ cells in the case of tissue containing inflammatory cells. Our findings regarding the dissociation between class I MHC expression on GFAP$^+$ cells in vivo and in vitro parallel previous reports in which either in vitro and in vivo cell properties only were examined.

Expression of class II MHC antigens was observed only on a minority of GFAP$^+$ cells in all donors tested, with levels of expression varying depending on culture conditions. Our initial data, indicating that 20–35% of GFAP$^+$ cells expressed MHC class II antigens, were obtained, as were those reported by Kim et al. (1985), using cultures supplemented with fetal bovine serum. In human AB serum-supplemented cultures, the proportion of cells expressing immunohistochemically detectable levels of class II antigens was < 10%. Our results suggest that fetal bovine serum (FBS) may contain factors inducing MHC expression or that human serum is inhibitory for MHC class II expression. We were unable to augment the proportion of MHC class II expressing astrocytes to > 40% by the addition of γ-interferon or in the

presence of allogeneic MNCs, although similar dosages of γ-interferon markedly enhanced expression of MHC class II antigens on macrophages–microglia in culture.

In agreement with previous reports we found that activated lymphocytes, stimulated in our study by co-culture of these cells with allogeneic dissociated neural cultures, augmented levels of MHC class II expression on astrocytes and macrophages–microglia. Again, however, only a proportion of the GFAP$^+$ cells expressed class II MHC antigens, whereas the majority of macrophages did. Lymphocytes were not stimulated when co-cultured with autologous glial containing cells, and little or no induction of class II MHC antigens on glial cells was observed. Fontana et al. (1986) have reported that γ-interferon treated fetal mouse astrocytes could induce reactivity by both syngeneic and allogeneic resting purified T-lymphocytes.

Based on reports that the MHC class II antigens are at times detected in situ on astrocytes surrounding inflammatory and neoplastic lesions, i.e. conditions where reactive astrocytes are found, we attempted to determine whether specific clinical or pathologic features of the individual cases correlated with their expression of class II MHC antigens. We did not observe a correlation between proportion of GFAP$^+$ cells in vitro expressing MHC class II antigens and the extent of gliosis observed histopathologically on tissue sections. Wong et al. (1984) found that direct injection of γ-interferon into 2-day-old murine brain in vivo, 36 h prior to sacrifice, did result in some increase in Ia$^+$ expression by astrocytes, examined immediately upon cell isolation. Suzumura et al. (1986) found that in vivo infection of newborn mice with mouse hepatitis virus strain A59 resulted in persistent in vitro expression of class I, but not class II, MHC antigens by astrocytes.

We were unable to find a correlation between the frequency of MHC class II expressing cells and any cell morphologic variable examined. Unlike for glial cells derived from developing rat, no standard system of morphologic classification applicable to adult human astrocytes in culture currently exists. We did observe a suggestive increase in the proportion of GFAP$^+$ cells with gemistocytic features (i.e., flat cells) in vitro in cultures derived from cases histopathologically demonstrating the most severe gliosis. With the present surgical techniques used to remove the brain specimen, it was not as yet possible to separate gliotic and normal appearing tissue from individual donors prior to cell isolation.

The oligodendrocyte cultures were derived from both corpus callosum and temporal lobe specimens. The oligodendrocytes appeared to grow best in the center of culture, where cell–cell contact occurred; this finding is previously described in human and murine culture systems. The OGCs in all cultures expressed MHC class I antigens, as has been observed previously in human cultures. We could not detect class II MHC antigens in our cultures, a finding similar to that reported by others, although Kim et al. (1985) did find a minority of OGCs with immunoreactivity. In keeping with Kim (1985), however, we did find the occasional cell in long-term culture which expressed both galC and GFAP. Our data would be consistent with data derived from murine fetal studies, and support the postulate of Suzumura et al. (1986) that OGCs can become susceptible to class I MHC-restricted cytotoxic T-cells.

The source of the macrophage–microglia in our cultures cannot be unequivocally defined, as to whether they are long-standing within the CNS tissue or are from the blood to which the specimen is exposed — a problem existent for all studies using CNS tissue to derive these cells. As well demonstrated by Hickey and Kumura (1988), the brain macrophages are blood-derived cells. As mentioned, the macrophage population in our material appeared to be the one most clearly expressing MHC class II antigens under 'basal' conditions, and being the most responsive to γ-interferon. Beller et al. (1984) have shown accessory cell capacity to be proportional to the amount of Ia antigen induced on the cell surface. These observations are of potential importance when considering the contribution of different cell types within the CNS to ongoing immune reactivity. In clinical (MS) or experimental (EAE) disorders in which MHC expressing astrocytes are present and postulated to be antigen-presenting cells, macrophages are a prominent cell type present at the lesion sites. MHC class II expression per se, however, may not indicate total immune competence

of a cell. Umetsu et al. (1986) demonstrated that fibroblasts induced by γ-interferon to express class II MHC antigens were able to present antigen and to stimulate allogeneic T cell clones, but not resting T cells; these data suggest that certain class II MHC expressing cells may not serve as primary antigen-presenting cells in vivo but may be able to perpetuate ongoing immune reactivity.

Our immunohistochemical data derived from study of surgically obtained young adult human CNS tissue, exhibiting a range of in situ gliosis, confirms the dissociation in MHC antigen expression by glial cells observed in vivo and in vitro suggested by studies of fetal murine tissue and autopsy material. In vitro expression of class I MHC antigens is observed on most astrocytes and oligodendrocytes, making these cells potential susceptible targets of antigen-specific immune effectors. MHC class II expression is noted on a minority of astrocytes; its up-regulation on astrocytes appears less prominent than for macrophages–microglia present in the same culture environment. Our results are consistent with data from studies of fetal murine astrocytes indicating class II MHC antigen expression and capacity of these cells to present antigen; however, their relative contribution to promoting immune reactivity in vivo disease states characterized by presence of macrophages–microglia needs further definition.

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