Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Neuroimmunology, 29 (1990) 203–211
Elsevier

JNI 00995

Adult human glial cells can present target antigens to HLA-restricted cytotoxic T-cells

Suhayl Dhib-Jalbut [1], Conrad V. Kufta [2], Marjorie Flerlage [1], Naoki Shimojo [1] and Henry F. McFarland [1]

[1] *Neuroimmunology Branch, and* [2] *Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

(Received 29 March 1990)
(Revised, received 1 May 1990)
(Accepted 1 May 1990)

Key words: Antigen presentation; Astrocyte; Microglia; Myelin basic protein

Summary

T-lymphocyte recognition of antigen either on antigen-presenting cells (APC) necessary for the generation of an immune response or on target cells during the effector phase of a cellular immune response requires expression of HLA molecules. Although immune mechanisms operate in many disease processes of the central nervous system (CNS), cells of the CNS generally express low levels of HLA molecules. In this study, the potential for upregulation of HLA molecules on adult human glial cells was examined. Moreover, the functional implication of this upregulation was assessed by the capacity of glial cells to process and present target antigens to HLA class I-restricted influenza-specific and class II-restricted myelin basic protein (MBP)-specific CTL lines. Glial cells cultured from adult human surgical brain specimens or cells from established glioblastoma multiforme cell lines were studied. Lysis by antigen-specific CTLs was dependent on treatment of the target cell with interferon-γ. The lysis was HLA restricted and antigen specific. The results indicate that adult human glial cells can process and present antigen to HLA-restricted CTLs but require the upregulation of HLA molecules. These findings have implications for infectious and autoimmune diseases of the CNS.

Introduction

Antigen-specific T-lymphocytes are believed to be involved in the production of immunopathological disease of the central nervous system (CNS) such as multiple sclerosis (MS) (Mokhtarian et al., 1984; Fontana et al., 1987). Direct damage of tissue by cytotoxic T-lymphocytes (CTLs) requires recognition of a processed antigen on the target cell in the context of the appropriate HLA determinants (Zinkernagel and Doherty, 1974; Sun et al., 1988). This process may be limited in the CNS, since brain cells express very low levels of HLA molecules (Williams et al., 1980). Expression

Address for correspondence: Dr. Suhayl Dhib-Jalbut, Bldg. 10, Room 5B-16, NIH, Bethesda, MD 20892, U.S.A.

of these molecules can be upregulated in murine glial cell cultures by interferon-γ (IFN-γ) (Wong et al., 1984) or virus infection (Massa et al., 1986) which allows these cells to function as antigen-presenting cells (APC). Animal experiments have demonstrated that mouse endothelial cells (McCarron et al., 1985) and rat astrocytes (Fontana et al., 1984) can present myelin basic protein (MBP) to encephalitogenic T-cell lines. These lines have been shown to lyse MBP-treated rat astrocytes in an antigen-specific and Ia-restricted fashion (Sun and Wekerle, 1986). In addition, inducibility of Ia on rat astrocytes has been found to be strain dependent (Massa et al., 1987). While these findings indicate that native cells of the newborn murine CNS can present self antigen to T-cells, it is uncertain if similar processes occur in cells of the adult human brain and whether these cells can process and present viral antigen to CTLs. Moreover, there is evidence that certain cells such as keratinocytes and epithelial cells can be induced to express Ia molecules but are incapable of processing and presenting antigen to T-cells (Geppert and Lipsky, 1985). In this study, the capacity of adult human glial cells to express HLA molecules and to acquire antigen-presenting function was examined by their ability to serve as targets for HLA class I- and class II-restricted CTLs.

Material and methods

Primary glial cultures

Adult human glial cell cultures were established from surgical brain specimens obtained from the temporal lobes of patients treated for intractable seizures. After dissecting the meninges, the tissue was washed in phosphate-buffered saline (PBS), minced into 2–3 mm pieces, and treated with 0.2% trypsin and 20 μg/ml DNase (Sigma) in a volume of 10 ml for 40 min at 37° C. The tissue was vortexed every 10 min for 1 min. The trypsin digestion was stopped by adding 10% fetal calf serum (FCS). The cell suspension was then transferred to a 25 cm^2 tissue culture flask or onto coverslips precoated with poly-D-lysine (10 μg/ml) (Sigma) and left undisturbed for 1 h in a CO_2 incubator. Dulbecco's modified Eagle's medium (DMEM) containing 15% heat-inactivated FCS, glutamine, Hepes buffer, MEM nonessential amino acids, MEM vitamins, and penicillin/streptomycin was then added to the cells. Medium was changed every 48 h for the first two changes, then every 4 days.

Glioblastoma multiforme cell lines

Glioblastoma multiforme cell lines U-251 MG and U-373 MG were a gift from Dr. Darrel Bigner (Duke University, NC, U.S.A.) and the characteristics of these cell lines have been previously described (Bigner et al., 1981; Wikstrand et al., 1985). U-251 MG is an established cell line derived from a human glioblastoma multiforme. This cell line continues to produce glial fibrillary acidic protein (GFAP) in culture which is suggestive of its glial origin. U-251 MG expresses high levels of class I HLA molecules but not class II (DR) molecules. U-373 MG is another established cell line derived from a human glioblastoma multiforme but does not produce GFAP. This cell line expresses both class I and class II (DR) HLA molecules.

Immunofluorescence microscopy

Cells grown on coverslips were washed with PBS and incubated with anti-HLA class I (W6/32), anti-HLA class II-DR (L243) or nonimmune hybridoma supernatant. The cells were then washed twice and a fluorescein-conjugated sheep anti-mouse IgG (Kappel) was applied. After two washes, the coverslips were fixed in 2% paraformaldehyde for 15 min followed by Triton X-100 (0.1%) treatment for 5 min. Rabbit antiserum to GFAP (Dako, CA, U.S.A.) or normal rabbit serum was then applied (1 : 250 dilution), followed by tetramethylrhodamine B isothiocyanate (TRITC)-conjugated goat anti-rabbit antibody (Sigma). All antibody incubations were carried for 45 min at 25° C. The specificity of the immunostaining was established by the negative results obtained when one of the primary antibodies was omitted or nonimmune serum was applied. Elimination of either sheep anti-mouse or goat anti-rabbit antiserum resulted in the appropriate lack of specific staining indicating absence of interspecies cross-reactivity. Cells were examined on a Leitz fluorescent microscope and photographed with Kodak Ektachrome p800/1600 film.

Generation of cytotoxic T-cells

Influenza CTL are predominantly restricted by HLA class I molecules (Biddison, 1982). These were used to examine class I-restricted antigen presentation by human glial cells. Influenza virus-specific CTL were generated as described (Dhib-Jalbut et al., 1989). Briefly, 4×10^6 peripheral blood lymphocytes (PBL) matched with the primary glial cell targets for class I HLA molecules were cultured with influenza virus (A/JAP) for 1 h in 2 ml RPMI 1640 supplemented with glutamine in a 24-well tissue culture plate. Five percent human AB serum was then added and the cultures were carried for 7 days in a 5% CO_2 humidified incubator. Generation of influenza virus-specific CTL lines Q115 and 1D3 has been previously described (Cowan et al., 1987; Nuchtern et al., 1989). The line Q115 recognizes a synthetic peptide (M1) which corresponds to amino acid sequences 55–73 of the matrix protein of influenza virus A/JAP, and is restricted by HLA-A2 molecules. The line 1D3 recognizes influenza virus A/JAP and is restricted by HLA-A3 molecules.

MBP-specific T-cell lines were generated from DR2 homozygous donors by repeated stimulation of PBL with MBP (10 μg/ml) in the presence of autologous irradiated feeders (6000 rad) in RPMI 1640 media containing 10% human AB serum. After two passages, 10% human T-cell growth factor (Cellular Products, Buffalo, NY, U.S.A.) was added to the cultures. These lines were $CD4^+$ by FACS analysis, and DR2 restricted as determined by lysis of DR2-transfected targets (Jaraquemada, D. et al., in preparation). These lines were used 6 days after stimulation to examine presentation of class II-restricted antigen by glial cell targets.

Generation of targets

Glial cell targets obtained from primary cultures were treated with IFN-γ (100 units/ml, Genzyme, MA, U.S.A.) for 3 days and then infected with influenza virus (A/JAP) for 16 h. Targets obtained from the cell lines U-251 MG, U-373 MG, and an EBV-transformed B-cell line (K4B) were infected either with influenza virus A/JAP strain or influenza virus B/AA strain or pulsed with the M1 peptide (5 μg/ml) for 16 h. MBP targets were pulsed with human MBP (100 μg/ml) for 16 h. Glial cells were removed from the tissue culture flasks by trypsinization and washed with RPMI 1640 containing 5% FCS. The targets were then suspended in 0.3 ml medium containing 100 μCi/ml of $Na[^{51}Cr]O_4$ (New England Nuclear, Boston, MA, U.S.A.) and incubated for 90 min in a 37°C water bath. The chromated targets were then washed twice with medium and counted.

CTL assay

Varying numbers of effector cells were cultured with 5000 ^{51}Cr-labelled targets in triplicate wells of a 96-well microtiter plate in a volume of 200 μl of DMEM containing 10% FCS supplemented with glutamine. After 4 h of incubation in a humidified, 5% CO_2 atmosphere, the plates were spun at 5000 rpm for 5 min, the supernatants were harvested and ^{51}Cr release measured using a gamma counter. Percent target lysis was calculated as (experimental release − spontaneous release)/(detergent release − spontaneous release) × 100.

Results

Characterization of cell types in the primary cultures

Cells cultured on coverslips were examined 2–3 weeks after tissue dissociation. 70–80% of the cells used in this study were astrocytes as determined by staining with rabbit antiserum to GFAP and visualization by immunofluorescence microscopy. The majority of the remaining cells reacted with the monocyte/macrophage marker (leu-M3) and less than 5% reacted with rabbit antiserum to fibronectin. Cells that reacted with leu-M3 had morphological characteristics consistent with microglia including irregular rod-like shapes and short branching processes. The presence of oligodendrocytes was not examined in the cultures used in this study. However, examination of subsequent cultures established from different brains under similar conditions indicated the presence of less than 5% of cells that react with antibodies to the oligodendrocyte markers galactocerebroside (gift from Dr. M. Dubois-Dalcq) and MBP (Dakopatts). Cells reacting with antibodies to Von Willebrand factor (an endothelial cell marker) were

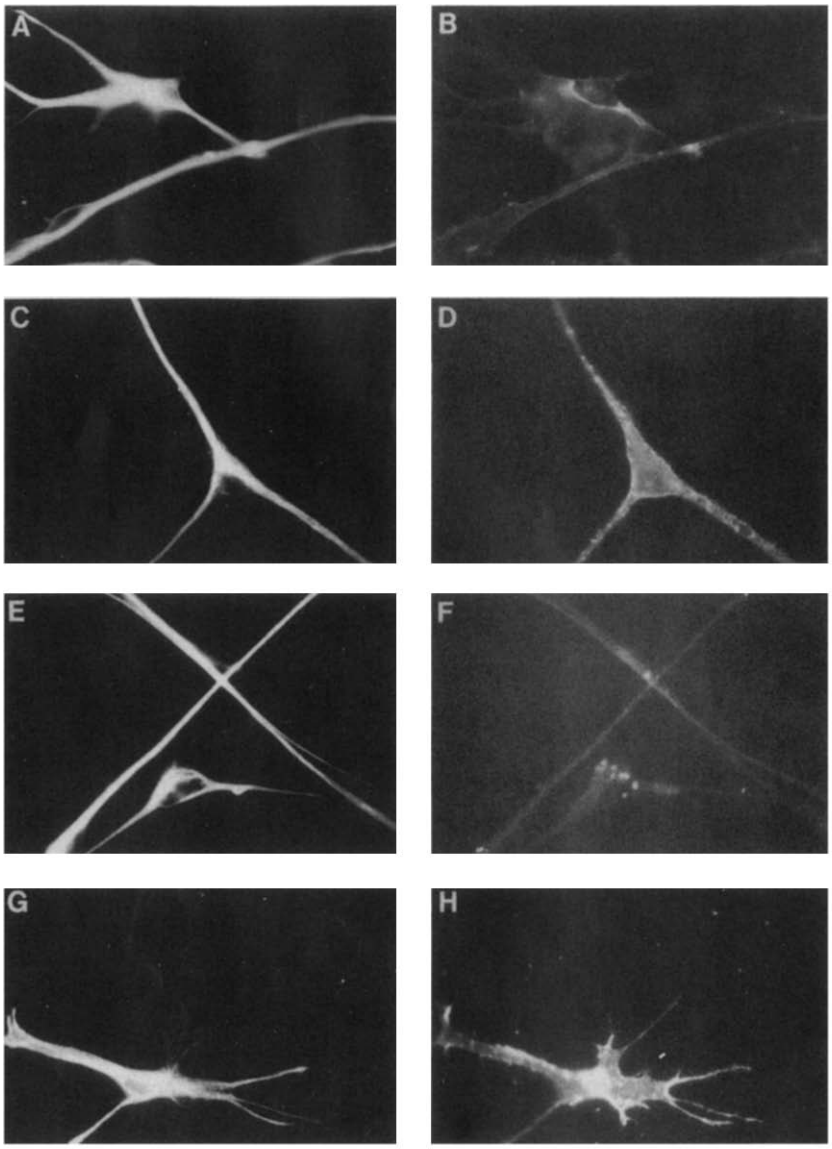

Fig. 1. Two-color immunofluorescence for GFAP (*A*, *C*, *E*, and *G*) and either class I or class II (DR) pre- and post-treatment with IFN-γ for 3 days. (*A* and *B*): Untreated cells costained for GFAP and HLA class I; (*C* and *D*): IFN-γ-treated cell contained for GFAP and HLA class I; (*E* and *F*): untreated cells costained for GFAP and HLA-DR; (*G* and *H*): IFN-γ-treated cells costained for GFAP and HLA-DR (×400).

not observed in these cultures. Approximately 50% of the GFAP-positive cells constitutively expressed low levels of HLA class I molecules (Fig. 1*A* and *B*) and less than 5% expressed HLA class II (DR) molecules (Fig. 1*E* and *F*), but expression could be upregulated on these cells up to 90% for both class I and class II DR by treatment with IFN-γ 100 units/ml for 3 days (Fig. 1*C*, *D*, and *G*, *H*). Upregulation of HLA class I and DR molecules was also observed on cells with a morphological appearance of macrophage/microglia.

Presentation of target antigens by primary cultures

The capacity of primary adult human glial cells (70% of which were GFAP positive) to present influenza A virus antigen to influenza virus-

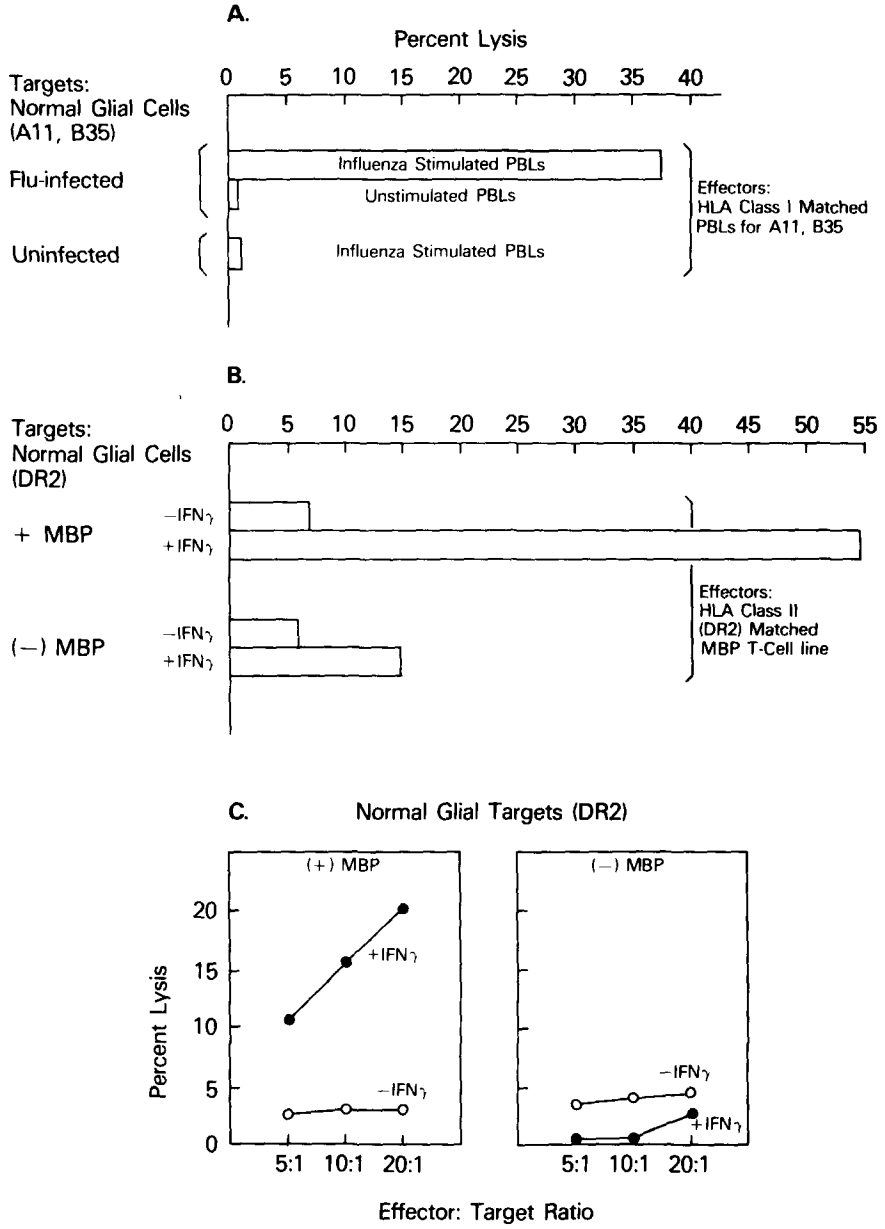

Fig. 2. (*A*) Lysis of primary adult human glial cells infected with influenza A/JAP virus by HLA class I-matched influenza-stimulated PBL from a healthy donor at effector to target ratio of 40:1. (*B*) Lysis of primary adult human glial cells obtained from an HLA DR2-positive individual by an MBP-specific DR2-restricted T-cell line at an effector to target ratio of 40:1. (*C*) Lysis of the same targets as in (*B*) with MBP-specific DR2-restricted T-cell line from a different donor at serial effector to target cell ratios.

specific class I HLA-restricted CTLs was examined. Following treatment with IFN-γ and subsequent infection with influenza A/JAP virus these glial cells were lysed by influenza virus-stimulated, HLA class I-matched PBL from a healthy donor. Lysis was not observed with the unstimulated PBLs or with the uninfected targets (Fig. 2*A*).

Lysis of MBP-pulsed targets by MBP-specific T-cell lines was used to examine HLA class II-restricted lysis. The MBP T-cell lines used in this

study are $CD4^+$ cells and are restricted by HLA class II molecules (Martin, R. et al., in preparation). Primary adult human glial cells (80% of which were GFAP positive) were obtained from a patient with an HLA-DR2 haplotype. Following treatment with IFN-γ and MBP these cells could be effectively lysed by two human MBP-specific DR2-restricted T-cell lines (Fig. 2*B* and *C*). The lysis was dependent on treatment of the target cells with IFN-γ. No lysis was obtained with targets treated with IFN-γ but not exposed to MBP (Fig. 2*B*). Similar results were obtained with another MBP-specific DR2-restricted T-cell line from a different donor at serial effector to target ratios (Fig. 2*C*).

Presentation of target antigens by $GFAP^+$ cells derived from glioblastoma multiforme

The primary adult human glial cell cultures also included nonastrocytic cell types, and therefore, the magnitude of ^{51}Cr release attributable to each cell population cannot be ascertained. Thus, the cell line U-251 MG which produces GFAP was used as a target in the subsequent experiment to establish that similar results could be obtained with cells presumably of glial origin. Moreover, the capacity of this glial target to be lysed by CTL in an HLA-restricted fashion was also examined.

U-251 MG cell line shares with normal adult astrocytes two relevant characteristics: it is GFAP positive and constitutively expresses HLA class I (A2) but not DR molecules. In contrast, U-373 MG does not express GFAP but expresses both HLA class I (A3) and DR molecules. U-251 MG was examined for its ability to present target antigens to the influenza virus-specific HLA-A2-restricted T-cell line Q115. An HLA-A2-positive EBV-transformed B-cell line (K4B) was used as a control target. As shown in Fig. 3, Q115 lysed U-251 MG and B-cell targets that were infected with the A/JAP strain of influenza virus (Fig. 3*A* and *I*) or pulsed with the M1 peptide (Fig. 3*B* and *J*) but not targets infected with a different strain of influenza virus (B/AA) (Fig. 3*C* and *K*) or uninfected targets (Fig. 3*D* and *L*). Lysis of U-251 MG target with the mismatched effectors (1D3) was not observed (Fig. 3*A–D*) indicating that the lysis of this target was restricted by HLA-A2 molecules. Similarly, no lysis was obtained with Q115 on the mismatched target (U-373 MG, HLA-A3 positive) but this target could be lysed by 1D3 (Fig. 3*E–H*) indicating that the lysis by these cell lines is HLA restricted.

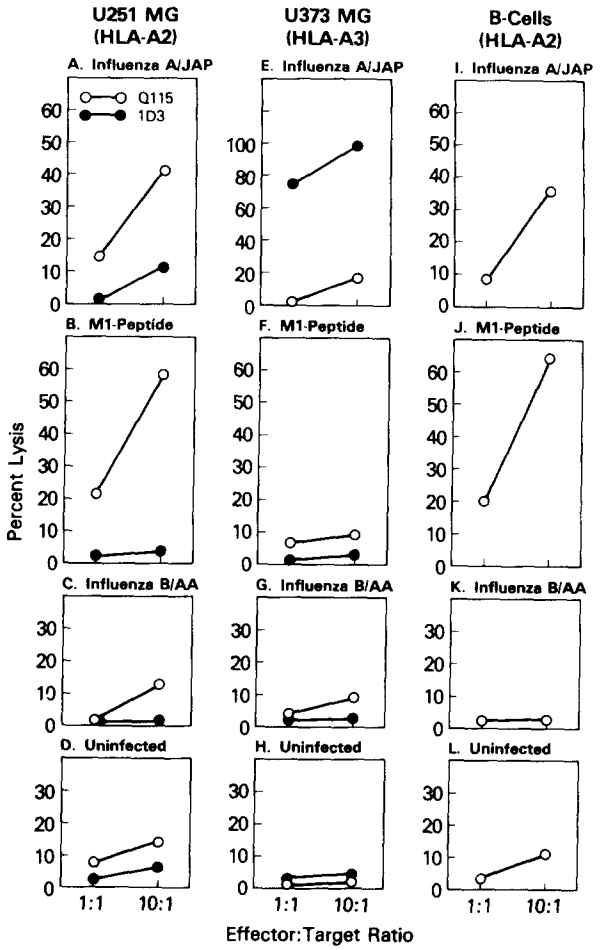

Fig. 3. Lysis of $GFAP^+$ glioblastoma multiforme cells (U-251 MG) by influenza virus-specific HLA-A2-restricted T-cell line Q115 (open circles). U-251 MG and EBV-transformed B-cells express HLA-A2. When infected with influenza virus, A/JAP strain or pulsed with the influenza virus M1 peptide, these targets were lysed by Q115 (*A*, *B*, *I*, *J*). No lysis was observed with targets infected with a different strain of influenza virus (B/AA) (*C*, *K*) or left uninfected (*D*, *L*). Glioblastoma U-373 MG targets which express HLA-A3 and infected with influenza virus were not lysed by T-cell line Q115 but could be lysed by the HLA-A3-restricted T-cell line 1D3 (*E*). Similarly, T-cell line 1D3 did not lyse U-251 MG targets (*A*).

Discussion

In this study, primary glial cell cultures were established from adult human brain. These cultures consisted primarily of astrocytes (70–80%) and microglia/macrophages (15–20%). The microglia/macrophage cells are likely to be derived from the brain rather than from peripheral blood contamination, since peripheral blood monocytes from the same patient cultured under similar conditions were morphologically different from the brain-derived microglia/macrophages. Fibroblasts were rarely detected in cultures less than 3 weeks old, but they increased in number, thereafter. Consequently, 2- to 3-week-old cultures were employed in order to study antigen presentation by glial cells. Under basal conditions, 50% of $GFAP^+$ cells expressed class I HLA molecules but only 5% expressed class II molecules. Both fluorescence intensity and number of cells expressing HLA molecules were increased by IFN-γ treatment. In general, these findings are in agreement with the observations reported in previous studies (Hirayama et al., 1986; Grenier et al., 1989) and personal communication with Dr. J.P. Antel.

The capacity of primary glial cells expressing class I and class II HLA molecules to present target antigens to CTL was examined. Since both viral and auto-antigens have been implicated in the development of autoimmune disease (Fontana et al., 1987), presentation of these antigens by primary human glial cells was studied. The present data suggest that primary mixed glial cell cultures can function as targets for antigen-specific CTL. As shown in Fig. 2*B* and *C*, the ability of these cells to function as targets for MBP CTL is dependent on treatment of the targets with IFN-γ which results in upregulation of HLA molecules. IFN-γ also has been shown to upregulate the expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) on murine astrocytes which could contribute to more effective lysis of these cells (Frohman et al., 1989). Since the primary adult human glial cells contained nonastrocytic cell types such as microglia, the extent of ^{51}Cr release attributable to each cell population was uncertain. Therefore, either astrocytes or microglia or both could have functioned as targets for CTL. Attempts to separate the astrocytes and microglial cells have been hampered by the limited number of cells that can be obtained from adult brain tissue.

For these reasons an established human glioblastoma cell line U-251 MG which produces GFAP was used as targets to establish that similar results could be obtained with cells that are likely to be derived from a glial origin. The study performed with this cell line indicated that U-251 MG can process and present viral antigens as efficiently as B-cells, since both targets produced comparable lysis with the influenza virus and the M1 peptide (which does not require processing). Moreover, the results obtained with the U-251 MG targets indicated that the lysis of these targets is antigen specific and HLA restricted.

In autoimmune disease of the CNS the evidence that native cells of the CNS can present antigen in situ or become targets for the cellular immune response is largely circumstantial. Such evidence is derived from the immunohistochemical demonstration of class II HLA molecules on astrocytes, microglia, and endothelial cells in lesions of brains affected with multiple sclerosis or EAE (McCarron et al., 1985; Hayes et al., 1987; Traugott, 1987; Grenier et al., 1989). Studies addressing the functional capacity of these CNS cells to present antigen to T-cells have been conducted in murine systems and have largely focused on MBP (Fontana et al., 1984; McCarron et al., 1985; Sun and Wekerle, 1986). The present study suggests that adult human astrocytes and/or microglia can present viral or auto-antigen to cytotoxic T-cells. In addition, the results obtained with the $GFAP^+$ cell line U-251 MG support a role for astrocytic cells in presenting antigen to T-cells. These findings have implications for pathogenic mechanisms involved in the development of autoimmune diseases of the CNS. HLA molecules induced on glial cells by IFN-γ released during inflammation could render these cells capable of presenting antigen to T-cells. Glial cells could then play a role in the induction of an immune response within the CNS or become targets for CTL.

In addition, clearance of a virus infection from the brain may be dependent, in part, on CTL lysis of infected cells (Oldstone et al., 1986). This, in turn, is dependent on the upregulation of HLA molecules on the infected brain cells and the

capacity of these cells to process viral antigens. While this study suggests that adult human glial cells expressing class I HLA molecules may process and present viral antigen to HLA class I-restricted CTLs, it is not known whether similar processes occur in other cell types of the CNS. The persistence of certain viruses in the CNS may be related to a failure to upregulate HLA molecules, and subsequently, failure of CTL recognition of infected cells.

Finally, there is increasing interest in intracerebral transplantation of fetal tissue for patients with Parkinson's disease. Experimental evidence in rats suggests that microglia may participate in the immunological reaction to xenografts by functioning as antigen-presenting cells (Poltorak and Freed, 1989). Moreover, adult human mixed glial cell cultures have been shown to induce an allogeneic T-cell response (Grenier et al., 1989). The present findings suggest that human glial cells potentially can participate in the immunological reaction to intracerebral transplants under conditions that would lead to upregulation of HLA molecules.

Acknowledgments

We would like to thank Drs. Carol Wikstrand and William E. Biddison for stimulating discussions, Ms. Toni Simonis for the HLA typing, the Leukophoresis Unit at the NIH, and Mrs. Millie Fenton for preparing the manuscript.

References

Biddison, W.E. (1982) The role of the major histocompatibility complex in cytotoxic T-cell responses to virus infected cells. J. Clin. Immunol. 2, 1–9.

Bigner, D.D., Bigner, S.H., Ponten, J., Westermark, B., Mahaley, M.S., Ruoslahti, E., Herschman, H., Eng, L.F. and Wikstrand, C.F. (1981) Heterogeneity of genotypic and phenotypic characteristics of fifteen permanent cell lines derived from human gliomas. J. Neuropathol. Exp. Neurol. 40, 201–229.

Caspori, A.A. and Katz, S.I. (1988) Induction and functional characterization of class II MHC (Ia) antigens on murine keratinocytes. J. Immunol. 140, 2956–2963.

Cowan, E.P., Jelachich, M.L., Coligan, J.E. and Biddison, W.E. (1987) Site directed mutagenesis of an HLA-A3 gene identifies amino acid 152 as crucial for major histocompatibility complex-restricted and alloreactive cytotoxic T-lymphocyte recognition. Proc. Natl. Acad. Sci. U.S.A. 84, 5014–5018.

Dhib-Jalbut, S., Jacobson, S., McFarlin, D.E. and McFarland, H.F. (1989) Impaired human leukocyte antigen-restricted measles virus-specific cytotoxic T-cell response in subacute sclerosing panencephalitis. Ann. Neurol. 25, 272–280.

Fontana, A., Fierz, W. and Wekerle, H. (1984) Astrocytes present myelin basic protein to encephalitogenic T cell lines. Nature 307, 273–276.

Fontana, A., Frei, K., Bodmer, S. and Hofer, E. (1987) Immune-mediated encephalitis: on the role of antigen-presenting cells in brain tissue. Immunol. Rev. 100, 183–201.

Frohman, E., Van den Noort, S. and Gupta, S. (1989) Astrocytes and intracerebral immune responses. J. Clin. Immunol. 9, 1–9.

Geppert, T.D. and Lipsky, P.E. (1985) Antigen presentation by interferon-γ-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression. J. Immunol. 135, 3750–3762.

Grenier, Y., Ruijs, T.C.G., Robitaille, Y., Olivier, A. and Antel, J.P. (1989) Immunohistochemical studies of adult human glial cells. J. Neuroimmunol. 21, 103–115.

Hayes, G.M., Woodroffe, M.N. and Cuzner, M.L. (1987) Microglia are the major cell type expressing MHC class II in human white matter. J. Neurol. Sci. 80, 25–37.

Hirayama, M., Yokochi, T., Shimokata, K., Iida, M. and Fujiki, N. (1986) Induction of human leukocyte antigen-A, B, C, and DR on cultured human oligodendrocytes and astrocytes by human gamma-interferon. Neurosci. Lett. 72, 369–374.

Massa, P.T., Dorries, R. and ter Meulen, V. (1986) Viral particles induce Ia antigen expression on astrocytes. Nature 320, 543–546.

Massa, P.T., Brinkman, R. and ter Meulen, V. (1987) Inducibility of Ia antigen on astrocytes by murine coronavirus JHM is rat strain dependent. J. Exp. Med. 166, 259–264.

McCarron, R.M., Kempski, O., Spatz, M. and McFarlin, D.E. (1985) Presentation of myelin basic protein by murine cerebral vascular endothelial cells. J. Immunol. 134, 3100–3103.

Mokhtarian, F., McFarlin, D.E. and Raine, C.S. (1984) Adoptive transfer of myelin basic protein-sensitized T cells produces chronic-relapsing demyelinating disease in mice. Nature 309, 356–358.

Nuchtern, J.G., Bonifacino, J.S., Biddison, W.E. and Klausner, R.D. (1989) Brefeldin A implicates egress from endoplasmic reticulum in class I restricted antigen presentation. Nature 339, 223–226.

Oldstone, M.B.A., Blount, P.J., Southern, P.J. and Lampert, P.W. (1986) Cytoimmunotherapy for persistent virus infection reveals a unique clearance pattern from the central nervous system. Nature 321, 239–243.

Poltorak, M. and Freed, W. (1989) Immunological reactions induced by intracerebral transplantation: evidence that host microglia but not astroglia are antigen-presenting cells. Exp. Neurol. 103, 222–233.

Sun, D. and Wekerle, H. (1986) Ia-restricted encephalitogenic T lymphocytes mediating EAE lyse autoantigen-presenting astrocytes. Nature 320, 70–72.

Sun, D., Meyermann, R. and Wekerle, H. (1988) Cytotoxic T cells in autoimmune disease of the central nervous system. Ann. N.Y. Acad. Sci. 532, 221–229.

Traugott, U. (1987) Multiple sclerosis: relevance of class I and class II MHC-expressing cells to lesion development. J. Neuroimmunol. 16, 283–302.

Wikstrand, C.J., Grahmann, F.C., McComb, R.D. and Bigner, D.D. (1985) Antigenic heterogeneity of human anaplastic gliomas and glioma-derived cell lines defined by monoclonal antibodies. J. Neuropathol. Exp. Neurol. 44, 229–241.

Williams, K., Hart, D., Fabre, J. and Morris, P. (1980) Distribution and quantitation of HLA-A, B, C, and DR (Ia) antigens on human kidney and other tissues. Transplantation (Baltimore) 29, 274–279.

Wong, G.M.W., Bartlett, P.F., Clark-Lewis, I., Battye, F. and Schrader, J.W. (1984) Inducible expression of H-2 and Ia antigens on brain cells. Nature 310, 688–691.

Zinkernagel, R.M. and Doherty, P.C. (1974) Restriction of in vivo T-cell mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogenic system. Nature 248, 701–70.