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Human oligodendrocytes are susceptible to cytolysis by major histocompatibility complex class I-restricted lymphocytes

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Summary

The majority of human oligodendrocytes in enriched glial cell cultures expresses class I major histocompatibility complex (MHC) antigens. We used a ^{51}Cr release assay to study the susceptibility of oligodendrocyte-enriched glial cells to MHC-restricted and non-restricted immune-mediated cytolysis. Mitogen-activated mononuclear cells induced significant lysis in a lectin-dependent cytotoxicity assay. Mononuclear cells allo-activated in a one-way mixed lymphocyte culture with E^- cells from the glial cell donor induced a significantly higher degree of oligodendrocyte cytolysis than mononuclear cells activated with E^- cells bearing MHC-class I antigens discordant with the glia. Cytolysis by alloactivated unfractionated lymphocytes and by purified $CD8^+$ lymphocytes was reduced by an anti-class I antibody (W6/32). Our findings suggest that human oligodendrocytes can be susceptible targets for MHC class I-restricted lysis.

Introduction

The human central nervous system (CNS) disease multiple sclerosis (MS) is characterized by focal areas of demyelination associated with inflammation. Whether the demyelination reflects a primary injury to the myelin sheath or to the oligodendrocyte (OGC), the myelin producing cell, remains speculative. A possible explanation for damage to myelin and/or the OGCs as observed in MS may be cellular immune-mediated injury. The involvement of such mechanisms is suggested by the presence of activated $CD8^+$ and $CD4^+$ T-lymphocytes and macrophages in the MS lesions and the demonstration that T-lymphocytes are required for the passive transfer of the animal model of MS, experimental allergic encephalomyelitis (EAE) (Mokhtarian et al., 1984).

A requirement for antigen-specific T-cell-mediated cytotoxicity is recognition of major histocompatibility complex (MHC) antigens in association with the antigen on the target cell surface. Most antigen-specific cytotoxic lymphocytes are

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contained in the $CD8^+$ suppressor/cytotoxic T-lymphocyte subset and are MHC class I restricted; however, cytotoxicity mediated by $CD4^+$ class II-restricted lymphocytes has also been described (Jacobson et al., 1984). Although in situ expression of MHC antigens is confined to endothelial cells in normal CNS, both MHC class I and class II antigens are detectable on astrocytes, endothelial cells and macrophages/microglia in and adjacent to the MS lesion (Traugott, 1987).

Immunohistochemical data on OGCs in situ are to date limited. We have recently demonstrated the expression of MHC class I antigens on human adult OGCs, in cultures of tissue derived from young adults undergoing surgery as a treatment for intractable seizures (Grenier et al., 1989). These findings were in accordance with studies on human glial cell cultures established from post-mortem tissue (Kim et al., 1985). We did not detect any MHC class II expression on the OGCs, whereas a proportion of the astrocytes in the cultures did express those antigens (Grenier et al., 1989).

In the current study we used a ^{51}Cr release assay to demonstrate the susceptibility of human OGCs to lysis by allo-reactive class I-directed cytotoxic T-lymphocytes.

Materials and methods

Human OGC-enriched cell culture

Tissue from temporal lobe or corpus callosum was removed in fragments by ultrasonic aspiration or resected en bloc as part of surgical treatment of young adult patients suffering from intractable seizures. The isolation procedure was adapted from that reported by Kim et al. (1983). Meninges and blood vessels were removed from the block specimens and the fragmented tissue was washed extensively to remove the blood. The tissue was enzymatically dissociated by use of 0.25% trypsin (Gibco Canada, Burlington, Ont., Canada) in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) for 1 h at 37°C in a humidified 5% CO_2 incubator. Trypsin was inactivated by addition of fetal calf serum (FCS; Gibco Canada) and the tissue was further mechanically dissociated by passage through two nylon meshes (210 and 132 μm pore size; Industrial Fabrics Corporation, Minneapolis, MN, U.S.A.). The obtained suspension was washed once and separated on a 30% Percoll (Pharmacia LKB, Montreal, Que., Canada) gradient in a Beckman J2-21M/E centrifuge with a fixed angle 17J rotor (15,000 rpm at 4°C for 30 min). The gradient resulted in formation of an upper layer of PBS, a layer of densely packed myelin, a clear layer of glial cell suspension and a narrow band of red blood cells at the bottom. Glial cells were recovered, diluted in PBS and washed 3 times at 1800, 1500 and 1200 rpm respectively in a Beckman IEC Centra-8R centrifuge with swinging bucket 216 rotor. Viable cell numbers, usually > 90%, were estimated under a haemocytometer by trypan blue exclusion and cells were resuspended in Dulbecco's modified Eagle medium (DMEM; Gibco Canada) supplemented with penicillin 2.5 U/ml, streptomycin 2.5 μg/ml, glutamine 2 mM (all from Gibco Canada) and 10% FCS and plated on 80 cm^2 tissue culture flasks (Nunclon, Gibco Canada). After 24–48 h, floating cells were removed and plated on poly-L-lysine (10 μg/ml, Sigma, St. Louis, MO, U.S.A.) coated cell culture flasks at a density of 10^6 per ml in DMEM supplemented with 5% human AB^+ serum (normal human serum, NHS).

Immunocytochemical staining

The proportion of OGC as well as the expression of MHC class I antigens was assessed by a double-immunofluorescence technique with the monoclonal pan-class I antibody W6/32 (gift from Dr. R. Sekaly, Montreal) and a polyclonal anti-2′, 3′-cyclic nucleotide phosphodiesterase (CNPase) antibody (gift from Dr. P. Braun, Montreal) as described previously (Grenier et al., 1989).

Chromium release assay

Glial cells were trypsinized and plated on a flat-bottom 96-well plate, at least 2 days prior to the assay (density of 5×10^4 per well in RPMI 10% NHS). The cells were labelled with ^{51}Cr (1 μCi per well) overnight. Average labelling with ^{51}Cr was approximately 5400 counts per minute (cpm) per well, with a range of approximately 1100–20,000 cpm/well over all experiments. No correlation was observed between total ^{51}Cr labelling and the percentage of OGCs in the culture

when assessed in a series of cultures containing 10–90% OGCs. On the day of the assay, cells were gently washed twice and allowed to stand for 30 min before lectin-activated or mixed lymphocyte culture (MLC)-activated lymphocytes (effector cells), prepared as described below, were added to triplicate or quadruplicate wells. In most studies a 10 : 1 effector/target ratio was used, except where indicated in the Results section. After a 5–6 h incubation with the lymphocytes, supernatants (A) were removed and counted in an LKB 1272 Clinigamma counter. Aliquots of 5 N NaOH were added to each well for 20 min and supernatants (B) removed and counted for remaining radioactivity. The percentage lysis was calculated by dividing the cpm of supernatant A by the total labelling (supernatants A + B). The value for specific lysis was obtained by subtracting the mean percentage of spontaneous release in ^{51}Cr-labelled control wells which had been incubated with media only.

Mononuclear cell isolation

Blood collected in heparinized tubes was separated on a Ficoll-Hypaque gradient (Pharmacia LKB). Mononuclear cells (MNCs) were recovered, washed 3 times in PBS and cultured in RPMI 1640 (Gibco Canada) supplemented with penicillin 2.5 U/ml, streptomycin 2.5 μg/ml, glutamine 2 mM and 10% FCS or NHS.

Lectin activation of MNCs

MNCs from a healthy donor were activated by incubation with concanavalin A (ConA) (10 μg/ml, Sigma) in RPMI containing 10% FCS for 2–3 days. The cells were then harvested, washed 3 times to remove free lectin and added to ^{51}Cr-labelled glial cells in the cytotoxicity assay, either in the presence of ConA (10 μg/ml) or in the presence of α-methyl-D-mannoside (2 mM, Sigma), to block ConA binding sites (Suzumura and Silberberg, 1985). Non-activated cells maintained in culture for 2–3 days were used as controls. The degree of lysis mediated by activated versus non-activated cells, in the presence or absence of ConA, was compared using the paired *t*-test. Proliferation rates of activated and non-activated lymphocytes were measured by incorporation of [^{3}H]thymidine (ICN-Radiochemicals, Montreal, Que., Canada) after a 5 h pulse with 1 μCi per 10^5 lymphocytes.

Mixed lymphocyte culture

MNCs from the glial cell donor were typed for all HLA-A, B, C epitopes (the MHC class I antigens) in the tissue typing laboratory, Royal Victoria Hospital (Dr. R.D. Guttmann). HLA class II typing was not performed.

Cytotoxic responder lymphocytes directed against the glial cell donor and against an HLA-A, B, C discordant control donor were generated in a one-way mixed lymphocyte culture (MLC), as follows. MNCs from the patient and from the control donor were depleted of T-lymphocytes by rosetting with neuraminidase-treated sheep erythrocytes (Frappier Diagnostic, Laval, Que., Canada). Non-rosetting cells (E^- cells, i.e. B-lymphocytes and macrophages) were recovered from a Ficoll-Hypaque gradient and irradiated (3000 Rad, AECL Gammacell 1000 irradiator). Whole MNCs isolated from peripheral blood from a second healthy donor, who was discordant with both the glial cell donor and with the other stimulator cell donor, were used as responder cells. These responder MNCs were incubated in a 3 : 1 ratio with irradiated E^- cells from patient or control donor, in RPMI supplemented with 10% NHS. Some MNCs were maintained in culture without stimulation. On day 7 viable cells were recovered from a Ficoll gradient and used as the source of cytotoxic cells in the ^{51}Cr release assay. Proliferation rates of the recovered cells were assessed by measurement of [^{3}H]thymidine uptake.

In two experiments, enriched $CD8^+$ and $CD4^+$ subsets were obtained from the MLC-derived lymphocytes by using OKT4 or OKT8 antibody (Ortho Diagnostic Systems, Don Mills, Ont., Canada) in a 'panning' technique for negative cell selection, as previously described (Rosenkoetter et al., 1984). This technique routinely yields > 85–90% enrichment of the desired T-cell subset as determined by fluorescence-activated cell sorting (FACS) analysis (Antel et al., 1986).

Results

Immunocytochemical properties of cultured human OGC

As shown in Fig. 1, MHC class I antigen expression was detectable on the soma and cell

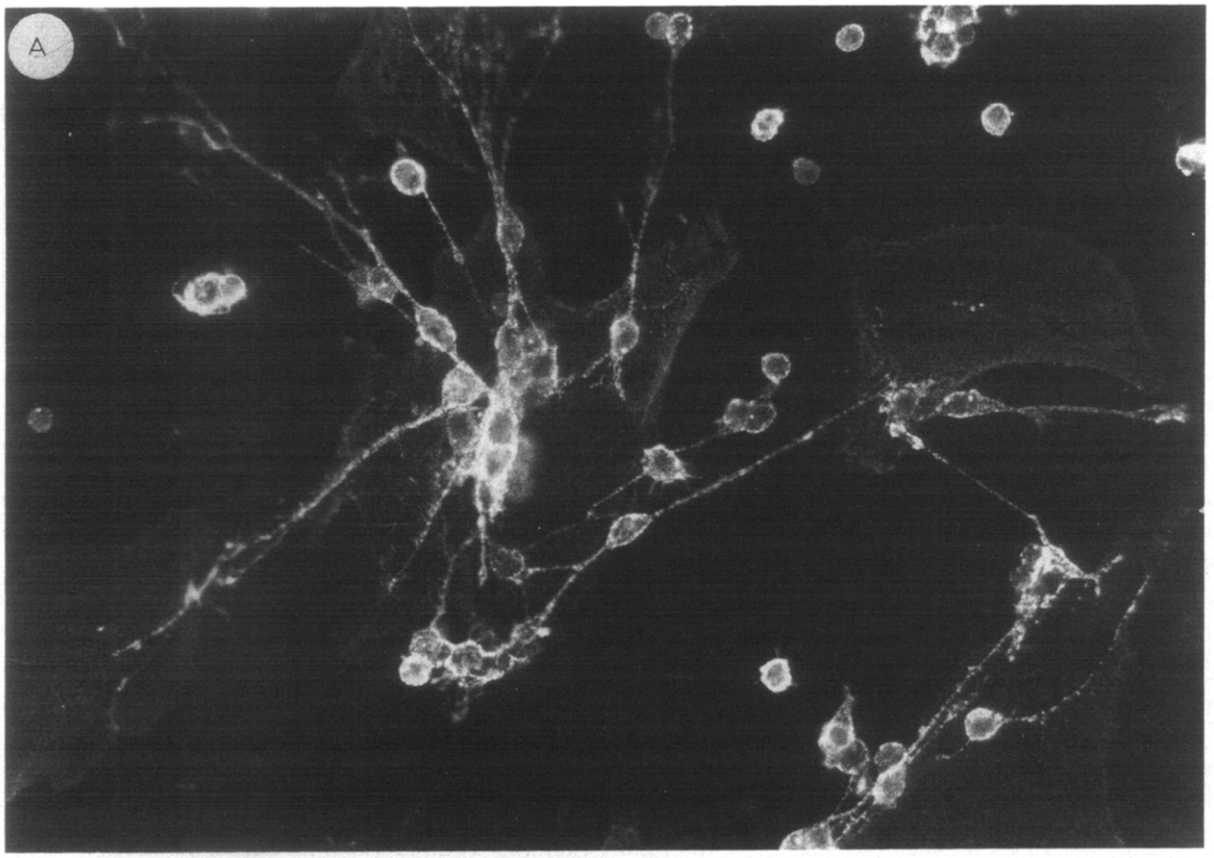

Fig. 1. Double immunolabelling of human cells in an oligodendrocyte-enriched culture. (*A*) Labelling with a mouse monoclonal antibody W6/32 against MHC pan-class I antigens (1 : 100 dilution), followed by fluorescein-conjugated rat anti-mouse IgG_κ antibody. (*B*) Same field as in (*A*), labelled with polyclonal rabbit antibody for the cytosol enzyme CNPase (1 : 150), followed by rhodamine-conjugated goat anti-rabbit IgG.

processes of CNPase-positive cells, even though the class I antigens were not detectable on brain tissue sections (Grenier et al., 1989). The percentage OGCs in the cultures ranged from approximately 60 to 90%.

Lectin-dependent cytotoxicity

Mean specific cytolysis mediated by ConA-activated lymphocytes at a 10 : 1 effector/target ratio, in the presence of the lectin was 24.3 ± 3.4%, $n = 12$ (range 15–52%). ConA-activated lymphocytes induced significantly less lysis when the lectin was deleted from the cytotoxicity assay and ConA-binding sites were blocked with α-methyl-D-mannoside (mean lysis 15.0 ± 4.1%, $p < 0.01$, paired *t*-test). Non-activated lymphocytes did not induce significant lysis of OGCs (0.7 ± 4.7%), even in the presence of ConA (7.2 ± 3.1%). No correlation was found between cytolytic activity and levels of [^{3}H]thymidine incorporation by activated lymphocytes (results not shown). The mean level of spontaneous lysis of OGC-enriched cultures was 22 ± 3%.

Mixed lymphocyte culture-activated cytotoxicity

In each of four experiments, responder lymphocytes allo-activated against the glial cell donor's E^- cells exhibited significantly higher in vitro cytotoxic activity against the glia than did the same responder lymphocytes activated by E^- cells from the donor discordant for MHC class I with the glial cell donor ($p < 0.02$ in the paired *t*-test; Fig. 2). With the latter, levels of glial cell lysis were not significantly different from those in-

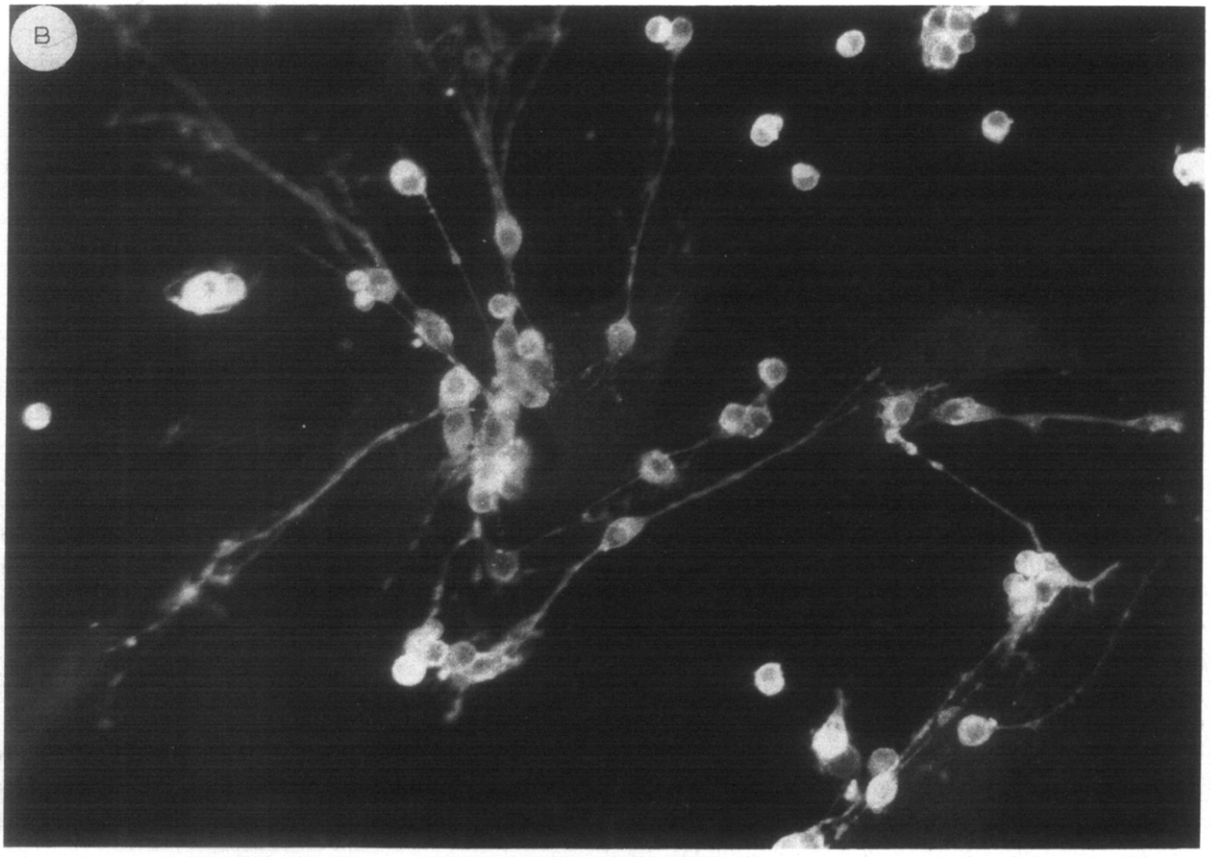

Fig. 1 (continued).

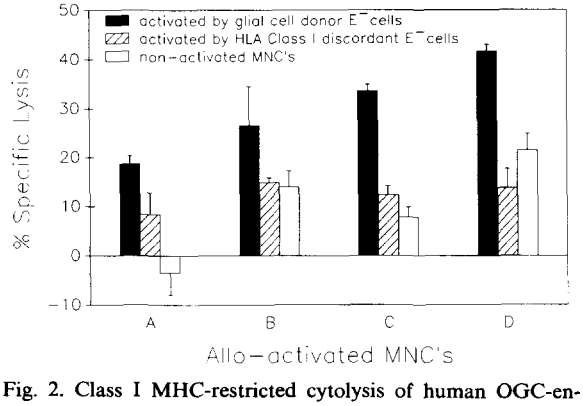

Fig. 2. Class I MHC-restricted cytolysis of human OGC-enriched cell cultures. Lymphocytes tested for cytotoxic activity were those allo-activated with E^- cells from either the glial cell donor or from a MHC class I-discordant donor. Results of four individual experiments (A, B, C, D) are presented. Non-activated MNCs were cultured for 7 days without stimulation. Effector/target ratio 10:1. Error bars: standard deviation for triplicate data points.

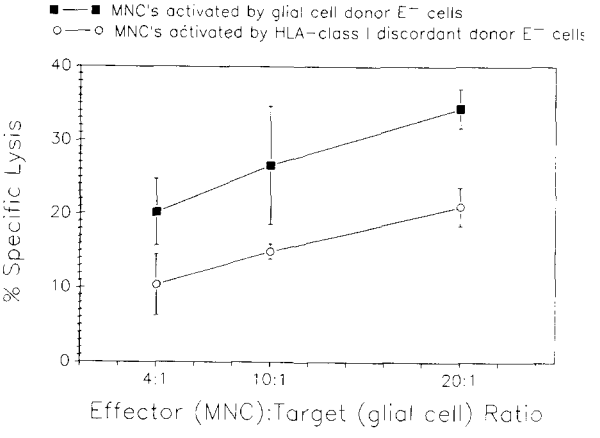

Fig. 3. Influence of effector to target ratio on MHC class I-restricted cytolysis of human OGC-enriched glial cell cultures. Three different effector/target cell ratios were used (20:1, 10:1, 4:1) in ^{51}Cr release assay. MNCs used correspond to experiment B in Fig. 2. Error bars: standard deviation for triplicate data points.

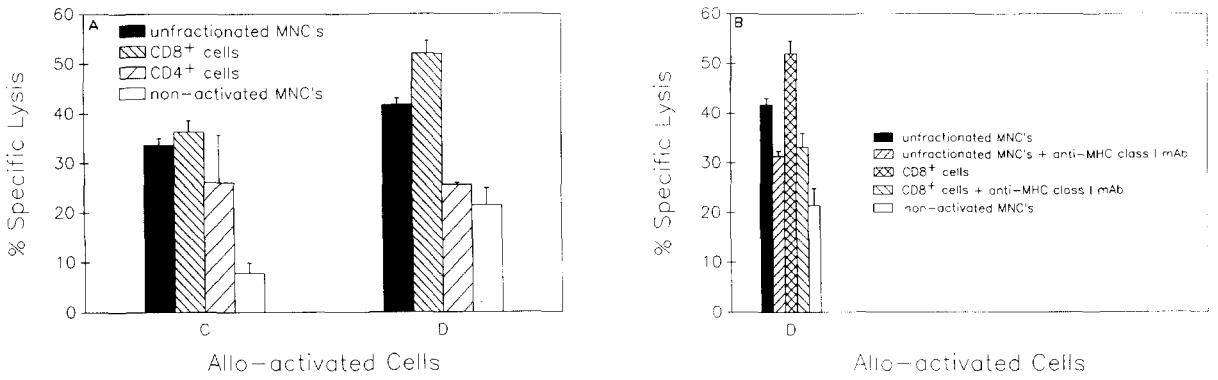

Fig. 4. T-lymphocyte subset-mediated cytolysis of human OGC-enriched cell cultures. (*A*) In two experiments (experiments C and D from Fig. 2), MNCs allo-activated by E^- cells from the glial donor were enriched for $CD8^+$ and $CD4^+$ subsets and applied in cytotoxicity assays. (*B*) In experiment D, the inhibitory effect of anti-class I antibody W6/32 on cytolysis mediated by activated unfractionated MNCs and by $CD8^+$ lymphocytes was determined.

duced with non-activated cultured lymphocytes from the responder donor. The levels of specific lysis varied as a function of effector/target ratio (Fig. 3). Lymphocyte activation as measured by [^{3}H]thymidine incorporation did not differ significantly between the responder MNCs allo-activated by E^- cells from glial cell and control donor (Table 1).

In an additional experiment (Table 2), the number of OGCs surviving after co-culture with allo-activated MNCs for 4 h was determined. In this experiment > 90% of the glial cells were $GalC^+$. Addition of the allo-activated MNCs resulted in a 45% reduction in the total number of $GalC^+$ cells. In parallel cultures using ^{51}Cr-labelled glial cells, the calculated percentage specific lysis was 41% after 5 h of incubation with the allo-activated MNCs.

Fractionation and specificity of MLC-activated cytotoxic lymphocytes

In two experiments (Fig. 4*A*), we observed that $CD8^+$ cell-mediated lysis exceeded that mediated by the $CD4^+$-enriched subset. The cultures used

TABLE 1

PROLIFERATION OF ALLO-ACTIVATED LYMPHOCYTES AS MEASURED BY [^{3}H]THYMIDINE INCORPORATION

MNCs were stimulated for 7 days in a mixed lymphocyte culture with irradiated allogenic cells, in four experiments as described in Fig. 2. The degree of activation of 10^5 MNCs was measured by incorporation of [^{3}H]thymidine after a 5 h pulse.

Experiment	Proliferation of lymphocytes (cpm)	
	Activated with irradiated E^- cells from glial donor	Activated with irradiated E^- cells from class I-discordant control donor
A	16,597	23,165
B	12,182	20,471
C	54,676	24,761
D	26,829	25,104

TABLE 2

QUANTITATIVE ASSESSMENT OF LYSIS OF OLIGODENDROCYTES ($GalC^+$ CELLS) BY ALLO-ACTIVATED MNCs

Cell number represents the number of $GalC^+$ cells counted in 15 high-power fields (magnification 250×) in cultures containing OGCs either with culture medium alone, or co-cultured with allo-activated MNCs (activated with the glial cell donor's E^- cells) or non-activated MNCs. $GalC^+$ cells accounted for 90–95% of cells in the culture as determined by double immunostaining with anti-GalC and anti-glial fibrillary acidic protein (GFAP) antibodies. MNCs were added to glial cells in a 10:1 ratio for 4 h. The percentage specific lysis mediated by the allo-activated MNCs in a parallel ^{51}Cr-labelled culture was 41%; specific lysis by non-activated MNCs was 10%

Treatment	Number of $GalC^+$ cells	Decrease (%) in $GalC^+$ cells after treatment
Media alone (control)	1,071	0
Allo-activated MNCs	585	45
Non-activated MNCs	855	20

for these studies contained 70% and 80% OGCs respectively. That the $CD8^+$-mediated cytolytic activity was dependent on class I expression on the target cells was suggested by the finding that $CD8^+$ cell-mediated cytotoxic activity could be blocked at least in part by a 30 min pre-incubation of the glia with the anti-class I MHC antibody W6/32 (Fig. 4*B*).

Discussion

In the present study we have demonstrated the cytotoxic activity of $CD8^+$ class I-directed lymphocytes against OGC-enriched glial cell cultures. We have previously shown that a large proportion of human OGCs in vitro express MHC class I antigens (Grenier et al., 1989), implying a potential susceptibility to lysis by cytotoxic T-lymphocytes (CTL). We have not detected expression of MHC class II antigens on OGCs, a finding consistent with that of others in studies on spontaneous and cytokine-induced expression of MHC antigens on murine glial cell cultures from immature animals (Wong et al., 1984; Suzumura et al., 1986b), on human glial cultures established from autopsy material (Lisak et al., 1983; Hirayama et al., 1986) and on human fetal glial cultures (Mauerhoff et al., 1988). In CNS tissue from the same patients whose cultured glial cells expressed MHC class I antigens, we could not detect MHC antigens on tissue sections, using immunohistochemical techniques (Grenier et al., 1989). Whether the differences between in situ and in vitro expression of MHC antigens on OGCs reflect technical or biological factors remains under study.

We applied a ^{51}Cr release assay to demonstrate cellular immune-mediated lysis of OGCs. This assay system has been used by others in studies of cytotoxicity to murine OGCs as mediated by human serum (Suzumura et al., 1986a) and by myelin basic protein-reactive $CD4^+$ cell lines (Kawai and Zweiman, 1988). Our studies indicated that OGCs and non-OGCs in our cultures were comparably labelled with ^{51}Cr.

The lectin-dependent cytotoxicity assay provided a means to assess susceptibility of cells in our OGC-enriched cultures to T-cell-mediated lysis which was neither antigen specific nor MHC restricted. The presence of an agglutinizing lectin, such as ConA, in the assay provides the required contact between cytotoxic-effector cell and target cell to permit cytolysis (Bevan and Cohn, 1975). α-Methyl-D-mannoside, a competitive blocker of ConA, was added in control assays in an attempt to prevent the agglutinizing effect of any residual ConA on stimulated lymphocytes. We did, however, observe that some glial cell lysis occurred in the absence of added lectin in the assay. Whether residual cell–cell binding occurred or whether soluble factors released by the activated effector cells induced the low level of lysis was not resolved by our study.

Effector lymphocytes allo-activated with the glial cell donor's E^- cells induced a higher degree of lysis of glial cells than did lymphocytes from the same effector cell donor, allo-activated by E^- cells bearing MHC class I eptitopes different from the glia. The levels of specific lysis observed in cultures which contained $> 70\%$ and $> 80\%$ OGCs ruled out the possibility that a population of non-OGC cells were exclusively the targets of MHC class I-dependent lysis. The observation was confirmed by separate studies showing quantitative reductions in OGCs following exposure to allo-activated MNCs. Our conclusion regarding the susceptibility of OGCs to MHC class I-restricted lysis was further supported by the findings that cytotoxicity by $CD8^+$ class I-dependent lymphocytes exceeded that by $CD4^+$ class II-dependent lymphocytes and specific cell lysis could be reduced by anti-MHC class I antibodies. The residual lysis by $CD8^+$ T-lymphocytes of OGC after pre-incubation with the anti-MHC class I antibody, together with the observation that lymphocytes allo-activated with MHC class I-discordant E^- cells induce some specific lysis of OGC, suggest that some degree of lysis mediated by $CD8^+$ cells is not MHC restricted. In previous studies with neonatal murine astrocytes, we have demonstrated that MHC class I-directed cytotoxic lymphocytes could lyse astrocytes. This cytotoxic effect was lost with target astrocytes from mutant animals which lacked the sensitizing MHC class I antigens (Skias et al., 1987). The observed class I-restricted lysis of OGCs suggests that MHC class I molecules on OGCs could contribute to

susceptibility of these cells to either antigen-specific cytotoxicity or to lysis by autoreactive cytotoxic cells (Birnbaum et al., 1984), if expression of MHC antigens occurred in vivo, as is the case in viral infection in mice (Suzumura et al., 1986c).

The results of the MLC experiments indicate that alloreactive $CD4^+$ lymphocytes may also induce glial cell lysis. The cytotoxic effect of $CD4^+$ lymphocytes may have reflected lysis of class II-expressing non-OGC cell types in the cultures, or may have resulted from the release of soluble intermediaries such as interferon-γ or tumor necrosis factor-α. Despite the lack of MHC class II expression, murine OGCs are susceptible to lysis by myelin basic protein (MBP)-specific, but not purified protein derivative (PPD)-specific cytotoxic $CD4^+$ cells in the presence of antigen-presenting cells, which may act as intermediaries in cytotoxicity (Kawai and Zweiman, 1988). MBP-reactive $CD4^+$ cell lines are essential for the passive transfer of EAE (Zamvil et al., 1985) and have been isolated from peripheral blood of normal human donors (Burns et al., 1983) and MS patients (Weber and Buurman, 1988).

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