Non-MHC-Restricted Cell-Mediated Lysis of Human Oligodendrocytes In Vitro: Relation with CD56 Expression

Jack P. Antel, Ellie McCrea, Uma Ladiwala, Yu-fen Qin and Burkhard Becher

J Immunol 1998; 160:1606-1611;
http://www.jimmunol.org/content/160/4/1606

References
This article cites 36 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/160/4/1606.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Non-MHC-Restricted Cell-Mediated Lysis of Human Oligodendrocytes In Vitro: Relation with CD56 Expression

Jack P. Antel, Ellie McCrea, Uma Ladiwala, Yu-fen Qin, and Burkhard Becher

Oligodendrocytes and their myelin membranes are the apparent target of the autoimmune response that characterizes the human adult central nervous system demyelinating disease multiple sclerosis. Human oligodendrocytes do not express MHC class II molecules, a requirement for MHC-restricted injury mediated by myelin-reactive CD4+ T cells, the cell type implicated in initiating the disease process. In this study we observed that human adult central nervous system-derived oligodendrocytes can be susceptible to non-MHC-restricted lysis mediated by myelin basic protein-reactive CD4+ T cell lines. Cytotoxicity was significantly greater (37 ± 4 vs 7 ± 3%) with cell lines in which a high proportion of cells (mean, 28 ± 6%) expressed CD56 compared with cytotoxicity mediated by low CD56 cell lines (8 ± 2%). High CD56 cell lines, when rested in IL-2, lost cytotoxic activity and had reduced expression of CD56 (mean, 5 ± 2%). CD4+ T cells isolated from short term (3-day) anti-CD3/IL-2-activated mononuclear cell cultures did not express CD56 and were not cytotoxic to oligodendrocytes unless lectin was added. In contrast, an enriched population of non-T cells extracted from the same activated MNC cultures expressed CD56 as well as other NK cell-associated surface molecules and was cytotoxic. These results indicate the potential susceptibility of human oligodendrocytes to non-MHC-restricted injury mediated by both Ag-reactive and nonspecific cellular immune effector cells, with CD56 expression being a common feature of the effector cells. The Journal of Immunology, 1998, 160: 1606–1611.

M

ultiple sclerosis (MS) is considered an immune-mediated disorder in which the central nervous system (CNS) myelin or its cell of origin, the oligodendrocyte (OL), is the primary target (1). Observations from the animal model of CNS-demyelinating disease, experimental autoimmune encephalomyelitis would implicate CD4+ myelin-reactive T cells as the cell type required to initiate the inflammatory response (2, 3). The precise basis for the progressive tissue injury that accounts for irreversible neurologic deficits in MS remains to be defined.

The presence of myelin-reactive T cells within the inflammatory infiltrates associated with active MS lesions is demonstrated by findings of TCR sequences identical with those of myelin basic protein (MBP)-reactive CD4+ T cell clones generated in vitro (4). MBP-reactive CD4+ T cell lines are shown to be cytotoxic to MHC class II histocompatible nonneural cell targets if the target cells are pulsed with MBP (5, 6). Human OLs, in contrast to other glial cells, either microglia or astrocytes, are not yet demonstrated to express MHC class II molecules in vivo or in vitro (7, 8). Vergelli et al. found that a portion of human MBP-reactive CD4+ T cells in vitro expressed CD56 (neural cell adhesion molecule), and that such cells could be cytotoxic to an array of CD56-expressing targets in the absence of added Ag (9).

CD56 expression was originally considered a marker for NK cells, but more recently has been detected on T cell lines that can mediate non-MHC-restricted cytotoxicity (10–12). Bovine OLs were reported to be resistant to lysis mediated by human NK and lymphokine-activated killer (LAK) cells (13, 14). CD56-dependent cytotoxicity requires homotypic interaction with CD56 expressed on the target cell (15).

The purpose of the current study was to determine whether human OLs were susceptible to non-MHC-restricted MBP-reactive CD4+ T cell-mediated cytotoxicity and whether the effect was dependent on CD56 expression on the effector and target cells. Since in active MS as well as in experimental autoimmune encephalomyelitis, many of the inflammatory cells infiltrating the CNS are not myelin Ag-specific (16, 17), we also examined the relation of cytotoxicity and CD56 expression of nonmyelin Ag-restricted T cells and non-T cells extracted from in vitro anti-CD3- and IL-2-activated peripheral blood MNCs. Our previous studies indicated that T cells, either CD4+ or CD8+, activated under these conditions could induce only low levels of promiscuous (non-MHC-restricted) killing of OLs even in long duration (18-h) 51Cr release assays (18).

Materials and Methods

Effector cells

All T cell lines and short term MNC cultures were derived from peripheral blood samples obtained from young healthy adult volunteers and from one untreated MS patient with relapsing disease. MNCs were isolated using a Ficoll-Hypaque density gradient.

MBP-reactive T cell lines

These lines were generated using a split well technique. MNCs from individual donors were resuspended at 2 × 10^6 cells/ml in RPMI 1640 culture medium (Life Technologies, Burlington, Canada) supplemented with 5% heat-inactivated autologous serum. One hundred microliters of cells were placed in individual wells of a 96-well U-bottom microtiter plate (Falcon, Franklin Lakes, NJ) together with 30 μg/ml (final concentration) of MBP, prepared by a standard technique (19). On day 4 of culture, 100 μl of culture medium containing 20 U/ml of recombinant human IL-2 (Genzyme, Cambridge, MA) was added. On day 7, 100 μl of medium was...
removed and replaced with fresh IL-2-containing medium. The first re-stimulation was performed on day 15. The cells were washed by first removing 100 μl of supernatant, adding 100 μl of fresh medium, spinning the plates, and removing another 100 μl of supernatant. Each well was then split into two by placing 50 μl from each well into a fresh microtiter well. Fifty microliters of irradiated (2500 rad) autologous MNCs, resuspended at 4 × 10^6 cells/ml, were added to each well as a source of feeder cells. MBP (30 μg/ml) was added only to the wells of the original plate. The procedure described for day 4 was repeated on day 18, and that for day 7 was repeated on day 21. A second restimulation was performed on day 24. Cells were collected from MBP-containing wells with visible growth if there was no growth in control wells. Cells from these wells were resuspended in fresh culture medium at 4 × 10^5 /ml and replated. An equal volume of fresh irradiated MNCs as feeders was added, resulting in a 10:1 feeder:MBP-reactive T cell ratio. MBP was then added. The day 4 and day 15 procedures were repeated on alternating weekly cycles. All studies were performed between the third and the sixth stimulation. With the exception of the studies involving resting cells, the MBP specificity, functional, and phenotypic studies were conducted 3 days after stimulation with MBP. The resting cell assays were conducted with cells maintained in IL-2 that had not been stimulated with MBP for 10 days.

Specificity assays were conducted by adding 2 × 10^4 MBP-reactive T cells (50 μl) and 2 × 10^5 irradiated feeders (50 μl) to individual microtiter wells along with either MBP or tetanus toxoid (10 μg/well) or no Ag. After 72 h, individual wells were pulsed for 5 h with 1 μCi of [3H]thymidine (Mandel, Guelph, Canada). Cells were harvested, and proliferation rates were determined in a scintillation counter. Data are presented as counts per minute ± SEM for triplicate aliquots. Additional aliquots of these cells were used in the cytotoxicity and phenotypic studies described below.

Short term MNC cultures

MNCs resuspended at 10^6 cells/ml were plated in 75-cm² flasks together with anti-CD3 mAb prepared from the OKT3 hybridoma cell line obtained from American Type Culture Collection (Rockville, MD) and 20 U of IL-2/ml. After 72 h in culture (day 3), cells were harvested, and subfractions were isolated using mAb-coated magnetic beads (Dynal, New Hyde Park, NY). CD4⁺ or CD8⁺ T cell fractions were isolated by positive selection using the corresponding mAb-coated beads. Cells were detached from the beads using anti-Ig Ab following the manufacturer’s instructions. Non-T cell-enriched fractions were obtained from the MNCs via negative
Table I. MBP reactive T cell-induced cytotoxicity of human OLs and U251 glioma cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Type</th>
<th>% CD3&lt;sup&gt;+&lt;/sup&gt;</th>
<th>% CD56&lt;sup&gt;+&lt;/sup&gt;</th>
<th>[%&lt;sup&gt;3&lt;/sup&gt;H]Thymidine Uptake (cpm × 10&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>% Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56 high</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>Activated</td>
<td>90</td>
<td>49</td>
<td>143</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Resting</td>
<td>93</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>G9</td>
<td>Activated</td>
<td>86</td>
<td>28</td>
<td>47</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Resting</td>
<td>88</td>
<td>8</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>H7</td>
<td>Activated</td>
<td>85</td>
<td>26</td>
<td>45</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Resting</td>
<td>89</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A3</td>
<td>Activated</td>
<td>84</td>
<td>12</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Resting</td>
<td>99</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>CD56 low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>Activated</td>
<td>78</td>
<td>10</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Activated</td>
<td>74</td>
<td>3</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Y1</td>
<td>Activated</td>
<td>92</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>C1</td>
<td>Activated</td>
<td>85</td>
<td>9</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>P2</td>
<td>Activated</td>
<td>94</td>
<td>7</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>P4</td>
<td>Activated</td>
<td>95</td>
<td>4</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>P1</td>
<td>Activated</td>
<td>95</td>
<td>3</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>P3</td>
<td>Activated</td>
<td>95</td>
<td>2</td>
<td>34</td>
<td>20</td>
</tr>
</tbody>
</table>

* Data indicate results of studies examining individual MBP reactive T cell lines with regard to expression of CD3, CD56, proliferation rate, and cytotoxic effects on either OLs or U251 glioma cells, at a 10:1 E:T ratio. Results of 5-hr and 18-hr <sup>31</sup>Cr release assays were pooled. Activated cell lines expressing >10% CD3<sup>+</sup>CD56<sup>+</sup> cells are considered CD56 high (lines C10–A3); lines M4–P3 are considered as CD56 low. Lines C10 and M4 were tested during two different cycles of activation. Line Y1 was tested in the presence and absence of the lectin, Con A.

Selection by incubating the cells either with a combination of anti-CD4- and anti-CD8-coated beads or with anti-CD3-coated beads. The derived cell fractions were washed and resuspended in fresh culture medium (RPMI plus 10% FBS (Life Technologies)). Aliquots of each of these fractions were assayed for proliferation rate by [%<sup>3</sup>H]thymidine labeling.

**Phenotypic analysis**

For both the MBP cell lines and short term cultures, aliquots of cells were either immunostained with a combination of FITC-conjugated anti-CD3 (Leu 4) and phycoerythrin (PE)-conjugated anti-CD56 and CD16 mAbs (Becton Dickinson, Mississauga, Canada) or double immunostained with anti-CD4 (Leu 3)-PE and anti-CD8-FITC mAbs. Additional studies were performed using anti-CD56-PE, anti-CD57-FITC, anti-CD11b-PE, and anti-CD14-FITC mAbs. Quantitative analysis was performed using a FACScan (Becton Dickinson).

**Target cells**

**OLs.** These cells were prepared from human adult temporal lobe tissue resected during surgery performed to treat medically refractory epilepsy. Our procedure, previously described in detail (20), involves initial trypsinization of the CNS tissue, passage through 130-mm pore size mesh sieves, and centrifugation on a 30% Percoll gradient (Pharmacia, Montreal, Canada). The initial mixture of dissociated glial cells is resuspended in MEM (Life Technologies) with 5% FBS and cultured for 48 h in 25-cm<sup>2</sup> flasks. Over this time period, microglia and astrocytes become adherent. The nonadherent fraction is then collected and plated onto poly-L-lysine-coated wells of a 96-well microtiter plate at 5 × 10<sup>4</sup> cells/microwell. The OLs were immunostained with either anti-galactocerebroside (gal C) mAb O1 (from Dr. Wee Yong, University of Calgary, Calgary, Canada) or anti-CD56 mAb, followed by biotin-conjugated goat anti-mouse IgG (1/100 dilution; Caltag Laboratories, Hornby, Ontario, Canada) or treated with concanamycin (CMA; Sigma Chemical Co.; 2 mm/well) for 2 h at 37°C before being added to the target cell cultures. In other studies, target cells were incubated with anti-Fas Ab M3 (10 mg/ml; Immunex, Seattle, WA) for 30 min at 4°C before addition of effector cells.

**U251 glioma cells.** These cells were provided by Dr. P. Collins (Karolinska Hospital, Stockholm, Sweden). This cell line does not express glial fibrillary acidic protein. Glioma cells were plated in microtiter plates at 1 × 10<sup>5</sup> cells/well on the evening before use in cytotoxicity assays. Glioma cells were also immunostained with PE-conjugated anti-CD56 Ab, followed by FACS analysis.

**Cytotoxicity assays**

To assess OL- and glioma-directed cytotoxicity, target cells were labeled overnight with <sup>31</sup>Cr (1 mCi/well; Mandel, Guelph, Ontario, Canada) and washed the next day. The effector cell populations described above were added to either OL or U251 glioma-containing microwells in numbers sufficient to achieve the E:T cell ratios indicated. OL-directed cytotoxicity assays using MBP-reactive T cells and non-T cell-enriched fractions were conducted at either 5:1 or 10:1 E:T cell ratios dependent on the number of effector cells available. For optimal survival, OLs could not be cultured in less than the indicated numbers of cells. For both OL- and glioma-directed cytotoxicity studies, culture supernatants were collected after either 5 or 18 h to determine <sup>31</sup>Cr release. Total <sup>31</sup>Cr release was determined after treatment of wells with 5 N NaOH. Spontaneous release was determined from microwells containing only target cells. <sup>31</sup>Cr was measured in a gamma counter (LKB Wallace, Montreal, Canada). The percent specific release was calculated as: (induced release – spontaneous release) × 100%.

In some studies, as indicated in Results, effector cells were either exposed to Con A (10 mg/ml for 15 min at 37°C; Sigma Chemical Co., Oakville, Canada) or treated with concanamycin (CMA; Sigma Chemical Co.; 2 mm/well) for 2 h at 37°C before being added to the target cell cultures.

**Statistics**

Individual cytotoxicity and proliferation assays were conducted in triplicate; mean values are presented. FACS analysis data were derived from counting at least 10,000 events. Group data are presented as the mean ± SEM and are compared using Student’s t test.

**Results**

**MBP-reactive T cell lines**

We derived between one and five cell lines per donor, using a single 96-well microtiter plate. Table I presents the results of <sup>31</sup>Cr release assays using these cell lines as effectors and human OLs as well as U251 glioma cells as targets. The effector cells were used either 3 days following restimulation with MBP, referred to as activated cells,
or after being maintained for an additional week in IL-2-containing culture medium, referred to as resting cells. CD56 expression on CD3$^+$ T cells was determined for each cell line tested by staining and FACS analysis (illustrated in Fig. 1A). The activated cell lines were divided into high (>10%) and low CD56 subgroups.

As summarized in Figure 2, high CD56-activated MBP T cell lines were able to induce significant lysis of OLs (mean, 37 ± 4%) in $^{51}$Cr release assays. The same cell lines under resting conditions were not cytotoxic. The extent of activated cell-mediated lysis was significantly greater than that mediated by activated CD56 low T cell lines (mean, 7 ± 3%; $p < .0001$). Similar results were obtained using the U251 cells as targets (Table I and Fig. 2). As shown in Table I, the low CD56 MBP T cell line (Y1) induced significantly enhanced cytotoxic activity in the presence of the lectin Con A (59 vs 0%). The two high CD56 CD4$^+$ T cell lines were obtained using the U251 cells as targets (Table I and Fig. 2). As shown in Figure 2, the high CD56-activated cell lines induced cytotoxicity greater than that mediated by activated CD56 low T cell lines and activated MBP T cell lines (mean, 79 ± 6% and 91%, respectively). The high and low CD56-activated cell lines were tested both also cytotoxic to nonmalignant human fetal astrocytes, prepared as previously described (21) (mean $^{51}$Cr release at 10:1 E:T ratios = 71 and 91%, respectively).

The mean proportion of CD56$^+$ CD3$^+$ cells in the activated high CD56 cell lines was 28 ± 6%. The mean percentage of CD56$^+$ CD3$^+$ cells in the same resting cell lines was 5 ± 2%. The proportion of CD3$^+$ CD56$^+$ cells in the activated high CD56 cell lines was 8 ± 2%. The high and low CD56-activated cell lines and the resting cell lines did not differ significantly with regard to the proportion of cells expressing CD3 (86 ± 2, 89 ± 3, and 92 ± 2%, respectively; Table I). Virtually all the CD3 cells were CD4$^+$ (illustrated in Fig. 1B). Although there was no significant correlation overall between cytotoxicity and cell proliferation in response to MBP, the mean proliferation in response to MBP for the high CD56 lines of $79 ± 23 × 10^3$ cpm (4 ± 2 × 10$^3$ cpm without Ag) was suggestively greater than for the low CD56 lines (25 ± 3 × 10$^3$ cpm; 2 ± 0.2 × 10$^3$ cpm without Ag; $p > 0.05$).

CD56 expression on the OLs was documented by staining the cells with anti-gal C and anti-CD56 mAbs (Fig. 3, A–D); the glioma cells were shown to express CD56 by FACS analysis (Fig. 3E).

### Nonmyelin-restricted cells

For these studies, we derived either T cell-enriched or non-T cell-enriched populations from MNC cultures following 3 days of stimulation with anti-CD3 and IL-2. The T cell population did not express CD16/CD56 (Fig. 1C). Highly enriched CD4 and CD8 T cell fractions (mean, 97%) induced only low levels of lysis of OLs, at a 10:1 E:T ratio unless Con A was added to the assay (Fig. 4).

As shown in Figure 1D, the non-T cell fraction, prepared by negative selection, was enriched for CD16/CD56-expressing cells. At least a proportion of these cells also expressed CD57 (Fig. 1E). CD11b expression could also be demonstrated in this fraction, which did not contain monocytes as assessed by the lack of CD14 expression (Fig. 1F). The non-T cell-enriched cell fraction showed high levels of cytotoxicity directed against OLs in 5-h $^{51}$Cr release assays (59 ± 5%) at 5:1 E:T ratios (Fig. 4). Results parallel to those found using OLs as targets were also observed using glioma cells as targets (Fig. 4). We could not inhibit either OL- or glioma cell-directed lysis induced by the CD56-enriched fraction using either anti-MHC class I mAb (W6/32; 1/10 dilution of hybridoma cell line; gift from Dr. R. Sekaly, Montreal, Canada) or anti-CD56 mAb (undiluted supernatant derived from hybridoma cell line; data not shown).
To examine whether cytotoxic effects mediated by the MBP-reactive T cell lines involved either a Fas- or perforin-mediated pathway, we used either anti-Fas Ab or the perforin inhibitor, CMA (22). With anti-Fas Ab M3 (10 µg/ml), we found incomplete inhibition of MBP CD4$^{+}$ T cell-mediated lysis. With glioma cells as targets, there was a mean percent inhibition of 33 ± 12% ($n = 4$). In the single study using OLs as targets, a 30% inhibition was also observed (23% 51Cr release with Fas Ab vs 33% without Ab). Treatment of the CD4$^{+}$ cells with CMA also only incompletely blocked lysis when using glioma cells as targets.

**Discussion**

The current study has defined cellular immune effector mechanisms that can induce injury of human OLs, the putative cell target in MS. Our studies with myelin (MBP)-reactive CD4$^{+}$ T cell lines have focused on the potential of these cells to induce non-MHC-restricted cytotoxicity of the OLs. Previous studies have shown that MBP-reactive CD4 T cell lines can exert MHC class II-restricted cytotoxicity if the target cells express the requisite MHC class II molecules and if MBP has been pulsed onto the cells (5, 6). OLs do not express MHC class II molecules in vitro or in vivo (7, 8). Unlike our recent studies showing that MBP-reactive CD8 T cells induced MHC class I-restricted cytotoxicity of human OLs in vitro (23), we could not demonstrate MHC-restricted lysis of OLs with MBP-reactive CD4$^{+}$ T cells even in the presence of exogenous MBP (18).

Our current results indicate that MBP-reactive CD4$^{+}$ T cells can acquire the capacity to induce non-MHC-restricted cytotoxicity. Notably, OL-directed cytotoxicity was associated with expression of CD56 on the T cells. We further found that our U251 glioma cell line, which was documented to express CD56 even in the absence of pretreatment with IFN-γ (9), was susceptible to lysis by CD56-expressing MBP-reactive T cell lines. Furthermore, we found that CD56 expression was dependent on the activation state of the T cells. Recently stimulated cells were highly activated, phenotypically showed increased CD56, and functionally were more cytotoxic than the same cells under resting conditions. Although we used a combination of CD56 and CD16 Abs to assess the T cell lines, previous studies indicate that MBP CD4$^{+}$ T cells express only CD56 (9).

The basis for the relation between cytotoxic effects and CD56 expression probably relates to enhanced effector-target interaction rather than the intrinsic cytotoxic capacity of the cells. Support for this view is derived by demonstrating that the low levels of cytotoxicity mediated by T cell lines with low proportions of CD56-expressing cells even when activated can be augmented by addition of lectin to the assay. In previous studies (9), CD56 Ab alone was not sufficient to block MBP-reactive CD4 T cell-mediated cytotoxicity, although a combination of anti-CD56 and intercellular adhesion molecule Abs did. Whether other adhesion molecules, which like CD56 seem to be associated with cell activation, are involved remains to be determined and could differ among cell types.

Our results using non-Ag-restricted MNC-derived cell fractions as mediators of OL injury further indicate the crucial role of cell surface molecules involved in effector-target cell interactions in determining whether non-MHC-restricted cellular immune-mediated injury will occur. CD4$^{+}$ and CD8$^{+}$ T cells, derived from anti-CD3- and IL-2-activated MNC cultures, did not induce OL injury unless a lectin was added. These results indicate that the cells do have the intrinsic capacity to induce injury. Neither CD4$^{+}$ nor CD8$^{+}$ T cells express significant levels of CD56. In contrast, we did find high levels of cytotoxicity with the non-CD3 T cell-enriched fraction, a cell population containing a significant proportion of CD56$^{+}$ cells. This population also expresses CD11b, and a proportion of these cells express CD57; these cells may be predicted to express other markers associated with NK cells. However, their cytotoxic ability was not inhibited by MHC class I-directed mAb. Previous reports had suggested that bovine OLs were resistant to the cytotoxic effects of NK and LAK cells (13, 14). In the latter study, LAK cells were generated by culturing macrophage-depleted mononuclear cells with IL-2 alone for 5 days; CD16 expression was documented on the cells, but CD56 expression could not be assessed.
With respect to the mechanisms used by the effector cells described in our study, the susceptibility of both OLs and malignant and nonmalignant astrocytes to these mediators differs from observations made using either TNF or Fas receptor-activating Ab as mediators of OL injury (25–28). TNF-α and lymphotoxin induce delayed (48–96 h) apoptosis of OLs, whereas other glial cells are resistant under the same culture conditions (25–27). Although both OLs and malignant glial cells are susceptible to Fas Ab-mediated lysis, only glialoma cells show concurrent evidence of DNA fragmentation (28). Fetal astrocytes, although expressing Fas, are resistant to Fas Ab- and soluble Fas ligand-induced cytotoxicity (28). All of the above glial cells were susceptible to lysis mediated by T χ/β cells, which induced their effects via a perforin-dependent mechanism (29, 30). In the current study, we found that we could only block OL-directed lysis mediated by MBP-reactive CD4 T cells by 30% with anti-Fas Ab. Fas Ab also only partially blocked CD4 lectin-dependent killing of glialoma cells and OLs. CMA alone also did not totally block glialoma-directed killing. These results suggest that both the perforin- and Fas-dependent pathways can be involved in OL-directed cytotoxicity (31–35). A recent report also indicates that reactive CD4 T cell lines can vary as to which mechanism is used to effect target cell injury (36).

Our results indicate that human OLs can be susceptible targets of myelin Ag-restricted and nonrestricted cellular immune mediators. Our current study implicates CD56 expression on these cells as one means to promote effector-target interaction. The CD56-associated effect was dependent on the activation state of the effector cells. Within the CNS in inflammatory disease states such as MS, infiltrating immune cells have an activated phenotype (37, 38). The above observations would support efforts to develop means to reduce activation and modulate adhesion molecule expression of potential immune effector cells as a therapy for MS.

Acknowledgments

We thank Trevor Owens, McGill University, for his advice throughout the project and his review of the manuscript. We also thank Manon Blain for her technical support and advice.

References
