MHC Class I-Restricted Lysis of Human Oligodendrocytes by Myelin Basic Protein Peptide-Specific CD8 T Lymphocytes

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MHC Class I-Restricted Lysis of Human Oligodendrocytes by Myelin Basic Protein Peptide-Specific CD8 T Lymphocytes[1]

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Multiple sclerosis (MS) is considered to be an autoimmune disease that is directed either at myelin or at its cell of origin, the oligodendrocytes (OL). The inflammatory lesions in the central nervous system contain multiple myelin Ag-restricted and non-restricted cell populations with the potential to mediate tissue injury. Previous studies indicate that it is possible to generate MHC class I-restricted myelin peptide-specific cytotoxic CD8 T cells, and that human adult OLs express MHC class I molecules in vitro. The purpose of this study was to demonstrate that myelin basic protein peptide-specific CD8 T cells could induce OL injury. We generated CD8 T cell lines from six healthy donors and five MS patients, and all cell lines were HLA-A2 positive. The obtained CD8 cell lines induced lysis of HLA-A2- but not HLA-A3-transfected HMy2.C1R cells in the presence of myelin basic protein peptide 110–118. In the absence of exogenous peptide, the CD8 T cell lines were cytotoxic to HLA-A2 but not to non-HLA-A2 OLs. Cytotoxicity was blocked with anti-MHC class I-blocking Ab. These results support the postulate that autoreactive CD8 cytotoxic T cells can contribute to the tissue injury in MS. *The Journal of Immunology,* 1998, 160: 3056–3059.

Multiple sclerosis (MS)[3] is a recurrent or progressive disorder of the central nervous system (CNS) characterized by multifocal areas of demyelination (1). The disease is considered to reflect an autoimmune response directed at CNS myelin or at its cell of origin, the oligodendrocyte (OL) (2, 3). Although the initial inflammatory events within the CNS in MS are attributed to myelin-reactive CD4 T cells in a manner analogous to that demonstrated in the induced animal model experimental autoimmune encephalomyelitis (4), the actual mediators of tissue destruction are not as well defined. The inflammatory infiltrate in active MS lesions contains multiple T cell populations including CD8 and CD4 α/β T cells and $\gamma\delta$ T cells as well as macrophages and microglia (5–8). Whether or how each of these cell types actually damage myelin and OLs is still largely unknown.

Although it has not yet been demonstrated that OLs in situ express either MHC class I or class II molecules by conventional immunohistochemical techniques, in vitro OLs, both human and murine, have been shown to express MHC class I molecules (9–12). This finding raises the question as to whether these cells could be vulnerable to MHC class I-restricted CD8 T cell-mediated cytotoxicity. Human OLs are susceptible to lysis mediated by cytotoxic cells that are directed at MHC class I alloantigens generated and activated in a mixed lymphocyte reaction (13).

Myelin-reactive MHC class I-restricted human CD8 T cells can be generated in vitro (14). Tsuchida et al. used a computer algorithm to predict the peptide sequences, which can form stable complexes with HLA-A2, and confirmed that most were stable in in vitro experiments (14). One of these peptides was myelin basic protein (MBP) peptide region 110–118, which is coded in exon 3 of the MBP (15). This exon is present in all known forms of MBP (15). Tsuchida et al. were able to generate CD8-specific cytotoxic T cell lines recognizing MBP peptide 110–118 from the PBMC of 1 of 10 healthy donors and 1 of 9 MS patients. These lines showed MHC class I-restricted cytotoxicity when tested using a transformed B cell line (HMy2.C1R) transfected with HLA-A2 pulsed with peptide as a target. HLA-A1-transfected cells were used as controls.

The purpose of the current study was to generate MBP peptide-specific CD8 T cell lines from HLA-A2 MS patients and normal donors, using the same MBP peptide (110–118) that was previously described by Tsuchida et al., and to assess the capacity of these cells to induce cytotoxicity to MHC class I- histocompatible OLs maintained in dissociated cell culture in the absence of any exogenous MBP.

Materials and Methods

Donors

Venous blood samples were obtained either from normal donors or from patients with the relapsing-remitting form of MS that were currently in remission. The donors were all tissue-typed using serologic techniques at the Tissue Typing Laboratory of the Royal Victoria Hospital (Montréal, Canada) and selected on the basis of HLA-A2 positive status.

CD8 T cell lines

CD8 T cells reactive to the 110–118 MBP peptide (SLSRFSWGA), (Department of Biochemistry, Queen's University, Kingston, Canada) were generated using a technique similar to that used in the study reported by Tsuchida et al. (14). A whole mononuclear cell (MNC) fraction was initially isolated on a Ficoll-Hypaque density gradient. Purified CD8 T cells were then obtained using magnetic beads coated with anti-CD8 mAb (Dynal, Great Neck, NY) according to the manufacturer's protocol. After washing, the CD8 T cells were suspended in culture medium, DMEM (Life Technologies, Burlington, Ontario) with 10% autologous serum containing streptomycin (50 μg/ml) and penicillin (50 U/ml) (Life Technologies) at 10^6 cells/ml. Irradiated (3000 R), autologous MNC feeder cells pulsed with MBP peptide (10 μg/ml) were added. On day 3, 5 U/ml of IL-2 (Genzyme, Cambridge, MA) was added to the CD8 T cell cultures. At 1 wk from the time of the initial culture and for each subsequent week, the CD8 T cells were provided with fresh irradiated autologous feeder cells and pulsed with

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[3] Abbreviations used in this paper: MS, multiple sclerosis; CNS, central nervous system; MBP, myelin basic protein; OL, oligodendrocyte; MNC, mononuclear cell; PE, phycoerythrin.
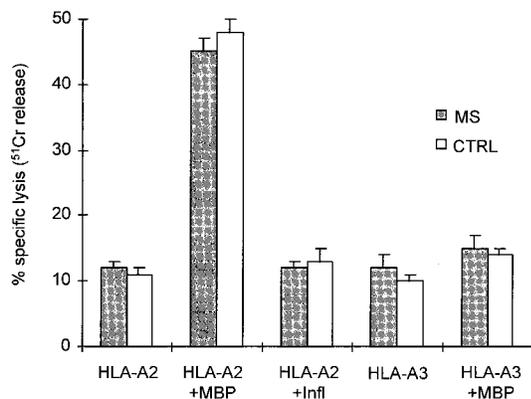
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FIGURE 1. Lysis of HLA-A2- or HLA-A3-transfected HMy2.C1R cell lines by HLA-A2 CD8 T cells in the absence or presence of pulsed MBP peptide 110–118 or influenza virus (Infl) peptide. Data indicate mean % specific lysis + SEM for a total of 10 T cell lines derived from 6 control donors and 5 T cell lines derived from 5 patients with MS.

FIGURE 2. Purity of MBP-specific CD8$^+$ T cells in culture assessed by double immunostaining with anti-CD4$^-$PE and anti-CD8$^-$FITC mAbs and FACS analysis.

peptide. On day 2 after every peptide stimulation, 5 U/ml of IL-2 was added to CD8 T cell cultures. The peptide and MHC class I specificity of the cell lines were assessed based on a chromium release assay (Na$_2$^{51}CrO$_4$), as described below.

Target cell lines

HMy2.C1R cells, which comprise the transformed B cell line HMy2.C1R transfected with HLA-A2 or HLA-A3, were prepared and maintained as previously described (14). OLs were prepared from human adult temporal lobe tissue resected as a surgical treatment of intractable epilepsy, as previously described (16). Our procedure involves initial trypsinization of the CNS tissue, passage through mesh, and centrifugation on a 30% Percoll gradient. The initial mixture of dissociated glial cells was suspended in MEM (Life Technologies) with 5% FCS (Medicorp, Montréal, Québec), streptomycin (50 μg/ml), and penicillin (50 U/ml) and cultured for 48 h in culture flasks. This step provides a means of separating adherent cells, such as microglia and astrocytes, from nonadherent cells, such as OLs. The nonadherent OL fraction was plated into poly-L-lysine-coated wells of 96-well microtiter plates at 5 × 10^4 cells/well. The HLA type of OL was determined by HLA-typing that was performed on MNC derived from the blood of the individual whose brain tissue was used as a source of OLs.

Cytotoxicity assays

To assess cytotoxic potential and the MHC class I restriction of the MBP peptide-reactive CD8 T cell lines we used, HLA-A2- or HLA-A3-transfected HMy2.C1R cell lines as targets. These cells were labeled with Na$_2$^{51}CrO$_4$ (5 HCi/ml) for 1 h and then washed to remove the label. The cells were pulsed either with MBP peptide 110–118 or with influenza virus peptide 58–66 (also presented in an HLA-A2-restricted manner) (17), or they were not pulsed with any peptide. Target cells (5 × 10^3) were plated in each well of a 96-well microtiter plate. CD8 T cells (5 × 10^4 per well) were added.

For human OL cytotoxicity assays, we labeled OLs with Na$_2$^{51}CrO$_4$ (1 μCi/well) overnight and washed them the next day. CD8 T cells (5 × 10^5) were added to each microwell to obtain a 10:1 E:T ratio. For both the transfected HMy2.C1R cells and the OL cytotoxicity studies, the supernatant was collected after 5 h to determine ^{51}Cr release. The total ^{51}Cr release was assessed after cell treatment with 1% Triton X-100. Spontaneous release was determined from microwells containing only target cells. ^{51}Cr release was read in a gamma counter (LKB Wallac, Gaithersburg, MD). The percentage of specific release was calculated as: ([induced release − spontaneous release]/[total release − spontaneous release]) × 100%. All assays were performed in triplicate.

To establish that OL-directed cytotoxicity was MHC class I restricted, we added pan-MHC class I-blocking Ab (W6/32) or control isotype Ab to some of the cultures.

Immunocytochemistry

The purity of OL cultures was assessed by immunostaining those cells recovered from microtiter wells with anti-galactocerebroside Ab (O1) (a gift from Dr. Wee Yong, University of Calgary, Calgary, Canada) followed
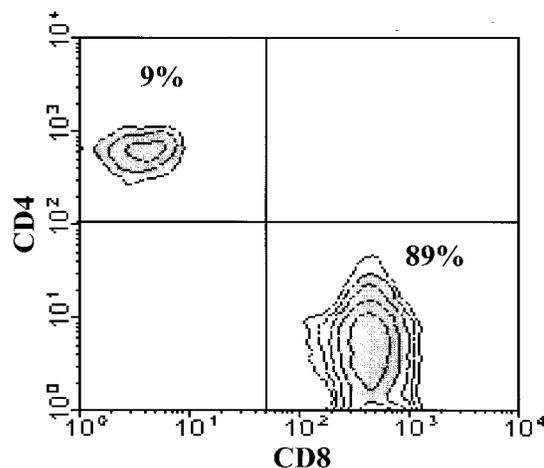
by goat anti-human Ig and FACS analysis. The expression of MHC class I on OLs was determined by preconjugated anti-MHC class I Ab (Serotec, Oxford, U.K.) staining and FACS analysis. Anti-keyhole limpet hemocyanin isotype-matched mAbs were used as controls. The purity of CD8 T cell cultures was assessed by double staining lymphocytes with phycoerythrin (PE)-conjugated anti-CD4 and FITC-conjugated anti-CD8 Ab (Becton Dickinson, Mountain View, CA) followed by FACS analysis.

Results

We were able to obtain MHC class I-restricted MBP peptide-reactive CD8 T cells from six of seven healthy donors and all MS patients using HMy2.C1R cells (transfected with HLA-A2 or HLA-A3) as targets (Fig. 1). Multiple cell lines were generated from some donors. The CD8 T cells were tested in cytotoxicity assays on day 5 after the last stimulation with MBP peptide and after the cells had undergone at least four rounds of stimulation. As shown in Figure 1, the CD8 T cells were significantly more cytotoxic to HLA-A2-transfected HMy2.C1R cells pulsed with peptide (overall mean for all donors was 48%) than they were to the same cells with no peptide (11%) or to cells pulsed with influenza virus peptide (13%). There was no difference in the killing of HLA-A3-transfected cells in the presence of MBP peptide (14%) compared with nonpulsed cells (10%). There was no significant difference in the cytotoxicity of CD8 T cells between healthy donors and MS patients.

The purity of the CD8 T cell lines, as tested by immunostaining and FACS analysis, was between 80 and 90% (Fig. 2). The contaminating cells were CD4 positive (5–10%). We obtained ~5 to 8 × 10^6 CD8 T cells per donor, which limited the number of experiments we were able to do with one line.

OL cultures were selected for cytotoxic assays based on the results of HLA tissue-typing and were categorized as HLA-A2-positive or -negative. The OL-enriched cultures contained 80 to 100% OLs (Fig. 3A), while the contaminating cells were astrocytes and fibroblasts. The presence of MHC class I was documented by FACS analysis following immunostaining with pan-MHC class I Ab conjugated with PE (Fig. 3B).

The results from individual functional cytotoxicity assays using HLA-A2 OL as a target are presented in Table I, and those using HLA-A2-negative OL are presented in Table II. Results are summarized in Figure 4. The CD8 T cells derived from HLA-A2 donors induced significantly greater lysis of OL derived from HLA-A2 donors compared with lysis of OL obtained from non-HLA-A2 donors. There was no difference between MS patients
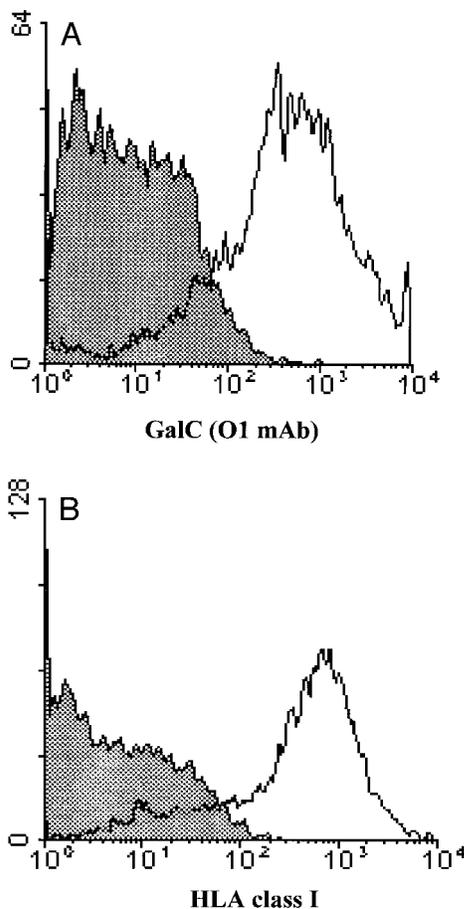
FIGURE 3. *A*, Purity of OLs in culture as assessed by indirect immunofluorescence using anti-galactocerebroside mAb (O1). An isotype-matched mAb was used as a control. *B*, Expression of MHC class I on OLs in culture as assessed by immunostaining with PE-conjugated anti-pan-MHC class I mAb. An isotype-matched mAb was used as a control.

and healthy donors. Pan-MHC class I Ab significantly inhibited the observed lysis of HLA-A2 OLs; no such effect was observed with control Ab. The extent of OL lysis was significantly less than that found using HMy cells as targets (Table I).

Table I. *MBP peptide-reactive CD8 T cell line-mediated cytotoxicity of HLA-A2 OL in absence of exogenous peptide[a]*

CD8 T Cell Line HLA-Haplotype	OL	OL + Pan-MHC Class I Ab	OL + IgG2a	HMy2 Cells + MBP Peptide
Control group CD8 T cell lines				
A2,32:B44,*	21%	5%	17%	65%
A2,25:B7,18	15%	3%	14%	47%
A2,-:B18,44**	18%	5%	15%	43%
A2,31:B60,	9%	2%	10%	43%
A2,24:B7,44***	14%	2%	14%	43%
MS patients CD8 T cell lines				
A2,1:B8,62	8%	2%	6%	38%
A2,-:B7,	20%	5%	18%	49%

[a] Data indicate the cytotoxic effect (% ^{51}Cr release) induced by MBP peptide 110-118-reactive CD8 T cell lines, generated from HLA-A2 normal donors and MS patients, on human OLs derived from an HLA-A2 donor (HLA-A2,28:B35,45). OL targets were utilized alone (OL) or in presence of anti-MHC class I mAb or IgG2a control mAb. Data present results from individual cell line experiments conducted at 10:1 E:T ratios. The corresponding extent of lysis of HLA-A2-transfected HMy2.C1R cells that were pulsed with MBP peptide 110-118 mediated by individual CD8 T cell lines is also shown.

*, **, and *** indicate which T cell lines are the same as those used in the studies presented in Table 2.

Table II. *Absence of lysis of non-HLA-A2 OLs mediated by HLA-A2 MBP peptide-reactive CD8 T cell lines[a]*

CD8 T Cell Line HLA-Haplotype	OL	OL + Pan-MHC Class I Ab	OL + IgG2a	HMy2 Cells + MBP Peptide
Control group CD8 T cell lines				
A2,-:B18,44**[b]	3%	5%	4%	57%
A2,-:B18,44**[b]	6%	5%	7%	52%
A2,32:B44,*	2%	2%	2%	44%
A2,24:B7,44***[c]	5%	5%	6%	45%
A2,24:B7,44***[c]	4%	5%	5%	42%
MS patients CD8 T cell lines				
A2,1:B8,27	5%	1%	2%	46%
A2,1:B8,57	3%	3%	3%	42%
A2,-:B18,51	5%	5%	7%	52%

[a] Data indicate the cytotoxic effect (% ^{51}Cr release) induced by MBP peptide 110-118-reactive CD8 T cell lines, generated from HLA-A2 normal donors and MS patients, on human OLs derived from a non-HLA-A2 donor (HLA-A28,33:B44,14). OL targets were utilized alone (OL) or in the presence of anti-MHC class I mAb or IgG2a control Ab. Data present results from individual cell line experiments conducted at 10:1 E:T ratios. The corresponding extent of lysis of HLA-A2-transfected Hmy.C1R cells that were pulsed with MBP peptide 110-118 mediated by individual CD8 T cells is also shown.

*, **, and *** indicate CD8 T cell lines that were used in the studies presented in Table 1.

[b] and [c] indicate cell lines derived from the same donor.

Discussion

The current study extends previous observations that MBP peptide-reactive CD8 T cells can be derived from both MS patients and control donors (14). Myelin-reactive CD4 T cells have repeatedly been derived from normal humans and animals, although they have not been used to adoptively transfer disease. We used the same MBP peptide (110–118) that was previously shown to bind to the HLA-A2 peptide groove. Unlike the initial study with this peptide, we could derive such cells from the majority of individuals sampled. As shown using the HMy cells as targets, cytotoxicity was restricted to recognition of the HLA-A2 molecule and was dependent on the presence of MBP peptide.

The studies using OLs as targets indicate that MHC class I-restricted lysis could be obtained in the absence of exogenous MBP peptide. Although the overall level of ^{51}Cr release by OLs was lower than that displayed by HLA-A2-transfected cells, the cytotoxicity was dependent upon the expression of the appropriate
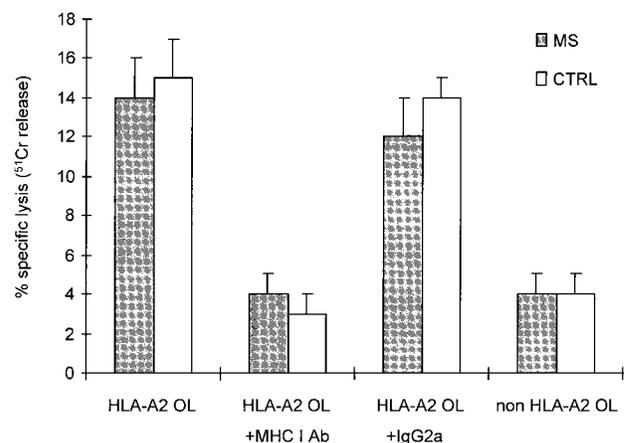

FIGURE 4. Lysis of human OLs by HLA-A2 MBP peptide-specific CD8 T cell lines. Vertical bars indicate mean % specific ^{51}Cr release from OLs induced by HLA-A2 MBP peptide-reactive CD8 T cells that were derived from MS patients and normal donors (at a 10:1 E:T ratio). Human OL targets were either HLA-A2$^+$ or non-HLA-A2 and were tested either alone or in the presence of anti-MHC class I Ab or IgG control Ab.

MHC class I molecule and inhibited by the anti-MHC class I Ab. The lower extent of ^{51}Cr could reflect a number of variables. The OLs are fully differentiated, nondividing cells compared with the proliferating HMy2.C1R cells. The transfected HMy2.C1R cells pulsed with peptide likely differ from the OLs with regard to the amount of MBP peptide present in the MHC class I groove. For the OLs, peptide presentation could result from endogenous Ag processing. The OLs also express MHC class I molecules other than HLA-A2, and their peptide grooves are probably occupied by multiple, different peptides. We did not have methods available to demonstrate whether the MBP peptide was present in the MHC class I groove of either OL or HLA-A2-transfected HMy2.C1R cells. Recent data indicate that the presentation of one molecule of Ag in the appropriate peptide groove is sufficient for T cell recognition and response (18).

The CD8 T cell-mediated injury of myelin targets has been postulated to contribute to tissue injury in a number of model and human diseases. Jewtoukoff et al. previously described a murine CD8 T cell clone specifically cytotoxic for OL but lacking in MHC class I restriction (19). In experimental autoimmune encephalomyelitis, depletion of CD8 T cells results in prolonged disease, but there was no mention of the extent of overall tissue injury (20, 21). In Theiler's virus encephalomyelitis model, susceptibility to demyelination is mapped within the MHC class I region (22). In human T cell lymphotropic virus type I myelopathy, a high frequency of CD8 T viral peptide-reactive T cells is found in blood and at lesion sites (23), but whether these cells can damage OLs is unknown.

The results of the present study expand the range of effector cells that are present in the inflammatory MS lesions and could mediate actual tissue injury. MBP-reactive CD4 T cells expressing CD56 can induce lysis of an array of cell targets in a non-MHC, non-Ag-restricted manner (24). $\gamma\delta$ T cells can directly induce lysis of human OLs, although the required ligand is not yet identified (25). An array of soluble molecules, including TNF and nitric oxide, which can be produced by inflammatory cells or endogenous microglia, can also be cytotoxic to OLs (26). Our studies with lectin-dependent CD8 and CD4 T cell-mediated lysis of OLs indicate that non-TNF- and non-Fas-dependent mechanisms mediate most of OL injury, suggesting that classical perforin-mediated injury would likely be the underlying mechanism of injury (27, 28). In none of these studies do we find a correlation between susceptibility to lysis and the HLA phenotype of the OLs. Understanding the events that link initial inflammation to ultimate tissue injury in MS will hopefully provide new opportunities to interfere with the disease process particularly in cases of already established disease.

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