Short Communication

Cytotoxic effect of myelin basic protein-reactive T cells on cultured oligodendrocytes

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Summary

To help clarify effector mechanisms in experimental allergic encephalitis (EAE), the cytotoxic effects of myelin basic protein (MBP)-reactive lymphocytes on oligodendrocytes were studied using a ^{51}Cr release assay. MBP-reactive encephalitogenic T cell lines were cytotoxic to ^{51}Cr-labeled oligodendrocyte target cells derived from Lewis rat fetal brain-dissociated culture, when incubated for 6 h in the presence of antigen-presenting cells (APC) and MBP (percent ^{51}Cr release = 65 ± 3% vs. spontaneous release = 22 ± 3% vs. normal lymph node cells + APC and MBP = 20 ± 3%). This reaction is time dependent, likely MHC restricted, and is not just a nonspecific toxic effect against any Lewis target cells since neither fibroblasts nor astrocytes were affected. Other (tetanus toxoid-reactive) lymphoblasts stimulated by specific antigen were not cytotoxic to the oligodendrocytes. These findings suggest that oligodendrocytes might be target cells for MBP-reactive lymphocytes in EAE if antigen presentation is appropriate.

Introduction. It has been long considered that cell-mediated immune mechanisms play a pathogenic role in experimental allergic encephalitis (EAE) induced in rats by sensitization to myelin basic protein (MBP). However, the exact effector

mechanisms underlying the clinical EAE manifestation in these animals are still not well defined.

In rat EAE induced with MBP, some investigators (Hoffman et al., 1973; Lasmann et al., 1979; Panitch et al., 1981) have found little or no pathologic evidence of demyelination or damaged oligodendrocytes, whereas others claim that such damage can be found if appropriate sampling and preparation of the suspect tissues are carried out (Pender, 1987). Demyelination can be seen in passively induced EAE after repeated transfer of MBP-reactive T lymphocytes (Vandenbark et al., 1986). Röyttä et al. (1985) have reported damage of oligodendrocytes in organotypic cultures induced by lymphoid cells obtained from rodents with EAE.

These findings prompted us to investigate the in vitro interaction of MBP-reactive lymphocytes and oligodendrocytes. To investigate this interaction, we carried out cytotoxic tests of MBP-reactive T cell lines to syngeneic oligodendrocytes by a ^{51}Cr release assay.

Materials and methods. *Oligodendrocyte culture:* Lewis rat oligodendrocytes were isolated from Lewis rat fetal brain-dissociation culture at 11–14 days in vitro by a modification of the method of Suzumura et al. (1984). The suspensions of isolated oligodendrocytes were plated on 12 mm diameter poly-L-lysine-coated coverglasses at a density of 1×10^5 cells per coverglass.

The mean percentage of galactocerebroside (GalC)-positive cells at the time they were used in experiments described below was 72% (range 65–91%).

Astrocytes were also isolated from primary brain cell culture at 21–28 days in vitro after oligodendrocytes had been isolated by mechanical agitation (McCarthy and de Vellis, 1970; Suzumura et al., 1984, 1986). The mean percentage of glial fibrillary acidic protein (GFAP)-positive cells was 93% (range 89–95%).

T cell line cultures: Lewis rats, 3–4 months old, were immunized with 50 µg of guinea pig MBP and/or tetanus toxoid (TT) (Connaught Laboratories) 1 Lfu/ml emulsified in complete Freund's adjuvant, containing 50 mg of *Mycobacterium tuberculosis* H37 RA.

Guinea pig MBP was prepared in our laboratory by a modification of the method of Deibler et al. (1972). The purity of MBP was confirmed with a single band seen on sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Nine to 10 days later the draining lymph nodes were removed and homogenized, washed three times and resuspended in RPMI 1640 medium supplemented with 2% of normal rat serum, 5% of NCTC 109 medium (MA Bioproducts), 0.05 mM 2-mercaptoethanol (Sigma Chemical Company, St. Louis, MO), 50 U/ml penicillin, 50 µg/ml streptomycin, 25 ng/ml Fungizone (Gibco Laboratories, Chagrin Falls, OH), 30 µg/ ml guinea pig MBP or 2 Lfu/ml TT.

After 5–7 days, lymphoblasts were separated from the small resting lymphocytes at the 1.050/1.065 interface of a discontinuous Percoll gradient. Lymphoblasts were then cultured in interleukin-2 (IL-2)-containing media. IL-2 was derived from conditioned medium of MLA 144 cells (Cohen et al., 1987). The line cells were restimulated every 7–14 days with MBP or TT for 3 days. Irradiated normal thymocytes (1000 R) were added as antigen-presenting cells (APC). After 2–4 weeks the cultured lymphocytes were restimulated as above and then used as effector cells

in the cytotoxic assay described below. The lymphocytes in these short-term cell lines were over 90% W3/25$^+$ (helper-inducer phenotype) as determined by immunohistochemical assay (Cohen et al., 1987). They will hereafter be referred to as 'line cells' or 'lymphoblasts'. We have found that intravenous injection of these lymphoblasts (1.6–3.0 × 10^7 cells) consistently transferred EAE.

Cytotoxicity assay — target cells: After determination of optimal ^{51}Cr labeling conditions in preliminary studies, 1 × 10^5 oligodendrocytes grown on 12 mm diameter, poly-L-lysine-coated coverglasses were incubated with 20 μCi of sodium ^{51}chromate (New England Nuclear, spec. act. 200–900 Ci/g chromium) for 2 h at 37°C, washed 3 times and placed into 16 mm diameter wells containing medium.

As control target cell populations, Lewis rat astrocytes, fibroblasts and Brown Norway rat oligodendrocytes were used. Fibroblast cultures (98% fibronectin positive) were obtained from Lewis rat connective tissue.

Cytotoxicity assay — effector cells: MBP or tetanus toxoid-reactive lymphoblasts from antigen-stimulated culture were separated by Percoll gradient centrifugation. Freshly isolated normal Lewis rat lymph node cells and TT-reactive lymphoblasts were used as control effector cell populations.

Analysis of cytotoxic activity: The supernatant medium was removed from the wells containing the target cells on coverglasses and replaced immediately with 1 ml of suspension of the MBP-reactive lymphoblasts or normal lymph node cells in concentrations of 1 × 10^6 or 2 × 10^6/ml in the oligodendrocyte culture medium. To replicate sets of these wells containing oligodendrocytes and lymphoid cells were added either: (a) irradiated normal Lewis rat thymocytes as APC, (b) APC and MBP, or (c) nothing. When TT-reactive lymphoblasts were assessed as a control effector cell population, they were added along with APC and 2 Lfu TT to the oligodendrocyte-containing wells. These cell mixtures were then incubated for varying periods up to 6 h at 37°C, depending on the protocol. Spontaneous ^{51}Cr release was assessed in other wells in which the same cultured oligodendrocytes were incubated without added reactants.

After the incubation period, the supernatant fluid was collected completely, centrifuged and the fluid portion was transferred to a test tube for gamma counting (Packard). 1 ml of 1 N NaOH was then added to each well to lyse the cells on the coverglasses. The cell lysate was then transferred to test tubes for gamma counting. Percent ^{51}Cr release was calculated as follows.

$$\text{Percent }^{51}\text{Cr release} = \frac{\text{cpm of supernatant}}{\text{cpm of supernatant} + \text{cpm of cell lysate}} \times 100$$

Results. In all experiments performed, MBP-reactive lymphoblasts were cytotoxic to the labeled oligodendrocyte target cells when incubated for 6 h at 37°C in the presence of irradiated thymocytes (APC) and MBP (Table 1). This was indicated by significantly greater percent ^{51}Cr release (mean ± SEM = 65.4 ± 3.3%) than the spontaneous ^{51}Cr release from the same target cells (22.1 ± 2.6%). The differences in individual experiments were highly significant ($P < 0.05$; Walsh test) (Table 1).

TABLE 1

CYTOTOXIC EFFECTS OF MBP-REACTIVE T CELLS ON OLIGODENDROCYTES

Each value represents the mean ± standard deviation of individual percent ^{51}Cr release ($n = 3$).

Expt. No.	MBP-reactive T cell	Percent ^{51}Cr release				
		Effector [a] +APC [b] +MBP [c]	Effector +APC	Effector alone	NLNC [d] +APC+MBP	Spontaneous release
1.	BT87-26	63.5± 7.2	14.5±1.4	13.2±0.42	11.7±0.42	11.9±0.41
	BT87-26 [e]	69.8±11	ND [f]	ND	ND	ND
2.	BT87-27	77.3± 7.9	32.8±0.78	31.4±2.5	34.1±7.7	21.6±1.8
3.	BT87-37	72.8± 1.5	25.8±1.3	39.0±2.9	20.7±1.0	28.2±4.1
4.	BT87-37	70.2± 3.9	26.1±0.91	31.1±1.5	22.1±3.1	29.0±7.3
5.	BT87-44	67.8± 8.6	23.6±3.2	26.9±3.4	25.1±8.0	20.5±0.75
6.	BT87-45	77.1± 6.9	20.4±1.2	ND	9.7±1.3	11.8±0.25
7.	BT87-46	42.6± 6.3	ND	ND	19.2±2.2	16.0±4.1
8.	B87-2	56.3± 2.9	33.2±4.4	30.2±6.3	ND	36.7±1.6
9.	B87-13	58.6± 1.4	ND	ND	ND	23.1±7.9

[a] Effector: MBP-reactive lymphoblasts (see text).
[b] Antigen-presenting cell.
[c] Guinea pig myelin basic protein: 30 µg/ml.
[d] Normal lymph node cells.
[e] Effector/target ratio: 10/1; others: 20/1.
[f] Not done.

Both MBP and APC were required for this cytotoxic effect, since the percent ^{51}Cr release induced by lymphoblasts alone (29.4 ± 3.4%) and that induced by lymphoblasts plus APC alone (25.2 ± 2.3%) were not significantly different from the spontaneous release from these target cells ($P > 0.109$, $P > 0.109$, respectively in Walsh test analysis of individual experiments). However, the combination of MBP and APC itself was not cytotoxic since the addition of these agents to normal lymph node cells did not make the latter induce increased ^{51}Cr release from the target cells (20.3 ± 2.7%, $P > 0.109$ when compared to spontaneous release in individual experiments) (Table 1).

Specificity of the cytotoxic effect: This cytotoxic action of the MBP-reactive lymphoblasts on the oligodendrocytes was not just a nonspecific effect on any Lewis target cells, as evidenced by the lack of any increased percent ^{51}Cr release from labeled Lewis rat astrocytes or fibroblasts incubated with these lymphoblasts in the presence of MBP and APC (22.8 ± 1.7% and 12.2 ± 0.8% release, respectively vs. 18.8% and 14.3% spontaneous release) (Table 2).

The cytotoxic effect of MBP-reactive Lewis rat lymphoblasts on labeled oligodendrocytes appeared to be histocompatibility restricted. Such lymphoblasts in the presence of MBP and syngeneic APC did not induce increased ^{51}Cr release from labeled oligodendrocytes obtained from nonhistocompatible Brown Norway rats (28.6 ± 3.6% vs. 32.8 ± 2.5% spontaneous release) (Table 3).

The cytotoxic effect by MBP-reactive lymphoblasts in the presence of MBP and APC was not simply a function of in vitro antigen stimulation of any sensitized

TABLE 2

EFFECTS OF MBP-REACTIVE T CELLS ON LEWIS RAT FIBROBLASTS AND ASTROCYTES

Target	MBP-reactive T cell	Percent ^{31}Cr release				
		Effector + APC + MBP	Effector + APC	Effector alone	NLNC + APC + MBC	Spontaneous release
Fibroblasts	BT87-43	11.0 ± 0.29	11.9 ± 0.45	11.6 ± 0.08	12.9 ± 2.3	12.4 ± 0.76
	BT87-46	12.2 ± 0.16	ND	ND	ND	16.3 ± 2.5
	B87-1	13.6 ± 0.85	ND	ND	ND	16.3 ± 2.5
Astrocytes	BT87-43	19.4 ± 7.3	18.6 ± 0.32	20.1 ± 2.8	19.5 ± 0.66	14.5 ± 4.0
	B87-1	24.3 ± 2.0	ND	ND	ND	23.1 ± 1.6
	BT87-46	24.8 ± 3.4	ND	ND	ND	23.1 ± 1.6

See Table 1 for abbreviations. Effector/target ratio is 20/1. Incubation time is 6 h.

TABLE 3

EFFECTS OF LEWIS RAT MBP-REACTIVE T CELLS ON BROWN NORWAY RAT OLIGO-DENDROCYTES

MBP-reactive T cell	Percent ^{51}Cr release				
	Effector + APC + MBP	Effector + APC	Effector alone	NLNC + APC + MBP	Spontaneous release
B87-2	25.6 ± 2.5	27.0 ± 3.0	34.4 ± 3.0	25.4 ± 2.7	30.3 ± 3.3
B87-8	29.3 ± 2.8	26.7 ± 5.7	32.9 ± 8.0	ND	35.3 ± 7.5
B87-12	34.3 ± 6.4	27.2 ± 2.9	21.3 ± 2.0	ND	35.3 ± 7.5
B87-13	25.3 ± 1.6	23.5 ± 1.6	18.7 ± 3.4	ND	35.3 ± 7.5

See Table 1 for abbreviations. Effector/target ratio is 20/1. Incubation time is 6 h.

lymphoblasts. When TT-reactive lymphoblasts obtained from animals sensitized to TT were incubated with labeled oligodendrocytes in the presence of TT and APC, there was a modest increased percent ^{51}Cr release (36.2% vs. 21.6% spontaneous

TABLE 4

EFFECTS OF TT-REACTIVE T CELL LINES ON ^{51}Cr-LABELED OLIGODENDROCYTES AS TARGET CELLS

TT-reactive line	Percent ^{51}Cr release				
	Effector + APC + TT	Effector + APC	Effector alone	Normal lymph node cells + APC + TT [a]	Spontaneous release
BT87-26	36.2 ± 2.1	39.2 ± 1.0	40.5 ± 1.2	34.1 ± 7.7	21.6 ± 1.7
TT-1	24.1 ± 0.45	20.7 ± 0.85	19.6 ± 0.70	ND	36.7 ± 1.5
TT-2	18.7 ± 1.8	24.9 ± 0.70	27.1 ± 1.70	ND	36.7 ± 1.5

See Table 1 for abbreviations. Effector/target ratio is 20/1. Incubation time is 6 h.
[a] Tetanus toxoid 2 lfu/ml.

TABLE 5

TIME DEPENDENCY OF THE CYTOTOXIC EFFECT OF MBP-REACTIVE T CELL LINES ON OLIGODENDROCYTES

MBP-reactive T cell	Incubation time			Spontaneous release (6 h)
	2 h	4 h	6 h	
BT87-37	34.8 ± 5.7	50.7 ± 5.4	70.2 ± 3.9	29.0 ± 7.3
BT87-44	20.9 ± 4.7	47.7 ± 1.4	67.8 ± 8.5	20.5 ± 0.75
BT87-45	6.9 ± 0.5	13.8 ± 1.2	77.1 ± 6.9	11.8 ± 0.25
BT87-46	14.6 ± 1.0	24.7 ± 3.1	42.6 ± 6.3	16.0 ± 4.1

MBP-reactive lines were incubated for 2, 4, 6 h with labeled oligodendrocytes in the presence of MBP and APC.

release) in one of three experiments and no increased release in the other two experiments (Table 4).

Time dependency of the cytotoxic effect: When MBP-reactive lymphoblast populations were incubated at 37°C with labeled oligodendrocyte target cells in the presence of MBP and APC, no increased ^{51}Cr release was seen after 2 h ($19.3 \pm 5.8\%$), modest increase at 4 h ($34.2 \pm 5.5\%$) and the expected release ($64.4 \pm 4.8\%$) after 6 h (Table 5).

Discussion. The findings reported here indicate that MBP-reactive Lewis rat lymphoblasts are cytotoxic in vitro to syngeneic oligodendrocytes. However, several questions about the mechanisms involved and in vivo relevance to the pathogenesis of EAE remain to be answered.

The cytotoxic effects of several reported T cell clones is restricted to target cells expressing the same major histocompatibility complex (MHC) antigens (Moretta et al., 1981; Krensky et al., 1982; Strassman et al., 1984; Sun et al., 1986). Although the cytotoxic reaction reported here appears MHC restricted, the oligodendrocytes used in this study expressed neither MHC I nor MHC II at the time they were exposed to the cytotoxic lymphoblasts (preliminary observations).

Another question relates to the necessity for both MBP and APC in the cytotoxic effect. APC could provide a source of cells expressing MHC. However, such expression is usually required on the target cell itself. Furthermore, we saw no cytotoxic effect by MBP-reactive lymphoblasts in the presence of APC but no MBP. Therefore, it is more likely APC may present MBP for in vitro stimulation of the lymphoblasts. However, the cytotoxic reaction cannot be due simply to any stimulated lymphoblasts or its secreted products (e.g., lymphokines, shed MHC antigen (Hale et al., 1981; Emeesen, 1987)) because TT-stimulated Lewis lymphoblasts were not cytotoxic.

During an initial and modest damage of the oligodendrocytes by MBP-reactive lymphoblasts, products released from the target cells themselves might enhance the cytotoxic effect over the next hours. Vass et al. (1984) have speculated that MHC antigens may be bound to such altered cells rendering them susceptible to further damage.

The relation of these findings to in vivo events in EAE is not yet clarified. The usual transient nature of clinical manifestations of acute MBP-induced EAE in the Lewis rat suggests that dysfunction rather than frank destruction of oligodendrocytes and/or myelin may be the predominant in vivo event, possibly because of a lower effector/target cell ratio than present in vitro as described here.

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