Oligodendrocyte-specific autoreactive T cells using an α/β T-cell receptor kill their target without self restriction

(cytotoxic T cells/glia cells/major histocompatibility complex/myelin/autoimmunity)

VOAHANGY JEWTOUKOFF, RENÉ LEBAR, AND MARIE-ANNE BACH*

Unité de Pathologie de l'Immunité, Institut Pasteur, 25, Rue du Dr Roux, 75724 Paris, Cedex 15, France

Communicated by Jean Dausset, January 23, 1989

ABSTRACT The oligodendrocyte (Od), a glial cell that produces myelin in the central nervous system, may be a target for autoreactive T cells in autoimmune demyelinating processes, although not expressing major histocompatibility complex (MHC) products. To analyze Od–T-cell interactions, we selected from normal SJL/J mouse splenocytes sensitized *in vitro* by Lewis rat Od a T-cell clone, named C2, exhibiting a surface phenotype of mature T cell (Thy 1⁺, CD3⁺, CD8⁺, CD4[–], asialo-GM1[–]). C2 T cells displayed a specific cytotoxicity to syngeneic Od as well as to rat Od, but not to astrocytes or lymphoblasts, or to YAC-1 cells, a target for natural killer and lymphokine-activated killer activity. The T-cell receptor of clone C2 was found to be a CD3-associated α/β -chain heterodimer similar to that usually expressed by antigen-specific MHC-restricted mature T cells. Attempts to block the C2-mediated cytotoxicity by a series of monoclonal antibodies showed that both the CD3–T-cell receptor complex and the CD8 accessory molecule were required for OD–T-cell interaction and confirmed the lack of involvement of polymorphic MHC products as epitope-presenting structures. Antibodies directed against a surface Od glycoprotein, previously shown to elicit demyelinating autoantibodies in experimental autoimmune encephalomyelitis, fully blocked the cytotoxicity of T-cell clone C2 to its Od target. These data suggest that an epitope of a surface Od glycoprotein may be directly and specifically recognized and killed by autoreactive T cells expressing an α/β receptor without conventional MHC restriction.

Organ-specific autoimmune diseases are thought to be favored by aberrant cellular expression of class II antigens of the major histocompatibility complex (MHC), allowing then autoreactive CD4 T cells to recognize tissue-specific autoantigens in an appropriate self MHC context and to develop a pathogenic autoimmune response (1). In the model of experimental autoimmune encephalomyelitis, myelin basic protein elicits a CD4 T-cell-mediated, self MHC-restricted, pathogenic autoimmune response (2, 3), for which astrocytes, microglial cells, and endothelial cells may serve as antigen-presenting cells, since they can express class II MHC products upon stimulation by γ interferon (4). On the other hand, surface antigens of the oligodendrocyte (Od), a glial cell that synthesizes the myelin sheath but does not express MHC products (5), also trigger strong cellular and humoral immune responses (6–9). Exploring further the role of Od as possible targets for an autoimmune process, we have selected from normal SJL/J mice a CD8 T cell, which exhibited a tissue-specific non-MHC-restricted cytotoxicity to autologous Od and used a CD3 α/β T-cell receptor (TCR) directed against a membrane-expressed Od glycoprotein called M2/MOG (myelin oligodendrocyte glycoprotein), previously shown to elicit demyelinating autoantibodies (8, 10).

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MATERIALS AND METHODS

Animals. SJL/J (H-2^s), C3H (H-2^k), and BALB/c (H-2^d) mice and Lewis (RT1^b) rats, obtained from the breeding unit of Pasteur Institute (Paris), were used at 4–7 weeks of age.

Glial Cell Preparation. Od-enriched glial cell suspensions were isolated from adult SJL/J mice or Lewis rats by the method of Hirayama *et al.* (11). In brief, meninges were removed and brains were dissected in ice-cold Ca²⁺- and Mg²⁺-free Hanks' balanced salt solution. After 30 min of 0.1% trypsin digestion, the resulting suspensions were filtered through a 63- μ m nylon screen mesh and Od were isolated over a 45% Percoll gradient (Pharmacia) by centrifugation at 23,000 $\times g$ for 45 min. Cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) (Boehringer Mannheim), supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 50 μ M 2-mercaptoethanol, penicillin-streptomycin (100 units/ml), bovine insulin (20 μ g/ml) (Sigma), and 10% heat-inactivated fetal calf serum, and were distributed into poly-L-lysine (PLL; Sigma) precoated tissue culture plates for *in vitro* culture at 37°C in a humidified incubator with 5% CO₂/95% air.

Astrocyte-enriched cell cultures were prepared from newborn rats and mice according to the method of McCarthy and De Vellis (12). Briefly, the brain was harvested and triturated in Hanks' balanced salt solution. After one washing, the resulting cell suspension was put into 75-cm² culture flasks in the culture medium described above. A monolayer of glial cells then established progressively, from which Od were removed 10–14 days later by vigorously shaking the flasks. The remaining cells were trypsin treated and replated for further culture cycles.

Obtaining an Od-specific T-Cell Clone. An Od-specific T-cell clone was obtained as described (13) from an Od-triggered polyclonal T-cell line. In brief, 2 $\times 10^6$ SJL/J mouse spleen cells suspended in 2 ml of culture medium were introduced into 24-well tissue culture plates, where 5 $\times 10^5$ Lewis rat Od had been cultured for 2 days, as described above. After a 10- to 12-day culture, supernatant of Con A-stimulated rat spleen cells was added as a source of interleukin 2 (IL-2). The T-cell lines were then maintained for *in vitro* long-term culture by replacing culture supernatant with IL-2-supplemented fresh medium every 2–3 days and restimulating with Lewis Od once a month. After 7 months of culture, a T-cell clone was obtained by a limiting dilution technique: T cells were distributed at a frequency of one cell per well in Terasaki plates, together with 5 $\times 10^4$ irradiated (50 Gy) Lewis Od in a final vol of 10 μ l. Seven to 10 days later, IL-2-supplemented fresh medium was added to each well.

Abbreviations: Od, oligodendrocyte(s); IL-2, interleukin 2; MHC, major histocompatibility complex; NK, natural killer; LAK, lymphokine-activated killer; PLL, poly-L-lysine; mAb, monoclonal antibody; GFAP, glial fibrillary acid protein; MOG, myelin oligodendrocyte glycoprotein; TCR, T-cell receptor; E/T ratio, effector/target cell ratio.

*To whom reprint requests should be addressed.

After another week of culture, growing colonies were transferred into flat-bottomed 96-well plates with 7×10^5 – 10^6 irradiated (50 Gy) Lewis Od and then into 24-well tissue culture plates. A single clone, C2, was obtained, which was further subcloned according to the same procedure, and expanded in long-term culture as described for the T-cell lines.

Antibodies. The following anti-T-cell monoclonal antibodies (mAbs) were used: mAbs directed against Thy-1.2 and CD8 (anti-Lyt 2-2) were obtained from NEN (France); anti-CD4 mAb (GK-1.5) (14) was generously provided by D. Portnoï (Pasteur Institute, Paris) and by C. Carnaud (Necker Hospital, Paris); anti-CD3 mAb (145-2C-11) (15) was kindly given by D. Primi (Pasteur Institute, Paris), as well as rabbit antisera against the α chain and the γ chain (constant regions) of the TCR, which were prepared by D. Primi according to the methods described by Traunecker *et al.* (16) and Lew *et al.* (17), respectively.

Several anti-rat or anti-mouse MHC mAbs were also used: 34-1-2S, a mouse (C3H) anti-K^dD^d antibody cross-reacting with K^s (kindly given by J. P. Abastado and P. Kourilsky, Pasteur Institute, Paris), and 3-83 P (18), a mouse (BALB/c) anti-K^dD^k cross-reacting with H-2^s (Bionetics Research Institute), as anti-SJL/J MHC class I; OX6 (19), a mouse (BALB/c) anti-rat MHC class II (monomorphic determinant), cross-reacting with SJL/J MHC class II (Ia specificities 17 and 18) (Sera-Lab, Crawley Down, U.K.); OX18 (20), a mouse (BALB/c) anti-rat MHC class I antibody (RT1¹ specific) (Sera-Lab).

A rabbit anti-mouse β_2 -microglobulin antiserum was given by F. Godeau (Pasteur Institute, Paris). A mAb directed against the glycolipid asialo-GM1, a surface marker of the natural killer (NK) cell lineage (21), was prepared and given by F. Jacquemart (Pasteur Institute, Paris) (22).

Anti-glial cell antibodies consisted of a rabbit antiserum anti-glial fibrillary acid protein (GFAP) from Dakopatts (Glostrup, Denmark); a rabbit antiserum anti-rat transferrin from Cappel Laboratories; a rabbit antiserum anti-galactocerebroside generously provided by P. Dupouey (Pasteur Institute, Paris); a rabbit antiserum anti-rat myelin basic protein kindly given by J. C. Antoine (Pasteur Institute, Paris); anti-M2 polyclonal IgG obtained from Hartley guinea pigs immunized with homologous brain homogenate as described elsewhere (9); a mouse mAb anti-MOG (8-18C5) (10), a gift from C. Linington (University of Wales College of Medicine). Both anti-MOG and anti-M2 antibodies were shown to immunoprecipitate two major bands of apparent molecular mass 27 and 54 kDa from the central nervous system myelin (9, 10).

Controls included normal mouse polyclonal IgG, an irrelevant anti-*Mycobacterium leprae* mAb (ML93) produced in our laboratory, normal guinea-pig IgG, and normal rabbit serum. Fluorescein isothiocyanate-conjugated goat anti-mouse, anti-rabbit, and anti-guinea pig IgG antibodies were used as second reagents for immunofluorescence studies.

Immunocytochemical Analysis of Glial Cells and T Cells by Indirect Immunofluorescence. Glial cells plated onto PLL-precoated chamber slides (Lab-Tek) were immunostained according to the method of Norton *et al.* (23). After washing in phosphate-buffered saline, cells were incubated in a solution containing 10% normal goat serum, 10% fetal calf serum, 40% phosphate-buffered saline, and 40% DMEM for 15 min. The first antibody, diluted 1:50–1:100 in the same medium, was then added for 30 min at room temperature. After extensive washings with phosphate-buffered saline, the second fluoresceinated antibody was added at 1:100 dilution. Finally, the cells were washed and fixed with cold 5% acetic acid/95% ethanol for 10 min at -20°C before their examination under a Leitz fluorescence microscope. For intracytoplasmic staining, cells were prefixed with 1% paraformaldehyde in phosphate-buffered saline and then treated as described above. Controls were run by using the second stage alone.

Immunofluorescence studies of T cells were conducted as described (13). Scoring of fluorescent cells was performed by flow cytometry (FACS-SCAN, Becton Dickinson) or by microscopy.

Immunoprecipitation Studies of TCR. ¹²⁵I-labeled T cells were detergent-solubilized and immunoprecipitated with anti-CD3–TCR complex antibodies and analyzed by one-dimensional NaDodSO₄/PAGE under reducing or nonreducing conditions as described (24). In brief, 5×10^7 viable T cells isolated by Ficoll/Hypaque gradient centrifugation were radioiodinated by the lactoperoxidase-catalyzed reaction (24). One aliquot of these labeled cells was solubilized in 300 μl of Tris-buffered saline (10 mM Tris·HCl/140 mM NaCl/1 mM EDTA, pH 7) containing 2 mM phenylmethylsulfonyl fluoride and 100 mM iodoacetamide with 1% digitonin, which preserves the CD3–TCR association (15). Immunoprecipitation with anti-CD3 was carried out with fixed *Staphylococcus aureus* protein A-Sepharose, after addition of a rabbit anti-mouse immunoglobulin as a second antibody to provide the protein A binding. The immune complexes were washed four times in Tris-buffered saline containing 1% digitonin and divided in two parts: one analyzed under reducing conditions by boiling with 2-mercaptoethanol for 5 min, and the second under nonreducing conditions. The second aliquot of the ¹²⁵I-labeled cells was solubilized in 1% Triton X-100 (which dissociates the CD3–TCR complex) and immunoprecipitated either with anti-TCR α chain (constant domain), anti-TCR γ chain (constant domain), or control normal rabbit serum directly bound on the protein A-Sepharose. All samples were analyzed on 10% NaDodSO₄/polyacrylamide gels exposed with Kodak XAR film for autoradiography.

Cell-Mediated Cytotoxicity Assays. Od-enriched glial cells were freshly isolated. Astrocytes in culture were collected by trypsinization after at least two passages of *in vitro* culture. Both cell types were replated onto PLL-precoated round-bottomed 96-well plates at 10^4 cells per well 2 days before the assay. The cells were labeled overnight with 0.037 MBq of ⁵¹Cr (9.25–18.5 GBq per mg of chromium; Amersham) in 100 μl of culture medium per well. Other target cells included B or T lymphoblasts (spleen cells cultured for 48 hr with either *Escherichia coli* lipopolysaccharide or Con A, respectively) and YAC-1 cells, a cell line serving as target for mouse NK or lymphokine-activated killer (LAK) activity (21, 25), kindly given by M. F. Saron (Pasteur Institute, Paris). These cells were labeled with ⁵¹Cr as described above, but for 1 hr only.

After washing the targets, effector C2 T cells were introduced at grading effector to target (E/T) ratios into target-containing triplicate wells in a final vol of 0.2 ml. Controls included target cells with medium alone and with detergent to determine the spontaneous and maximal release, respectively.

In some experiments, effector cells other than the C2 T cells were used. BALB/c mouse splenocytes precultured for 48 hr in IL-2-supplemented medium served as positive controls for NK/LAK activity. C3H spleen cells cultured for 6 days with irradiated (30 Gy) SJL/J splenocytes used as stimulating cells served as a source of H-2^s-specific cytotoxic T lymphocytes to test the efficiency of cytolysis inhibition by anti-H-2^s mAb.

After a 4-hr incubation at 37°C , ⁵¹Cr release was measured in 0.1 ml of supernatant in a γ radioactivity counter (Kontron, Zürich). Results were expressed as percentages of specific lysis: (experimental release cpm – spontaneous release cpm / maximum release cpm – spontaneous release cpm) $\times$ 100.

Blocking Cell-Mediated Cytotoxicity by Antibodies. Antibodies were used at an approximate concentration of 10 $\mu\text{g}/\text{ml}$ for most experiments. mAbs directed at the effector cells were added to graded numbers (10^4 – 10^5) of effector T cells. The mAb/effector cell mixtures were incubated for 4 hr at 37°C

(except when otherwise stated) and were then introduced into culture wells containing ^{51}Cr -labeled target cells. Antibodies directed against the target cells were incubated with the ^{51}Cr -labeled targets 4 hr at 37°C prior to the addition of the effector cells. All target cell/effector cell/antibody mixtures were further incubated 4 hr at 37°C , and the ^{51}Cr release by targets was then measured as described above.

RESULTS

Cellular Composition of Glial Cell Suspensions. Od-enriched glial cell suspensions, separated over a Percoll gradient from adult Lewis rat white matter, were analyzed for the expression of surface or cytoplasmic markers using specific polyclonal or monoclonal antibodies after the cells had been plated onto PLL-coated chamber slides for at least 24 hr. A large majority of these cells (85–95%) expressed surface galactocerebroside, an Od-specific marker (26). A similar percentage of cells (70–95%) was also labeled by a mouse mAb and by guinea pig polyclonal antibodies directed at the MOG/M2 glycoprotein, a recently described surface antigen of Od (9, 10). A slightly lesser percentage expressed detectable amounts of intracytoplasmic myelin basic protein (40–76%), a molecule synthesized by Od only, and of transferrin (70–78%), which, among glial cells, is also expressed by Od but not by astrocytes (27). On the other hand, GFAP, an astrocyte-specific intracytoplasmic marker (28), was seen in <5% of the cells for up to 96 hr of culture. The percentage of cells expressing surface class I or class II MHC antigens remained below 1%. Glial cell cultures similarly prepared from SJL/J mouse white matter also contained 90–93% of galactocerebroside-positive cells and <4% GFAP-positive cells. Since the glial cell suspensions appeared to contain a large majority of cells expressing the surface and cytoplasmic markers of Od and very few contaminating astrocytes, they are referred to as Od throughout the paper.

Astrocyte cultures prepared from newborn Lewis rat or SJL/J mouse brain contained 90–99% GFAP-positive cells, and <2% galactocerebroside-positive cells.

Cytolytic Activity and Specificity of an Od-Triggered T-Cell Clone (C2). A T-cell clone was selected by limiting dilution from Od-specific long-term polyclonal T-cell lines, obtained as described earlier by sensitizing normal SJL/J mouse spleen cells with Lewis rat Od and expanding these activated T cells by periodic restimulation with the same antigen in IL-2-containing medium (13). Earlier preliminary data indicated that this clone, C2, could mount a tissue-specific

proliferative response to Od (13). In the present study, clone C2 was tested for its cytotoxicity toward various ^{51}Cr -labeled target cells in a 4-hr ^{51}Cr release assay and was shown to exhibit killer activity toward both Lewis rat Od, the cell type used for T-cell selection, and, to the same extent, syngeneic SJL/J mouse Od as shown in Fig. 1A. Interestingly, this cytotoxic activity was found to be tissue antigen restricted, since no killing was observed when other targets were tested, such as astrocytes, the other major cell type among glial cells, or B or T lymphoblasts.

To exclude the possibility that Od could be exquisitely sensitive to a putative LAK-like activity of T-cell clone C2, we tested the cytolytic activity of this clone toward YAC-1 cells, a highly sensitive target for NK/LAK activity. As shown in Fig. 1B, the Od-reactive clone killed Od but not YAC-1 cells, showing thus no demonstrable LAK activity, whereas IL-2-stimulated mouse spleen cells, used as a source of LAK cells, killed YAC-1 cells but not Od, indicating that the latter are actually relatively resistant to LAK activity.

Phenotypic Analysis of Clone C2. The clone C2 appeared to display a set of surface markers normally expressed by mature T cells, being Thy-1^+ , CD3^+ , CD8^+ , and CD4^- . Conversely, C2 cells were not labeled by antibodies to asialo-GM1, a marker of the NK cell lineage (21).

TCR Analysis of the T-Cell Clone C2. Since the Od-specific T-cell clone C2 (antigen specific, autoreactive, and non-MHC restricted) displayed unusual features, it was important to determine whether it expressed a "classical" α/β TCR, as is usually found in antigen-specific mature T cells, or a γ/δ type TCR, as is often displayed by T cells expressing NK activity (17, 29). Immunoprecipitation by an anti-CD3 mAb was conducted under conditions that preserve the CD3-TCR association. As shown in Fig. 2, NaDodSO₄/PAGE of the immunoprecipitates, run under reducing versus nonreducing conditions, showed two disulfide-linked polypeptides of 40–50 kDa that correspond to the TCR heterodimer, associated with a smaller polypeptide (27 kDa) corresponding to the CD3 complex itself (29, 30). Immunoprecipitation performed in parallel with rabbit anti-TCR α chain and γ chain (constant regions) antisera indicated that one of these polypeptides corresponded to the TCR α chain, whereas no γ chain was detected.

Inhibition of Cell-Mediated Cytolysis by Antibodies. To further analyze the mechanism of effector–target interaction, attempts were made to block the cytolytic activity of clone C2 toward rat Od by various antibodies directed at membrane components of either the effector or the target cell. As shown

FIG. 1. Specific cytolytic activity of mouse T-cell clone C2 against rat and syngeneic Od. (A) C2 T cells were assayed for their cytotoxicity to Od from Lewis rat (□) and SJL/J mouse (■), to astrocytes from Lewis rat (◇) and SJL/J mouse (◆), and to lipopolysaccharide-activated lymphoblasts from Lewis rat (△) and SJL/J mouse (▲). (B) C2 T cells (solid lines) and IL-2-activated mouse spleen cells used as NK/LAK cells (dashed lines) were assayed for their cytolytic activity against Lewis rat Od (□) and YAC-1 cells (+). ^{51}Cr release was measured after a 4-hr incubation of the effector cells with the target cells.

FIG. 2. Immunoprecipitation of TCR from C2 T-cell clone. 125 I-labeled C2 T cells were detergent solubilized and immunoprecipitated with anti-CD3-TCR complex antibodies and analyzed by one-dimensional NaDodSO₄/PAGE under reducing (R) or nonreducing (N) conditions. Lanes: A, anti-CD3 (N); B, anti-CD3 (R); C, anti-TCR α chain (R); D, anti-TCR γ chain (R); E, normal rabbit serum (R).

in Table 1, cytotoxicity was blocked by both anti-CD3 and anti-CD8 mAbs (but not by anti-CD4), suggesting that both the TCR and the CD8 accessory molecule were required for effector-target interaction.

The possibility existed, however, that Od antigens would have been released and taken up by T cells, which could then present these antigens to themselves in association with self MHC products and secrete lymphotoxins that could eventually kill Od through a mechanism that would not involve direct TCR-target binding. Attempts were therefore made to block the cytotoxicity by mAbs directed at MHC products of the effector C2 cell. Two mAb anti-class I MHC products of the SJL/J strain, which were shown in immunofluorescence studies to label >90% of the C2 T cells, were used. Incubating C2 T cells with these antibodies did not alter their cytolytic activity to Od, although both antibodies were shown in a control experiment to block efficiently the cytolytic activity of C3H anti-H-2^s cytotoxic T lymphocytes to SJL/J T lymphoblasts. A rabbit anti- β_2 -microglobulin antiserum did not decrease the cytolytic activity of the clone either. An anti-MHC class II mAb that happened to recognize both the

FIG. 3. Inhibition of the cytotoxicity of the Od-specific T-cell clone C2 by antibodies to the M2/MOG glycoprotein. 51 Cr-labeled Od were preincubated with the indicated antibodies prior to the addition of the effector cells. ▲, Anti-M2 guinea-pig IgG; ◆, anti-MOG mouse mAb; □, medium alone; △, normal guinea pig IgG; ◇, control mouse mAb.

rat species and the SJL/J mouse strain also failed to inhibit C2 cell-mediated killing of Od, in agreement with the lack of expression of MHC class II products by either the effector mouse T cell or the rat Od target.

Antibodies to Od surface antigens were also assessed. Anti-galactocerebroside failed to block cytotoxicity (data not shown). On the other hand, a mouse mAb and guinea pig polyclonal IgG, both directed against the MOG/M2 glycoprotein expressed at Od membrane, fully blocked the cytolytic activity of clone C2, suggesting that this protein bears an epitope recognized by the TCR of clone C2 (Fig 3). As mentioned above, we did not detect either class I or class II MHC products on Od membranes and failed accordingly to block the cytolytic activity of clone C2 with mAb directed at either MHC class I or class II products of the rat Od targets (data not shown).

DISCUSSION

By culturing normal mouse spleen cells over a monolayer of Lewis rat Od, we could select a cytolytic T-cell clone that displayed unusual features. Indeed, the cytolytic activity of the clone appeared to be tissue specific (only directed against Od targets), autoreactive (killing to the same extent syngeneic Od and rat Od), and non-MHC restricted, since it recognizes both mouse and rat Od targets on which we, as

Table 1. Inhibition of cytolytic activity of T-cell clone C2 by mAbs

Exp.	Effector cell	Target cell	Antibodies							
			None	Control (mouse IgG)	Anti-CD3	Anti-CD4	Anti-CD8	Anti-SJL MHC		
								Class I (mAb 3-83P)	Class I (mAb 34-1-2S)	Class II
I	T-cell clone C2	Lewis rat Od	89	—	—	100	0	—	—	—
II	T-cell clone C2	Lewis rat Od	92	—	0	77	0	92*	—	90*
III	T-cell clone C2	Lewis rat Od	100	100	22	—	—	77†	—	86
IV	T-cell clone C2	Lewis rat Od	—	100	—	—	—	72	—	100
V	T-cell clone C2	Lewis rat Od	29	36	—	—	—	—	58	—
VI	T-cell clone C2	Lewis rat Od	100	100	—	—	—	—	100*	—
VII	Anti-H-2 ^s C3H T cell	SJL/J T blast	99	100	—	—	—	0	0	—

Percentage specific lysis obtained for optimal E/T ratio is shown; i.e., 2.5:1 for Exp. I, 5:1 for Exps. II–VI, and 20:1 for Exp. VII. Percentages significantly lower than that of controls are underlined. ML93, an anti-*M. leprae* mAb produced in our laboratory, as well as polyclonal mouse IgG, were used as controls.

*Antibodies added to the Od target together with the effector cell without preincubation.

†Antibody used at 50 μ g/ml.

others (5), could not detect any MHC class I or class II polymorphic antigen.

The tissue specificity and the lack of MHC restriction of this clone had already been indicated by earlier preliminary results that we had obtained from proliferative response studies, showing that various rat or syngeneic tissues such as kidney cells or islets of Langerhans cells failed to activate the clone, whereas Od from several mouse strains differing by their H-2 haplotype did (13). Moreover, in the present study, we have also excluded the possibility that the T cells would serve themselves as antigen-presenting cells for Od-released antigens in an MHC-restricted manner: indeed, anti-MHC class I and class II antibodies directed at the effector T cells could not block the cytolytic activity of the clone.

Non-MHC-restricted killing by NK and LAK cells has already been extensively described (21, 25), and it does not manifest any tissue specificity being directed against various tumoral target cells. The Od-specific T-cell clone described here does not express any cytotoxicity toward YAC-1 cells, highly sensitive to NK/LAK activity. Moreover, it bears surface markers characteristic of the T-cell lineage (Thy-1, CD3, and CD8) and not of the NK cell lineage (it is asialo-GM1-negative). Finally, the TCR of this clone, a CD3-associated α/β heterodimer, was found quite similar to the TCR of other antigen-specific MHC-restricted T cells so far described (28) and differed from that of CD3⁺ NK/LAK cells, which were shown to express a γ/δ chain TCR (17, 29). Blocking experiments by anti-CD3 and anti-CD8 mAbs indicated that this TCR was used for target-T-cell interaction, as well as the CD8 accessory molecule.

Antigen-specific non-MHC-restricted helper T-cell clones directed at self- or non-self-soluble antigens have been already described (31) but are not a common observation. Rare reports also suggested the existence of cytotoxic non-MHC-restricted T cells directed against self target cells. Thus, bulk lymphocytes from diabetic patients or from melanoma-bearing mice displayed tissue-specific lytic activity toward xenogeneic islets of Langerhans and melanomas, respectively (32, 33). In neither case, however, was the molecular mechanism of effector-target interaction explored. In our study, the Od epitope recognized by the Od-specific T-cell clone is most likely a part of the glycoproteins MOG/M2 expressed at the Od membrane. The structure of these molecules, with apparent molecular mass of 27 and 54 kDa (9, 10), is unknown, but they might behave as MHC molecules to present their specific epitopes to TCR. Indeed, some minor histocompatibility antigens have been reported to be tissue restricted—that is, to bear epitopes recognized only by tissue-specific T cells and/or antibodies (34). Alternatively, the M2/MOG epitope could be presented to T cells in association with some monomorphic class I antigens encoded by the Tla complex genes shown to be expressed in a broad variety of tissues including brain (35).

The physiologic and/or pathogenic role of these autoreactive Od-specific cytotoxic T cells remains unknown. Antibodies to the glycoprotein MOG/M2 were shown to act in synergy with myelin basic protein-specific T cells in passively transferred experimental autoimmune encephalomyelitis (8). Cytotoxic T cells directed at the same molecule might play a similar role in the pathogeny of this disease. Alternatively, they might act as suppressor cells involved in the perpetuation of self-tolerance: indeed suppressor T cells of the CD8 phenotype have been described that also displayed both antigen specificity and lack of MHC restriction (36). Whether the non-MHC-restricted autoreactive Od-specific T cell described here belongs to a broader category of “natural” autoreactive T cells directed at various organ-specific epitopes remains questionable. Since myelination only develops in postnatal life, T cells directed at late-appearing

myelin antigens may have escaped the mechanisms of selection responsible for self tolerance. On the other hand, we do not know yet whether Od-specific autoreactive T cells exist in mouse strains other than the SJL/J strain which is highly susceptible to the induction of autoimmune diseases and to immunoregulation disorders (37).

We are grateful to Dr. D. Primi for help and advice in TCR immunoprecipitation studies; to Dr. C. Linington for the gift of mAb 8-18C5; to Dr. F. Jacquemart for the gift of anti-asialo-GM1 mAb; to Drs. J. P. Abastado, F. Godeau, and P. Kourilsky for the gift of mAb 34-1-2S and anti- β_2 -microglobulin antiserum; to Dr. P. Dupouey for the gift of anti-galactocerebroside antiserum; and to G. Dumas for skillful technical assistance. This work was supported by Association pour la Recherche sur la Sclérose en Plaques.

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