Antigen-Specific Blockade of Lethal CD8 T-Cell Mediated Autoimmunity in a Mouse Model of Multiple Sclerosis[1]

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Increasing evidence implies CD8 T cells in tissue-specific autoimmune diseases including multiple sclerosis. mAbs specific for MHC class I molecules presenting a dominant autoantigenic peptide may allow selective immunotherapy in such settings. We demonstrate the prophylactic and therapeutic efficacy of such a mAb in a transgenic mouse model of lethal demyelinating disease in which a neo-self Ag expressed by oligodendrocytes is targeted by CD8 T cells with transgenic Ag receptors. Mechanistic studies performed in vitro and in vivo indicate that it is the low expression of MHC class I on oligodendrocytes, which makes this form of Ag-specific intervention possible. ***The Journal of Immunology,*** **2009, 182: 6569–6575.**

In multiple sclerosis (MS),[3] autoreactive CD8 T cell responses to various myelin Ags have been identified (1–3), and Ag-triggered cytokine production as well as in vitro killing of human oligodendrocytes by such CD8 T cells has been reported (2, 4). Furthermore, the persistence of expanded myelin-Ag-specific CD8 T cell clones in MS patients underlines their potential importance for disease development (5–9). The target cells expressing these myelin Ags, i.e., the oligodendrocytes, are deficient in MHC class II but express low levels of MHC class I molecules (10), which are a prerequisite for CD8 T cell-mediated autoimmune attack. Indeed, several recent studies performed in mice have illustrated the destructive potential of CD8 T cells specific for natural or model Ags expressed in oligodendrocytes (11–15).

Ideally, therapies for autoimmune diseases are Ag-specific, thereby avoiding generalized immunosuppression. A conceivable strategy to interfere with organ-specific CD8 T cell-mediated autoimmunity is the selective masking of complexes formed by dominant peptides from the targeted tissue-specific Ag (TSA) with the presenting MHC class I (MHC I) molecules. As shown for mAb specific for complexes of mouse MHC I molecules formed with viral or model Ags, the generation of such mAb is feasible (16–18). MHC/peptide-specific blockade of T cell recognition is likely to be constrained, however, by the small number of recognition events required for T cell activation (which for triggering of CTL can approach one; Ref. 19). Therefore, protection of tissue cells expressing high levels of MHC I molecules may be difficult to address by such a therapeutic strategy, whereas cells expressing only low levels, such as oligodendrocytes, may provide an opportunity for mAb-mediated interference.

The best-characterized MHC I/peptide specific mAb is 25-D1.16 (D1), which is specific for the OVA-derived SIINFEKL ("OVA8") peptide presented by the mouse MHC I molecule H-2K^b (K^b/OVA8). D1 binds to K^b/OVA8 with an affinity around 7×10^{-8} M, which is in the low range for Abs (18). By comparison, however, TCR-MHC/peptide affinities are even considerably lower, suggesting a window for blocking effects (18). Specifically, the 42.12 TCR, which is expressed by the OT-I CD8 T cells used in the present study (20), binds to immobilized K^b/OVA8 with a K_d of 6.83×10^{-6} M (21).

We have developed a new mouse model for MS in which OVA is exclusively expressed in the cytosol of oligodendrocytes (ODC-OVA mice) (22). This sequestration leads to antigenic ignorance by OVA-specific CD4 T cells (which recognize Ags presented by MHC II), whereas coexpression in these mice of the OT-I TCR derived from K^b/OVA8-specific CD8 T cells leads to spontaneous fulminant disease characterized by extensive demyelination and MS-like lesions (22, 23). The availability of a well-characterized mAb with specificity for the K^b/OVA8 target Ag therefore prompted us to investigate its blocking efficacy in this system.

Materials and Methods

Mice and Ab treatments

ODC-OVA transgenic (tg) mice on the C57BL/6 background, expressing OVA under the oligodendrocyte-specific myelin basic protein promoter (22), OT-I mice (20), luciferase transgenic mice (24), and Thy1.1 congenic C57BL/6 mice (Jackson ImmunoResearch Laboratories) were kept in a specified pathogen-free animal facility at the Institute for Virology and Immunobiology Würzburg.

For mAb therapy, ODC-OVA/OT-I mice were treated i.p. with 100, 200, or 500 μg of D1 (18) or isotype control Ab (PPV) produced in a low-endotoxin format. Mice were scored for clinical signs of experimental autoimmune encephalomyelitis (EAE) on a daily basis using a scale from 0 to 5: 0, normal; 1, limp tail; 2, partial hind leg paralysis; 3, total hind leg paralysis; 4, hind and front limb paralysis; 5, moribund or dead.

Cell preparations and in vitro assays

EG.7 (25) or EL-4 cells were labeled with 2.5 μM CFSE for 5 min at RT as taget cells, and EL-4 cells were further pulsed with 100 to 0.01 nM SIINFEKL for 2 h at 37°C. Unpulsed EL-4 cells labeled with 10 μM CFSE cells were used as internal controls. As CTL, MACS-purified (Miltenyi

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[3] Abbreviations used in this paper: MS, multiple sclerosis; TSA, tissue-specific Ag; MHC I, MHC class I; tg, transgenic; EAE, experimental autoimmune encephalomyelitis; ODC-OVA mice, mice expressing ovalbumin in oligodendrocytes; WT, wild type.

Biotec) OT-I cells were activated for 6 days with anti-CD3 plus anti-CD28 coated beads (Dynal Biotech) in RPMI 1640 (Invitrogen) supplemented with 10% FCS and T cell growth factor (α-methylmannopyranoside-treated supernatant from concavalin A-stimulated rat splenocytes). In brief, 1/1 mixed indicator and target cells were incubated in triplicates with CTL at various E:T ratios in the presence or absence of D1 Ab for 6 h at 37°C and analyzed by flow cytometry. Percent specific lysis was calculated as $(1 - \%\ \text{targets}/\%\ \text{control cells}) \times 100$.

For oligodendrocyte CTL assay, oligodendrocytes from luciferase or ODC-OVA/luciferase mice were isolated as described (23). Four $\times 10^4$ ODC/well were cultured in 96-well plates in replicates of 10 for 6 days before 4×10^5 activated OT-I cells with or without 20 μg/ml D1 Ab were added. Twenty-four hours later, 1 μM luciferin was added and luminescence was measured by MicroLumat (EG & G Berthold).

Cell transfer

Purified OT-I CD8 T cells from OT-I/Thy1.1 tg mice were labeled with 10 μM CFSE for 5 min at room temperature and 5×10^6 cells were transferred i.p. into 7 days old wild-type (WT) or ODC-OVA mice with 500 μg D1 or isotype control Ab. Where indicated, mice also received 200 μg soluble OVA. Three days later, lymphocytes from cervical lymph node were isolated and analyzed by flow cytometry.

Flow cytometry

OT-I cells were stained for FACS using the following Abs (all from BD Pharmingen): anti-CD8-PE, anti-CD8α-allophycocyanin, anti-CD69-FITC and anti-CD90.1-PE. For K^b/SIINFEKL staining EL-4 or EG.7 cells were treated with Fc block (anti-CD16) for 10 min and stained with D1 or isotype control Ab followed by donkey anti-mouse IgG PE Ab (Jackson ImmunoResearch Laboratories). Data were acquired on a FACScalibur (BD Biosciences) and analyzed by FlowJo software (Tree Star).

Preparation of acute living brain slices and coculture experiments with OT-I cells

Six- to 8-wk-old transgenic ODC-OVA mice were anesthetized with isoflurane and decapitated, the brain was transferred in oxygenated ice-cold saline containing (mM): sucrose, 200; PIPES, 20; KCl, 2.5; NaH_2PO_4, 1.25; $MgSO_4$, 10; $CaCl_2$, 0.5; dextrose, 10 (pH 7.35), adjusted with NaOH. Slices were prepared as coronal sections on a vibratome. For incubation, slices were placed each into a well of a 12-well plate filled with standard artificial cerebrospinal fluid containing (mM): NaCl, 125; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 24; $MgSO_4$, 2; $CaCl_2$, 2; dextrose, 10 (pH adjusted to 7.35 by bubbling with a mixture of 95% O_2 and 5% CO_2). Each slice was incubated with 5×10^5 $CD8^+$ T cells (OT-I) and either D1 Ab or an isotype control for 6 h. Afterward, slices were harvested and embedded using OCT compound tissue-tek (Sakura Finetek) and frozen in liquid nitrogen. Ten micrometer coronary cryo-sections were obtained using a cryostat (Leica CM 1950).

Immunfluorescence staining and confocal microscopy of brain slices

Immunohistochemical staining was performed on 10-μm coronary sections. For double labeling, slices were postfixated in 4% paraformaldehyde for 10 min and incubated in blocking solution (PBS containing 5% BSA, 1% normal goat serum, and 0.2% Triton X-100). Slices were then incubated simultaneously or consecutively with Abs NogoA (1/750, Chemicon) and activated caspase-3 (1/200, Cell Signaling Technology) overnight at 4°C. Secondary Abs were Alexa Fluor 488-coupled goat Abs recognizing mouse IgG, Cy3-coupled goat Abs recognizing rat or rabbit IgG. Negative controls were obtained by either omitting the primary or secondary Ab (data not shown).

For quantification of cell densities, sections were examined with an Axiophot2 microscope (Zeiss) with a charge-coupled device camera (Visitron Systems). Cell density was determined within preselected fields at specific sites (Dentate gyrus of the hippocampus; piriform cortex (cortex)). Cells were counted using MetaVue Software (Molecular Devices).

Histopathology of CNS sections

Preparation and staining of paraformaldehyde fixed sections was performed as described (23). Quantification of demyelination was conducted on Luxol fast blue stained spinal cord sections using the Cell^D imaging software (Olympus). Six sections each from three mice per group were analyzed.

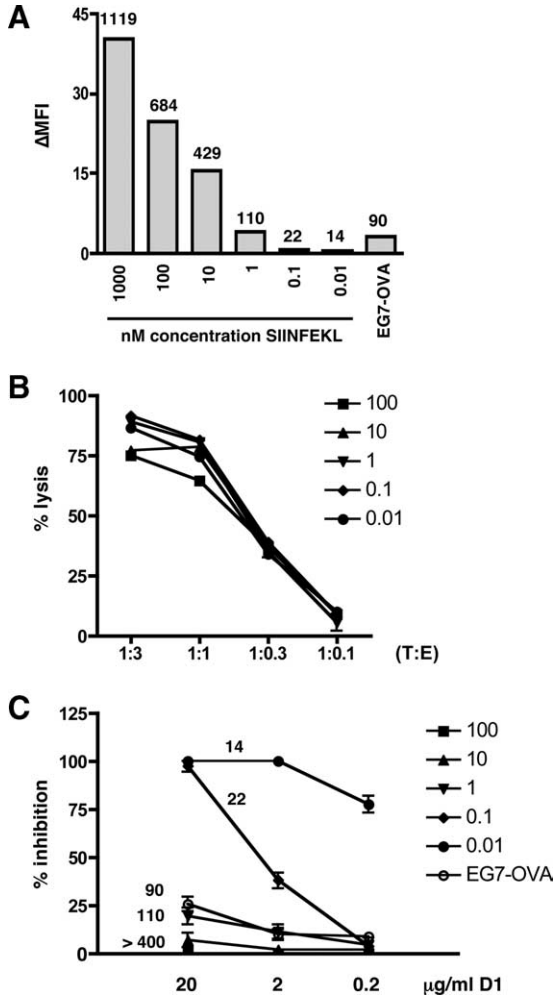

FIGURE 1. Effect of Ag density on mAb D1-mediated blockade of killing by OT-I cells in vitro. *A*, Quantification of K^b/SIINFEKL complexes on SIINFEKL pulsed EL-4 cells. Cells were loaded for 2 h at 37°C with the peptide concentrations indicated, and indirectly stained with D1. *B*, Lysis of SIINFEKL pulsed EL-4 cells by OT-I CTL. Activated OT-I cells were coincubated with differentially CFSE-labeled pulsed and unpulsed EL-4 cells for 6 h before flowcytometric determination of specific lysis. *C*, Blockade of OT-I mediated lysis by D1. D1 was included in the CTL assay as in *B*, using an E:T ratio of 1:1.

Statistical analysis

All statistics were performed using GraphPad Prism 4.0 (GraphPad Software). Data are presented as mean ± SEM, p values are included in the figures.

Results

Inhibition of K^b/OVA8-directed target cell lysis by mAb D1 at low Ag density

Initially, we tried to estimate the maximum number of cognate MHC/peptide complexes on a target cell that would allow blockade by mAb at concentrations that are also achievable in vivo. EL-4 T-lymphoma cells were loaded for 2 h at 37°C with the OVA8 peptide over a range of 5 $\log_{10}$ dilutions. OVA-transfected EL-4 cells (E.G7 cells), which express ~90 K^b/OVA8 complexes (26, 27), were included for calibration, and measurements were performed by indirect fluorescence and FACS analysis using mAb D1 to detect K^b/OVA8. The target cells generated in this fashion expressed between 14 and 1,119 K^b/OVA8 complexes on average (Fig. 1*A*). All of these OVA8-loaded targets were lysed by

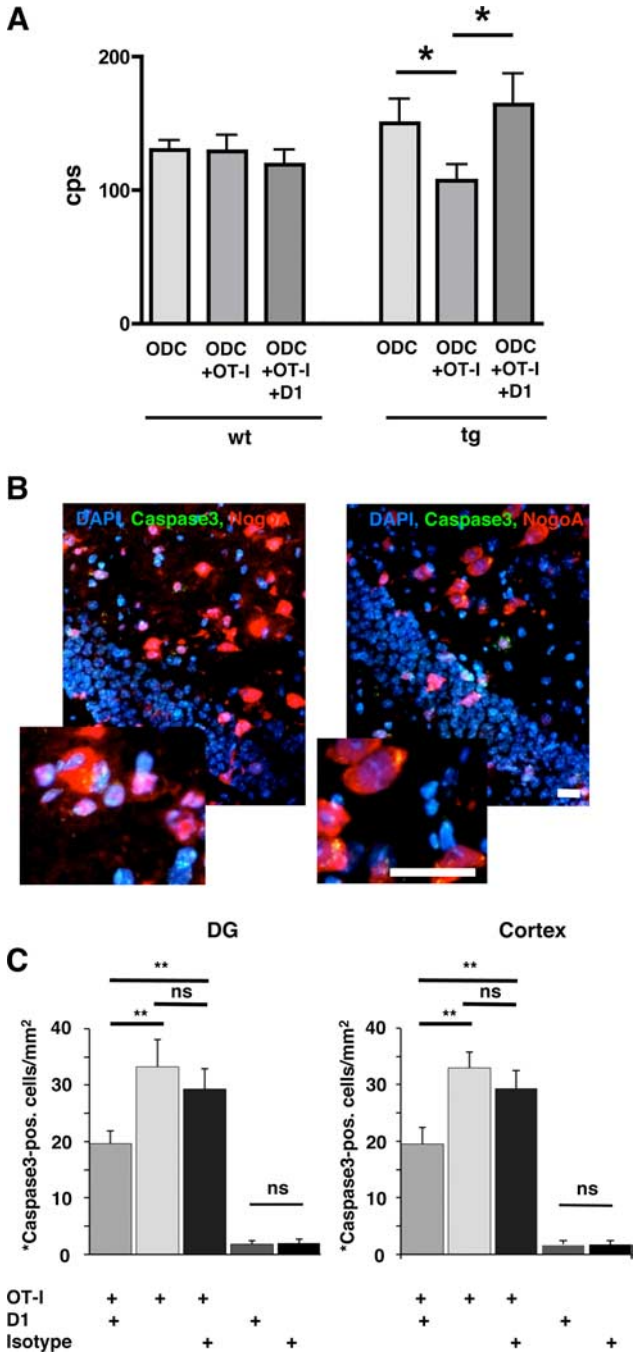

FIGURE 2. mAb D1 blocks lysis of OVA-transgenic oligodendrocytes. *A*, Lysis of ODC-OVA oligodendrocytes by OT-I CTL and blockade by mAb D1. Luciferase single-transgenic (WT) or luciferase/OVA double transgenic (tg) oligodendrocytes were prepared as described in *Materials and Methods*. Activated OT-I cells were added for the last 24 h of culture. Results are given as net cps of chemoluminscence after addition of luciferin. One of two experiments with similar results is shown. *B* and *C*, Induction of apoptosis in oligodendrocytes by OT-I cells is blocked by mAb D1 in organotypic slice cultures. Acute living brain slices from hippocampus (dentate gyrus, DG) or cortex of ODC-OVA mice were incubated for 6 h with effector OT-I T cells. Multicolor-fluorescence immunohistochemistry was applied to staining for ODCs (NogoA, red), markers of apoptosis (activated caspase-3 (*caspase-3), green) and nuclei (DAPI, blue). Scale bars represent 20 μm. $n = 6$ for each group. In *C*, numbers of NogoA$^+$ *caspase-3$^+$ cells per square mm are given.

activated OT-I cells with indistinguishable efficiency (Fig. 1*B*), in keeping with the high sensitivity of T cell triggering to Ag presented at low density.

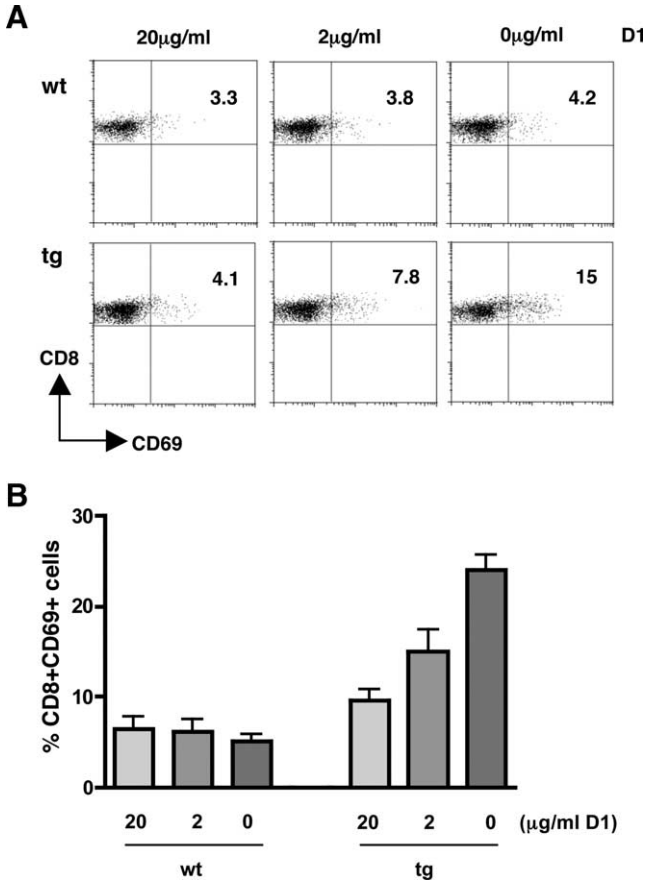

FIGURE 3. mAb D1 blocks activation of naive OT-I cells by OVA-expressing oligodendrocytes. *A*, Cultured oligodendrocytes from WT or ODC-OVA mice were cocultured with naive OT-I cells for 4 days in the presence or absence of D1. Activation was measured as CD69 expression on gated CD8 positive cells. Numbers give percentage of positive cells. *B*, Statistical analysis of pooled data from five individual experiments.

When killing was monitored in the presence of 20, 2, or 0.2 μg/ml D1 Ab, targets expressing 400 or more cognate peptide/MHC complexes were not protected by the mAb at any concentration used (Fig. 1*C*). Targets with an average of only 14 complexes were fully protected at 20 and 2, and partially by 0.2 μg D1/ml. Of note, an increase in K^b/OVA8 complexes to an average of 22 per cell still allowed full protection at 20 μg D1/ml, but less than 50% at 2 and none at 0.2 μg/ml. It is apparent from these data that inhibition of target cell lysis by the mAb does not follow a linear relationship between the number of cognate Ag complexes expressed per cell and the concentration of mAb required for blockade. Rather, even in the low range of peptide loading, a 15-fold increase of D1 was required to offset an only 1.5-fold increase in K^b/OVA8 complexes (Fig. 1*C*), and full blockade of lysis by target cells with 100 or more complexes (such as E.G7) by mAb seems unrealistic at any D1 concentration that would also be achievable in vivo. These results show that mAb blockade of CTL lysis is feasible as long as the number of cognate complexes available at any given time is pushed below the number required for triggering, which can be as little as one (19). In the present example, these conditions are met if 20 μg/ml D1 are used to block a target with around 20 K^b/OVA8 complexes.

Lysis of OVA-expressing oligodendrocytes is blocked by mAb D1 in vitro

Oligodendrocytes, the primary targets for OT-I cells in ODC-OVA/OT-I double transgenic mice, express low amounts of MHC

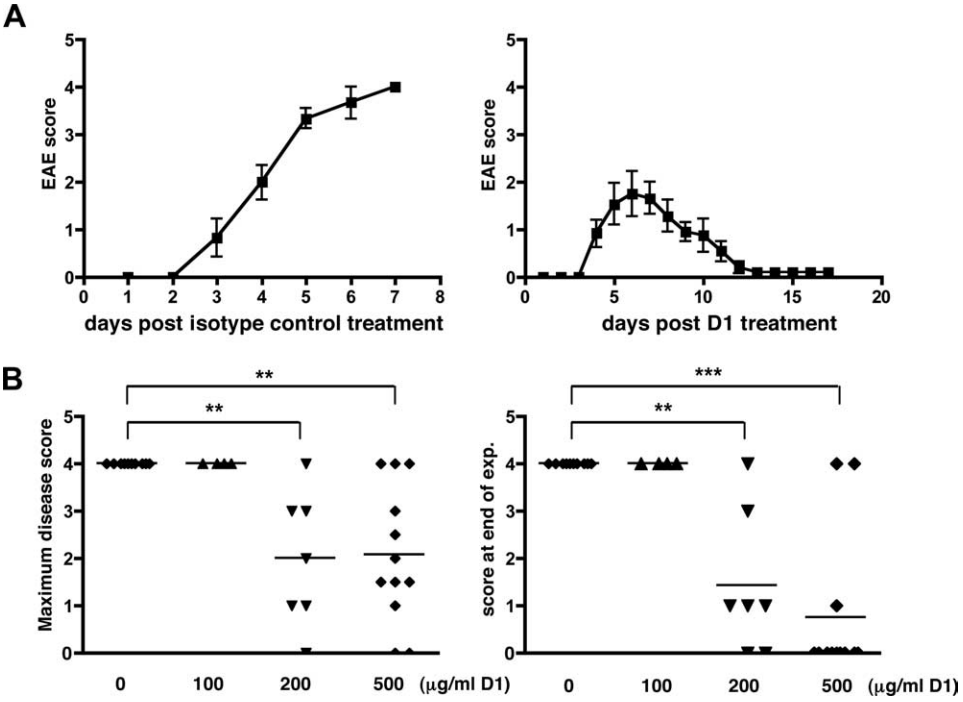

FIGURE 4. mAb D1 prevents lethal EAE of ODC-OVA/OT-I mice. *A*, Disease course in ODC-OVA/OT-I double transgenic mice treated with 500 μg mAb D on day 7. *Left*, Control mice (isotype control; $n = 10$); *Right*, mAb D1-treated mice (beginning: $n = 12$, end: $n = 10$). Mice that had to be euthanized were not included in further scoring. *B*, Dose-dependent suppression of EAE in day 7-treated ODC-OVA/OT-I double transgenic mice. *Left*, Maximum score; *Right*, Score at end of experiment (day 7–17 after treatment).

I (23). We generated mature oligodendrocytes by in vitro culture from precursor cells obtained from ODC-OVA mice and nontransgenic controls as previously described (23). Flow cytometric determination of rare K^b/OVA8 complexes with D1 gave unsatisfactory results due to the background noise generated by these large cells and the ensuing small signal-to-noise ratio. Expression of H-$2K^b$ was, however, readily measured and found to be 4.4-fold lower than on E.G7 cells (10 vs 44 ΔMFI). Assuming a similar contribution of cytosolic OVA to K^b loading as in EG.7 cells, this result translates into ~20 K^b/OVA8 complexes per oligodendrocyte.

To asses cytotoxic activity of OT-I CTL against OVA-expressing oligodendrocytes, we introduced a luciferase transgene (24) into ODC-OVA mice and measured chemoluminescence after 6 and 24 h of coincubation of these double transgenic oligodendrocytes with a 10-fold excess of preactivated OT-I cells. No significant lysis was observed after 6 h. As shown in Fig. 2*A*, however, significant destruction of luciferase/OVA double transgenic, but not of luciferase single transgenic oligodendrocytes was observed at 24 h. As compared with EL-4 lymphoma cells expressing an estimated similar number of cognate Ag molecules (Fig. 1*B*), lysis was, however, much less efficient. Importantly, mAb D1, included at 20 μg/ml, fully blocked target cell destruction. This result establishes that murine oligodendrocytes can be lysed by CTL specific for a cytosolic TSA, and that lysis can be blocked by MHC I/peptide specific mAb.

mAb D1 reduces OT-I mediated oligodendroglial cell death in living brain slices

In a second in vitro approach, which preserves the complex architecture of the brain, activated OT-I cells were transferred onto acute living brain slices from ODC-OVA mice. In this system, OT-I CTL are able to accumulate in areas of oligodendrocyte-related Ag expression (Göbel, K., N. Melzer, A. M. Herrmann, C. Wang, T. Hünig, S. G. Meuth, and H. Wiendl, submitted for publication). Furthermore, they are capable of inducing apoptosis in oligodendrocytes in an Ag-dependent manner as read out by high levels of activated cytosolic caspase-3 within 6 h of incubation (Göbel, K., N. Melzer, A. M. Herrmann, C. Wang, T. Hünig, S. G. Meuth, and H. Wiendl, submitted for publication, and Fig. 2, *B* and *C*), in keeping with our unpublished observations of extensive TUNEL staining in areas undergoing demyelination in ODC-OVA/OT-I double transgenic mice. Inclusion of mAb D1 in the culture medium at

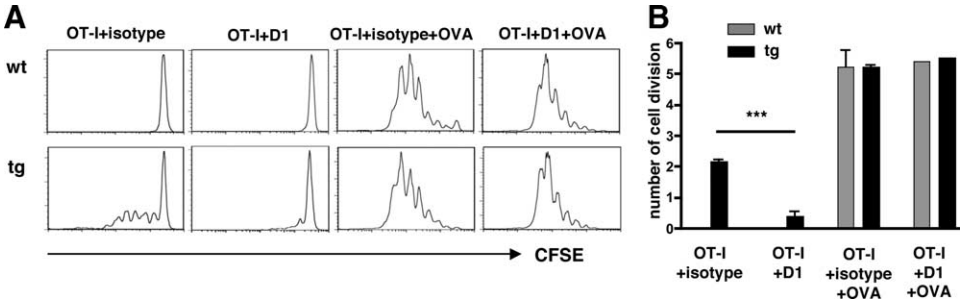

FIGURE 5. mAb D1 prevents activation of resting OT-I cells in vivo. Ag-driven division of CFSE-labeled OT-I cells recovered from cervical lymph nodes of ODC-OVA mice 3 days after transfer. *A*, Representative experiment. *B*, Average number of cell divisions of OT-I cells recovered from cervical lymph nodes of ODC-OVA mice. Five $\times$ 10^6 CFSE-labeled Thy1.1 congenic OT-I cells were transferred to WT or ODC-OVA mice with or without 500 μg D1 or isotype control ($n = 5$), and Thy1.1-gated cells were analyzed. Where indicated, mice received 200 μg OVA in PBS i.p. ($n = 3$).

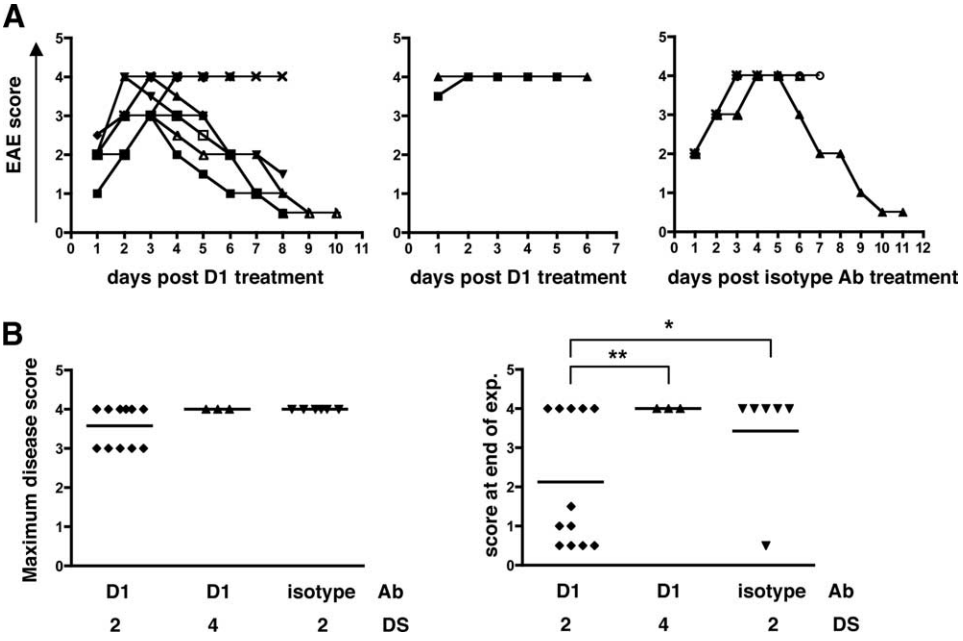

FIGURE 6. Therapeutic intervention with ODC-OVA/OT-I EAE with mAb D1 at grade 2, but not at grade 4 of EAE. *A*, Disease course in individual ODC-OVA/OT-I double transgenic mice treated with 500 μg mAb D1 at score 2 (left, $n = 12$) or 4 (center, $n = 3$), or with 500 μg control mAb at score 2 (right, $n = 6$). *B*, Maximum disease scores (*left*, differences ns) and endpoints (days 5–12 after treatment) of groups shown in *A*. DS indicates disease score at time of application of either D1 or isotype control mAb.

10 μg/ml significantly reduced the number of *caspase-3$^+$ oligodendrocytes in ODC-OVA brain slices undergoing cytotoxic attack by OT-I cells, confirming the results obtained with cultured oligodendrocytes in a more natural setting.

mAb D1 blocks activation of naive OT-I cells by OVA-expressing oligodendrocytes

In OT-I mediated EAE of ODC-OVA mice, initial recognition of the cognate Ag does not occur on cross-presenting APC in draining lymph nodes, but on the oligodendrocytes themselves (23). We therefore also tested the ability of D1 to prevent activation at that stage, using cultured oligodendrocytes from ODC-OVA and wild type mice as stimulator cells for resting OT-I cells. Pilot experiments showed that activation does occur but is slow and ineffecient, with induction of the activation marker CD69 measured on day 4 of coculture yielding the best results. As expected (Fig. 3), oligodendrocytes from WT mice did not activate OT-I cells, whereas those from ODC-OVA mice were stimulatory. mAb D1 was able to fully block activation at a high (20 μg/ml), and partially at a low concentration (2 μg/ml).

Taken together, the results obtained with D1-mediated interference with the initial activation and CTL lysis of OT-I cells suggested that the inefficient Ag presentation by oligodendrocytes may open a window for the blockade of autoimmunity in the ODC-OVA/OT-I system.

mAb D1 prevents lethal EAE of ODC-OVA/OT-I mice

Untreated ODC-OVA/OT-I double transgenic mice synchronously develop disease around day 12 and are euthanized by day 18 when a disease score of four (front and hind limb paralysis) or five (moribund) is reached. When double transgenic mice were treated on day 7 with a single injection of purified D1 mAb, a dose-dependent reduction in disease severity was observed (Fig. 4, *A* and *B*). Thus, at 100 μg/mouse, the disease course was indistinguishable from that observed in control mice, whereas at 500 μg, 10/12 mice survived, and about half of the animals experienced only mild or no symptoms. An intermediate phenotype was observed at 200 μg/mouse. Histopathologic comparison of mice that recovered under D1 therapy showed mild residual vacuolization, T cell infiltration (45.3 $\pm$ 4.7 CD3+ cells/mm^2 vs 512 $\pm$ 36) and macrophage/microglia activation (230.7 $\pm$ 21.34 MAC-3+ cells/mm^2 vs 3411 $\pm$ 295.9), and less demyelination in cerebellum and spinal cord (31.6 $\pm$ 1.0% myelin loss in white matter vs 55.9 $\pm$ 2.3%) as compared with double transgenic mice without D1 treatment, which showed extensive inflammation and myelin destruction (supplemental Fig. S1).[4]

mAb D1 prevents the induction of proliferation of OT-I cells in ODC-OVA mice

To test whether as suspected, D1 treatment inhibited OT-I Ag recognition in vivo, we used the CFSE dye-dilution method. Allotype-marked CFSE labeled OT-I cells were injected into 7 days old single transgenic ODC-OVA mice with or without 500 μg D1 or isotype control Ab. As previously reported, OT-I cells recovered 3 days later had proliferated in the absence of the blocking Ab (Fig. 5, *A* and *B*). In contrast, OT-I cells failed to divide when mice were coinjected with D1. Importantly, additional i.p. injection of soluble OVA protein resulted in enhanced proliferation that was insensitive to D1 treatment, indicating that efficient OVA cross-presentation by "professional" APCs is unaffected by this reagent.

D1 therapy of ongoing EAE

We also investigated the therapeutic potential of D1 when applied after disease onset. Although triggering of preactivated CTL occurs at a lower threshold than activation of their naive precursors, the ability to block lysis of OVA-transgenic oligodendrocytes in vitro (Fig. 1) suggested that this might be feasible. Based on our experience with prophylactic treatment, we only used the highest dose (500 μg/mouse) to treat double transgenic mice at grades 2 and 4 of EAE. Grade 4 mice ($n = 3$) did not improve during the following days and had to be euthanized (Fig. 6). In contrast, 7/12 mice with grade 2 EAE recovered after transient exacerbation, while 5/12 mice progressed to full-blown disease (Fig. 4). Five of six mice treated with control mAb had to be euthanized, while 1 mouse recovered. Such spontaneous remission is a rare event observed in

[4] The online version of this article contains supplementary material.

ODC-OVA/OT-I mice with intact RAG genes, as were used in the present experiments (23).

Discussion

Our results show that murine oligodendrocytes expressing a cytosolic TSA can trigger activation of and lysis by TSA-specific CD8 cells in vitro, and that triggering can be blocked by a mAb specific for the cognate MHC/peptide complex at a concentration achievable also in vivo. Indeed, fulminant EAE observed in double transgenic mice expressing both the TSA in oligodendrocytes and a transgenic TCR conferring TSA-specificity to CD8 cells can be prevented and, in part, treated with such mAb.

We believe that this unexpected efficiency of MHC-I/peptide-specific mAb in the blockade of autoimmunity is related to the poor APC function of oligodendrocytes which, at least in part, is explained by their low MHC I density. The actual threshold number of MHC/peptide complexes up to which interference with CD8 T cell recognition is feasible in vivo will, obviously, not only depend on the affinity of the mAb used for blockade, but on additional parameters such as TCR affinity for the complex in question and target cell-intrinsic properties such as cell size and sensitivity of its apoptotic machinery to the triggers used by the CTL, i.e., CD95 ligation and attack by perforin and granzymes.

Most likely, another important factor contributing to the successful interference of mAb D1 in the ODC-OVA/OT-I system is the absence of costimulatory ligands on the target cells, a scenario likely to hold true for MS as well and which may extend to other CD8 T cell mediated autoimmune diseases such as autoimmune polymyositis (28).

Of note, all mice which had survived under D1 therapy remained without further symptoms throughout an observation period of up to 3 mo, indicating that once an initial attack by OT-I cells had been sufficiently blocked to contain overt disease, tolerance was established. It remains to be investigated to which extent regulatory and deletional mechanisms contributed to this self-tolerance, and to which extent it depended on the closing of the blood brain barrier during this phase in life.

There are a number of anticipated problems in translating the present results into treatment of MS patients. One is the requirement for the mAb to pass the blood-brain barrier, but this may vary with the degree of acute inflammation, as has been suggested as an explanation for the successful depletion of B-cells from the CSF by anti-CD20 therapy (29). Under conditions of an intact blood-brain barrier, application into the CSF, as has been practiced with IFNβ (30), may even increase the half-life of mAb available on site. Another obstacle is the polymorphism of the human MHC class I loci, which together with the likely importance of several TSAs for CD8-mediated autoimmune attack restricts the usefulness of one given MHC I/peptide specific mAb. On the other hand, dominant clonal expansions of CD8 T cells exist in MS (5–9) and, at least for some well-studied, frequent class I alleles (2, 4), can be characterized with regard to Ag specificity and affinity using tetramer technology (31). Using a panel of class I tetramers loaded with candidate target Ags, patients may therefore be identifiable who could profit from shielding the cognate MHC-peptide complexes on their oligodendrocytes from autoimmune attack with the appropriate mAb.

The list of oligodendrocyte-specific TSAs has recently been extended to a soluble cytosolic protein, transaldolase, an enzyme involved in energy supply for lipid biosynthesis during myelination (31). Prevalence of HLA-A2 restricted CD8 T cells specific for a transaldolase peptide was more closely associated with MS than that of CD8 cells recognizing the abundant classical myelin Ags myelin basic protein or myelin oligodendrocyte glycoprotein, suggesting that CD8-mediated autoimmunity to such sequestered cytosolic TSA could be of pathological relevance in MS (31). Our model system using cytosolic expression of OVA may thus be particularly useful to assess mAb-mediated experimental strategies combating CD8 responses to such cytosolic TSA which direct the autoimmune attack at oligodendrocytes and other target cells with low MHC class I expression.

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Disclosures

The authors have no financial conflict of interest.

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