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Heather A. Wasserman; ... et. al

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Induction of Anergy by Antibody Blockade of TCR in Myelin Oligodendrocyte Glycoprotein-Specific Cells¹

Heather A. Wasserman and Brian D. Evavold²

Previous studies have found that a 95% reduction in TCR expression does not adversely affect response to foreign Ags, indicating that T cells have an excess of TCR for Ag recognition. Because self-reactive T cells may have low affinity for peptide:MHC, we investigated whether myelin-reactive T cells require these excess TCR for optimal response. To test this concept, mAb were used to effectively reduce the TCR of V α 3.2 and V β 11 TCR transgenic mice (referred to as 2D2). After masking the TCR with either continuous or prepulsed anti-V α 3.2 Ab, 2D2 cells were immediately stimulated with myelin oligodendrocyte glycoprotein (MOG)_{35–55}. These cells have a dramatic Ab dose-dependent reduction in proliferation, with a small reduction in TCR expression leading to a 50% reduction in proliferation in vitro. Additionally, 2D2 cells, treated with anti-V α 3.2 Ab and peptide for 7 days, were re-stimulated with MOG and continue to have a dose-dependent reduction in proliferation. TCR quantitation identified the same amount of TCR on the Ab/peptide treatment compared with the peptide-only control. These results point out that the combination of reduced TCR and peptide challenge leads to a phenotypic change resulting in T cell anergy. Importantly, adoptive transfer of these anergic T cells upon autoimmune disease induction had a marked reduction in disease severity compared with untreated MOG-specific CD4⁺ T cells, which had significant autoimmune disease manifested by optic neuritis and death. Thus, reduction of TCR expression may provide a potential therapy for self-reactive T cells involved in autoimmune diseases through the induction of anergy. *The Journal of Immunology*, 2008, 180: 7259–7264.

The interaction between MHC:peptide and TCR is a finely tuned interplay of molecules that is critical for activation of the effector responses of adaptive immunity. The activation of T cells by high-affinity non-self peptides requires few TCR on the cell surface to respond to Ag (1, 2); however, the spectrum of peptide signals ranges from weakly, to partially, to fully agonistic, as well as antagonistic. Our laboratory and others have previously demonstrated a direct relationship between strength of signal and density of TCR on foreign Ag-specific T cells (3, 4), suggesting that TCRs are an example of a receptor/ligand system governed by the spare receptor theory (5). According to this theory of receptor response, agonists achieve maximal responses by occupation of a small number of receptors, whereas weaker ligands require engagement of many more receptors (5, 6). The power and utility of a spare receptor type of response is that the excess of receptors extends the spectrum of ligands to which the system can respond. This type of system aptly describes TCR, where contact is required over a range of peptide potencies from weak (self) to strong (pathogen). Consistent with such a model, we found that a weak agonist required significantly more receptors to induce complete T cell activation compared with a full agonist, which required 5% or less of available receptors (3, 6).

Experimental allergic encephalitis (EAE)³ is an autoimmune disease that targets myelin-producing cells of the CNS and is initiated by autoreactive CD4⁺ T cells (7, 8). Myelin oligodendrocyte glycoprotein (MOG)_{35–55} is an agonist that has been identified as a target Ag of encephalitogenic T cells in the C57BL/6 mouse model (9). These MOG-specific T cells have undergone positive/negative selection in the thymus, which has been shown to express MOG_{35–55} (10, 11). Therefore, MOG T cells with high-affinity receptors may have been clonally deleted leaving only those MOG-specific T cells with low-affinity receptors to enter the periphery. Hypothetically, these autoimmune T cells, which recognize their cognate Ags with low affinity, should require more available TCR to respond to self-Ag if these cells conform to the spare receptor paradigm. Importantly, the spare receptor model suggests a potential therapy, because reduction of TCR may hamper autoreactive T cell effector responses while leaving the response to foreign Ags intact.

In this report, autoreactive MOG-specific CD4⁺ T cells preincubated with anti-V α 3.2 Ab displayed a hypoproliferative response accompanied by reduced IL-2 production. TCR quantitation of these T cells found that a slight reduction in available TCR induced a marked decrease in proliferation, a phenotype in direct contrast to that observed for T cell responses triggered by foreign Ags. Further, these MOG-specific T cells had a stable hypoproliferative phenotype, as cells restimulated after the initial culture in the presence of TCR masking Ab still had significantly decreased proliferation compared with non-Ab masked T cells, indicating an anergic phenotype. Importantly, the anergic phenotype was maintained in vivo as the adoptive transfer of hyporesponsive T cells induced a mild form of disease compared with untreated MOG-specific CD4⁺ T cells, which developed optic neuritis and had

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³ Abbreviations used in this paper: EAE, experimental allergic encephalitis; MOG, myelin oligodendrocyte glycoprotein.

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significant mortality. We conclude that treatment of myelin-reactive T cells with Ab and Ag leads to an anergic phenotype even with its full contingent of TCR, whereas a similar reduction in a pathogen-specific T cell's TCR would not affect the cell's response to that high-affinity Ag. This indicates that self-reactive T cells do not have spare receptors but indeed require their full contingent of TCR to respond to their weak cognate ligand.

Materials and Methods

Peptides

MOG₃₅₋₅₅ (MEVGWYRSPFSRVVHLYRNGK) was synthesized by the Microchemical Core Facility at Emory University (Atlanta, GA). Peptides were purified using size exclusion HPLC and analyzed for purity by mass spectrometry.

Mice

2D2 transgenic mice, on a C57BL/6 background, have V α 3.2/V β 11 TCR with specificity for the MOG₃₅₋₅₅ peptide (12). These mice were a gift from V. Kuchroo (Harvard University, Boston, MA). C α ^{-/-} (B6.129S2-Tcr^{tm1Mom}/J) and SCID (*B6.CB17-Prkdc^{scid}/SzJ*; The Jackson Laboratory) female mice were used as adoptive transfer recipients in these experiments. All mice were housed at Emory University Department of Animal Resources facility according to approved Institutional Animal Care and Use Committee protocols. Mice were used at 5–8 wk of age.

Cells and reagents

For the generation of 2D2 cell lines, 2.5 × 10⁶ splenocytes were cultured in 24-well flat-bottom plates with 1 μM MOG₃₅₋₅₅ and 1 pg/ml IL-2 for 7 days. Culture medium was composed of RPMI 1640 medium supplemented with 10% FBS (Mediatech), 2 mM L-glutamine, 0.01 M HEPES buffer, 100 μg/ml gentamicin (Mediatech), and 2 × 10⁻⁵ M 2-mercaptoethanol (Sigma-Aldrich). Supernatants from the B cell hybridoma RR3-16 were harvested, and anti-V α 3.2 Ab was purified on an IgG column (protein A column; Amersham Biosciences) (13). Anti-V α 3.2 Ab Fab fragments were produced using an Immunosorbent Fab preparation kit (Pierce). FACS wash consisted of PBS, 0.05% sodium azide, and 0.1% BSA.

Blocking assay

In all experiments, live cells were purified over a Ficoll gradient and counted, and viability was assessed in trypan blue. Activated 2D2 T cells were Fc blocked, and continuously incubated with or pretreated for 1 h with anti-V α 3.2 Ab or Fab fragments in a 96-well flat-bottom plate. Irradiated C57BL/6 splenocytes (5 × 10⁵ cells, 3000 rads) and the indicated concentration of peptide were added. For proliferation assay, after 48 h in culture in a 96-well plate cells were labeled with 0.4 μCi/well [³H]thymidine, and 18 h later cells were harvested on a FilterMate harvester and counted on a Perkin Elmer 1450 LSC and Luminescence Counter Microbeta Trilux. Activated 2D2 T cells were also incubated for 7 days continuously with or pretreated with anti-V α 3.2 Ab, 1 μM MOG₃₅₋₅₅, and irradiated syngeneic splenocytes. Cells were then separated on a Ficoll gradient and re-stimulated in a proliferation assay as described above.

TCR quantitation

TCR expression levels were enumerated as previously described (3, 6). In brief, Quantum FITC-labeled beads (13,000–350,000 molecules of equivalent soluble fluorophores; Bangs Laboratories) were used as a standard to quantify the absolute number of 2D2 V α 3.2^T receptors. Activated 2D2 T cells were pretreated with anti-V α 3.2 Ab for 1 h at 37°C and then surface stained with anti-V α 3.2 FITC, or pretreated or continuously incubated with anti-V α 3.2 Ab for 7 days with irradiated syngeneic splenocytes and 1 μM MOG₃₅₋₅₅ before being surface stained with anti-V α 3.2 FITC. Flow cytometry was performed on a BD Biosciences FACSCalibur. The data were analyzed using the platform function of FlowJo software.

IL-2 ELISA

Activated 2D2 T cells were incubated in a 24-well plate with the indicated concentrations of peptide, irradiated syngeneic splenocytes, and anti-V α 3.2 Ab as described. After 24 h, supernatants were removed and placed on microtiter plates coated with purified anti-IL-2 (5 μg/ml clone JES6-1A12; BD Pharmingen) overnight at 4°C. Recombinant IL-2 (BD Pharmingen) was used as a standard. Captured cytokines were detected using biotinylated anti-IL-2 (100 μg/ml JES6-5H4, 100 μl per well; BD Pharmingen)

and detected using alkaline phosphatase-conjugated avidin (Sigma-Aldrich) and *p*-nitrophenyl phosphate substrate (Bio-Rad). Colorimetric change was measured at dual wavelengths of 405 and 630 nm on a Microplate Autoreader (Bio-Tek Instruments).

Induction and assessment of EAE and optic neuritis

We incubated 3 × 10⁴ activated 2D2 T cells for 7 days continuously with 2 μg of anti-V α 3.2 Ab, 1 μM MOG₃₅₋₅₅, and irradiated syngeneic splenocytes. Forty-eight hours after the last stimulation, live cells were purified over a Ficoll gradient and counted, and viability was assessed in trypan blue. Naive female C α ^{-/-} or SCID mice were injected i.v. with 4 × 10⁶ live cells, as described (14). At the same time as the adoptive transfer, mice received 250 ng of pertussis toxin i.p. and 100 μg of MOG₃₅₋₅₅ emulsified in 1 mg/ml CFA s.c. in the hind flank. Disease severity was monitored according to the following scale: 0, no disease; 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; 5, moribund.

Development of optic neuritis was assessed by similar criteria as reported by Bettelli et al. (12, 15). In brief, mice that presented had eyelid swelling, as well as partial to complete atrophy of the eye.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism (Software for Science). Mean clinical scores were analyzed using the Mann-Whitney non-parametric *t* test. Disease incidence was analyzed using a one-tailed *t* test.

Results

Reduction of TCR expression limits the response of myelin-specific T cells

To determine whether a reduction in TCR expression was important for the response of self-reactive T cells that we hypothesize to have a lower affinity for ligand, we used anti-V α 3.2 Ab to mask the TCR of the 2D2 monoclonal, myelin-specific T cells and then examined the ability of these cells to proliferate to the full agonist MOG₃₅₋₅₅ (Fig. 1). Activated 2D2 T cells that were incubated continuously with the full agonist showed maximal proliferation: in contrast, 2D2 T cells masked with the TCR Ab had a dose-dependent decrease in proliferation with increasing amounts of TCR Ab (Fig. 1A). Because we observed a decrease in proliferation, we wanted to determine whether the Ab induced a decrease in available TCR for the ligand to bind. Addition of as little as 1.25 μg/ml Ab reduced the available TCR by 31% or 8072 TCR (Fig. 2), and resulted in a 25-fold shift in the EC₅₀ value from 0.19 to 5 μM (1.25 μg/ml Ab) (Fig. 2B), as shown by a marked decrease in proliferation of Ab masked 2D2 T cells (Fig. 1A). These data indicate that a slight decrease in TCR molecules can dramatically reduce proliferation to the agonist peptide in self-specific T cells; therefore there are few excess or spare TCR on these myelin-reactive T cells during recognition of MOG₃₅₋₅₅.

We next wanted to determine whether the decrease in TCR and the resultant decrease in proliferation was from Ab continuously binding to the TCR and out-competing the ligand or whether the Ab initially bound to the TCR, reduced the number of TCR, and at the same time also changed the phenotype of the cell to have a lower affinity for ligand. Therefore, 2D2 T cells were pretreated with TCR Ab to address whether the continuous presence of Ab was required to physically mask the receptor (Fig. 1). Continuous and pretreated conditions in the presence of Ag (Fig. 1, A and B) had very similar trends in proliferation; however, the continuous presence of Ab led to a more rapid shift in the dose curve (more potent inhibition of response). The two conditions, at low Ab concentrations, have similar half-maximal responses to the agonist in the pretreated (Ab = 1.25 μg/ml, EC₅₀ = 3 μM) and continuous (Ab = 1.25 μg/ml, EC₅₀ = 5 μM) experiments. With very high amounts of Ab, the continuous treatment produced a greater shift in the EC₅₀ values (Ab = 5.0 μg/ml, EC₅₀ = 70 μM) as compared with the pretreated (Ab = 5.0 μg/ml, EC₅₀ = 10 μM) condition.

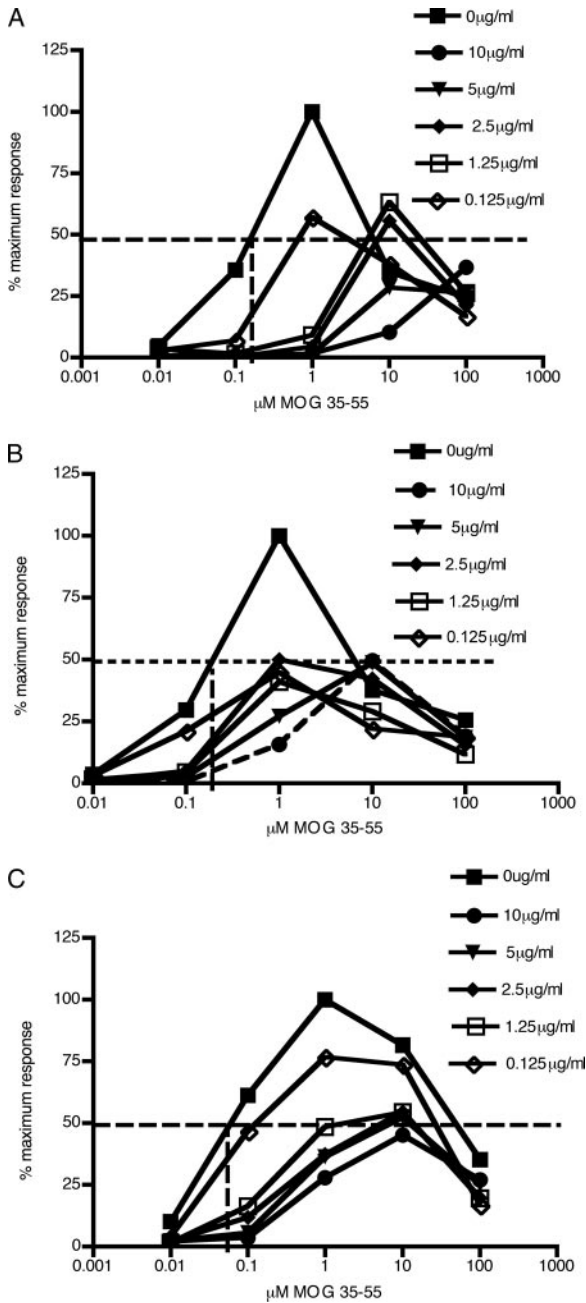


FIGURE 1. Reduction of TCR expression limits the response of myelin-specific T cells. 2D2 splenocytes were passed on 1 μ M MOG₃₅₋₅₅ for 1 wk. Live cells were purified over a Ficolll gradient and counted, and viability was assessed in trypan blue. 2D2 T cells were incubated continuously (A) or pretreated (B) with anti-V α 3.2 Ab and then washed 3 times in 10 ml media, or were incubated with Fab fragments (C). Live cells were then re-challenged with MOG₃₅₋₅₅ and a proliferation assay was performed. These results are representative of at least three separate experiments and shown as percentage of maximal response. Maximal cpm for the above experiments were 47,393, 74,166, and 120,191, for A, B, and C, respectively.

Regardless of these differences, both continuous incubation and pretreatment of TCR Ab with myelin-reactive T cells in the presence of Ag have a dampening effect on the reactivity of these self-specific T cells.

To exclude the possibility of effects via Ab cross-linking on this phenotype, Fab fragments were made out of the anti-V α 3.2 Ab. The anti-V α 3.2 Fab fragments incubated with the 2D2 T

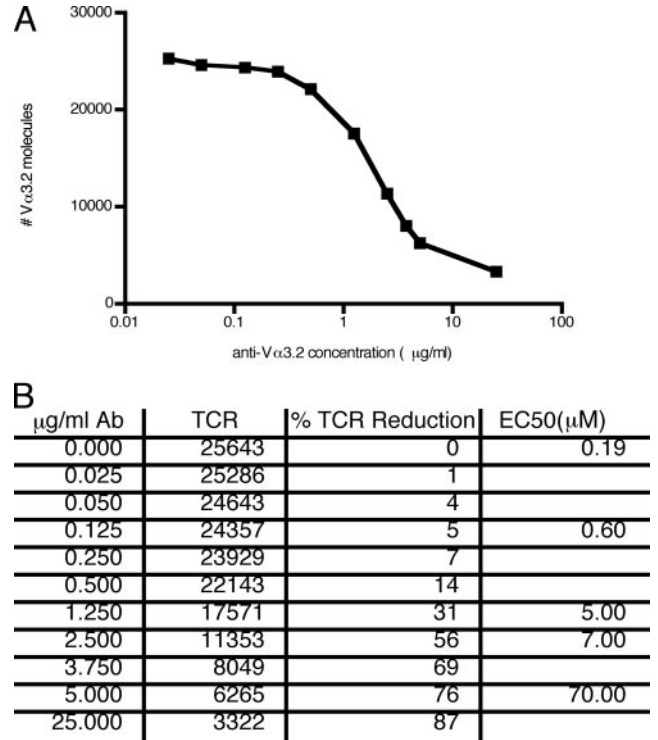


FIGURE 2. A decrease in available receptors greatly reduces the half-maximum response of myelin-specific T cells to the self-peptide. 2D2 splenocytes were passed on 1 μ M MOG₃₅₋₅₅ for 1 wk. Live cells were purified over a Ficolll gradient and counted, and viability was assessed in trypan blue. These cells were then incubated with anti-V α 3.2 Ab. Cells were then surface stained with anti-V α 3.2 Ab. Quantum FITC-labeled beads were used as a standard to quantify the absolute number of 2D2 V α 3.2⁺ receptors. A, A best fit curve of the number of available V α 3.2 molecules on the cell surface after incubation with the Ab. B, A table comparing concentration of Ab, percentage of TCR reduction, and EC₅₀ after 2D2 T cells are incubated with anti-V α 3.2 Ab. These results are representative of three separate experiments.

cells and Ag also had a dose-dependent decrease in proliferation, with results similar to the continuous and pretreated proliferation experiments (Fig. 1C). Therefore, any of the continuous, pretreated, or Fab treatments decreased the levels of available TCR and inhibited the ability of the myelin-reactive T cell to respond to Ag.

Reduction of TCR interaction with the self-peptide leads to a reduction in IL-2 secretion

To further characterize the phenotype of the 2D2 T cells incubated continuously or pretreated with TCR masking Ab, the amount of IL-2 produced after stimulation was assessed. There was a dose-dependent decrease in IL-2 in both culture conditions (Fig. 3); however, 2D2 T cells continuously incubated with Ab had a more dramatic decrease in IL-2 compared with 2D2 T cells pretreated with Ab. These results are consistent with the proliferation assay that showed a more rapid increase in the EC₅₀ of the continuous condition as compared with the pretreated cells (Fig. 1, A and B). 2D2 T cells have a reduced response to agonist upon masking of the TCR, resulting in decreased proliferation and IL-2 secretion that could be classified as a hypoproliferative or anergic phenotype. Therefore, an initial reduction in available TCRs on myelin-specific T cells can induce T cell anergy in the presence of MOG₃₅₋₅₅.

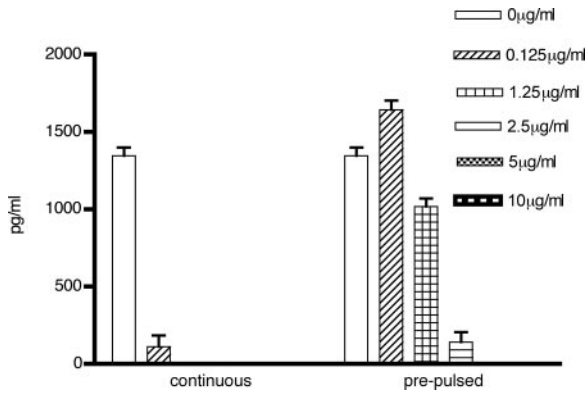


FIGURE 3. Masking the myelin-specific TCR markedly affects IL-2 secretion. 2D2 splenocytes were passed on $1 \mu\text{M}$ MOG₃₅₋₅₅ for 1 wk. Live cells were purified over a Ficoll gradient and counted, and viability was assessed in trypan blue. 2D2 T cells were incubated continuously with anti-V α 3.2 Ab or pretreated with anti-V α 3.2 Ab. Live cells were then challenged with MOG₃₅₋₅₅. After 24 h in culture, cells were stained with the appropriate Ab by ELISA. These results are representative of two separate experiments.

Autoreactive T cells treated with TCR-blocking Ab have a stable phenotype

From our initial data, we wanted to determine the mode of action in this newly described form of T cell anergy. Therefore, activated 2D2 T cells were passed for 7 days on Ab with MOG₃₅₋₅₅ and irradiated syngeneic splenocytes, and the live cells were subsequently re-stimulated on MOG₃₅₋₅₅ (Fig. 4A). Results indicated the TCR Ab, with the ability to induce low but detectable amounts of proliferation in the presence of peptide, rendered MOG₃₅₋₅₅-specific T cells hypoproliferative upon peptide restimulation. Additionally, there was a significant increase in the EC₅₀ upon addition of increasing doses of TCR Ab. For example, the EC₅₀ value with treatment of $2.5 \mu\text{g/ml}$ of Ab was $0.8 \mu\text{M}$; however, there

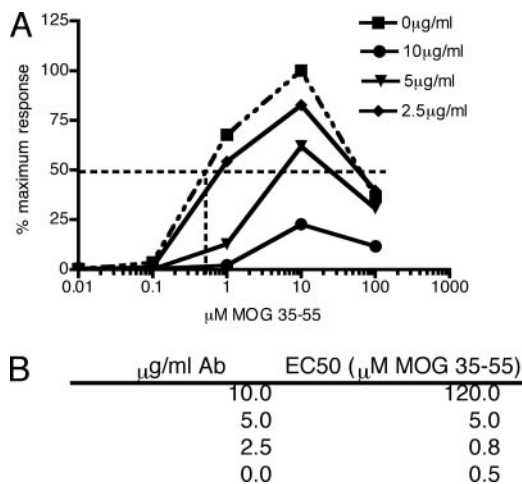


FIGURE 4. Myelin-specific T cells passed on TCR-blocking Ab retain a dramatically reduced response to re-activation with the self-peptide. 2D2 splenocytes were passed on $1 \mu\text{M}$ MOG₃₅₋₅₅ for 1 wk. Live cells were incubated continuously (A) with anti-V α 3.2 Ab, syngeneic irradiated APCs, and $1 \mu\text{M}$ MOG₃₅₋₅₅ for an additional 7 days. Live cells were purified over a Ficoll gradient and counted, and viability was assessed in trypan blue. T cells were then re-challenged with MOG₃₅₋₅₅ and a proliferation assay was performed. These results are representative of two separate experiments. B, A table comparing the concentrations of Ab and EC₅₀ after 2D2 T cells were incubated with anti-V α 3.2 Ab.

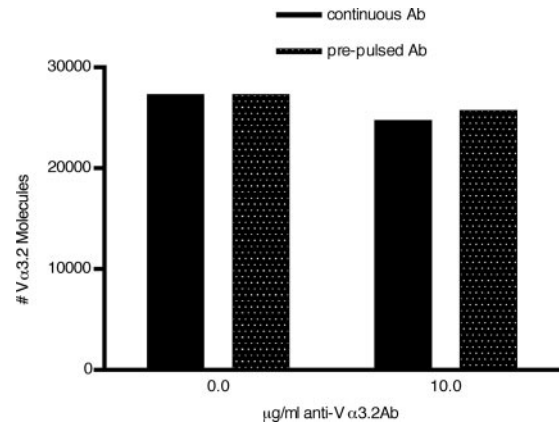


FIGURE 5. Passed myelin reactive T cells reconstitute receptor numbers to basal levels. 2D2 splenocytes were passed on $1 \mu\text{M}$ MOG₃₅₋₅₅ for 1 wk. Live cells were purified over a Ficoll gradient and counted, and viability was assessed in trypan blue. Live cells were incubated continuously with anti-V α 3.2 Ab or pretreated with anti-V α 3.2 Ab, syngeneic irradiated APCs, and $1 \mu\text{M}$ MOG₃₅₋₅₅ for an additional 7 days. Live cells were once again purified and counted, and viability was assessed in trypan blue. Quantum FITC-labeled beads were used as a standard to quantify the absolute number of 2D2 V α 3.2 receptors. This histogram shows the number of available V α 3.2 molecules on the cell surface after incubation with the Ab. These results are representative of two separate experiments.

was a dramatic increase to an EC₅₀ of $100 \mu\text{M}$ upon addition of $10 \mu\text{g/ml}$ Ab. This suggests that incubation of Ab and peptide with the MOG₃₅₋₅₅-specific T cells induces a permanent change in phenotype that is reflected in reduced proliferation and a decreased half-maximal response to the full agonist. Various possibilities for this stable hypoproliferative phenotype include “carry over” Ab, a permanent reduction in TCR, or a permanent change in cell signaling (anergy).

We next determined whether the hypoproliferative phenotype was induced by a lack of TCR to respond to agonist or, as in the case of an anergic phenotype, the TCR masking Ab altered the activation state of the T cell, such that the receptor now has a reduced ability to respond to the agonist. After 7 days on TCR masking Ab, 2D2 T cell TCR levels were quantified. As shown in Fig. 5, TCR levels in the 2D2 T cells treated with TCR Ab returned to basal levels, yet the cells failed to proliferate at maximal levels. Taken together, these results suggest these hypoproliferative T cells still recognize the agonist but now respond to the peptide as a weak agonist.

Anti-TCR treatment mediates protection from EAE

Because 2D2 T cells treated with TCR Ab were hypoproliferative in vitro, the ability of these cells to mediate EAE in vivo was assessed (Fig. 6A). 2D2 T cells were activated with MOG₃₅₋₅₅ for 7 days. The live T cells were then treated with anti-V α 3.2 Ab and stimulated with MOG₃₅₋₅₅ for 7 days. Following a 48-h restimulation with MOG₃₅₋₅₅, cells were adoptively transferred into T cell-deficient recipient mice to assess the ability of these cells to induce EAE. Mice were also given one injection of MOG₃₅₋₅₅ in CFA and pertussis toxin. These mice were scored for clinical signs of EAE and optic neuritis. Previously, 2D2 transgenic mice challenged with MOG₃₅₋₅₅ and given pertussis toxin developed optic neuritis in addition to EAE, and had higher mortality compared with non-transgenic mice challenged with the same protocol (12, 15). In our adoptive transfer model into $\text{C}\alpha^{-/-}$ hosts, we observed optic neuritis in 50% of the mice receiving untreated 2D2 cells.

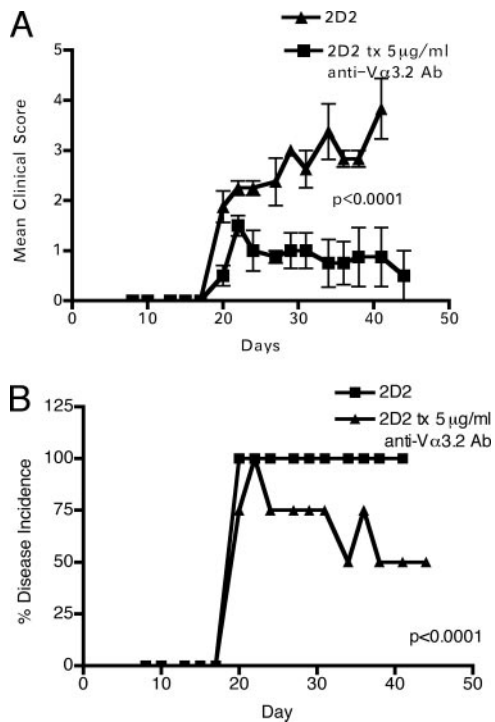


FIGURE 6. Reduced disease severity of myelin reactive T cells masked with TCR-blocking Ab upon adoptive transfer. Naive $C\alpha^{-/-}$ mice received 2D2 ($n = 8$) or 2D2/anti-V α 3.2Ab ($n = 6$) treated T cells (4×10^6), pertussis toxin, and MOG_{35–55} in CFA. Mice were then scored for disease induction of EAE. Recipients of 2D2/anti-V α 3.2Ab treated T cells exhibited a significant reduction ($p < 0.0001$) in disease severity (A), but a similar disease onset (B) compared with mice that received 2D2 T cells not masked with Ab. These results are representative of two separate experiments.

These mice rapidly progressed to a severe disease phenotype resulting in mortality or cessation of the study (score >4). In contrast to the progressive paralysis described above, mice receiving 2D2 T cells treated with TCR Ab and MOG_{35–55} to induce anergy had an average cumulative clinical EAE score of 9.12 ± 1.75 compared with mice that received untreated 2D2 cells, which had an average cumulative clinical score of 28.6 ± 3.4 . These two treatments were statistically different ($p < 0.0001$). Mice receiving these hypoproliferative T cells showed, at most, clinical signs of hind limb weakness compared with mice that received normal 2D2 T cells, which showed almost immediate onset of hind limb and forelimb paralysis, as well as death within 14 days of disease onset. These mice also had a complete absence of optic neuritis as compared with a 50% incidence in mice receiving untreated 2D2 T cells. Disease onset in both treatments coincided (Fig. 6B); however, disease incidence was a significant 25–50% lower in recipient mice receiving Ab-treated cells ($p < 0.0001$). Together, these data demonstrate that autoreactive T cells require the maximal number of TCR to mediate disease. Therefore, lessening Ag recognition by decreasing the availability of TCR results in an anergic phenotype.

Discussion

In this study, we explored whether self-reactive T cells, which may have a lower affinity for their ligands, need all or most of the available TCR to mount a response. Most T cells have an average of 20–30,000 TCR, yet at most a few thousand ($<10\%$) of available TCRs are needed to recognize Ag (2). Our previous work found that a 95% TCR reduction did not adversely affect the re-

sponse of foreign Ag-specific T cells (3); however, these excess TCR were absolutely needed for response to weaker stimuli. For example, we observed that a weak agonist required expression of at least 40% of available TCR for a response, compared with the full agonist, which needed only a small fraction of these TCRs (3, 6). Because autoreactive T cells may have a significantly lower affinity for receptor compared with pathogenic T cells, we hypothesized that reduction in TCRs, or overall signal strength, on autoreactive T cells would result in a loss of response to self-peptide. We found that reductions in TCR availability caused by Ab treatment resulted in a decrease of TCR levels and proliferation (Fig. 1). Additionally, the continuous physical presence of the TCR-blocking Ab was not necessary for this effect. Rather, initial binding of the TCR by the blocking Ab in the presence of MOG peptide was sufficient to permanently reduce proliferation (Fig. 1).

As discussed previously, the spare receptor theory posits that a strong agonist requires only a small amount of receptors, or TCR in this case, to achieve a maximal response (5); however, we found that decreasing available TCR on autoreactive T cells dramatically affects their proliferation to self-Ag (Fig. 1). Therefore, our self-Ag, MOG_{35–55}, seems to be acting as a weak ligand, which may indicate that a stronger ligand, which requires fewer receptors for response, could exist for this T cell as well as other self-reactive T cells. In fact, several superagonists for encephalitogenic and diabetogenic T cells have been identified using either single amino acid substitutions of their parent peptide or combinatorial peptide libraries (mimotopes) (16–19). We believe that self-reactive T cells are amenable for identification of superagonists because they use such a high percentage of their available TCRs for activation and should therefore be of lower affinity from the outset. In contrast, model foreign Ag or pathogenic T cells require very few of their available TCRs for activation, and one would therefore be less likely to identify a superagonist for those cells, because TCR use equates to Ag potency.

Our results indicate that a reduction of available TCRs using specific Abs in autoreactive T cells leads to a phenotypic change. Previously, Abs to CD3 have been used as treatments in models of diabetes and transplantation and proven efficacious at regulating the unwanted T cell responses (20, 21). There have even been reports of the use of anti-CD3 or anti-TCR in multiple sclerosis patients and EAE models, although with somewhat less impressive results (22–24). Many of these preliminary Ab treatment trials suffered some setbacks as a result of the initial CD3 Abs (OKT3 in humans and 145-2C11 in mice) being potent activators, causing rapid activation and deletion of all T cells. More recent studies have made use of non-activating forms of the Abs, which avoid the massive side effects of T cell activation but still rely on the deletion of T cells before their repopulation (20, 21). Importantly, our proposed modulation of TCR levels does not depend on global depletion of T cells, but instead results from a hyporesponsive state.

Upon Ag re-challenge with the agonist peptide, we found that MOG-specific T cells masked with TCR Ab had a dose-dependent decrease in IL-2 production compared with untreated control T cells (Fig. 3). The cause of the reduced response was not due to a long-term “carry over” of Ab (Fig. 4) or a reduction in TCR, because MOG-specific T cells treated 7 days previously with TCR-blocking Ab were found to have the same absolute number of TCRs as unmanipulated MOG-specific T cells. Hence, the hypoproliferative, Ab-treated T cells had a normal basal number of TCR, indicating that the changes may have occurred in receptor signaling. Several studies have previously demonstrated the induction of T cell anergy using other methods to partially stimulate T cells or prevent a complete T cell response. These have included costimulation blockade (25–28), low doses of peptide (29–32),

altered peptide ligands (33, 34), and MHC variant peptides (35, 36). Although all these methods result in a T cell hyporesponsive state, the signaling mechanisms behind these effects vary. We are currently investigating the signaling pathways following Ab mediated TCR-reduction to identify potential therapeutic targets.

To further the possibility of treatment for autoimmune diseases using TCR-blocking Ab, we wanted to determine whether our hypoproliferative T cells could still mediate EAE *in vivo*. To this end, the monoclonal myelin-specific T cells were anergized with Ab and peptide and adoptively transferred into naive recipient mice lacking endogenous T cells. These mice were also given MOG_{35–55} peptide emulsified in CFA and pertussis toxin at the time of transfer. Our results demonstrate that the hypoproliferative T cells induced a very mild form of EAE compared with mice that received untreated T cells. Recipients that received untreated T cells had optic neuritis, rapid progression of EAE symptoms, and significant mortality as compared with those given Ab-treated cells, which had no mortality.

In conclusion, this hyporesponsive phenotype of the Ab-treated MOG-specific T cells leads to a reduction in disease severity in our EAE model. Although these experiments using a monoclonal transgenic model provide the basis of the concept that manipulations of TCR levels can decrease the efficacy of autoimmune T cells, future experiments will focus on the role of cell signaling in anti-TCR Ab induction of anergy to discern whether this phenotype is an alteration in signaling proteins, signaling pathways, or threshold of activation that can be exploited as a treatment. Notably, altering TCR expression may provide a potential therapeutic window, that one can use to differentiate foreign Ag-specific T cells from self-reactive T cells and allow for inhibition of autoimmunity without inducing global immune suppression.

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Disclosures

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