Monoclonal Antibody against T-Cell Receptor $\alpha\beta$ Induces Self-Tolerance in Chronic Experimental Autoimmune Encephalomyelitis

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Abstract

The therapeutic effect of monoclonal antibody (H57-597 MoAb) against T-cell receptor (TCR) $\alpha\beta$ has been investigated on MOG$_{35-55}$-induced experimental autoimmune encephalomyelitis (EAE), as a model system for T-cell-mediated chronic inflammation in the central nervous system (CNS). Short-term administration of the anti-TCR $\alpha\beta$ immediately after immunization protected the mice from EAE. Furthermore, anti-TCR $\alpha\beta$ treatment on an established disease restored the self-tolerance which led to a complete remission of EAE and a dramatic reduction of inflammatory cells in the CNS, while treatment with control antibody (hamster IgG) was ineffective. The remission was durable and not associated with disappearance of autoreactive T cells as measured by persistence of MOG-reactive T-cell proliferation in vitro. However, MOG-reactive T cells from anti-TCR-treated animals produced significantly lower amounts of inflammatory TNF-$\alpha$ and IFN-$\gamma$. In addition, while a transient deletion of CD4$^+$ and CD8$^+$ T cells was observed, a population of T cells expressing CD3, NK1.1 and CD69 (NKT cells) were expanding. By transfer of spleen cells from anti-TCR MoAb-treated animals, we could show that the tolerogenic capacity can be transferred to untreated recipients with EAE. The data indicate therapeutic effect of anti-TCR $\alpha\beta$ MoAb (H57-597), which represents a promising approach in treatment of T-cell-mediated autoimmune diseases.

Introduction

The objective of this study was to determine if anti-T-cell receptor (TCR) $\alpha\beta$ treatment has the capacity to inhibit the development of the disease in experimental autoimmune encephalomyelitis (EAE) when given early in the course of the disease.

The components of the immune system that are important for initiation of an immune response are becoming well understood but less is known about how immune and inflammatory reactions once started are regulated. Strategies used successfully for modulating the initiation of immune responses have had variable success when applied therapeutically. Exceptions to this rule are findings with antibodies directed to the TCR complex, where dramatic therapeutic activity has been demonstrated in several cases. Anti-CD3 antibody induces a long-term remission of diabetes in NOD mice [1, 2]; antibody to disease-associated V$\beta$ region is therapeutic in EAE [3]; antibody to TCR $\alpha\beta$ is therapeutic in acute EAE [4] and antibody to TCR $\alpha\beta$ is therapeutic in models for arthritis [5–7]. The effects have generally been attributed to the physical elimination of T cells. However, other possibilities exist and there are examples where the treatment has been successful in the absence of T-cell elimination. In most cases, such treatments have met with little success when applied therapeutically. Exceptions to this rule are findings with antibodies directed to the TCR complex, where dramatic therapeutic activity has been demonstrated in several cases. Anti-CD3 antibody induces a long-term remission of diabetes in NOD mice [1, 2]; antibody to disease-associated V$\beta$ region is therapeutic in EAE [3]; antibody to TCR $\alpha\beta$ is therapeutic in acute EAE [4] and antibody to TCR $\alpha\beta$ is therapeutic in models for arthritis [5–7]. The effects have mostly been related to general T-cell depletion or functional blockade of T cells involved in inflammation. However, there are several findings in these studies indicating that this may not be the case. Studies in the diabetes model suggest that signalling through the TCR is critical for the effect [2]. In the collagen-induced arthritis model, therapeutic action did not
correlate to T-cell elimination [8]. In fact, it was shown in this model that anti-TCR αβ treatment resulted in a strong antibody response to the injected antibody, suggesting activation rather than deletion of T cells [5].

The aim of the current study was to investigate the therapeutic effect of anti-TCR MoAb in a chronic model for autoimmune disease. We show how anti-TCR αβ treatment in chronic EAE was tolerogenic. We also show that the underlying mechanism behind the anti-TCR-induced restoration of self-tolerance was a transferable T-cell-mediated tolerance involving an NKT-cell population.

Materials and methods

Animals. C57BL/6 female mice, 8–9 weeks old, were purchased from Bommece (Bomhult Gård Breeding and Research Center, Ry, Denmark). The mice were fed a standard diet and allowed to acclimatize for at least 1 week before the experiments. All the animals were marked, randomly mixed in the cages and scored by investigators in a blinded fashion. Animal experiments followed the European Community regulations for animal experiments and were approved by the regional animal ethics committee.

EAE model. Chronic EAE was induced in the mice according to Mendel et al. [9] with some modifications. An encephalogenic peptide from myelin oligodendrocyte glycoprotein (MOG) was used to induce EAE. The synthetic peptide, amino acids 35–55 (MEVGWYRSPFSRVVHL YRNGK–COOH), with a purity of 98%, was purchased from Ake Engstrom (The Institute of Medical Biochemistry and Microbiology, Uppsala, Sweden).

The mice were immunized with an emulsion containing 100 μg peptide in complete Freund’s adjuvant H37RA (Difco laboratories, Detroit, MI, USA). The immunization was done under enflurane anaesthesia by an intradermal injection at the base of the tail with 0.1 ml of the emulsion. Immediately following and 48 h after immunization, the mice were given an intraperitoneal injection of 0.1 ml of 4 μg/ml pertussis toxin (ICN Biochemicals Inc., Aurora, OH, USA). The mice were weighed and examined for clinical signs of EAE daily until the last day of treatment and afterwards 6 days/week throughout the experiment. The signs of EAE were scored into eight categories: 0 = no signs of clinical disease, 1 = weakness in the tail, 2 = paralysed tail, 3 = paresis and gait disturbance, 4 = paralysis of one limb, 5 = paralysis of two limbs, 6 = two limbs paralysed and paresis of a third limb, the mouse still able to move forward, 7 = quadriplegia, no mobility and moribund state, 8 = dead. Food was placed on the cage floor when any mouse showed a grade 5 (or higher) clinical sign of disease. Mice that scored 6 were given, once a day, 0.5 ml of physiological saline solution subcutaneously, to avoid dehydration. When a mouse scored 7, it was killed for ethical reasons. At the end of the experiments, animals were killed by carbon dioxide inhalation and different organs were dissected.

MoAb treatment. Hybridoma cells producing hamster MoAb against mouse TCR αβ (H57-597) were purchased from American Type Culture Collection, and were cultured in serum-free medium. The MoAb was purified on a Hi-Trap protein G column (Amersham Pharmacia Biotech, Solna, Sweden). The antibody was tested for the presence of endotoxin with the E-TOXATE® kit (Sigma, St. Louis, MO, USA) and contained less than 2 U in 0.5 mg (MoAb)/ml.

For inhibition of the development of EAE, the MoAb were administrated on day 0, one hour prior to immunization with MOG, 1 and 2. For the treatment of ongoing EAE, MoAb were administrated individually for three consecutive days, starting 24 h after the mouse had been scored 3 or higher clinical signs of EAE. Control mice received an identical amount of purified normal hamster IgG (Jackson’s Immunoresearch, West Grove, PA, USA). Another group of animals induced for EAE remained untreated to exclude the possible side-effects of stress caused by injections, or immunoglobulins, on disease development. Each group contained six to 10 mice.

Cell transfer studies. Mice with developed EAE were treated with either anti-TCR or hamster IgG as described above. Splenocytes were collected from both groups. Single-cell suspensions were prepared and washed extensively. Erythrocyte-free splenocytes (20 × 10⁶) were then administered intravenously (i.v.) into recipients (C57BL/6 female mice, 8–9 weeks old). EAE was induced 24 h later by immunization with MOG35–55. The animals were handled and observed for clinical signs of EAE as mentioned above.

T-cell proliferative response. Splenocytes from three animals per group were isolated for in vitro analysis. The animals were selected randomly with clinical signs of EAE (control groups), no signs of EAE (mice prophylactically treated with anti-TCR αβ MoAb) and totally recovered from EAE (mice therapeutically treated with anti-TCR αβ MoAb). Spleen cells were cultured in DMEM with Glutamax-1 (Life Technologies, Taby, Sweden) supplemented with 10 mM HEPES buffer, 10% heat-inactivated FCS, 0.16 mM penicillin, 0.03 mM streptomycin, and 50 μM 2-mercaptoethanol; from now on, this will be called cDMEM. The cells were stimulated with purified protein derivative (PPD) from Mycobacterium tuberculosis (Statens Serum Institut, Copenhagen, Denmark) at 10 μg/ml and MOG35–55 peptide at 6–50 μg/ml. Spleen cells were cultured in complete medium (cDMEM) at a concentration of 5 × 10⁶ cells in 200 μl, at a humidified
atmosphere containing 7.5% CO₂ at 37 °C. Cells were stimulated in triplicates with MOG₃₅₋₅₅ to investigate the Ag-specific response where PPD was used as positive control, and medium as negative control. The cells were cultured for 72 h with additional 16 h after addition of 1 μCi of [³H]thymidine. Cells were then harvested and counts per minute (CPM) were determined by a gas-flow beta counter (Matrix 96 Direct beta counter; Packard, Meriden, CT, USA).

ELISA for cytokines. To analyse the cytokine production, supernatant from spleen cell cultures were collected at different time points (24, 48, and 72 h) after in vitro challenge with 50 μg/ml MOG₃₅₋₅₅ peptide (as described above). Supernatants from triplicates were pooled and assayed in a classical sandwich ELISA. The following capture antibodies were used: anti-IFN-γ, R6A2 (5 μg/ml), anti-IL-4, 11B11 (2 μg/ml) and anti-TNF-α, (6 μg/ml) (all from BD Biosciences). The following detection biotinylated antibodies were used: anti-IFN-γ, Ani8 (0.6 μg/ml) and anti-TNF-α MP6-XT3 (1 μg/ml) (all from BD Biosciences). Anti-IL-4, BVD6-24.G2 (0.5 μg/ml) was used from our hybridoma collection. Briefly, FluoroNunc Maxisorp plates (Nunc, Roskilde, Denmark) were coated with antibodies as described above, and after washing, the plates were incubated with 10% FCS in PBS to block unspecific binding. Subsequently, samples or recombinant cytokines (BD PharMingen) were added and left at 4 °C overnight, and after washing, the plates were incubated with detection antibodies. Finally, the plates were incubated with europium-labelled streptavidin followed by enhancement buffer according to the manufacturer’s instructions and measured for fluorescence intensity (Wallac Oy, Turku, Finland). The cytokine content in supernatants was determined when data were within the linear region of the standard curve calculated from values of the recombinant cytokines.

Flow cytometric analysis (FACS). Cells were incubated with anti-CD16/CD32 (clone: 2.4G2) to block Fcγ III/II receptors and reduce non-specific labelling of cells. For detection of surface markers, the following biotinylated, FITC- or PE-labelled MoAb (all purchased from BD Biosciences) against CD4 (clone: RM4–5), CD8 (clone: 53–6.7), CD3 (clone: 145–2C11), NK1.1 (clone: PK136) and CD69 (clone: H1.2F3) were used. Streptavidin-Per CP (Becton Dickinson, Mountain View, CA, USA) was used to detect biotinylated antibody. Cells were analysed on a FACSort (Becton Dickinson) and a viable gate was used to exclude dead and fragmented cells.

Immunohistochemistry. After sacrificing, cerebrum, and cerebellum were dissected out from mice and divided into two halves by a medial/sagittal cut, and snap-frozen in isopentan pre-chilled in liquid nitrogen. A mid-section was collected from the lumbar region of spinal cord. The tissues were then stored in −70 °C freezer. Cryosections (sagittal) were prepared containing both cerebellum and cerebrum. After fixation in acetone, the sections were blocked for endogenous avidin/biotin activity (Vector, Burlingame, CA, USA) and incubated with the primary antibody, rat anti-mouse CD4 (BD Biosciences), at a concentration of 5 μg/ml and were incubated for 1 h. The secondary antibody, a biotinylated goat anti-rat IgG (Jackson’s Immunoresearch, West Grove, PA, USA) was used at 4 μg/ml and was applied for 30 min. Three washes with 0.1 M Tris/0.15 M NaCl pH 7.0 were carried out between all steps. After incubation in streptavidin–biotin/peroxidase (Dako, Copenhagen, Denmark) for 30 min, the immunoreaction was developed in diaminobenzidine (Saveen, Malmö, Sweden) and the sections were counterstained in methyl green. The degree of leukocyte infiltration was calculated by using a PC-based image analysis system (Leica Q500, Cambridge, UK). With this equipment, the area of stained ‘positive’ cells was measured and compared with the entire analysed area.

Statistical evaluation. Statistical evaluation was performed using StatView software (SAS Institute, Cary, NC, USA). For analysing differences in clinical scores, Mann–Whitney tests were used. When analysing differences in cytokine production, T-cell proliferation, and cell infiltration in central nervous system (CNS), Student’s unpaired t-tests were used.

Results

Protective effect of anti-TCR zβ treatment on EAE

A short-term treatment with anti-TCR zβ MoAb applied to mice immediately after the immunization completely inhibited the development of EAE. Briefly, the female mice received i.p. treatment with either TCR zβ MoAb for three consecutive days or an identical dose of normal hamster Ig. The induction of EAE was completely blocked by anti-TCR treatment given from the day of immunization (Fig. 1A). The animals were observed for clinical signs of EAE 48 days after immunization. No signs of the disease were noticed in animals treated with anti-TCR.

To investigate the protective effect of anti-TCR zβ on antigen-driven T-cell activation, the cellular infiltration into the brain and spinal cord of the treated mice was examined by immunohistochemistry. Treatment of mice with anti-TCR zβ prevented the appearance of inflammatory lesions in the brain and the spinal cord (data not shown).

Effects of anti-TCR zβ on spleen CD4⁺ and CD8⁺ T cells

Treatment with the anti-TCR MoAb led to a transient depletion of both CD4⁺ and CD8⁺ T cells in the spleen (Fig. 1B). Disappearance of CD4⁺ T cells was shown in
mice treated with anti-TCR αβ already 7 days after the last injection. At this time, however, a considerable higher proportion of CD8+ T cells were seen, which showed a delay in both maximum deletion, up to 16 days, and recovery. More than 90% of CD4+ T cells were recovered 35 days after the last injection anti-TCR MoAb injection, whereas up to 70% of the CD8+ T cells were retrieved. Collectively, these results indicate that, anti-TCR MoAb inhibit induction of EAE by temporary depleting autoreactive T cells.

Despite the fact that the CD4+ and CD8+ T-cell subsets both express TCR αβ on their surface, they respond differently to anti-TCR treatment in vivo. The therapeutic effect of anti-TCR on EAE

Experimental autoimmune encephalomyelitis-affected animals were treated individually 24 h after they reached score 3 or higher, for 3-day period with 100 µg anti-TCR αβ MoAb/day (n = 12) or an irrelevant hamster Ig (n = 12) while a third control group remained untreated (n = 10) before induction of EAE (A). The figure shows average clinical score (0–8) during 48 days. A kinetic flow cytometric analysis of proportions of CD4+ (B) or CD8+ (C) T cells in spleen at days 7, 16 and 35 after treatment with TCR αβ MoAb or an irrelevant control antibody hamster Ig. The figures show pooled result from two different experiments with balanced groups; error bars represent SEM of these experiments.

Therapeutic effects of anti-TCR on EAE

Experimental autoimmune encephalomyelitis-affected animals were treated individually 24 h after they reached a score of 3 (paralysed tail, paresis and gait disturbance) or higher, by administration of anti-TCR αβ for three consecutive days. An identical dose of normal hamster Ig was used in control EAE-mice, while a third group of animals induced for EAE remained untreated. Treatment with anti-TCR αβ suppressed chronic EAE completely (Fig. 2A). The therapeutic effect of TCR-specific treatment was established rapidly (5–7 day after the last MoAb injection). Observing the animals for a period of 36 days showed no sign of the disease in anti-TCR-treated animals after recovery.
A transient body weight loss (data not shown) was also observed in anti-TCR-treated animals. EAE in hamster Ig-treated mice was slightly suppressed compared with that in untreated mice.

A kinetic flow cytometric analysis at days 3, 7, respective 17 after the first MoAb treatment, revealed a transient depletion of T lymphocytes including CD4+ T cells in the spleens of anti-TCR-treated mice (Fig. 2B).

**Dose-dependent effect of anti-TCR αβ**

Anti-TCR αβ MoAb exhibited a dose-dependent therapeutic effect with a minimally effective concentration of 100 μg of MoAb with repeated i.p. administration to mice with established EAE. The EAE mice receiving 100 respective 300 μg of TCR-specific MoAb per day were totally restored from disease (six of six treated animals), while lower doses of 10, respective 30 μg/day treatment had no or only partial restoration (one to two of five treated animals).

**Anti-TCR αβ treatment reduced inflammatory cells in CNS**

Twenty days after the last treatment, sections of the brain and the spinal cord from mice with EAE were stained for inflammatory cells and when analysed for the presence of inflammatory CD4+ T cells, the main infiltration was found in cerebellum (Fig. 3A and C). A significant decrease of all inflammatory cells, including the disease inducing CD4+ lymphocytes was seen in brain and spinal cord of mice treated with anti-TCR αβ (Fig. 3B and D). In the CNS of these animals, no or very few infiltrating cells were recorded. These data demonstrated that TCR-specific antibody treatment resulted in a strong reduction of inflammatory cells in CNS which remained for at least 20 days after therapy.

**Effects of anti-TCR αβ treatment on autoreactive T cells**

Spleens were collected 20 days after the therapy and spleen cells were assayed for in vitro proliferation against the disease-inducing MOG35–55 peptide. MOG-specific responses were detected in all groups and no difference could be detected between anti-TCR αβ and hamster Ig-treated control animals, despite the fact that anti-TCR-treated animals were totally recovered from EAE (Fig. 4A). The in vitro proliferation to PPD, as a positive control showed no difference between groups (data not shown).

Supernatants from antigen-stimulated spleen cell cultures were analysed after 24, 48, and 72 h of in vitro stimulation for cytokine production using an ELISA. Spleen cells from anti-TCR αβ-treated mice produced significantly lower amounts of inflammatory TNF-α and IFN-γ compared with cells from hamster Ig-treated animals (Fig. 4B and C). Spleen cells from anti-TCR αβ-treated animals also produced significantly lower concentrations of IL-4 (Fig. 4D).

**Tolerance can be transferred from anti-TCR αβ-treated mice**

Significant suppression of EAE in recipient mice was obtained by transferring splenocytes from EAE animals treated with anti-TCR αβ (Fig. 5A). As shown in the figure, the tolerogenic capacity of spleen cells from anti-TCR αβ-treated mice inhibited progression of EAE, whereas cells from hamster Ig-treated EAE mice showed no suppressive effect but rather an enhancement of the
disease development. MOG-induced chronic EAE was slightly suppressed in all immunized mice after day 21 (Figs 1A and 2A), but mice receiving splenocytes from hamster Ig-treated EAE mice developed a more aggressive disease after this date.

**CD3**<sup>+</sup> NK1.1<sup>+</sup> lymphocytes in antibody-treated mice

To investigate the mechanisms underlying the in vivo effects of anti-TCR αβ, we examined different T-cell populations in spleens collected from treated animals 20 days after treatment using flow cytometry. Interestingly, an expansion of CD3<sup>+</sup> T cells expressing NK1.1 was observed (Fig. 5B). Also, co-expression of CD69 was present on the NK1.1<sup>+</sup> T cells.

**Discussion**

A principal theme in autoimmunity is the breakdown of central tolerance resulting in the persistence and activation of autoreactive T cells [10]. As CD4<sup>+</sup> T cells are key contributors responsible for the onset and progression of multiple sclerosis (MS), they have been used as a target for therapeutic interventions. Various approaches have been designed to block autoreactive CD4<sup>+</sup> T-cell function by targeting different cell surface proteins [11–13]. These treatment strategies often resulted in either non-specific immune suppression or other undesirable side-effects. The TCR has been an interesting target for CD4<sup>+</sup> T-cell-specific therapy. It is already known that prophylactic treatment with antibodies against TCR blocks the development of disease by eliminating a large proportion of the T cells [3, 7]. Depletion of disease-related Vβ2 T cells by using monoclonal antibodies has been therapeutic in mice, but showed less success when tested in humans [14]. These data show a need for a better understanding of the in vivo mechanism of action of anti-TCR antibodies, which could help to create a therapy targeting a broad spectrum of T cells.

In our approach, the immunosuppressive and therapeutic capacities of anti-TCR αβ MoAb (H57-597), with specificity for a general TCR αβ framework, were evaluated in an autoimmune model for T-cell-mediated chronic inflammation in the central nervous system (EAE). The protective activity of anti-TCR antibodies on acute EAE has already been reported where Vβ-specific antibodies showed only a partial protection in mice [3], and not long lasting in rats when administered before the immunization [4]. In the present...
study, a complete and durable protection from EAE was achieved by administration of anti-TCR αβ immediately after the immunization. In fact, anti-TCR αβ not only prevented the development of EAE completely but also abolished the infiltration of inflammatory cells into the CNS. A transient depletion of T cells was observed, and more than 90% of the CD4+ T cells were restored 35 days after the treatment. Moreover, the persistence of the autoreactive T cells in the protected animals argues for induction of a regulatory mechanism suppressing their effector function.

The immunosuppressive capacity of anti-TCR αβ MoAb (H57-597) was further examined to induce tolerance in animals with established EAE. Diseased mice treated with short-term administration of anti-TCR MoAb recovered completely from signs of EAE shortly after the treatment. TCR-specific antibody treatment induced a transient depletion of T cells. The majority of the T cells were restored 17 days after the treatment but the tolerance against autoreactive T cells remained. Depletion of CD8+ T cells was delayed compared with CD4+ T cells, indicating a distinct in vitro mechanism of action of this antibody on different T-cell populations. The in vitro proliferation of splenocytes from the anti-TCR αβ-treated animals showed a clear MOG peptide reactivity. Immunohistochemical analysis revealed a significantly lower inflammation in the CNS of anti-TCR-treated animals compared with control mice. Thus, these data showed that anti-TCR treatment not only depletes the autoreactive T cells but also reduces all inflammatory cells in the CNS. Treatment with anti-TCR αβ MoAb caused a transient body weight loss which, as previously been shown with CD3-specific antibodies [15] had no effect on the antibody-induced immunosuppression. This is a transient side-effect caused by local release of IL-6, TNF-α and IFN-γ [16, 17]. This syndrome is self-limiting, and resolves by the second to third day of treatment.

Administration of hamster IgG resulted in a minor suppression of EAE compared with untreated diseased mice. Except the stress caused by the injections, this could also be explained by a general immunosuppressive effect of antibodies, as intravenous immunoglobulins have been shown to suppress EAE when given during the initial phases of the disease [18, 19].
As the cytokine profile of the MOG_{35-55}-specific T cells could be an important determining factor for EAE development, collected supernatants from the MOG_{35-55}-stimulated spleen cell cultures after in vitro stimulation were assayed for cytokine production. Cells from anti-TCR αβ-treated animals showed a significantly lower synthesis of the inflammatory cytokines IFN-γ and TNF-α, but also of IL-4. This led us to suggest that the suppressive effect of TCR-specific MoAb treatment might not be due to of an improvement and shift to an antigen-specific Th2 response, but rather to a different immunoregulatory mechanism.

Monoclonal antibodies against the TCR complex have been shown to act as agonists both in vitro and in vivo. CD3-specific antibody has been used to suppress unwanted immune reactions, but the real mechanism of action of this antibody was unknown until recently. Anti-CD3 MoAb treatment is therapeutic in mice with diabetes [2], and despite a transient depletion of T cells the tolerogenic effect has been attributed CD4^{+} CD25^{+} regulatory T cells [20]. We observed an increased proportion of CD3^{+} NK1.1^{+} CD69^{+} cells, which are specific for regulatory natural killer (NK) T cells, in the spleen after anti-TCR αβ treatment. NKT cells play a regulatory role during an immune response. Upon TCR ligation, NKT cells have been shown to very rapidly secrete high amounts of IFN-γ and IL-4, while CD69^{+} NKT cells in spleen are potent producers of IL-4 [21]. Emerging evidence indicates their suppressive effect on autoimmunity [22]. Thus, the therapeutic effect of the anti-TCR αβ MoAb might be related to its agonist activity by activating NKT cells in the periphery indicating a possible immunotherapeutic potential of this pathway.

Lastly, we investigated whether we could transfer the anti-TCR αβ-induced tolerance to other immunized animals. We could, in fact, successfully show that transfer of spleen cells from anti-TCR αβ-treated mice have the capacity to potently suppress the development of EAE in recipients, while animals receiving cells from hamster Ig-treated mice developed a more severe EAE. MOG-reactive T cells in spleens of hamster Ig-treated EAE mice seemed to be more encephalitogenic which could synergize the effect of active immunization with MOG peptide and finally induced a more aggressive EAE.

In summary, our finding reveals that anti-TCR αβ treatment induces transferable T-cell-mediated tolerance involving populations of immune regulatory T lymphocytes. The details of this mechanism needs further study.

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