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ESC-derived thymic epithelial cells expressing MOG prevents EAE by central and peripheral tolerance mechanisms

Min Su^{1,2}, Yujun Lin¹, Cheng Cui¹, Xiaohong Tian¹, Xiuling Lu³, Zhixu He^{2,4}, and Laijun Lai^{1,5}

¹Department of Allied Health Sciences, University of Connecticut, Storrs, CT

²Tissue Engineering and Stem Cell Research Center, School of Basic Medical Sciences, Guizhou Medical University, Guizhou, China

³Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT

⁴Pediatric Department of the Affiliated Hospital, Guizhou Medical University, Guizhou, China

⁵University of Connecticut Stem Cell Institute, University of Connecticut, Storrs, CT

Abstract

Experimental autoimmune encephalomyelitis (EAE) is an animal model for multiple sclerosis (MS), and is induced by immunization with disease-causative self-antigens such as myelin oligodendrocyte glycoprotein (MOG). We have previously reported that transplantation of MOG expressing thymic epithelial progenitors (TEPs) derived from 129S6SvEv Tac mouse embryonic stem cells (mESCs) prevented the development of EAE. In this study, we expand our previous studies to show that transplantation of MOG expressing mESC-TEPs derived from C57BL/6 mice also prevents EAE development. Furthermore, by using a MOG-specific T cell receptor (TCR) transgenic mouse model, we demonstrate that both central and peripheral tolerances are involved in the prevention of EAE induced by MOG expressing mESC-TEPs. Our results suggest that transplantation of human ESC-TEPs expressing MOG may provide an effective approach for the induction of MOG-specific immune tolerance, thereby the prevention and treatment of MS.

Keywords

multiple sclerosis; experimental autoimmune encephalomyelitis; thymic epithelial cells; embryonic stem cells; tolerance induction; T cells

Corresponding authors: Laijun Lai, M.D., Department of Allied Health Sciences, University of Connecticut, 1390 Storrs Road, Storrs, CT 06269, USA. Phone: (860) 486-6073; Fax: (860) 486-0534; laijun.lai@uconn.edu. Zhixu He, M.D. Tissue Engineering and Stem Cell Research Center, Guizhou Medical University, Guiyang 550004, China. Phone: 86 13595017670; hzx@gmc.edu.cn.

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1. Introduction

MS is a devastating autoimmune disease of the central nervous system [1–6]. EAE, induced by immunization with disease-causative self-antigens such as MOG, is the most commonly used animal model for human MS [3, 7]. MS is currently incurable and remains a major cause of disability in both young and older populations [1, 2]. Therefore, development of new strategies to prevent and treat MS is in an urgent need.

We have reported that mESCs (TC-1 line) derived from 129S6SvEv Tac mice can be selectively induced to generate TEPs *in vitro*. When placed *in vivo*, these mESC-derived TEPs differentiate into TECs, reconstitute the normal thymic architecture, and support T cell generation in mice [8, 9]. We have also demonstrated that transplantation of the mESC-TEPs expressing MOG prevented the development of EAE in syngeneic 129S6SvEv Tac mice [10]. However, C57BL/6 (B6) mice are the most commonly used animal strain in EAE induction. Recently, we established a new protocol to induce B6 mESCs to differentiate into TEPs *in vitro* that further develop into TECs to support T cell development *in vivo* [11].

In this study, we extend our previous studies to demonstrate that transplantation of B6 MOG/mESC-TEPs into B6 mice also prevents the development of EAE. Furthermore, we also investigate the mechanisms by which transplantation of MOG/mESC-TEPs prevents EAE development by using a MOG-specific TCR transgenic mouse model (2D2 mice). Our data show that transplantation of MOG/mESC-TEPs into the mice results in the deletion of MOG-specific autoreactive T cells and the generation MOG-specific regulatory T cells (Tregs). In addition, we show that deletion of Tregs in the mice partly abrogates the EAE preventive effect induced by MOG/mESC-TEP transplantation. Our results suggest that both central tolerance and peripheral tolerance are involved in the prevention of EAE in MOG/mESC-TEP-transplanted mice.

2. Materials and Methods

2.1. Mice

Four- to 6-week-old female B6 and C57BL/6-Tg (Tcra2D2, Tcrb2D2)1Kuch/J (2D2 TCR) mice were purchased from Jackson laboratory (Bar Harbor, ME, USA). All animal experiments were performed to minimize animal suffering and discomfort in accordance with NIH guidelines and approved by the University of Connecticut Animal Care and Use Committee.

2.2. Generation of mESCs expressing MOG and induction of the differentiation of the mESCs into TEPs

B6 mESCs were cultured in ESGRO Complete Plus Serum-free Clonal Grade Medium with GSK3 β inhibitor supplement (Millipore, Temecula, CA). The mESCs were transfected with a pEF1a-IRES-AcGFP vector with or without MOG gene using Lipofectamine® 2000 Transfection Reagent (Invitrogen, Carlsbad, CA) [10]. It has been reported that the transfection efficiency of this reagent in mESCs was more than 70% [12]. The mESCs were then screened in medium containing 200 μ g/ml of G418 (Clontech Laboratories, Inc.,

Mountain View, CA) to obtain mESC lines that stably expressed the vector with MOG (MOG/mESCs) or without MOG (control mESCs).

The MOG/mESCs and control mESCs were first induced to differentiate into definitive endoderm, and then TEPs in the presence of BMP-4, FGF 7, FGF10, and EGF (BFFE), as well as rFOXP1 and rHOXA3 protein as we previously described [11].

2.3. Immunomagnetic Cell Separation

Single-cell suspensions from MOG/mESC-derived cells were stained with rat anti-mouse EpCAM1 antibody (eBioscience Inc., San Diego, CA), and followed by anti-rat IgG MicroBeads (Miltenyi Biotec, Auburn, CA). EpCAM1⁺ cells and EpCAM1⁻ cells were isolated using a magnetic-activated cell sorter immunomagnetic separation system (Miltenyi Biotec).

2.4. Induction and assessment of EAE

A total of 200 µg of mouse encephalitogenic peptide MOG_{35–55} (GL Biochem, Shanghai, China) in 100 µl of PBS was emulsified in 100 µl of complete Freund's adjuvant (CFA) (Sigma-Aldrich, St Louis, MO, USA) supplemented with 400 µg Mycobacterium tuberculosis H37Ra (Difco Laboratories, Detroit, MI). Mice were injected subcutaneously with the MOG_{35–55} in CFA on day 0 and intraperitoneally with 500 ng of purified Bordetella pertussis toxin (Sigma-Aldrich, St. Louis, MO) on days 0 and 2.

The mice were observed for clinical scores based on the following scale: 0, normal; 0.5, partially limp tail; 1, paralyzed tail; 2, loss in coordinated movement, hind limb paresis; 2.5, one hind limb paralyzed; 3, both hind limbs paralyzed; 3.5, hind limbs paralyzed, weakness in forelimbs; 4, forelimbs paralyzed; 5, moribund or dead. As required by animal ethics, mice with a score of 4 and beyond were euthanized.

2.5. Intrathymic (i.t.) injection

Mice were anesthetized and injected with cells in 10–30 µl PBS into the thymus posterior to the upper sternum using a 26–28 gauge needle [13].

2.6. Western blot analysis

Cells or tissues were collected and lysed. Equal amounts of denatured proteins were loaded onto a 4–12% Bis-Tris gel (Invitrogen, Carlsbad, CA), electrophoresed and transferred onto a PVDF membrane (Invitrogen, Carlsbad, CA). The membranes were blocked with 5% nonfat milk in TBST (mixture of Tris-Buffered Saline and Tween 20), and then incubated with primary antibody against MOG (Abcam, Cambridge, MA) or actin (Santa Cruz Biotechnology, Inc., Dallas, TX) overnight at 4°C. After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibody and developed with a SuperSignal West Pico chemiluminescence substrate (Thermo Scientific, Rockford, IL).

2.7. Flow cytometric analysis

The single-cell suspension was stained with fluorochrome-conjugated antibodies directly or indirectly as described [14]. For intracellular staining, the cells were first permeabilized with

a BD Cytofix/Cytoperm solution for 20 minutes at 4°C. The following antibodies were used: CD4, CD8, CD25, FOXP3, EpCAM1, Vα3.2 and Vβ11 (BioLegend, or BD Biosciences, San Diego, CA), Keratin (K) 5 (Covance, Dallas, TX), and K8 (US Biological, Salem, MA). The samples were analyzed on a FACSCalibur or LSRFortessa X-20 Cell Analyzer (BD Biosciences).

2.8. Histopathology

Spinal cords were removed from mice and fixed with 10% formaldehyde for 24 hours. Segments of the tissues were embedded in paraffin, and sections (4 to 7 μm) were prepared. The sections were stained with hematoxylin-eosin (H&E), Luxol fast blue (LFB) and Bielschowski silver impregnation (BSI) to assess inflammation, demyelination, and axonal damage, respectively. All histological stained sections were semiquantitatively scored blind as described [6, 15].

2.9. Immunofluorescence staining

Cultured cells were fixed with 4% paraformaldehyde, permeated with 0.25% Triton X-100, and blocked with 5% BSA. The cultured cells were incubated with primary antibodies. The following primary antibodies were used: anti-SSEA1, Sox2 (Cell Signaling Technology, Inc., Danvers, MA), and MOG (Abcam, Cambridge, MA). After washing, the sections were incubated with fluorochrome-conjugated secondary antibody, counterstained with 4', 6'-diamidino-2-phenylindole (DAPI) and observed under a Nikon AIR Spectral Confocal microscope (Nikon, Kanagawa, Japan).

2.10. Cytokine production detection

Splenocytes were stimulated with MOG *in vitro* for 3 days. Samples of supernatant were collected and measured for cytokine content using ELISA kits for IFNγ, TNFα, and IL-17A (Biolegend) according to the manufacturer's instructions.

2.11. Statistical analysis

P-values were based on the two-sided Student's *T* test. A confidence level > 95% (*p*<0.05) was determined to be statistically significant.

3. Results

3.1. Characterization of B6 mESCs expressing MOG and induction of the mESCs to differentiate into TEPs *in vitro*

We have demonstrated that transplantation of MOG expressing TEPs from TC-1 mESCs that were derived from 129S6SvEv Tac mouse prevented the development of EAE in syngeneic mice [10]. However, because B6 mice are the most commonly used animal strain in EAE induction, and because 2D2 TCR mice that will be used in the studies of MOG tolerance mechanisms are in the B6 background, we established B6 mESCs expressing MOG. To this end, B6 mESCs were transfected with a mammalian expression vector pEF1a-IRES-AcGFP containing MOG gene as described [10]. For controls, the mESCs were transfected with the pEF1a-IRES-AcGFP vector without the MOG gene. The mESCs were then cultured with the

antibiotic G418 to screen cell lines that stably expressed the vector containing MOG (MOG/mESCs) or without MOG (control mESCs).

We evaluated the expression of GFP protein in the transfected mESCs since the pEF1a-IRES-AcGFP expression vector contains the GFP gene. Both MOG/mESCs and control mESCs expressed GFP (Figure 1A), indicating that the mESCs had been successfully transfected with the expression vector. We then analyzed the expression of MOG with a MOG antibody. As shown in Figure 1A, MOG/mESCs expressed MOG protein, whereas control mESCs did not. The results were further confirmed by Western blot showing that MOG/mESCs, but not control mESCs, expressed MOG protein (Figure 1B). No morphological difference was observed between MOG/mESCs and control mESCs. Both MOG/mESCs and control mESCs were positive for alkaline phosphatase (AP) activity (Figure 1C) and expressed the pluripotent markers Sox2 and SSEA1 (Figure 1D), suggesting that the mESCs were in an undifferentiated state.

We have recently developed a new protocol to induce the differentiation of B6 mESCs into TEPs *in vitro* [11]. We then determined whether, like their parent B6 mESCs, MOG/mESCs and control mESCs can be selectively induced to differentiate into TEPs *in vitro*. It has been shown that EpCAM1⁺K5⁺K8⁺ cells contain or represent TEPs [11, 16, 17]. After TEP differentiation, both MOG/mESC-, and control mESC-derived cells contained a similar number of EpCAM1⁺K5⁺K8⁺ TEPs as their parent B6 mESC-derived cells (Figure 1E). These results suggest that the transfection did not alter the TEP differentiation ability of the mESCs.

We then purified EpCAM1⁺ mESC-TEPs from the cultures, injected them into the thymus of B6 mice, and detected MOG protein expression in the thymus over time. We found that MOG expression could still be detected on day 90 after the transplantation (Figure 1E), consistent with our previous data that transplantation of TC1 MOG/mESC-TEPs resulted in a long-term MOG expression in the thymus [11]. In contrast, no MOG expression was detected in the thymus of control mESC-TEP-transplanted mice (Figure 1F).

3.2. Transplantation of B6 MOG/mESC-TEPs prevents EAE development in mice

To determine whether transplantation of the B6 MOG/mESC-TEPs could prevent the development of EAE, B6 mice were injected i.t. with these cells. Control mESC-TEP- or PBS-injected mice and untreated naïve B6 mice were used as controls. Two months after the injection, EAE was induced in the mice with immunization of MOG peptides emulsified in CFA. EAE development was monitored over time. As shown in Figure 2A, transplantation of MOG/mESC-TEPs resulted in a significant reduction in disease severity as compared with the control mice. In addition, the number of mice with a disease-free condition was increased in the MOG/mESC-TEP-transplanted group (Figure 2B).

To confirm that transplantation of MOG/mESC-TEPs resulted in a significant reduction in EAE development, we performed histopathological evaluations. Forty two days after EAE induction, the spinal cords were harvested and stained with H&E, LFB and BSI to evaluate inflammatory cell infiltrates, myelin integrity, and axonal loss, respectively. As shown in Figure 2C, transplantation of MOG/mESC-TEPs resulted in a reduction in inflammatory cell

infiltrates, demyelination, and axonal loss or damage. Consequently, the histological scores were significantly reduced in MOG/mESC-TEP-treated mice, as compared with those in control-treated mice (Figure 2D). The results suggest that transplantation of B6 MOG/mESC-TEPs prevents the development of EAE.

3.3. Transplantation of MOG/mESC-TEPs results in the deletion of MOG autoreactive T cells

To determine the mechanisms by which transplantation of MOG/mESC-TEPs prevents EAE development, we first determined whether MOG autoreactive T cells had been deleted in the mice. To this end, we used a 2D2 TCR transgenic (Tg) mouse model in which the majority (>95%) of CD4⁺ T cells in this mouse line are V α 3.2 and V β 11 TCR double positive, and recognize MOG_{35–55} in the context of H-2^b [6, 18]. This animal model allows the tracking and quantification of MOG autoreactive T cells throughout development or during immune responses using anti-V α 3.2 and V β 11 antibodies [6, 18]. 2D2 TCR Tg mice were injected i.t. with the MOG/mESC-TEPs, control mESC-TEPs, or PBS. Untreated 2D2 TCR Tg mice were also used as controls. Two months later, the percentages of thymocyte subsets were analyzed by flow cytometry. Although the percentages of CD4⁺CD8⁺ double positive (DP), and CD4⁺CD8⁺ single positive (SP) thymocytes were not significantly different in each group, the percentage of CD4⁺CD8⁺ SP thymocytes was reduced ~3-fold in MOG/mESC-TEP-transplanted mice (Figure 3A). MOG/mESC-TEP-transplanted mice also had a ~2-fold reduction in the percentage of mature CD4⁺ cells in the spleen, as compared with control mice (Figure 3A).

To evaluate MOG autoreactive T cells, we analyzed clonotypic CD4⁺V α 3.2⁺V β 11⁺ cells. As shown in Figure 3B and 3C, there was a significant reduction in the percentage and number of this population in the thymus and spleen in MOG/mESC-TEP-transplanted mice, as compared with those in control mice. Taken together, our results suggest that MOG-specific autoreactive T cells have been, at least partly, deleted in MOG/mESC-TEP-transplanted mice.

3.4. The role of MOG-specific Tregs in the prevention of EAE in MOG/mESC-TEP-transplanted mice

Studies have shown that TECs support the development of Tregs [19, 20]. We then determined whether MOG-specific Tregs were generated in MOG/mESC-TEP-transplanted mice. 2D2 TCR Tg mice were injected i.t. with the MOG/mESC-TEPs, control mESC-TEPs or PBS as in Figure 3. Two months later, the thymus and spleen were analyzed for Tregs. We first gated on clonotypic CD4⁺V α 3.2⁺V β 11⁺ cells, and then analyzed the percentage and number of MOG-specific CD25⁺Foxp3⁺ Tregs. As shown in Figure 4A and B, both the percentage and number of MOG-specific Tregs in the thymus of MOG/mESC-TEP-transplanted mice were significantly increased, as compared with control mice. The percentage of MOG-specific CD25⁺Foxp3⁺ Tregs in the spleen of MOG/mESC-TEP-transplanted mice was also significantly increased. In addition, the number of MOG-specific Tregs in the spleen of MOG/mESC-TEP-transplanted mice was increased although the difference did not reach statistical significance (Figure 4A, B), probably because of the

massive depletion of CD4⁺ V α 3.2⁺ V β 11⁺ conventional T cells. Our results suggest that transplantation of MOG/TEPs results in increased number of MOG-specific Tregs.

To determine the role of Tregs in MOG/mESC-TEP-transplanted mice, C57BL/6 mice were i.t. injected with MOG/mESC-TEPs. Six weeks later, one group of the animals was injected with anti-CD25 antibody to delete Tregs, and other group was injected with IgG isotype as a control. Three days later, the mice were immunized with MOG_{35–55} to induce EAE. As shown in Figure 4C, mice that received the anti-CD25 antibody had a significantly higher increase in EAE clinical scores as compared to isotype-treated mice. The results indicate that Tregs play an important role in the prevention of EAE in MOG/mESC-TEP-transplanted mice.

3.5. Reduced generation of proinflammatory cytokines in MOG/mESC-TEP-transplanted mice

Since both Th1 and Th17 T cells play an important role in MS pathology [21–23], we analyzed cytokine production profiles in MOG/mESC-TEP-transplanted 2D2 TCR Tg mice. To this end, 2D2 mice were injected i.t. with the MOG/mESC-TEPs, control mESC-TEPs or PBS. Two months later, the mice were immunized with MOG_{35–55} to induce EAE. The spleen was harvested on day 14 after the immunization. A single-cell suspension of the spleen was stimulated with MOG_{35–55} *in vitro* for 3 days. The supernatants of the splenocytes were analyzed for the content of IFN- γ , TNF α , and IL-17A. As shown in Figure 5, the levels of these cytokines were significantly reduced in MOG/mESC-TEP-transplanted mice as compared to the control mice, consistent with our previous data obtained from non-transgenic mice [10]. These results suggest that transplantation of MOG/mESC-TEPs results in reduced production of proinflammatory cytokines.

4. Discussion

We have previously demonstrated that transplantation of TC-1 mESC-derived TEPs expressing MOG prevents the development of EAE in syngeneic 129S6SvEv Tac mice [10]. As an extension of our previous observations, we show here that transplantation of B6 MOG/mESC-TEPs into syngeneic B6 mice also prevents the development of EAE. In addition, we have used 2D2 TCR transgenic mice to study the underlying mechanisms.

Our previous *in vitro* T cell proliferation assays showed that splenocytes from control-treated mice proliferated well in response to MOG stimulation *in vitro*, whereas splenocytes from MOG/mESC-TEP-treated mice did not [10]. In contrast, splenocytes from all animal groups were able to mount an immune response to TCR-mediated signals [10]. The results suggest that MOG-specific immune tolerance had been established in MOG/mESC-TEP-treated mice, which might be due to MOG autoreactive T cells being deleted (or inactivated) in the mice. By using the 2D2 TCR transgenic mouse model, we demonstrate here that MOG/mESC-TEP-transplanted mice had a significant reduction in the percentage and number of MOG-specific autoreactive T cells. The data suggest that MOG-specific autoreactive T cells have been, at least partly, deleted in the MOG/mESC-TEP-transplanted mice. Therefore, central tolerance induction plays a role in the prevention of EAE development induced by the transplantation of MOG/mESC-TEPs.

Our previous studies also showed that transplantation of TC1 mESC-TEPs either expressing MOG or without MOG led to an increased number of Tregs, but the therapeutic benefit was only observed in the mice that had been transplanted with mESC-TEPs expressing MOG. By using the 2D2 TCR transgenic mouse model, we show here that transplantation of MOG/mESC-TEPs resulted in an increased number of MOG-specific Tregs, whereas transplantation of control mESC-TEPs did not. Our results are in agreement with other published reports that the generation of MOG-specific Tregs was sensitive to the expression of MOG in the thymus [6, 24]. It is possible that MOG expressing TECs plays an instructive role in the differentiation of thymocytes into MOG-specific Tregs [6, 24–26]. Our data also show that deletion of Tregs before EAE induction partly abrogated the EAE preventive effect in MOG/mESC-TEP-transplanted mice. Therefore, peripheral tolerance induction also plays an important role in the EAE prevention in MOG/mESC-TEP-transplanted mice.

Although our data indicate that MOG/mESC-TECs contribute to both negative selection and generation of Tregs, whether the MOG is directly presented by the mESC-TECs to developing T cells remains to be determined. Some studies have shown that TECs, especially mTECs, can directly present antigens to developing T cells [19, 27]. However, other studies showed that dendritic cells capture antigens from TECs, and then process and cross-present the antigen epitopes to developing T cells [28–30]. Our future studies will determine whether the tolerance induction is mediated directly by antigen presentation via MOG/mESC-TECs or indirectly by cross-presentation via thymic bone marrow-derived cells.

In summary, our data suggest that both central tolerance and peripheral tolerance are involved in the prevention of EAE in MOG/mESC-TEP-transplanted mice. Therefore, transplantation of human ESC- or induced pluripotent stem cell-derived TEPs expressing MOG may provide an effective strategy to induce MOG-specific immune tolerance, thereby preventing or treating patients with MS. However, there are limitations to this study. For example, there are some differences in pathology and immunology between EAE and human MS. Since ESC-TEPs need to be injected intrathymically, safety issues need to be addressed before the use of this approach in patients.

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Abbreviations

EAE	experimental autoimmune encephalomyelitis
MS	Multiple sclerosis
MOG	myelin oligodendrocyte glycoprotein
TEPs	thymic epithelial progenitors

TECs	thymic epithelial cells
ESCs	embryonic stem cells
mESCs	mouse embryonic stem cells
TCR	T cell receptor
Tregs	regulatory T cells
B6	C57BL/6J
i.t	intrathymic
AP	alkaline phosphatase
H&E	hematoxylin-eosin
LFB	luxol fast blue
BSI	Bielschowski silver impregnation
DN	double negative
DP	double positive
SP	single positive

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Highlights

- Transplantation of MOG/mESC-TEPs prevents the development of EAE in mice.
- MOG-specific autoreactive T cells are deleted in MOG/mESC-TEP-transplanted mice
- MOG-specific Tregs were generated in MOG/mESC-TEP-transplanted mice.

Figure 1.

Characterization of B6 MOG/mESCs and induction of the differentiation of the cells into TEPs *in vitro*. (A) MOG/mESCs and control mESCs were analyzed for GFP and MOG expression by immunofluorescence with an anti-MOG antibody. The expression of GFP (green) and MOG (red) was observed under a confocal microscope. Scale bars: 50 μ m. (B, C) MOG/mESCs and control mESCs were examined for the expression of (B) MOG protein by Western blot, and pluripotent markers (C) AP by AP staining and (D) Sox2 and SSEA1 by immunofluorescence. (E) Parent B6 mESC, MOG/mESCs and control mESCs (5×10^5 cells/well) were induced to differentiate into definitive endoderm and then TEPs in the presence or absence of BFFE, rFOXN1 and rHOXA3 proteins. The mESC-derived cells were analyzed for the expression of EpCAM1, K5 and K8 proteins on day 16 by flow cytometry. The number of EpCAM1⁺K5⁺K8⁺ cells was shown. (F) B6 mice were injected i.t. with EpCAM1⁺ MOG/mESC-TEPs (5×10^4), or control mESC-TEPs (5×10^4). MOG protein expression in the thymus was examined by Western blot on day 90 after the injection. The data are presented from 3 independent experiments.

Figure 2.

Transplantation of B6 MOG/mESC-TEPs prevents the development of EAE. B6 mice were injected i.t. with MOG/mESC-TEPs (5×10^4), control mESC-TEPs (5×10^4), or PBS. Two months later, EAE was induced in the mice, and EAE development was monitored over time. (A) Mean clinical scores, and (B) a Kaplan-Meier plot of the proportion of disease-free mice are shown. (C, D) Forty two days after the EAE induction, spinal cords were harvested from the mice and stained with H&E, LFB and BSI. (C) Representative stained histological sections (the magnification was $200 \times$), and (D) histological scores for inflammation, demyelination, and axonal damage. The data are presented as mean + SD and representative of 2 independent experiments with 4–7 mice per group. * $P < 0.05$ compared with PBS-treated mice.

Figure 3.

Transplantation of MOG/mESC-TEPs leads to the deletion of MOG autoreactive T cells. 2D2 TCR Tg mice were injected i.t. with MOG/mESC-TEPs (5×10^4), control mESC-TEPs (5×10^4), or PBS. Untreated 2D2 TCR Tg mice were also used as controls. Two months later, the thymus and spleen were harvested, stained with antibodies against (A) CD4 and CD8, (B, C) CD4, CD8, Vα3.2 and Vβ11, and analyzed by flow cytometry. (A) Representative flow cytometric profiles showing the percentages of DN, DP, CD4 SP, and CD8 SP thymocytes, as well as CD4 SP and CD8 SP splenocytes. (B) Representative flow cytometric profiles showing the percentages of Vα3.2⁺Vβ11⁺ after gating on CD4⁺CD8⁻ cells. (C) The

number of clonotypic CD4⁺Vα3.2⁺Vβ11⁺ cells in the thymus and spleen. The data are expressed as mean + SD and representative of 2 independent experiments with 4–7 mice per group. * P<0.05 compared with untreated 2D2 TCR Tg mice.

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Figure 4.

MOG-specific Tregs play an important role in the prevention of EAE in MOG/mESC-Treg-transplanted mice. (A–B) 2D2 TCR Tg mice were injected i.t. with MOG/mESC-TEPs, control mESC-TEPs, or PBS as in Figure 3. Two months later, the thymus and spleen were harvested, stained with antibodies against CD4, CD8, CD25, FoxP3, Vα3.2 and Vβ11, and analyzed by flow cytometry. (A) Representative flow cytometric profiles showing the percentages of CD25⁺FoxP3⁺ Tregs in the thymus and spleen after gating on CD4⁺CD8⁻Vα3.2⁺Vβ11⁺ cells. (B) The number of MOG-specific CD4⁺CD25⁺FoxP3⁺ Tregs in the thymus and the spleen. (C) B6 mice were injected i.t. with MOG/mESC-TEPs (5×10^4) as

in Figure 2. Six weeks later, the mice were injected with 750 μ g anti-CD25 antibody or IgG isotype as a control. Three days after the antibody injection, EAE was induced in the mice as in Figure 2, and EAE development was monitored over time. Mean clinical scores were shown. The data are expressed as mean + SD and representative of 2 independent experiments with 4–7 mice per group. * $P < 0.05$ compared with untreated 2D2 TCR Tg mice.

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Figure 5.

Transplantation of MOG/mESC-TEPs results in reduced production of proinflammatory cytokines. 2D2 TCR Tg mice that had been injected i.t. with the MOG/mESC-TEPs, control mESC-TEPs or PBS for 2 months were immunized with MOG35–55 to induce EAE. The splenocytes were harvested on day 14 after the immunization and stimulated with MOG35–55 *in vitro* for 3 days. The supernatants of the cultures were analyzed for the content of IFN γ , TNF α , and IL-17A by ELISA. The data are expressed as mean + SD and representative of 2 independent experiments with 3–7 mice per group. * $P < 0.05$ compared with PBS-treated mice.