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TCR vaccination in aluminum adjuvant protects against autoimmune encephalomyelitis

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Abstract Experimental Allergic Encephalomyelitis (EAE) is a neuroinflammatory, autoimmune disorder in which myelin-reactive Th1 T cells with a restricted TCRVβ repertoire play a pathogenic role. Here, I show that an engineered single-chain TCR containing dominant TCRVα/Vβ encephalitogenic elements, when administered in aluminum adjuvant, generates a marked anti-TCR humoral response that correlated with protection against the development of EAE in Vβ8-expressing B10.PL but not in Vβ8-deficient SJL mice. sc-TCR/Al vaccination was highly efficient in preventing murine EAE in a TCR-specific manner through a mechanism involving anti-TCR B cells and/or antibodies. Collectively, these data have important implications for designing preventive or therapeutic strategies combining TCR vaccination with the use of aluminum adjuvant in the treatment of multiple sclerosis and other human autoimmune inflammatory diseases.
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Introduction

Experimental autoimmune encephalomyelitis (EAE) is a CD4$^+$ T cell-mediated inflammatory disease of the CNS induced by immunization with myelin antigens, and it can be considered a surrogate model of human multiple sclerosis (MS) [1]. The EAE autoimmune CD4$^+$ T cells have a restricted Vβ TCR repertoire [2–4]. TCR oligoclonality was also observed in MBP-specific T cells from MS patients [5,6]. Therefore, the EAE model is valuable for testing experimental immune therapies for MS based on the specific control of encephalitogenic T cells. Several observations support the existence of a peripheral regulatory network that prevents activation or expansion of pathogenic T cells, suggesting that specific preventive and/or therapeutic immunomodulatory approaches could be of benefit in tackling the disease [7]. Experimental model demonstrates that immunization with attenuated encephalitogenic T cells protects from subsequent induction of EAE and induces remission of the disease [8]. This activates T cells specific for a clonotype-specific determinant of encephalitogenic T cells, suppressing its pathogenic activity [9]. The results of successful of T cell vaccination in animal models lead to clinical trial with MS patients [10]. The results with these clinical trials demonstrate that T cell vaccination is safe and induces a moderate

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clinical improvement in some patients with respect to reduced rate of exacerbations and EDSS scores [10]. Vaccination with recombinant Vβ regions [11] or peptides [12] of TCRs derived from encephalitogenic T cells, emulsified in complete Freund's adjuvant (CFA), were also able to induce protection in experimental models. These therapies involved anti-TCR regulatory T cells that suppress [12], delete [13] or deviate to a Th2 phenotype [14], the encephalitogenic T cells. However, the utilization of CFA is forbidden for human and veterinary vaccination.

T cell vaccination and TCR vaccination induce anti-TCR antibody response in experimental models [15–18]. Purified anti-TCR antibodies are able to prevent the establishment and promote the recovery from EAE when passively transferred [16,17]. Protection is, in some experiments, independent of MBP-specific T cell depletion, and appeared to involve modifications of encephalitogenic T cell functionality. Despite the regulatory property of anti-TCR antibodies, the therapeutic potential in regulating autoreactive T cells has not been thoroughly studied. In the present study, it is demonstrated for the first time, a vaccination protocol based in the generation of anti-TCR antibody response that efficiently protects mice from EAE. The vaccination procedure consisted the injection of an encephalitogenic single chain Vα–Vβ TCR fragment (sc-TCR) [19] adsorbed in aluminum hydroxide (sc-TCR/Al). Aluminum hydroxide is an adjuvant that selectively induces a strong Th2 and antibody responses [20,21] but it is not an efficient delayed-type hypersensitivity (DTH) inducer and it is not able to generate MHC class I-restricted cytotoxic T cells easily [21]. Sc-TCR/Al vaccination prevented EAE induction in B10.PL mice and increased their levels of circulating anti-Vβ8 antibodies without affecting either the frequencies of $V\beta8^+$ T cells or on the MBP-reactive T cell response. The same protocol of sc-TCR/Al vaccination was unable to prevent EAE in Vβ8-deficient SJL and in B cell-deficient mice, indicating that protection is TCR-specific and dependent on anti-TCR B cells and/or antibodies.

Materials and methods

Mice

Six- to eight-week-old female B10.PL, SJL, C57BL/6 B cell-deficient (B6μmt) and F1 (B6 × B6μmt) ($B6^{+/-}$) mice were used in these experiments. In B10.PL, the majority of encephalitogenic T cells use Vβ8.2 [2] while SJL mice, which were used as control, have their Vβ8 genes deleted [22] and their MBP-specific T cells use Vβ17a and Vβ4 [3]. In the B6 mice immunized with MOG_{35-55}, the majority of encephalitogenic T cells are also $V\beta8^+$ [4]. B10.PL were obtained from the animal house of the Instituto de Ciências Biomédicas of Universidade de São Paulo, Brasil, and SJL were obtained from CEMIB (Campinas, São Paulo). Mice were housed in our own facilities in filter-top cages until the end of the experiments. The study was approved by the institutional ethical board for animal studies.

sc-TCR vaccination and EAE induction

For vaccination, mice were injected i.p. with 10 μg of recombinant single-chain TCR fragment (Vα4.2-sc-Vβ8.2; kindly provided by E.S. Ward, University of Texas Southwestern Medical Center, Dallas, USA)[19] adsorbed in 1.5 mg $Al(OH)_3$ (sc-TCR/Al). The control group was treated with 1.5 mg of $Al(OH)_3$ alone. EAE was induced by a single injection of myelin antigen emulsified in complete Freund's adjuvant (CFA; Life Technologies, Paisley, UK) supplemented with 400 μg BCG (Instituto Butantã, São Paulo, SP). For EAE induction in B10.Pl and SJL mice, 200 μg of guinea pig myelin basic protein (MBP; Sigma-Aldrich, St. Louis, MO) in 100 μl of PBS was emulsified in an equal volume of CFA, and injected subcutaneously into the base of the tail 7 days after vaccination. For induction of EAE in mice of C57/BL6 (B6) background, MBP was substituted by 200 μg of myelin oligodendrocyte glycoprotein MOG_{35-55} peptide (MEVGWYRSPFSRVVHLYRNGK). MOG_{35-55} peptide was synthesized in the Laboratory of Clinical Immunology (ICB, USP, São Paulo, SP) using a Pioneer peptide synthesizer (PE Biosystems, Foster City, CA, USA). Mice also received 200 ng of pertussis toxin (Life Technologies) intravenously on days 0 and 2. Clinical symptoms were monitored daily after the first immunization. The clinical score was graded as follows: 0, no disease; 1, tail limpness; 2, hind limb weakness; 3, hind limb paralysis; 4, fore limb weakness; 5, quadriplegia or death.

In vitro proliferation assay

Individual cell suspensions were prepared from the spleen cells (3 × 10^6), were cultured for 72 h at 100 μl/well with 50 μg/ml of MBP or purified recombinant Vβ8.2 fragment [19] in RPMI 1640 (Life Technologies), supplemented with 5% FCS (Life Technologies), 50 μM 2-ME (Life Technologies), 100 μg streptomycin (Life Technologies) and 100 U/ml penicillin (Life Technologies). Spleen T cells were also stimulated in F23.1 (0.5 μg/well) coated wells. Cultures were pulsed with 0.5 μCi [^{3}H]-thymidine/well (Amersham Biosciences, Buckinghamshire, UK) for the last 8 h, followed by cell harvesting and determination of [^{3}H]-thymidine incorporation by liquid scintillation β-counter. Percentage of response was calculated as follows: % of response = (cpm of sc-TCR/Al-vaccinated / cpm of sham-vaccinated) × 100.

Flow cytometric analysis

For two-color flow cytometric analyses, 1 × 10^6 cells in 30 μl PBS with 0.5% BSA (Life Technologies) were incubated with each monoclonal antibody for 20 min at 4°C in the dark. Double-labeling experiments were performed with saturating concentrations of the following fluorescein isothiocyanate (FITC) and biotin-conjugated monoclonal antibodies (PharMingen, San Diego, CA, USA): anti-CD4, anti-CD8 and anti-Vβ8. Phycoerythrin-conjugated streptavidin (PharMingen) were used to detect biotinylated antibodies.

Cytokine assay

IFN-γ, IL-2 and IL-4 in culture supernatants were measured by ELISA using purified and biotinylated antibody pairs, standards and streptavidin-HRP according to the manufacturer (PharMingen); and *o*-phenylene-diamine (Sigma-Aldrich) as substrate. Supernatants were diluted, when necessary, to fit

in the range of the standard curve. Concentrations were calculated on the basis of standard curves using recombinant IFN-γ, IL-2 and IL-4.

Assessment of antibody response

Anti-Vβ8.2, anti-Vβ8.3 and anti-MBP antibodies in serum were assessed by standard ELISA. Briefly, diluted sera (1/500 or 1/1000 for samples before or after EAE induction, respectively; as determined by previous titration) from vaccinated and control mice were incubated in 96-well Maxisorb (Nunc) plates coated with 2 μg/well of purified recombinant Vβ8.2, Vβ8.3 [19] or MBP, and bound antibodies were detected spectrophotometrically with peroxidase-labeled rabbit anti-mouse Ig, IgG1 or IgG2a (Southern Biotechnology, Birmingham, AL, USA) and *o*-phenylene-diamine (Sigma) as substrate.

Statistical analysis

The significance of differences between means was determined using the Mann–Whitney and Student's *t* tests as appropriate. *P* values less than 0.05 were considered statistically significant.

Results

Effects of sc-TCR/Al vaccination in immune responses of B10.PL and SJL mice

First, I evaluated the effects of sc-TCR/Al and sham vaccination (aluminum adjuvant alone) in immune responses of B10.PL and SJL mice. Sc-TCR/Al-vaccinated B10.PL and SJL mice yielded higher levels of circulating anti-Vβ8.2 antibodies than did sham controls, while the in vitro anti-Vβ8.2 proliferative response of splenic T cells was similar in both sham- and sc-TCR/Al-vaccinated mice (Fig. 1A). The analysis of circulating antibodies against Vβ8.3, an irrelevant Vβ chain, demonstrates that sc-TCR/Al vaccination specifically induces increased levels of anti-Vβ8.2 antibodies (Fig. 1A). Frequencies of $CD4^{+}V\beta8^{+}$ and $CD8^{+}V\beta8^{+}$ T cells were similar in sham- and sc-TCR/Al-vaccinated B10.PL mice (Fig. 1B). Mean Vβ8 fluorescence was also similar in the two groups. I do also evaluate the response of spleen Vβ8 cells in F23.1-coated plates. The result demonstrates that sc-TCR vaccination does not affect the ability of these cells to proliferate when stimulated (Fig. 1C). Spleen cells from SJL mice were unable to proliferate upon F23.1 stimulation (Fig. 1C) because these mice have the Vβ8 gene deleted [22]. Together, these data indicate that circulating anti-Vβ8.2 antibodies induced by sc-TCR/Al vaccination cause neither

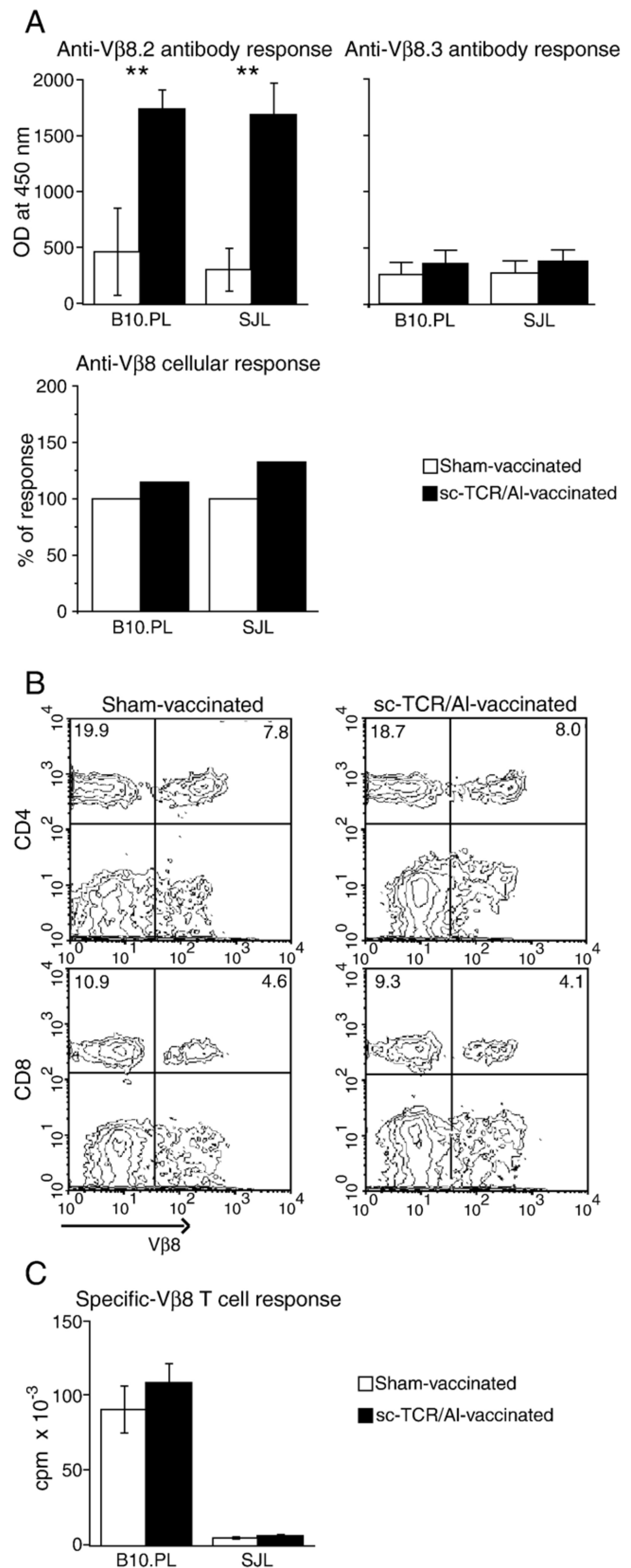

Figure 1 Vaccination with sc-TCR/Al induces an anti-TCR humoral, but not a cellular, response, resulting in neither deletion nor anergy of $V\beta8^{+}$ T cells. Fifteen days after sc-TCR/Al- or sham-vaccination, B10.PL and SJL mice were bled and the sera tested for the presence of anti-Vβ8.2 and anti-Vβ8.3 antibodies. Spleen cells from the same animals were assayed for their ability to proliferate to TCRVβ8.2 stimulation (A). Anti-Vβ8.2 cellular response was calculated considering that of sham-vaccinated mice as 100%. B10.PL splenocytes were double-stained for Vβ8 and CD4 or CD8 T cell subpopulations (B). Proliferative Vβ8 T cell response induced with anti-Vβ8 antibody F23.1(C). The results presented in panels A–C are the mean ± SD of four mice analyzed individually and they are representative of two independent experiments. $^{**}P < 0.01$.

deletion of Vβ8$^+$ T cells nor down modulation of TCRVβ8 surface expression or suppression of Vβ8 T cell response.

sc-TCR/Al vaccination specifically prevents EAE and reduces brain inflammation

In order to determine the effect of sc-TCR vaccination on EAE, groups of B10.PL and SJL mice, sham- or sc-TCR/Al-vaccinated 7 days earlier, were immunized with myelin antigen to induce EAE. Thirty days post-EAE induction, almost all sham-vaccinated, 18 out 19 (95%), developed the disease, while only 5 out 22 (23%) sc-TCR/Al-vaccinated B10.PL mice present EAE symptoms (Fig. 2A). Whenever sc-TCR/Al-treated B10.PL mice developed disease, this had a lower clinical score (Fig. 2B) and delayed onset (Fig. 2C, b) than sham-vaccinated (Fig. 2C, a) mice. In fact, all sc-TCR/Al-vaccinated B10.PL mice survived, while 16% of sham-immunized mice died during the course of the experiment. In contrast with these results, sc-TCR/Al vaccination did not protect SJL mice against the induction of EAE (Fig. 2A). The maximum clinical score (Fig. 2B) and EAE clinical course were similar in sham- (Fig. 2C, c) and sc-TCR/Al-vaccinated (Fig. 2C, d) SJL mice. These results indicate that the protective effect of sc-TCR/Al vaccination against EAE is TCR-specific.

Brain histological analyses in sham-vaccinated B10.PL mice revealed the presence of an invasive perivascular inflammatory infiltrate disrupting the tissue barriers and destroying adjacent nervous tissue (Fig. 2D, a). A higher magnification showed the presence of blastic cells, with frequent polymorphonuclear and plasma cells (Fig. 2D, c). In contrast, sc-TCR/Al-vaccinated mice had few or no CNS inflammatory lesions (Fig. 2D, b). Infiltrating cells were restricted to interstitial perivascular areas and consisted of small, mononuclear cells (Fig. 2D, d).

Modulation of the MBP-specific immune response by sc-TCR/Al vaccination

I next evaluated the effect of sc-TCR/Al vaccination in the response to MBP. For this, splenocytes and lymph node cells from sham- and sc-TCR/Al-vaccinated B10.PL mice were stimulated in vitro with MBP 28 days after in vivo MBP inoculation to assess MBP-specific proliferation and cytokine responses. Splenocytes from sc-TCR/Al-vaccinated mice proliferated more than those from sham-vaccinated mice upon in vitro MBP-stimulation ($P < 0.001$) (Fig. 3A). In addition, MBP-specific IL-2 production by splenic cells was

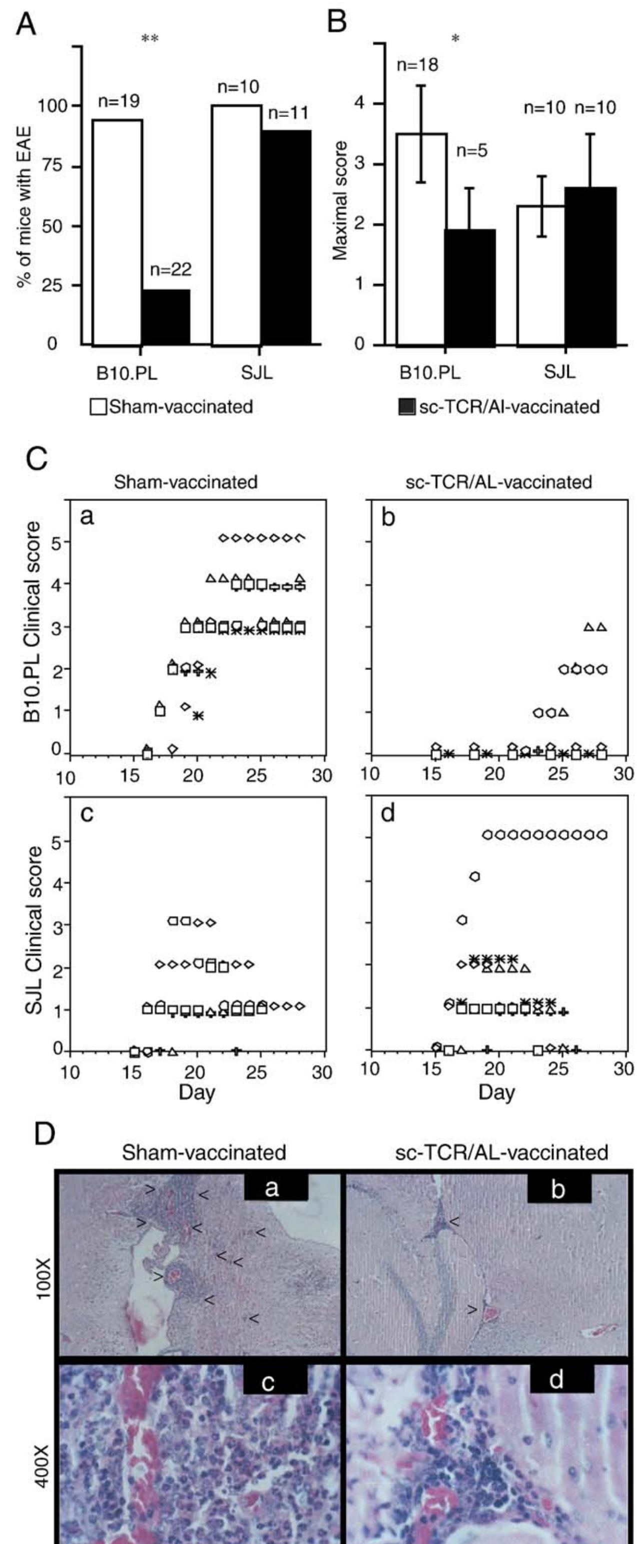

Figure 2 Vaccination with sc-TCR/Al protects B10.PL but not SJL mice from MBP-induced EAE. EAE was induced in sham-vaccinated and sc-TCR/Al-vaccinated B10.PL or SJL mice by immunization with MBP in CFA. Disease incidence (A), maximal clinical score (B) and daily clinical scores (C) are shown. In panels A and B, calculations were made considering all mice in four or two distinct experiments for B10.PL and SJL mice, respectively. In panel B, maximal scores were the mean ± SD of mice which developed disease; $^*P < 0.05$ and $^{**}P < 0.01$. In panel C, each symbol represents an individual mouse within the group. Results are representative of four or two independent experiments for B10.PL and SJL mice, respectively. Perivascular inflammation in brains of sham- and sc-TCR/Al-vaccinated B10.PL mice (D). Twenty-eight days after EAE induction, brains were collected, fixed, cross-sectioned and stained with hematoxylin and eosin. Arrowheads point to sites of perivascular inflammation (a, b). At the time of brain collection, sham-vaccinated and sc-TCR/Al-vaccinated mice scored 4 and 0, respectively. 100× (a, b) and 400× (c, d) magnifications are shown. Data are representative of two distinct experiments.

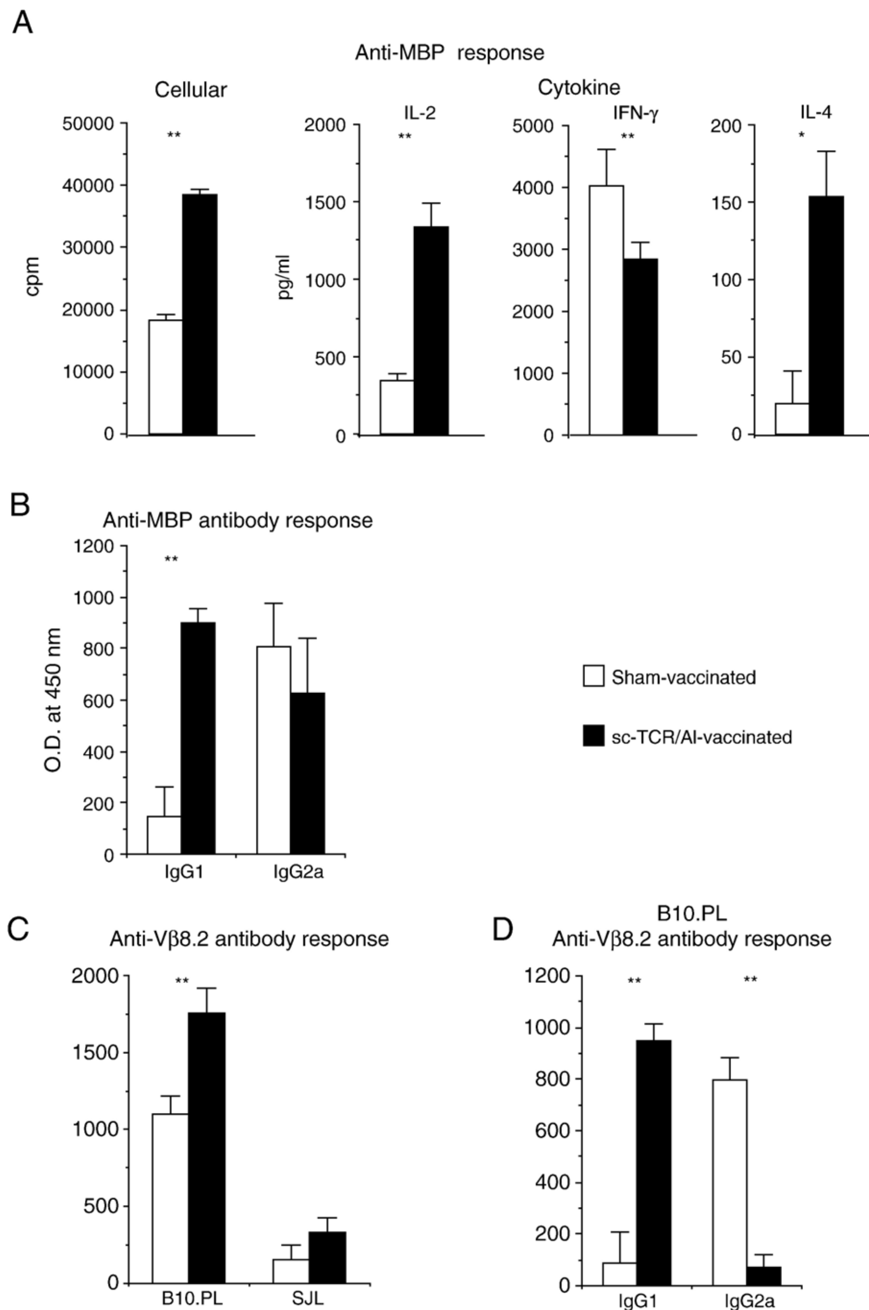

Figure 3 Effects of sc-TCR/Al vaccination on proliferative, cytokine and antibody responses after EAE induction. Twenty-eight days after EAE induction, spleen cells from sham- or sc-TCR/Al-vaccinated B10.PL mice were stimulated with MBP to assess anti-MBP proliferative and cytokine responses (A). Mean background counts were 1240 ± 260 cpm, while positive control stimulation with Concanavalin A yielded values of 135,000 ± 12,300 cpm. IL-2, IFN-γ and IL-4 concentrations in culture supernatants of MBP-stimulated splenocytes were determined by ELISA. MBP-specific IgG_1 and IgG_{2a} antibodies were measured by ELISA in 1/1000 dilution of the serum samples (B). Total anti-Vβ8.2 antibody response (C). Anti-Vβ8.2-specific IgG1 and IgG2a response (D). Results are the mean ± SD of four-to-six mice per group. *$P < 0.01$ and **$P < 0.001$.

greater in sc-TCR/Al-vaccinated than in sham-vaccinated mice ($P < 0.001$) (Fig. 3A). These results indicate that MBP-specific T cells are not anergic in sc-TCR/Al-vaccinated mice. However, IFN-γ secretion was suppressed in splenocytes ($P < 0.001$) of sc-TCR/Al-vaccinated mice relative to sham controls (Fig. 3A). Higher MBP-induced IL-4 production was observed in splenocytes from vaccinated mice than in sham-vaccinated mice ($P < 0.01$) (Fig. 3A). Collectively, these findings indicate that MBP-specific, including encephalitogenic, T cells are neither deleted nor anergic in sc-TCR/Al-vaccinated mice. They show specific suppression of Th1 cytokines, such as IFN-γ, and a bias toward the production of the Th2-type IL-4 cytokine in their specific anti-MBP response.

The relationship between circulating IgG_1 and IgG_{2a} serum levels is a valuable index for assessing Th2 and Th1

responses, respectively [23]. Consistent with the above results, sc-TCR/Al-vaccinated mice had increased levels of anti-MBP IgG_1 antibodies ($P < 0.001$), while IgG_{2a} levels were not significantly different when compared with sham-vaccinated mice ($P < 0.2$) (Fig. 3B).

I also analyzed the presence of anti-Vβ8 in the serum of B10.PL and SJL mice at day 28 after EAE induction, and compared between sham- and sc-TCR/AL-vaccinated groups and mice strains. The establishment of EAE in sham-vaccinated mice induces the production of anti-Vβ8.2 antibodies. However, sham-vaccinated mice had lower levels of anti-Vβ8.2 antibodies than sc-TCR/AL-vaccinated mice at the same time point (Fig. 3C). This result clearly demonstrates that sc-TCR/AL vaccination primes B10.PL mice to produce a stronger anti-Vβ8.2 antibody response than sham-vaccinated mice. EAE did not interfere with the level of circulating anti-Vβ8.2 antibodies in the SJL strain (Fig. 3C). Without activation of $V\beta8^+$ T cells during disease, as in SJL mice, sc-TCR/AL vaccination was unable to sustain the level of anti-Vβ8 antibodies.

Since aluminum hydroxide as adjuvant promotes the development of Th2 responses, I should expect that sc-TCR/AL-vaccinated mice produce mainly anti-Vβ8.2 antibodies of the IgG1 isotype. As expected, the analysis of isotype used at anti-Vβ8.2 antibodies response at day 28 after EAE induction demonstrated that sc-TCR/AL-vaccinated mice respond with IgG1 antibodies while sham-vaccinated mice respond preferentially with IgG2a antibodies (Fig. 3D). This result demonstrates that despite the absence of anti-Vβ8.2 T cell proliferation, sc-TCR vaccination primes T cells for helping anti-Vβ8.2 antibody response.

sc-TCR/AL vaccination did not prevent EAE in B cell-deficient mice

The results described above demonstrated that sc-TCR vaccination induces antibody but not T cell-mediated response against $V\beta8^+$ T cells that protects B10.PL mice against EAE, suggesting an important role for anti-Vβ8 antibodies in the protection. To further dissect the mechanism involved in sc-TCR/Al-mediated protection, I evaluate the effect of sc-TCR/Al vaccination in the establishment of EAE in B cell-deficient mice. Myelin oligodendrocyte glycoprotein (MOG) is postulated to be a target autoantigen in MS. In the C57BL/6 background, 40% of MOG_{35-55}-specific T cells are $V\beta8^+$ [4]. Therefore, in order to analyze the role of B cells in sc-TCR/AL vaccination, I injected $B6^{+/-}$ and B6μmt mice with sc-TCR/Al. A week later, mice were inoculated with MOG_{35-55} to induce EAE. As before, sc-TCR/Al vaccination of B6 mice induced increased anti-Vβ8 serum antibodies but no specific proliferative responses (Table 1). Compared with their sham-vaccinated counterparts, sc-TCR/Al-vaccinated mice were less susceptible to EAE and presented less severe clinical symptoms (Table 1). In contrast, both sham- and sc-TCR/Al-vaccinated B6μmt mice were equally susceptible to MOG_{35-55}-induced EAE. The disease incidence and maximum clinical score were equal in the two groups (Table 1). These results demonstrate that anti-TCR B cells and/or their secreted antibodies play an important role in sc-TCR/Al-induced protection from EAE.

Discussion

T cell vaccination and TCR-based vaccination were extensively studied in the last decade and have demonstrated their potential in the prevention of T cell-mediated autoimmune diseases in experimental models of, for example, collagen-induced arthritis [24] and EAE [11,25,26]. This has encouraged the development of trial assays to examine therapeutic applications in the treatment of multiple sclerosis patients [10]. T cell vaccination induces a moderate clinical improvement followed by relapses in some patients. TCR-based vaccination protocols were successfully used in experimental models where TCR or their peptides were emulsified in CFA. However, CFA is not allowed for human or veterinary uses. In contrast, aluminum hydroxide is innocuous and already included in many vaccines for human and animal applications [20]. Contrasting with CFA which induces a strong antibody response, delayed-type hypersensitivity (DTH) and promotes MHC class I-restricted cytotoxic T cells, aluminum hydroxide as an adjuvant promotes a strong antibody response and the differentiation of T cells into a Th2 phenotype, but no DTH or cytotoxic T cells [21]. The results shown here demonstrate the beneficial effects of including aluminum hydroxide as adjuvant during TCR vaccination for the prevention of murine EAE, and suggest a major role for anti-TCR B cell and/or antibodies in the regulation of immune responses to myelin antigens and protection against EAE.

Vaccination with sc-TCR/Al protected B10.PL mice from EAE induced by immunization with myelin antigens (Fig. 2). The protective effect was not related to deletion of dominant, pathogenic $V\beta8^+$ T cells (Fig. 1B), despite increased levels of circulating anti-Vβ8 antibodies (Fig. 1A). Splenic T cells from sc-TCR/Al-vaccinated mice secreted IL-2 and proliferated upon antigen stimulation (Fig. 3A)

Table 1 Vaccination prevents EAE in B6 but not in B6μmt mice

Mice	Treatment	Anti-sc-TCR response		Incidence	Maximum	
		Humoral[a]	Cellular		Clinical score[b]	Mortality
B6	Sham-vaccinated	230 ± 53	15,425 ± 2150	10/11	3.0 ± 1.4	3/11
	Vaccinated	1251 ± 134*	18,425 ± 1754	7/12	1.1 ± 1.2*	1/12
B6μmt	Sham-vaccinated	—	287 ± 194	11/11	2.6 ± 0.9	0/11
	Vaccinated	—	650 ± 120	11/11	2.3 ± 1.1	0/11

[a] Vβ8-specific antibodies were measured by ELISA in 1/500 dilution of the serum samples.
[b] Mean ± SD.
* $P < 0.01$.

and upon stimulation with anti-Vβ8 antibodies (Fig. 1A), further indicating that anergy and suppression were not involved in the protection.

Sc-TCR/Al vaccination induced an increase in the level of circulating anti-Vβ8.2 antibody in B10.PL and SJL mice (Fig. 1A). I observed that EAE induction leads to increased level of anti-Vβ8 antibody in sham-vaccinated B10.PL mice. Yet, sc-TCR/Al-vaccinated B10.PL mice showed even higher levels of anti-Vβ8.2 antibodies than sham-vaccinated mice (Fig. 3A). This antibody response was also qualitatively different between these groups. While sham-vaccinated B10.PL mice responded mainly with IgG2a antibodies, sc-TCR/Al vaccination preferentially induced IgG1 antibodies (Fig. 3D). The anti-Vβ8.2 antibody response in sc-TCR-vaccinated B10.PL mice clearly demonstrates that vaccination was able to drive the anti-Vβ8.2 T cell response into Th2 pattern. Further, upon Vβ8.2 T cell activation during EAE, sc-TCR/Al-vaccinated B10.PL, but not SJL, mice generated a secondary-like antibody immune response. Taken together, these results clearly demonstrate that vaccination primes for anti-Vβ8.2 antibody response and that this antibody response correlates with the protection of the B10.PL strain, in which encephalitogenic T cells express mainly Vβ8 chain in the TCR, but not in SJL mice in which the Vβ8 family is deleted (Figs. 2A–C).

It is well known that the cytokine pattern of myelin-specific T cell responses plays an important role in EAE as well as in MS. Studies in IL-4 knockout mice have revealed a role for IL-4 in modulating the severity of the encephalitogenic process [27]. This is corroborated by IL-4 gene therapy [28], generation of Th2 clones by copolymer 1 [29] or by altered peptide inoculation [30], all of which significantly ameliorate clinical and pathological EAE signs through immune deviation. As shown in Fig. 3A, spleen cells from sc-TCR/Al-vaccinated B10.PL mice produce much more IL-4 in response to in vitro MBP stimulation than those from sham-vaccinated B10.PL mice. This indicates that sc-TCR/Al vaccination induces a shift of myelin-specific T cells into a Th2 cytokine response, which in turn could control brain inflammation (Fig. 2D).

Although the mechanisms underlying the beneficial effects of sc-TCR/Al vaccination in the prevention and treatment of autoimmune EAE remain to be defined, at least two may explain the protective effect of sc-TCR vaccination. First, the fact that this vaccine elicits a potent anti-TCR antibody response that protects B10.PL, but not SJL, mice and its inability to protect B cell-deficient mice against EAE, strongly suggest that TCR-specific B cells and/or antibodies play a relevant role. Initially, sc-TCR vaccination induces a primary anti-Vβ8.2 antibody response (Fig. 1A), which upon activation of encephalitogenic Vβ8 T cells during EAE induction generates a stronger and faster antibody response able to prevent EAE establishment (Figs. 3C and D). This is further supported by previous studies showing that anti-TCR antibodies are increased during the recovery phase of EAE [15], and that their passive transfer prevents EAE development [15,16]. Also, anti-TCR antibodies prevented collagen-induced arthritis in rats [31]. In MS patients, T cell vaccination induces anti-TCR antibodies that showed regulatory properties on encephalitogenic T cells [32]. Anti-TCR antibodies have regulatory effects also on myasthenia gravis [33,34]. In a less specific way, polyclonal immunoglobulin preparations (IVIg) have been shown to be effective in the prevention and/or suppression of full-blown murine EAE [35], as well as in relapsing-remitting MS patients [36]. IVIg also appears beneficial in the treatment of acute relapses and in prevention of new relapses [37]. The mechanism of action of IVIg is still poorly understood, but it may be dependent on natural anti-TCR antibodies present in IVIg preparations [38,39], which may induce a bias toward a Th2 response [35,40,41]. Therefore, anti-Vβ8.2 B cells and/or antibodies induced after sc-TCR/Al vaccination may induce a Th2 phenotype in encephalitogenic T cells, thus interfering with their ability to produce brain inflammation.

Another possibility is that sc-TCR/Al vaccination induces specific anti-TCR Th2 regulatory T cells that did not proliferate in response to in vitro antigenic stimulation. Anti-Vβ8 regulatory T cells could be unable to prevent EAE in SJL mice because their encephalitogenic T cells preferentially use Vβ17a and Vβ4. Further, the impairment of proper T cell activation in the absence of B cells could explain the failure of sc-TCR vaccination to protect B cell-deficient mice [42,43]. Thus, putative regulatory T cells may not be properly activated in the absence of B cells, leaving B cell-deficient mice susceptible to EAE induction. However, the role of B cells in T cell activation is still controversial. In fact, some studies indicate that, in the absence of B cell, T cells can be normally activated [44,45]. There are also evidences that B cell-deficient mice are able to recover from EAE, indicating that even in the absence of B cells, a regulatory T cell network is achieved [46]. Another point to be considered is that aluminum hydroxide recruits mainly macrophages to the site of injection, suggesting an important role of these cells for antigen presentation in vaccines including this adjuvant [47]. In any case, the results still indicate that protection induced by sc-TCR/Al vaccination is dependent on B cells.

In conclusion, the present work demonstrates that sc-TCR/Al vaccination is highly efficient in the prevention of murine EAE. The protective effect of sc-TCR/Al appears to be independent of T cell anergy, depletion or suppression, but requires anti-TCR B cells and/or antibodies. The immunoglobulin- and/or B-cell-dependent change in the Th phenotype of myelin-specific T cells and the subsequent clinical outcome are strongly related to the use of aluminum adjuvant in the TCR vaccine. In contrast with other adjuvants, aluminum is permitted for clinical use in humans and animals. Although the molecular mechanisms by which B cells and/or antibodies induce protection from EAE have not yet been identified, I believe that TCR vaccination with aluminum could be used to treat MS in humans. Similar approaches might be taken with immunopathological diseases in which oligoclonality or relative restriction of Vβ gene products is related to T cell pathogenicity.

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