Journal of Neuroimmunology 234 (2011) 63–70

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

A natural anti-T-cell receptor monoclonal antibody protects against experimental autoimmune encephalomyelitis

Edgar Fernández-Malavé [a,*], Luiz Stark-Aroeira [b,**]

[a] Inmunología, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain
[b] Instituto de Pesquisas Energéticas e Nucleares, Laboratório de Biologia Molecular, São Paulo, SP-CEP 05508-900, Brazil

ARTICLE INFO

Article history:
Received 18 October 2010
Received in revised form 21 December 2010
Accepted 8 February 2011

Keywords:
Natural antibody
TCR
Immunomodulation
EAE

ABSTRACT

The therapeutic potential of natural anti-T-cell receptor (TCR) antibodies is largely unknown. We investigated whether passive administration of C1-19, a novel natural anti-TCRVβ8 monoclonal antibody, could interfere with the development of EAE. Treatment with C1-19 prevented myelin basic protein (MBP)-induced EAE in Vβ8-sufficient B10.PL but not in Vβ8-deficient SJL mice. Furthermore, C1-19 reduced disease severity when administrated shortly after disease onset. These protective effects of C1-19 correlated with a Th2 bias of the cytokine response, in the absence of T-cell deletion or anergy. Together, these findings indicate that natural anti-TCR antibodies could function as therapeutic tools in autoimmune inflammatory diseases.

1. Introduction

Natural antibodies refer to any type of immunoglobulin present in the serum of an individual in the absence of deliberate immunization or infection. These antibodies can be of the IgM, IgG or IgA isotypes (Lacroix-Desmazes et al., 1998), and recognize several self and foreign antigens in mice (Baccala et al., 1989; Guilbert et al., 1982; Haury et al., 1994). Natural auto-antibodies also occur in healthy humans, as well as in patients with autoimmune disease, where they react with a wide range of evolutionary conserved cell surface, intracellular and circulating antigens including DNA, thyroglobulin, senescent cell antigens, and constant and variable regions of immunoglobulin and the T cell antigen receptor (TCR) (Lacroix-Desmazes et al., 1998). However, the precise role of natural antibodies in physiological and pathological immunity remains unclear.

Experimental autoimmune encephalomyelitis (EAE) is a neuroinflammatory disease in which anti-myelin $CD4^+$ T helper (Th) cells have a key contribution by recruiting and stimulating peripheral macrophages and resident microglia, which are the ultimate responsible for demyelination, oligodendrocyte death and axonal dysfunction (Merrill et al., 1992). EAE is a surrogate model of human multiple sclerosis (MS) that has contributed to understand the mechanisms underlying the disease and its potential treatment (Steinman and Zamvil, 2005). In EAE as well as in MS, the anti-myelin $CD4^+$ T cells responsible for disease development express an oligoclonal Vβ TCR repertoire (Acha-Orbea et al., 1988; Kotzin et al., 1991; Oksenberg et al., 1993; Padula et al., 1991; Urban et al., 1988; Zaller et al., 1990). During EAE, animals develop both T-cell (Kumar and Sercarz, 1993) and humoral (Hashim et al., 1990; Hashim et al., 1991) immune responses against the TCR of encephalitogenic T cells; and these T cells and antibodies protected from EAE when passively transferred into naïve mice. The mechanisms of action of regulatory anti-TCR T cells have been analyzed extensively and may involve anergy (Kumar et al., 1995), deletion (Madakamutil et al., 2003) or immune deviation of the cytokine response (Kumar and Sercarz, 1998) of encephalitogenic T cells. In contrast, although the role of induced anti-TCR antibodies in regulating encephalitogenic T cells has been addressed in some studies (Aroeira, 2006; Hashim et al., 1990; Zhou and Whitaker, 1993), the precise mechanisms underlying their regulatory properties remain to be clearly defined.

Natural anti-TCR antibodies are present in the serum of healthy mice and humans (Marchalonis et al., 1994), and at higher levels in patients with rheumatoid arthritis, systemic lupus erythematosus (Marchalonis et al., 1992) and myasthenia gravis (Jambou et al., 2003). Some natural anti-TCR monoclonal antibodies have been generated and characterized (Marchalonis et al., 1994; Robey et al., 2000). However, their therapeutic potential remains to be determined. In the present study, we tested the capability of a novel natural anti-TCRVβ8 monoclonal antibody to protect against autoimmune encephalomyelitis, and characterized its effects on the T-cell response during disease development.

* Corresponding author. Tel.: +34 913947220; fax: +34 913941641.
** Correspondence to: L. Stark-Aroeira, Laboratorio de Nefrología, Unidad de Investigación, Hospital La Paz, Paseo de la Castellana 261, 28048 Madrid, Spain. Tel.: +34 917277221; fax: +34 917277524.
E-mail addresses: edfernan@med.ucm.es (E. Fernández-Malavé), lstark.hlpr@salud.madrid.org (L. Stark-Aroeira).

0165-5728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2011.02.006

2. Materials and methods

2.1. Mice

Six- to eight-week-old female B10.PL and SJL mice were used in the experiments. Encephalitogenic T cells in B10.PL mice use mainly Vβ8.2 (Acha-Orbea et al., 1988) while SJL mice, which were used as control, have their Vβ8 genes deleted (Behlke et al., 1986) and their encephalitogenic T cells bear Vβ17a and Vβ4 (Padula et al., 1991). B10.PL and SJL mice were obtained from Instituto de Ciências Biomédicas of Universidade de São Paulo, Brasil, and CEMIB (Campinas, São Paulo), respectively. The study was approved by the institutional ethical board for animal studies.

2.2. EAE induction

For EAE induction, 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 complete Freund's adjuvant (CFA; Life Technologies, Paisley, UK) supplemented with 400 μg BCG (Instituto Butantã, São Paulo; SP), and injected subcutaneously into the base of the tail on day 0. Mice also received 200 ng of pertussis toxin (Life Technologies) intravenously on days 0 and 2. Clinical symptoms were monitored daily after 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; and 5, quadriplegia or death.

2.3. C1-19 preparation and treatment

C1-19 is a monoclonal IgM/*k* antibody obtained by fusion of spleen cells from a normal non-immunized BALB/c mouse with the non-secreting P3X63Ag8.653 myeloma line. C1-19 was selected based on its ability to recognize recombinant Vβ8.2 in ELISA and stain the TCRVβ8-expressing Yac-1 cell line in flow cytometric assays. For further analysis of antibody specificity, primary spleen cells from a B cell-deficient (B6μmt) mouse (Kitamura et al., 1991) were used to avoid nonspecific staining by endogenous antibodies. The C1-19-secreting hybridoma was cultured in RPMI 1640 (Life Technologies) containing 7% B6μmt mouse serum. IgM concentration in the culture supernatant was determined by standard ELISA. For antibody treatment, culture supernatant containing 10 μg of C1-19 or control irrelevant IgM immunoglobulin (Southern Biotechnology, Birmingham, AL, USA) diluted in RPMI plus 7% B6μmt mouse serum were injected i.p. every other day, starting at day 4 after MBP immunization, or when first signs of disease were detected (mice showing clinical score 1).

2.4. Proliferation assay

Spleen cells (5×10^6) were cultured for 72 h at 100 μl/well with 50 μg/ml of MBP in RPMI 1640 supplemented with 5% FCS, 50 μM 2-ME, 100 μg streptomycin and 100 U/ml of penicillin, all from Life Technologies. Cultures were pulsed with 0.5 μCi [^{3}H]-thymidine/well (Amersham Biosciences, Buckinghamshire, UK) for the last 8 h. Incorporation of [^{3}H]-thymidine was measured as counts per minute (cpm) using a liquid scintillation β-counter.

2.5. FACS staining and analysis

For two-color surface staining, 1×10^6 cells in 30 μl PBS with 0.5% BSA (Life Technologies) were incubated with each monoclonal antibody on ice for 20 min. Staining was performed with saturating concentrations of the following fluorescein isothiocyanate (FITC) and biotin-conjugated monoclonal antibodies (BD Biosciences, San Diego, CA, USA): anti-CD3, anti-CD4, anti-CD8, anti-CD45RB, anti-CD69, anti-TCRVβ3, anti-TCRVβ8 and anti-TCRVβ9. Phycoerythrin-conjugated streptavidin (BD Biosciences) was used to detect biotinylated antibodies. For intracellular cytokine staining, cells were cultured in the presence of Golgi transport inhibitor for the final 4 h of culture. Cells were then surface-stained with anti-CD4-PerCP, followed by fixation and permeabilization using Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's protocol. Finally, cells were stained with anti-IFN-γ-FITC and anti-IL-4-PE and analyzed by flow cytometry.

2.6. Cytokine assays

IFN-γ and IL-4 in culture supernatants were measured by ELISA using purified and biotinylated antibody pairs, standards and streptavidin-HRP according to the manufacturer (BD Biosciences); and o-phenylene-diamine (Sigma-Aldrich) as substrate. Concentrations

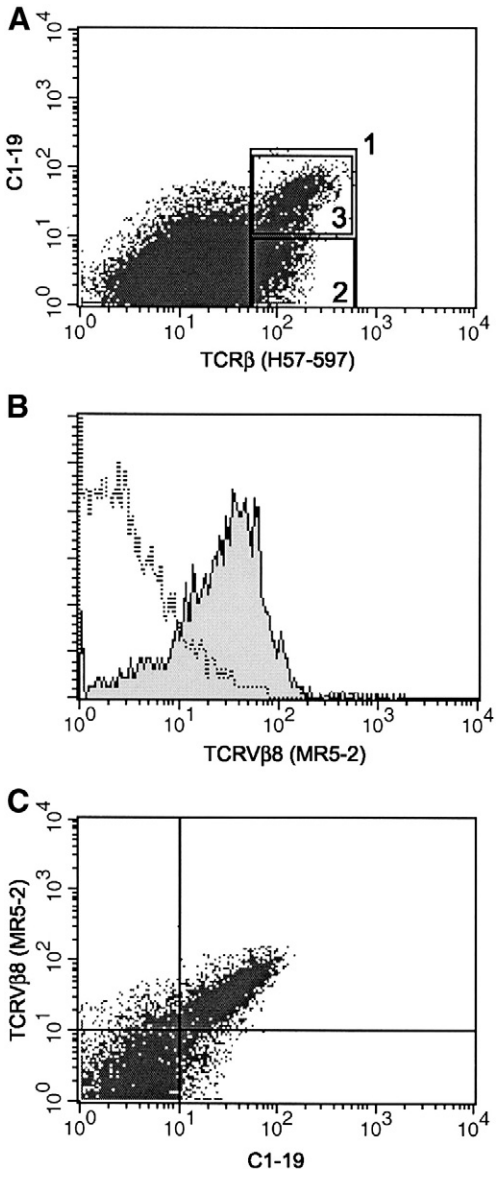


Fig. 1. C1-19 recognizes surface TCRVβ8 in mouse primary T cells. Spleen cells from B6μmt mice were analyzed by three-color flow cytometry after staining with FITC-conjugated anti-TCRCβ (H57-597) and C1-19 developed with PE-conjugated anti-mouse IgM, followed by biotinylated anti-TCRVβ8 (MR5-2) and streptavidin-PE-Cy5 staining. TCRCβ and C1-19 staining of total spleen cells (A). TCRCβ$^+$ cells gated as indicated in A were analyzed for MR5-2 staining of C1-19$^-$ (gate 2; dotted line) and C1-19$^+$ (gate 3; shaded solid line) cells (B). MR5-2 and C1-19 staining of TCRCβ$^+$ cells (gate 1; as indicated in A) (C).

Table 1
C1-19 treatment induces activation but not deletion of TCRVβ8$^+$ T cells.

Treatment	% of CD3$^+$ cells		
	TCRVβ8$^+$	TCRVβ8$^+$/CD69$^+$	TCRVβ8$^+$/CD45RB^{low}
None	26.6 ± 4.1[a]	19.7 ± 3.5	19.7 ± 2.0
Control IgM	27.0 ± 3.3	21.8 ± 2.1	19.8 ± 2.1
C1-19	25.4 ± 1.8	30.4 ± 1.4	28.6 ± 5.3

B10.PL mice were treated with C119 or control IgM every other day during a week, as described in Materials and methods. Two hours after the last injection, splenocytes were collected, double or triple stained for the indicated surface molecules, and analyzed by flow cytometry. Results are shown as the percentage of cells within the CD3$^+$ population.

[a] Mean ± SD for four mice in each group and representative of three independent experiments with similar results.

were calculated on the basis of standard curves using recombinant IFN-γ and IL-4.

2.7. Statistical analysis

Statistical analysis was performed using the Mann–Whitney non-parametric test. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. C1-19 recognizes TCRVβ8 on mouse primary T cells

C1-19 is a monoclonal IgM antibody generated by hybridoma technology using spleen cells from a non-immunized BALB/c mouse.

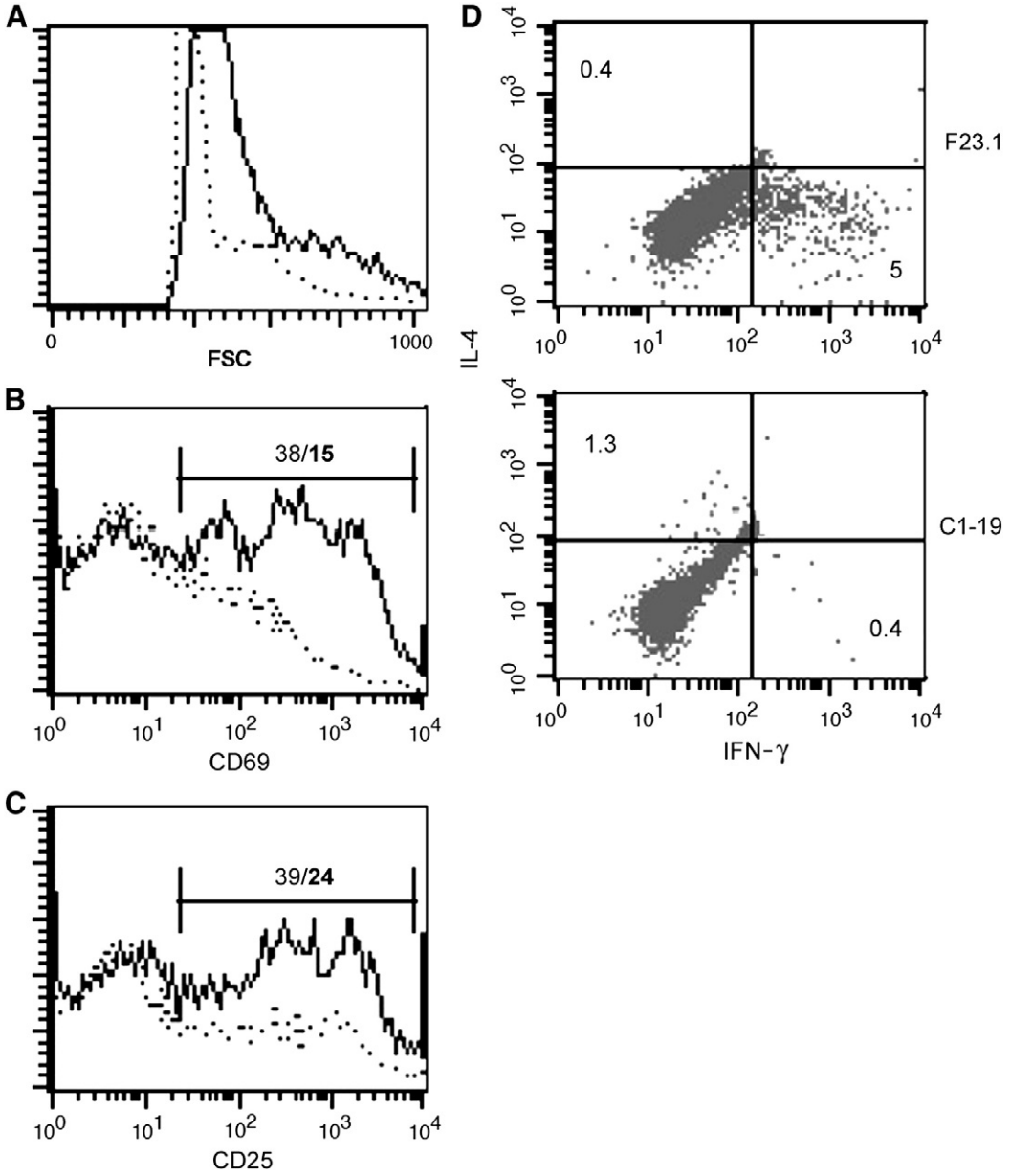


Fig. 2. C1-19 induces weak T-cell activation in vitro. C57BL/6 splenocytes were stimulated with immobilized (1 μg) anti-TCR Vβ8 C1-19 or F23.1 antibodies for 48 h, followed by determination of cell size (forward scatter; FSC) (A), CD69 (B) and CD25 (C) surface expression in C1-19 (dotted line) or F23.1-stimulated (continuous line) CD4$^+$ T cells by flow cytometry. Numbers within histograms represent the percentage of positive cells in the marked region (upper value: F23.1; lower (bold) value: C1-19). Cytokine induction in CD4$^+$ T cells stimulated with immobilized F23.1 (1 μg) or C1-19 (10 μg) was determined by intracellular cytokine staining and flow cytometry (D). Numbers within dot plots represent the percentage of cells in the quadrant.

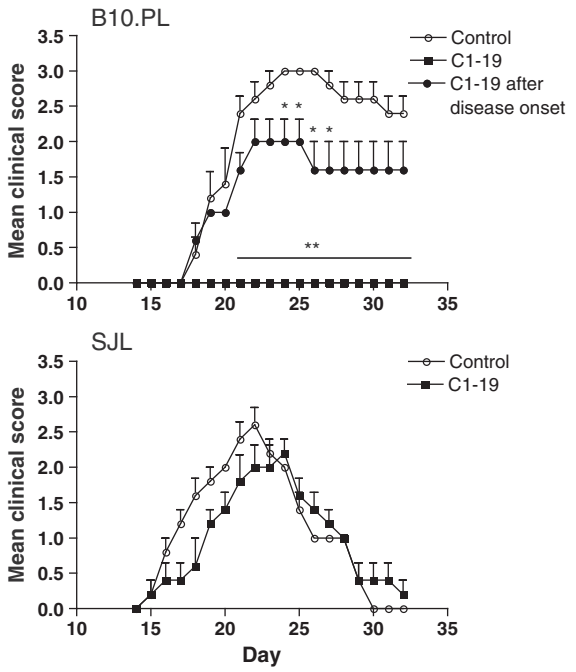


Fig. 3. C1-19 protects B10.PL but not SJL mice from EAE. EAE was induced in B10.PL or SJL mice by immunization with MBP in CFA. C1-19 treatment was started at day 4 after immunization or at the onset of disease (mice showing clinical score 1). Mean ± SD clinical scores ($n=5$) are shown. Data are representative of three independent experiments. *$P<0.05$; **$P<0.001$.

Table 2
C1-19 protects B10.PL mice against EAE.

	Control	C1-19	C1-19 after onset
Maximal score	3[a]	0	2 ($P=0.013$)[b]
Cumulative score	35	0	24.6 ($P=0.043$)
Incidence	5/5	0/5	5/5

Values are for the experiment in Fig. 3.
[a] Mean of five mice per group.
[b] *P* values versus control group.

C1-19 was selected on the basis of its reactivity for recombinant TCRVβ8 and the TCRVβ8$^+$ Yac-1 cell line in ELISA and flow cytometry assays, respectively (data not shown). To determine whether C1-19 was also able to recognize TCRVβ8 on the surface of primary T cells, we performed three-color staining of spleen cells from B6μmt mice using a combination of anti-TCRCβ (H57-597), anti-TCR Vβ8.1 and Vβ8.2 (MR5-2) and C1-19 monoclonal antibodies. C1-19 stained a fraction of splenic TCRCβ$^+$ cells (Fig. 1A) which were also positive for staining with the anti-TCRVβ8 antibody MR5-2 (Fig. 1B), while TCRCβ$^+$C1-19$^-$ T cells were not stained by MR5-2. Moreover, the analysis of C1-19 and MR5-2 staining of TCRCβ$^+$ spleen cells showed that all T cells stained by MR5-2 were also reactive with C1-19 (Fig. 1C), indicating further that C1-19 recognizes TCRVβ8, and that C1-19 and MR5-2 bind to non-overlapping epitopes on TCRVβ8. Together, these data demonstrate that C1-19 is an anti-TCRVβ8 antibody capable of detecting this particular TCRVβ element on the surface of primary mouse T cells.

3.2. C1-19 induces activation of TCRVβ8$^+$ T cells

Initially, we analyzed the effect of low-dose antibody on the frequency and phenotype of TCRVβ8$^+$ spleen T cells from B10.PL mice treated with 10 μg of C1-19, irrelevant IgM or vehicle i.p. every other day during a week. The frequency of splenic TCRVβ8$^+$ cells within the CD3$^+$ (i.e. T-cell) population was similar in mice treated with C1-19, control IgM or vehicle alone (Table 1), indicating that C1-19 did not cause deletion of TCRVβ8$^+$ T cells. In contrast, TCRVβ8$^+$ T cells expressing CD69 or low levels of CD45RB (CD45RB^{low}), indicative of T cell activation, were more abundant in mice treated with C1-19 than in control mice treated with irrelevant IgM or vehicle alone (Table 1). Together, these data indicate that C1-19 acts in vivo as a non-deleting but activating antibody for TCRVβ8$^+$ T cells.

Next, we examined the capability of C1-19 to induce T-cell activation in vitro compared with F23.1, a non-natural anti-TCRVβ8 monoclonal (IgG2a) antibody (Staerz et al., 1985). For this, we stimulated splenocytes with immobilized (plastic-adsorbed) C1-19 or F23.1 antibodies. C1-19 was less effective than F23.1 for induction of cell size increase (Fig. 2A) and surface upregulation of the early activation markers CD69 (Fig. 2B) and CD25 (Fig. 2C). Furthermore, C1-19 was a poor inducer of IFN-γ even when used at ten-fold the amount of F23.1 (Fig. 2D), while provoking a slight but consistent increase in the frequency of activated T cells expressing IL-4. Thus, compared with the non-natural F23.1 antibody, C1-19 is a weaker inducer of early T-cell activation.

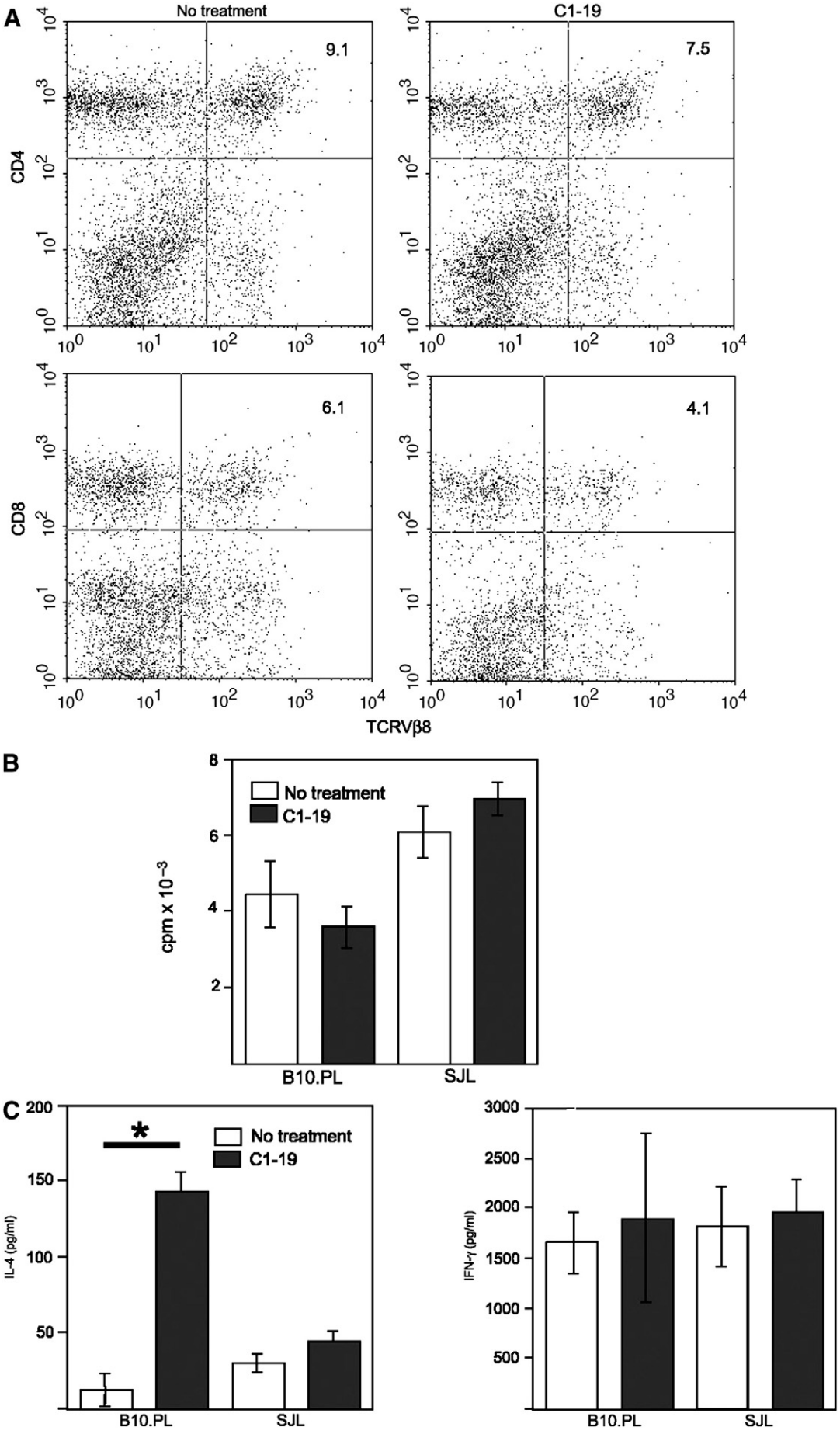
3.3. C1-19 prevents EAE induction in B10.PL but not SJL mice

We next evaluated the effect of C1-19 on the development of EAE induced by MBP immunization in B10.PL mice, in which encephalitogenic T cells are mainly TCRVβ8$^+$ (Acha-Orbea et al., 1988), or SJL mice that have all the Vβ8 gene family deleted and their encephalitogenic T cells use preferentially Vβ17a and Vβ4 (Padula et al., 1991). Mice were immunized with MBP in CFA (day 0), and subsequently treated with low-dose C1-19 or vehicle every other day from day 4. In another group of B10.PL mice, C1-19 treatment was started at the onset of disease (mice showing clinical score 1). As shown in Fig. 3, both B10.PL and SJL mice developed EAE in response to MBP immunization. Strikingly, treatment with C1-19 completely prevented the establishment of EAE in B10.PL, but not in SJL mice. Furthermore, C1-19 reduced the clinical score of and prevented disease progression when administered after the first signs of disease (Fig. 3; upper panel). These results demonstrate that continuous low-dose administration of C1-19 can prevent EAE specifically in mice in which disease is mediated by anti-MBP TCRVβ8$^+$ T cells, such as B10.PL. In addition, C1-19 could partially treat ongoing EAE in B10.PL mice, significantly reducing the worsening of disease when administered after the appearance of the first clinical signs (Table 2).

3.4. C1-19-mediated protection from EAE is independent from deletion or anergy, but correlates with deviation of the cytokine response of MBP-reactive T cells

To confirm that the protective effect of C1-19 on EAE establishment was not dependent on deletion of TCRVβ8$^+$ cells, we determined the frequency of these cells in B10.PL mice treated or not with C1-19. On day 10 after EAE induction, the frequency of CD4$^+$Vβ8$^+$ and CD8$^+$Vβ8$^+$ T cells in spleen (Fig. 4A) and lymph nodes (not shown) were only slightly reduced in mice treated with C1-19 compared with untreated mice.

Fig. 4. Prevention of EAE by C1-19 correlates with deviation of the cytokine response but not with deletion or anergy of encephalitogenic T cells. Ten days after EAE induction, spleen cells from untreated or C1-19-treated B10.PL mice were isolated and analyzed for TCRVβ8 expression within CD4 and CD8 T-cell subsets by flow cytometry (A). Total cell numbers were 35 ± 1.9 and 24.6 ± 3.9 ($n=5$; $P=0.2$) for untreated and C1-19-treated mice, respectively. Spleen cells from B10.PL and SJL mice were stimulated in vitro with MBP to assess anti-MBP proliferative responses by [^{3}H]-thymidine incorporation (B) and cytokine responses by ELISA (C). Results are the mean ± SD of five mice per group and representative of two independent experiments. *$P<0.001$.


A
No treatment
C1-19
9.1
7.5
6.1
4.1
CD4
CD8
TCRVβ8
B
No treatment
C1-19
cpm x 10⁻³
B10.PL
SJL
C
*
No treatment
C1-19
IL-4 (pg/ml)
IFN-γ (pg/ml)
B10.PL
SJL

Thus, similarly to non-immunized mice (Table 1), C1-19 does not seem to cause major deletion of TCRVβ8$^+$ T cells in mice developing EAE.

Next, we tested the effect of C1-19 on the in vitro recall response to MBP. For this, B10.PL and SJL splenocytes were harvested on day 10 after EAE induction, and re-stimulated in vitro with MBP for assessment of proliferation and cytokine responses. T cells from C1-19-treated B10.PL and SJL mice exhibited MBP-induced proliferation comparable to that of splenocytes from untreated mice (Fig. 4B), indicating that MBP-reactive T cells in mice treated with C1-19 are not anergic. In contrast to this, secretion of the signature Th2 cytokine, IL-4, in response to antigen was significantly augmented by C1-19 in B10.PL but not SJL mice (Fig. 4C, left). Curiously, MBP-induced production of IFN-γ, a signature Th1 cytokine, was not affected by C1-19 treatment in either B10.PL or SJL mice (Fig. 4C, right). Collectively, these results indicate that C1-19-mediated protection is independent from deletion or anergy of MBP-specific T cells, but could be a consequence of deviation of the MBP-specific response towards a Th2-like cytokine profile.

3.5. Interruption of C1-19 treatment results in attenuated EAE

Since C1-19 did not cause deletion or anergy of MBP-specific T cells during EAE, we were prompted to evaluate the effect of interrupting C1-19 treatment on the course of the disease. Treatment of B10.PL with C1-19 was started on day 4 and discontinued on day 20. Five days after stopping C1-19 administration, three out of five mice developed disease but with reduced clinical scores compared to untreated animals (Fig. 5), suggesting a residual therapeutic action of C1-19 treatment. Thus, C1-19-mediated protection against EAE seems to be dependent on immune regulatory mechanisms requiring the continuous presence of the antibody.

4. Discussion

In the present study, we evaluated the therapeutic potential of a novel natural anti-TCRVβ8 antibody, named C1-19, in the prevention and treatment of autoimmune neuroinflammation. C1-19 is an IgM antibody and because this isotype is a strong complement activator, C1-19 could therefore be potentially cytolytic for TCRVβ8$^+$ T cells. This was not likely the case, as treatment with the antibody did not affect the relative abundance of TCRVβ8$^+$ T cells in B10.PL mice. Instead, C1-19 induced the activation of TCRVβ8$^+$ T cells, as judged by their up-regulated expression of CD69 and reduced expression of CD45RB. The activating effect of C1-19 was specific for cells bearing TCRVβ8, as the frequency of TCRVβ3$^+$ and TCRVβ9$^+$ T cells expressing an activated surface phenotype was not altered by in vivo C1-19 treatment (data not shown).

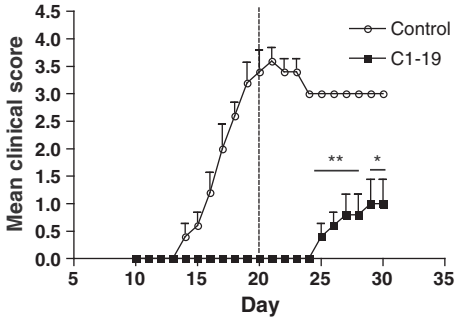


Fig. 5. Interruption of C1-19 treatment results in development of attenuated EAE. EAE was induced in B10.PL mice by immunization with MBP in CFA. C1-19 treatment was started on day 4 after immunization and stopped on day 20 (dashed line). Mean ± SD clinical scores ($n=5$) are shown. Data are representative of two independent experiments. *$P<0.01$; **$P<0.001$.

C1-19 prevented EAE in B10.PL but not in SJL mice, which indicates that the regulatory actions of the antibody result from interaction with T cells bearing the TCR involved in the anti-MBP response, i.e. TCRVβ8, and not from nonspecific effects of administrating IgM immunoglobulin. Apart from preventing EAE induction, C1-19 also displayed therapeutic potential, reducing the severity of EAE when provided upon disease onset.

CD4$^+$ Th cells are the main contributors to the pathogenic mechanisms responsible for the onset and progression of autoimmune diseases. CD4$^+$ T-cell infiltration of the central nervous system is required for the development of autoimmune encephalomyelitis, with infiltrating macrophages and resident microglia acting as the ultimate effectors causing demyelination, oligodendrocyte death and loss of axonal function (Merrill et al., 1992). Activation of macrophages and microglia depends critically on pro-inflammatory Th1 cytokines, such as IFN-γ, thus explaining the essential role of Th1 cells in the initiation and establishment of the neurological disease (Jee and Matsumoto, 2001; Kuchroo et al., 1993; Willenborg et al., 1999). Recently, it was demonstrated that IL-17-secreting T (Th17) cells also play a critical role in the development of EAE (Langrish et al., 2005). On the contrary, Th2/Th3 cytokines such as IL-4, IL-10 and TGF-β have a preventive effect on EAE (Racke et al., 1994). These regulatory cytokines antagonize pro-inflammatory cytokines, in particular IFN-γ, by reducing their expression or suppressing their effects (Murphy and Reiner, 2002; O'Shea and Paul). Therefore, the deviation of the anti-MBP response towards an IL-4-dominated Th2-like cytokine profile could, at least partly, explain the beneficial effects of C1-19 in the prevention and/or amelioration of EAE (Tran et al., 2001). Although the precise molecular mechanisms underlying the protective effects of C1-19 in EAE remain to be further determined, our results clearly indicate that C1-19, in contrast to previously reported non-natural antibodies against constant or variable regions of TCR components (Kohm et al., 2005; Lavasani et al., 2007; Zaller et al., 1990) acts through mechanisms not involving down-modulation of surface TCR, deletion or unresponsiveness of antigen-specific T cells. Yet, the immune regulatory mechanisms and therapeutic effect triggered by C1-19 seemed to require the continuous presence of the antibody, at least at the dose and administration protocol tested in the study. This latter feature could be partly related to the use of a relatively low dosage of the antibody, compared with previous studies with non-natural anti-TCR antibodies (Acha-Orbea et al., 1988; Urban et al., 1988; Zaller et al., 1990). Alternatively, the higher clearance rate for IgM compared to IgG antibodies could be underlying the need for continued administration of C1-19.

Interestingly, mice treated with C1-19 presented as FCS-containing culture supernatant developed a strong IgE response against bovine albumin, ultimately leading to anaphylactic shock (Stark-Aroeira, unpublished data). Because IgE responses are Th2-dependent (Romagnani, 2000; Yazdanbakhsh et al., 2001), the latter finding further supports that C1-19 specifically induces immune deviation of antigen-specific T cells towards a Th2-like cytokine profile (Falcone and Bloom, 1997), an effect that could be secondary to sub-optimal induction of TCR cross-linking and signaling by C1-19 (Leitenberg and Bottomly, 1999; Nakayama and Yamashita). This latter property of C1-19 could in turn be associated with the intrinsic low affinity of natural antibodies and/or a more restricted cellular expression of FcRs for IgM (Boes, 2000) compared to their counterparts for IgG. Importantly, due to their intrinsic low affinity, natural antibodies are unlikely to induce the strong release of pro-inflammatory cytokines which results in what is known as "first dose" syndrome (Alegre et al., 1990; Ferran et al., 1990), an undesirable side-effect restricting the use of anti-TCR antibodies as therapeutic agents.

In conclusion, our study demonstrates that C1-19, a natural anti-TCR monoclonal antibody, can protect against autoimmune encephalomyelitis by, at least partly, skewing the global response to self-antigen in a Th2 direction and independently from T-cell deletion or

anergy. Thus, C1-19 could be a useful tool in mouse models of pathology for understanding the immune regulatory properties of natural anti-TCR antibodies (Boes, 2000; Marchalonis et al., 2002). Furthermore, our study establishes a proof-of-concept for a therapeutic value of natural anti-TCR antibodies and for development of natural antibody-based therapies (Holmoy and Vartdal, 2007; Vollmers and Brandlein, 2005; Zhang et al., 2003) applicable in human autoimmune inflammatory diseases (Adelman et al., 2007; Jambou et al., 2003; Schluter et al., 2003).

Acknowledgments

We thank Concepción Mouton for helping with C1-19 preparation. We also thank Sabela Díaz-Castroverde for support with in vitro experiments. This work was supported by grants 98/115195 from FAPESP to L.S-A., and FIS PI080102 from Instituto de Salud Carlos III and CCG08-UCM/SAL-4215 to E.F-M.

References

Acha-Orbea, H., Mitchell, D.J., Timmermann, L., Wraith, D.C., Tausch, G.S., Waldor, M.K., Zamvil, S.S., McDevitt, H.O., Steinman, L., 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. Cell 54, 263–273.

Adelman, M.K., Schluter, S.F., Robey, I.F., Marchalonis, J.J., 2007. Natural and autoantibodies to human T-cell receptor Vbeta segments: potential roles in immunomodulation. Crit. Rev. Immunol. 27, 221–232.

Alegre, M., Vandenabeele, P., Flamand, V., Moser, M., Leo, O., Abramowicz, D., Urbain, J., Fiers, W., Goldman, M., 1990. Hypothermia and hypoglycemia induced by anti-CD3 monoclonal antibody in mice: role of tumor necrosis factor. Eur. J. Immunol. 20, 707–710.

Aroeira, L.S., 2006. TCR vaccination in aluminum adjuvant protects against autoimmune encephalomyelitis. Clin. Immunol. 119, 252–260.

Baccala, R., Quang, T.V., Gilbert, M., Ternynck, T., Avrameas, S., 1989. Two murine natural polyreactive autoantibodies are encoded by nonmutated germ-line genes. Proc. Natl Acad. Sci. USA 86, 4624–4628.

Behlke, M.A., Chou, H.S., Huppi, K., Loh, D.Y., 1986. Murine T-cell receptor mutants with deletions of beta-chain variable region genes. Proc. Natl Acad. Sci. USA 83, 767–771.

Boes, M., 2000. Role of natural and immune IgM antibodies in immune responses. Mol. Immunol. 37, 1141–1149.

Falcone, M., Bloom, B.R., 1997. A T helper cell 2 (Th2) immune response against non-self antigens modifies the cytokine profile of autoimmune T cells and protects against experimental allergic encephalomyelitis. J. Exp. Med. 185, 901–907.

Ferran, C., Sheehan, K., Dy, M., Schreiber, R., Merite, S., Landais, P., Noel, L.H., Grau, G., Bluestone, J., Bach, J.F., et al., 1990. Cytokine-related syndrome following injection of anti-CD3 monoclonal antibody: further evidence for transient in vivo T cell activation. Eur. J. Immunol. 20, 509–515.

Guilbert, B., Dighiero, G., Avrameas, S., 1982. Naturally occurring antibodies against nine common antigens in human sera. I. Detection, isolation and characterization. J. Immunol. 128, 2779–2787.

Hashim, G.A., Vandenbark, A.A., Galang, A.B., Diamanduros, T., Carvalho, E., Srinivasan, J., Jones, R., Vainiene, M., Morrison, W.J., Offner, H., 1990. Antibodies specific for VB8 receptor peptide suppress experimental autoimmune encephalomyelitis. J. Immunol. 144, 4621–4627.

Hashim, G.A., Vandenbark, A.A., Offner, H., 1991. Antibodies against T-cell receptor peptide arrest autoimmune rejection of normal central nervous system myelin. Transplant. Proc. 23, 83–85.

Haury, M., Grandien, A., Sundblad, A., Coutinho, A., Nobrega, A., 1994. Global analysis of antibody repertoires. 1. An immunoblot method for the quantitative screening of a large number of reactivities. Scand. J. Immunol. 39, 79–87.

Holmoy, T., Vartdal, F., 2007. The immunological basis for treatment of multiple sclerosis. Scand. J. Immunol. 66, 374–382.

Jambou, F., Zhang, W., Menestrier, M., Klingel-Schmitt, I., Michel, O., Caillat-Zucman, S., Aissaoui, A., Landemarre, L., Berrih-Aknin, S., Cohen-Kaminsky, S., 2003. Circulating regulatory anti-T cell receptor antibodies in patients with myasthenia gravis. J. Clin. Invest. 112, 265–274.

Jee, Y., Matsumoto, Y., 2001. Two-step activation of T cells, clonal expansion and subsequent Th1 cytokine production, is essential for the development of clinical autoimmune encephalomyelitis. Eur. J. Immunol. 31, 1800–1812.

Kitamura, D., Roes, J., Kuhn, R., Rajewsky, K., 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. Nature 350, 423–426.

Kohm, A.P., Williams, J.S., Bickford, A.L., McMahon, J.S., Chatenoud, L., Bach, J.F., Bluestone, J.A., Miller, S.D., 2005. Treatment with nonmitogenic anti-CD3 monoclonal antibody induces CD4+ T cell unresponsiveness and functional reversal of established experimental autoimmune encephalomyelitis. J. Immunol. 174, 4525–4534.

Kotzin, B.L., Karuturi, S., Chou, Y.K., Lafferty, J., Forrester, J.M., Better, M., Nedwin, G.E., Offner, H., Vandenbark, A.A., 1991. Preferential T-cell receptor beta-chain variable gene use in myelin basic protein-reactive T-cell clones from patients with multiple sclerosis. Proc. Natl Acad. Sci. USA 88, 9161–9165.

Kuchroo, V.K., Martin, C.A., Greer, J.M., Ju, S.T., Sobel, R.A., Dorf, M.E., 1993. Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clones to mediate experimental allergic encephalomyelitis. J. Immunol. 151, 4371–4382.

Kumar, V., Sercarz, E., 1998. Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR peptide-specific regulatory CD4 T cells. J. Immunol. 161, 6585–6591.

Kumar, V., Sercarz, E.E., 1993. The involvement of T cell receptor peptide-specific regulatory CD4+ T cells in recovery from antigen-induced autoimmune disease. J. Exp. Med. 178, 909–916.

Kumar, V., Tabibiazar, R., Geysen, H.M., Sercarz, E., 1995. Immunodominant framework region 3 peptide from TCR V beta 8.2 chain controls murine experimental autoimmune encephalomyelitis. J. Immunol. 154, 1941–1950.

Lacroix-Desmazes, S., Kaveri, S.V., Mouthon, L., Ayouba, A., Malanchere, E., Coutinho, A., Kazatchkine, M.D., 1998. Self-reactive antibodies (natural autoantibodies) in healthy individuals. J. Immunol. Methods 216, 117–137.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., Cua, D.J., 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J. Exp. Med. 201, 233–240.

Lavasani, S., Dzhambazov, B., Andersson, M., 2007. Monoclonal antibody against T-cell receptor alphabeta induces self-tolerance in chronic experimental autoimmune encephalomyelitis. Scand. J. Immunol. 65, 39–47.

Leitenberg, D., Bottomly, K., 1999. Regulation of naive T cell differentiation by varying the potency of TCR signal transduction. Semin. Immunol. 11, 283–292.

Madakamutil, L.T., Maricic, I., Sercarz, E., Kumar, V., 2003. Regulatory T cells control autoimmunity in vivo by inducing apoptotic depletion of activated pathogenic lymphocytes. J. Immunol. 170, 2985–2992.

Marchalonis, J.J., Kaveri, S., Lacroix-Desmazes, S., Kazatchkine, M.D., 2002. Natural recognition repertoire and the evolutionary emergence of the combinatorial immune system. FASEB J. 16, 842–848.

Marchalonis, J.J., Kaymaz, H., Dedeoglu, F., Schluter, S.F., Yocum, D.E., Edmundson, A.B., 1992. Human autoantibodies reactive with synthetic autoantigens from T-cell receptor beta chain. Proc. Natl Acad. Sci. USA 89, 3325–3329.

Marchalonis, J.J., Schluter, S.F., Wang, E., Dehghanpisheh, K., Lake, D., Yocum, D.E., Edmundson, A.B., Winfield, J.B., 1994. Synthetic autoantigen of immunoglobulins and T-cell receptors: their recognition in aging, infection and autoimmunity. P. S. E. B. M. 207, 129–147.

Merrill, J.E., Kono, D.H., Clayton, J., Ando, D.G., Hinton, D.R., Hofman, F.M., 1992. Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. Proc. Natl Acad. Sci. USA 89, 574–578.

Murphy, K.M., Reiner, S.L., 2002. The lineage decisions of helper T cells. Nat. Rev. Immunol. 2, 933–944.

Nakayama, T., Yamashita, M., 2010. The TCR-mediated signaling pathways that control the direction of helper T cell differentiation. Semin Immunol 327, 303–309.

O'Shea, J.J., Paul, W.E., 2010. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science 327, 1098–1102.

Oksenberg, J.R., Panzara, M.A., Begovich, A.B., Mitchell, D., Erlich, H.A., Murray, R.S., Shimonkevitz, R., Sherritt, M., Rothbard, J., Bernard, C.C., et al., 1993. Selection for T-cell receptor V beta-D beta-J beta gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. Nature 362, 68–70.

Padula, S.J., Lingenheld, E.G., Stabach, P.R., Chou, C.H., Kono, D.H., Clark, R.B., 1991. Identification of encephalitogenic V beta-4-bearing T cells in SJL mice. Further evidence for the V region disease hypothesis? J. Immunol. 146, 879–883.

Racke, M., Bonomo, A., Scott, D., Cannella, B., Levine, A., Raine, C., Shevach, E., Rocken, M., 1994. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. J. Exp. Med. 180, 1961–1966.

Robey, I.F., Schluter, S.F., Yocum, D.E., Marchalonis, J.J., 2000. Production and characterization of monoclonal IgM autoantibodies specific for the T-cell receptor. J. Protein Chem. 19, 9–21.

Romagnani, S., 2000. T-cell subsets (Th1 versus Th2). Ann. Allergy Asthma Immunol. 85, 9–18 quiz 18, 21.

Schluter, S.F., Adelman, M.K., Taneja, V., David, C., Yocum, D.E., Marchalonis, J.J., 2003. Natural autoantibodies to TCR public idiotopes: potential roles in immunomodulation. Cell. Mol. Biol. 49, 193–207.

Staerz, U.D., Rammensee, H.G., Benedetto, J.D., Bevan, M.J., 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. J. Immunol. 134, 3994–4000.

Steinman, L., Zamvil, S.S., 2005. Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. Trends Immunol. 26, 565–571.

Tran, G.T., Carter, N., He, X.Y., Spicer, T.S., Plain, K.M., Nicolls, M., Hall, B.M., Hodgkinson, S.J., 2001. Reversal of experimental allergic encephalomyelitis with non-mitogenic, non-depleting anti-CD3 mAb therapy with a preferential effect on T(h)1 cells that is augmented by IL-4. Int. Immunol. 13, 1109–1120.

Urban, J.L., Kumar, V., Kono, D.H., Gomez, C., Horvath, S.J., Clayton, J., Ando, D.G., Sercarz, E.E., Hood, L., 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy [see comments]. Cell 54, 577–592.

Vollmers, H.P., Brandlein, S., 2005. The "early birds": natural IgM antibodies and immune surveillance. Histol. Histopathol. 20, 927–937.

Willenborg, D.O., Fordham, S.A., Staykova, M.A., Ramshaw, I.A., Cowden, W.B., 1999. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. J. Immunol. 163, 5278–5286.

Yazdanbakhsh, M., van den Biggelaar, A., Maizels, R.M., 2001. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol. 22, 372–377.

Zaller, D.M., Osman, G., Kanagawa, O., Hood, L., 1990. Prevention and treatment of murine experimental allergic encephalomyelitis with T cell receptor V beta-specific antibodies. J. Exp. Med. 171, 1943–1955.

Zhang, X., Hupperts, R., De Baets, M., 2003. Monoclonal antibody therapy in experimental allergic encephalomyelitis and multiple sclerosis. Immunol. Res. 28, 61–78.

Zhou, S.R., Whitaker, J.N., 1993. Specific modulation of T cells and murine experimental allergic encephalomyelitis by monoclonal anti-idiotypic antibodies. J. Immunol. 150, 1629–1642.