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Regulation of experimental autoimmune encephalomyelitis by $CD4^+$, $CD25^+$ and $CD8^+$ T cells: analysis using depleting antibodies

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Abstract

Experimental Autoimmune Encephalomyelitis (EAE) can be induced in mice of the C57BL/6 strain by subcutaneous immunization with myelin/oligodendrocyte glycoprotein (MOG) peptide p35–55 in CFA, administered twice at an interval of one week and supplemented with *Bordetella pertussis* toxin given IV. Here, we studied the effect on the induction of EAE of depleting antibodies to CD4, CD8, or CD25 administered before either the first or the second dose of MOG p35–55. We found that anti-CD4 abolished EAE when given before the first immunization; anti-CD4 did not affect the disease when it was given before the second immunization. Anti-CD8 enhanced EAE induction when given before either of the two immunizations. Anti-CD25 enhanced EAE to the same degree as anti-CD8 when given before the first immunization, but anti-CD25 was even more effective in enhancing EAE when given before the second immunization. The anti-CD25 treatment led to significantly enhanced IFNγ production by T cells responding to MOG p35–55 and persisting anti-MOG antibodies detectable 56 days after the first immunization. Administration of anti-CD8 or anti-CD25 abolished the need for pertussis toxin to induce EAE. These findings are compatible with the idea that CD4 T cells are required for the initial induction of EAE and that the disease is down-regulated by T cells expressing CD8 or CD25. These regulatory T cells exist prior to MOG immunization, but the $CD25^+$ regulators appear to be further amplified by immunization.

Keywords: CD25; CD8; MOG; Immunoregulation; EAE

1. Introduction

Experimental Autoimmune Encephalomyelitis (EAE) is an experimental model of autoimmune inflammatory disease of the central nervous system that shares many features with human multiple sclerosis [1]. Myelin/oligodendrocyte glycoprotein (MOG) is a myelin antigen and its p35–55 peptide is capable of inducing chronic progressive EAE in C57BL/6 (H-2^b) mice [2,3]. The induction of EAE in this model requires that the MOG p35–55 peptide be administered in CFA, and pertussis toxin is usually needed to facilitate induction [4]. MOG p35–55 peptide induces both T-cell and antibody responses, and the severity of EAE correlates with the presence of MOG-specific autoantibodies and the secretion of Th1 cytokines such as IFNγ [5–7].

Different regulatory cells have been implicated in controlling EAE, including CD8 T-cell suppressors and $CD4^+CD25^+$ T-cell suppressors [8–13]. Here, we investigated the effects on EAE induction of depleting antibodies to CD4, CD8 or CD25 administered before either the first or the second MOG p35–55

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MOG, myelin/oligodendrocyte glycoprotein; mAb, monoclonal antibody; IV, intravenously; CFA, complete Freund's adjuvant; PPD, mycobacterium-derived purified protein derivative.

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peptide immunization. We also tested whether depleting the CD8 or CD25 regulators could enhance EAE in the absence of pertussis toxin.

2. Materials and methods

2.1. Mice

Female C57BL/6 H-2^b mice, aged 8–12 weeks, were raised in the Animal Breeding Center of The Weizmann Institute of Science. The experiments were performed under the supervision and guidelines of the Animal Welfare Committee.

2.2. Antigen

MOG peptide p35–55 (MEVGWYRSPFSRVVH-LYRNGK) was synthesized at The Weizmann Institute of Science as described [14]. The purity of the peptide was greater than 90%.

2.3. Monoclonal antibodies

Rat mAb was prepared from tissue culture supernatants containing depleting anti-CD4a (IgG2b) from the rat YTS 191.1.1.2 hybridoma; anti-CD8a (IgG2b) from rat YTS 169.4.2.1 hybridoma; and anti-CD25 (IgG1) from rat PC61 hybridoma. The specific depleting effects of these antibodies have been reported [15,16]. Antibodies were purified by precipitation with 50% saturated ammonium sulphate followed by dialysis into saline solution (pH 7.2), filtered by 0.2 μm and stored at 2–8 °C. The mAb concentration was adjusted to 5 mg/ml as estimated by absorbance at 280 nm. For $CD4^+$, $CD8^+$ and $CD25^+$ T-cell depletion, mice were given one intravenous injection of 1 mg of monoclonal antibody per dose in 0.2 ml by the tail vein. Controls received an equal amount of purified normal rat IgG, or saline. We documented by FACS analysis the degree of depletion of splenic populations following the administration of these antibodies: in general, we observed a reduction of >80% by 72 h of a single intravenous administration. Recovery was about 50% at one week, and full recovery occurred after 3–4 weeks (data not shown).

2.4. EAE induction

Mice were immunized subcutaneously on days 0 and 7 at two sites with 200 μg MOG p35–55 peptide emulsified in complete Freund's adjuvant (CFA; Difco) supplemented with heat-killed *Mycobacterium tuberculosis* H37 RA (500 μg per mouse; Difco) in a total volume of 100 μL [2]. Mice, as noted, did or did not receive 200 ng of *B. pertussis* toxin (List Biological, Campbell, California, USA) in 200 μL PBS in the tail vein immediately after the first immunization and again 72 h later.

2.5. Assessment of clinical EAE

Mice were examined daily for disease, assessed clinically on a scale of 0–5: 0 = no disease; 1 = tail weakness or tail paralysis; 2 = hind leg paraparesis or hemiparesis; 3 = hind-leg paralysis or hemi-paralysis; 4 = complete paralysis; 5 = moribund state, or death.

2.6. Flow cytometric analysis

To check the efficiency of T-cell depletion protocols and to follow lymphocyte repopulation, mice were bled individually from the retro-orbital plexus (approximately 0.2 ml) into tubes containing 10 μl of EDTA. Peripheral blood lymphocytes were stained with anti-CD4 mAb conjugated with FITC or R-PE, anti-CD8 conjugated with R-PE, or anti-CD25 conjugated with FITC (Pharmingen, San Diego, CA). Samples were incubated for 30 min on ice with 1 μl of labelled mAb. The cytofluorimetric analysis was performed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA), and data were analysed by Lysys II software.

2.7. Antibody assay

Antibodies were determined by an ELISA assay using flat-bottom microtiter plates (Maxisorb, Nunc, Denmark) coated overnight with 500 ng/well of MOG p35–55 peptide in carbonate buffer at 4 °C. The plates were blocked with 1% skim milk for 1 h at 37 °C. To detect serum antibodies, sera were diluted 1/100 in the skim milk. The diluted sera were added to the plates and incubated for 3 h at 37 °C. Bound antibodies were detected using alkaline phosphatase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, USA) together with Sigma's substrate for alkaline phosphatase. After 30–60 min, absorbance at 405 nm was measured using an ELISA plate reader.

2.8. T-cell proliferation

T-cell proliferation was assayed in vitro using unfractionated spleen cells. Spleen cells were taken 56 days after the first immunization, and 3×10^5 cells per well were cultured as previously described [2,17] in quadriplicates in round-bottom plates (Nunclon, Nunc, Denmark) for 72 h in the presence of different concentrations of either MOG p35–55 peptide or BSA. [^{3}H]thymidine (0.5 μCi of 5 mCi/mmol; Amersham,

England) was added to the cultures for the last 18 h of incubation. Concanavalin A (Con A; Sigma) 1.25 μg/ml was used as a positive control. Thereafter, cells were harvested and the cpm were counted. The results are expressed as the stimulation index, the mean cpm of cultures incubated with antigen divided by the mean cpm of cultures incubated in the absence of antigen. The background cpm in the absence of antigen varied from about 1500 to 2000 cpm.

2.9. IFNγ determination

The supernatants of T-cell proliferation cultures were collected and analysed for IFNγ content by ELISA using an appropriate pair of capture and detecting monoclonal antibodies (Pharmingen, San Diego, USA) according to the manufacturer's instructions. The amounts of IFNγ were determined in pg/ml, based on a calibration curve constructed using known amounts of recombinant IFNγ (Pharmingen). The results are expressed here as an index of the amount of IFNγ produced by stimulation divided by the amount produced in the absence of MOG p35–55 peptide.

2.10. Statistical analysis

The InStat 2.01 program was used for statistical analysis. Student's *t*-test and the Mann–Whitney test were carried out to assay significant differences between the different experimental groups.

3. Results

3.1. Effect on EAE of depleting antibodies to CD4, CD8 or CD25

Mice were immunized twice with MOG p35–55 at an interval of one week, and pertussis toxin was administered immediately after the first immunization and 72 h later to induce EAE. One dose of 1 mg of depleting mAb or control IgG were administered IV using either of two different schedules: 24 h before the first immunization or 24 h before the second immunization. The effects on the development of EAE are shown in Fig. 1; each panel of the figure compares the effects of the two times of administration of depleting mAb (Fig. 1B–D) or control mAb shown in Fig. 1A.

3.1.1. CD4 T cells

Anti-CD4 given before the first immunization completely prevented EAE (open symbols, Fig. 1B). However, anti-CD4 given once before the second immunization had no effect on the clinical course of EAE to day 30 compared to the control group (filled symbols in Fig. 1B compared to Fig. 1A). Thus, the difference between administering anti-CD4 before the

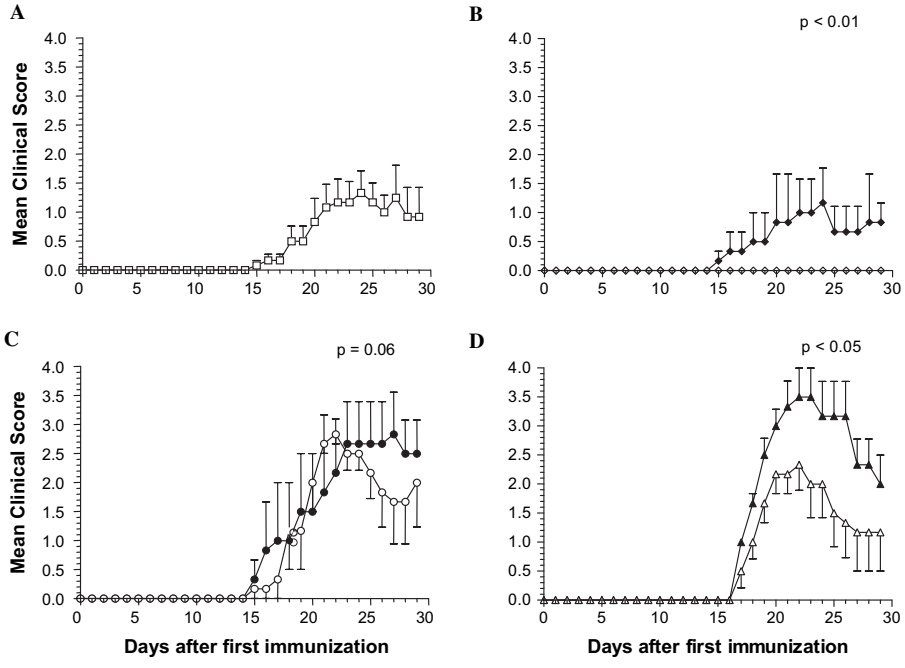

Fig. 1. Anti-CD4, anti-CD8 or anti-CD25 affect the induction of EAE. C57BL/6 mice were immunized on days 0 and 7 with 200 μg MOG peptide emulsified in CFA and received 200 ng of *B. pertussis* toxin immediately after the first immunization and 72 h later. One dose of 1 mg of depleting mAb or control IgG was administered IV using two different schedules: 24 h before the first immunization (open symbols) or 24 h before the second immunization (filled symbols). (A) Control EAE (no antibody); (B) anti-CD4; (C) anti-CD8; and (D) anti-CD25 treatment. Results for ANOVA statistical test are shown.

first or the second immunization was highly significant ($p < 0.01$) (Fig. 1B).

3.1.2. CD8 T cells

Anti-CD8 given before either the first or the second immunization (Fig. 1C) equally enhanced EAE induction compared to the control group (Fig. 1A) ($p < 0.001$). And there was no significant difference between the anti-CD8 schedules ($p = 0.06$) (Fig. 1C).

3.1.3. CD25 T cells

Anti-CD25 given before either the first or the second immunization enhanced EAE (Fig. 1D) compared to the control induction (Fig. 1A) ($p < 0.001$). Unlike anti-CD8 treatment, however, anti-CD25 given before the second immunization induced more severe EAE than did anti-CD25 given before the first immunization (Fig. 1D) ($p < 0.05$).

In summary, anti-CD4 before the first immunization prevented EAE, while anti-CD8 or anti-CD25 before the first immunization equally enhanced EAE. Moreover, anti-CD25 given before the second immunization caused significantly more severe EAE than did any other treatment ($p < 0.05$ vs. anti-CD8 before the second immunization and $p < 0.001$ vs. any of the other treatments).

3.2. Effects of pertussis toxin and of one or two doses of anti-CD25 or anti-CD8

In view of the finding that depletion of CD8 or CD25 T cells before the second immunization enhanced EAE, we tested the effects on the induction of EAE of this schedule of antibody administration in the absence of pertussis toxin. Fig. 2A shows that immunization with MOG p35–55 peptide in CFA without pertussis toxin induced only marginal EAE. Anti-CD4 made no difference. However, anti-CD8 or anti-CD25 given before the second immunization facilitated the development of EAE, despite the absence of pertussis toxin. Note, however, that the antibody-mediated enhancement of EAE tended to remit by day 30.

Fig. 2B shows the effects of giving two doses of antibody before the second immunization—the first dose 24 h and the second 1 h before the second immunization. In this experiment, the control group was immunized using pertussis toxin; the groups receiving the antibodies did not receive pertussis toxin after the first immunization with MOG p35–55 peptide. It can be seen that two doses of anti-CD4 prevented EAE, even when given before the second immunization. In contrast, two doses of anti-CD8 or anti-CD25 markedly enhanced the EAE ($p < 0.05$) with no tendency for remission at day 30 (without pertussis toxin). Thus, two doses of antibody were more effective than a single dose.

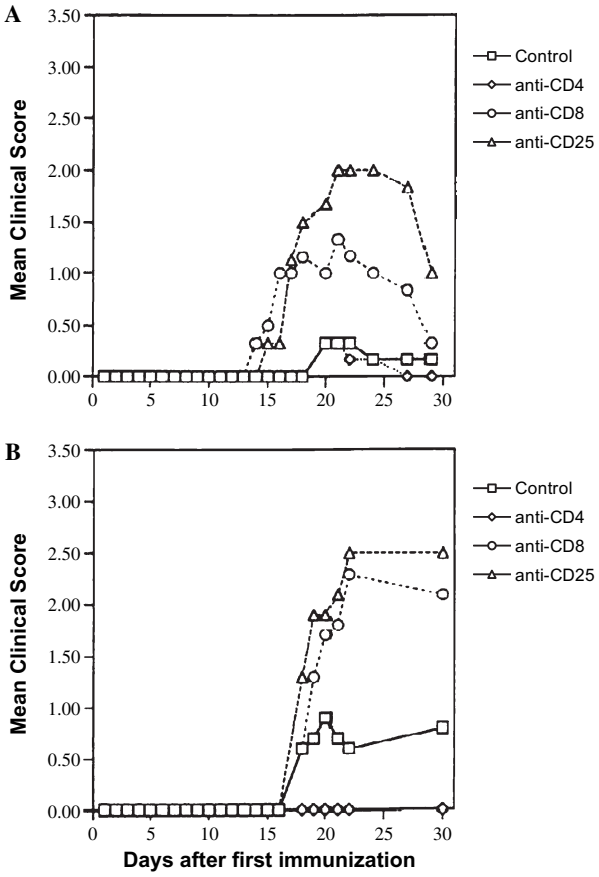

Fig. 2. Effects of anti-CD4, anti-CD8 and anti-CD25 without pertussis toxin. (A) One dose of 1 mg of depleting mAb or control IgG was injected IV 24 h before the second immunization. None of the groups received *B. pertussis* toxin. (B) Two doses of 1 mg of depleting mAb were injected 24 h and again 1 h before the second immunization; only the control group received 200 ng of *B. pertussis* toxin immediately after the first immunization and 72 h later.

3.3. Effect of the depleting antibodies on the late T-cell response to MOG peptide

To document an effect on the immune response to MOG p35–55 peptide, we measured T-cell proliferation on day 56 to MOG p35–55 peptide and to PPD (present in the CFA) detectable in the spleen cells of immunized mice. Two control groups were included: "naïve" mice immunized with CFA and pertussis toxin but without MOG p35–55 peptide and mice immunized with MOG p35–55 peptide in CFA who received pertussis toxin immediately after the first immunization and 72 h later ("control" mice). The mice receiving the depleting antibody treatments were given two doses of antibodies (24 and 1 h) before the second immunization, without either pertussis toxin injection.

Fig. 3A shows that, as expected, there was no T-cell proliferative response to MOG p35–55 peptide manifested by the mice of control group 1. The mice in control group 2, which had been immunized to

MOG p35–55 peptide, showed a mild response to the peptide, as did the immunized groups that had received anti-CD4 or anti-CD8. However, the group that received anti-CD25 showed a significantly enhanced response to MOG p35–55 peptide ($p < 0.05$). In contrast to the differences manifested to MOG p35–55 peptide, none of the groups showed any difference in their T-cell proliferative response to the foreign antigens in PPD (Fig. 3B). Thus, the response to a self-antigen, MOG, may be more sensitive to depletion of regulatory cells than the response to a foreign antigen (PPD).

3.4. Anti-CD25 enhances IFNγ production

To assay the effects of the depleting antibodies on cytokine secretion, the media from the T-cell proliferation assay described in Fig. 3A were collected at 72 h and tested for the presence of IFNγ, a major Th1 cytokine associated with EAE [6,7]. Fig. 4 shows that the group of mice that had been given anti-CD25 manifested a significantly enhanced index of IFNγ production ($p < 0.001$) in response to MOG p35–55 peptide; the other groups did not significantly vary from the background response shown by the mice of control group 1 (not immunized with MOG p35–55 peptide). Thus the anti-CD25 treated group, above the others, was characterized by T-cell reactivity to MOG p35–55 peptide persisting for at least 56 days.

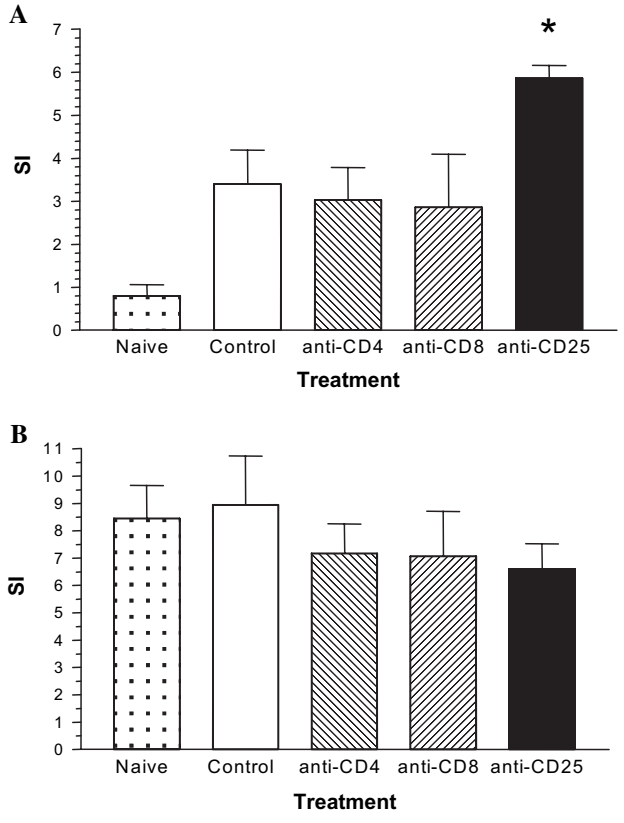

Fig. 3. Anti-CD25 enhances anti-MOG T-cell proliferation but not anti-PPD at day 56. (A) T-cell proliferation to MOG p35–55 peptide was evaluated using unfractionated spleen cells from C57BL/6 mice immunized to MOG p35–55 peptide in CFA (compared to naïve mice which received CFA alone) and given two doses of 1 mg of depleting mAb 24 h and 1 h before the second immunization. Only the control group received pertussis toxin immediately after the first immunization and 72 h later. The experiment was performed on day 56 after the first immunization (SI, stimulation index). (B) Anti-PPD proliferation response was evaluated as a control antigen.

3.5. Anti-MOG antibody response persists following anti-CD25 treatment

We evaluated the contribution of $CD4^+$, $CD8^+$ and $CD25^+$ T cells in the anti-MOG antibody response. C57BL/6 mice were immunized on days 0 and 7 with 200 μg MOG p35–55 peptide emulsified in CFA and given two doses of 1 mg of depleting mAb injected IV 24 h and 1 h before the second immunization; only the control group received pertussis toxin.

We found a significant increase of anti-MOG p35–55 IgG antibody in the mice treated with anti-CD25, compared with other treatment groups ($p < 0.05$), persisting for more than 8 weeks after the first immunization (Fig. 5).

4. Discussion

EAE is a prototypic autoimmune disease model in which Th1 cells are major effectors [6,7]. MOG p35–55 peptide is the inducing antigen and the target for autoimmune attack in EAE in C57BL/6 mice [2,3]. The chronic progressive form of demyelinating EAE manifested in this model is reminiscent of some aspects of human multiple sclerosis [17,18], and makes the model attractive for investigating mechanisms in the pathogenesis and regulation of autoimmune disease.

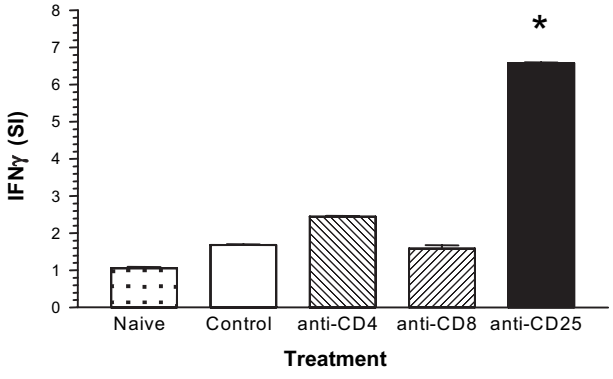

Fig. 4. IFNγ production is enhanced significantly in the group treated with anti-CD25 at day 56. IFNγ was measured by ELISA in supernatants collected at 72 h from cultures of unfractionated spleen cells from C57BL/6 mice incubated with MOG p35–55 peptide, as described in the legend to Fig. 3.

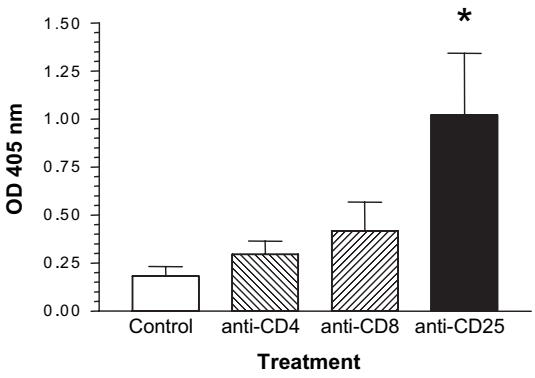

Fig. 5. Anti-CD25 antibody treatment enhances anti-MOG antibody response at day 56. Groups of mice were immunized and treated as described in the legend to Fig. 3, and anti-MOG IgG antibody was measured in sera taken at day 56 after the first immunization.

Autoimmunity to MOG is part of the natural autoimmune repertoire of C57BL/6 since these mice produce IgG anti-MOG antibodies naturally without disease [19]. It is reasonable to conclude that mechanisms must exist to control the expression of intrinsic MOG autoimmunity [20–22]. The induction of EAE by immunization to MOG p35–55 peptide would seem to require the transition of benign autoimmunity into an unregulated, pathogenic type of immune inflammation.

Here, we studied the effects of depleting antibodies to CD4, CD8 and CD25 on the induction of EAE. The most likely explanation for the effects of the antibodies would be the transient depletion of $CD4^+$, $CD8^+$ or $CD25^+$ T cells, and our present interpretation of the results will be based on this assumption.

$CD4^+$ depletion before the first immunization completely prevented the development of clinical EAE (Fig. 1B). This was to be expected considering that CD4 T cells mediate EAE [7,11,23]. Indeed, two administrations of the anti-CD4 antibody aborted the disease even when given before the second immunization (Fig. 2B).

$CD8^+$ T cells have been reported recently to mediate effector functions in EAE [24]. The results here, however, would suggest that the regulatory effect of $CD8^+$ T cells may be more critical than their pathogenic effects since depleting anti-CD8 enhanced disease (Figs. 1C, 2A and B). $CD8^+$ T cells have been found to function as target antigen-specific suppressor cells [25], but also as idiotype-specific regulators in EAE T-cell networks [26]. Transient depletion of either or both of these types of $CD8^+$ T cells could account for enhancement of EAE. It is noteworthy that the anti-CD8 antibody allowed the expression of EAE without the need for pertussis toxin adjuvant (Fig. 2A); this alludes to the possibility that pertussis toxin may inhibit suppressor mechanisms, as well as open the blood–brain barrier [27].

The most marked enhancement of EAE followed administration of anti-CD25; administration before the second immunization led to amplified enhancement (Fig. 1D) and long-term persistence of T-cell proliferation (Fig. 3A), IFNγ production (Fig. 4), and IgG antibodies to MOG (Fig. 5). These findings are compatible with the key role played by $CD4^+CD25^+$ T cells in the regulation of autoimmune disease [28–31]. These regulatory T cells would seem to exist before immunization with MOG p35–55 peptide, because anti-CD25 given before the first immunization enhanced EAE (Fig. 1D). Immunization apparently amplifies these regulatory T cells because the anti-CD25 was even more effective when given before the second immunization (Fig. 1D). Thus, $CD4^+CD25^+$ T cells probably can function both as natural regulators and as induced regulators [32,33]. Additional work must be done to learn why anti-CD4 antibody apparently did not affect the $CD4^+CD25^+$ T-cell regulators as much as did the anti-CD25 antibody. Perhaps, the CD25 molecule is more accessible than the CD4 molecule in this class of regulators. Another question in need of further research is the relative lack of enhancement by anti-CD25 on the T-cell response to PPD compared to the effect on the anti-MOG autoimmune response (Fig. 3). Others too have reported a similar phenomenon in human subjects; anti-CD25 antibody in vitro could preferentially enhance the T-cell response to MOG but not to PPD, tetanus toxin or Candida in some individuals [13]. Such findings suggest that immune responses to self and foreign antigens are under different forms of regulation [34,35]. The structured natural autoimmunity to certain self-antigens, the immunological homunculus, apparently has its own regulatory mechanisms [22,36,37].

The enhancement of autoimmune inflammation obviously is not desirable in the case of clinical autoimmunity. This is not the case with regard to tumor immunotherapy; here it might be helpful to abolish the down-regulation of immunity enjoyed by a tumor and activate a tumor-specific autoimmune rejection [38,39]. We are currently investigating this possible approach to tumor immunotherapy [40].

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References

[1] Raine CS. Biology of disease. Analysis of autoimmune demyelination: its impact upon multiple sclerosis. Lab Invest 1984; 50:608–35.

[2] Mendel I, Kerlero de Rosbo N, Ben-Nun A. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. Eur J Immunol 1995;25:1951–9.
[3] Slavin A, Ewing C, Liu J, Ichikawa M, Slavin J, Bernard CC. Induction of a multiple sclerosis-like disease in mice with an immunodominant epitope of myelin oligodendrocyte glycoprotein. Autoimmunity 1998;28:109–20.
[4] Ben-Nun A, Mendel I, Kerlero de Rosbo N. Immunomodulation of murine experimental autoimmune encephalomyelitis by pertussis toxin: the protective activity, but not the disease-enhancing activity, can be attributed to the nontoxic B-oligomer. Proc Assoc Am Physicians 1997;109:120–5.
[5] Bernard CC, Johns TG, Slavin A, Ichikawa M, Ewing C, Liu J, et al. Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis. J Mol Med 1997;75:77–88.
[6] Brosnan CF, Raine CS. Mechanisms of immune injury in multiple sclerosis. Brain Pathol 1996;6:243–57.
[7] Yura M, Takahashi I, Serada M, Koshio T, Nakagami K, Yuki Y, et al. Role of MOG-stimulated Th1 type "light up" (GFP+) CD4+ T cells for the development of experimental autoimmune encephalomyelitis (EAE). J Autoimmun 2001;17:17–25.
[8] Jiang H, Zhang SI, Pernis B. Role of CD8+ T cells in murine experimental allergic encephalomyelitis. Science 1992;256:1213–5.
[9] Koh DR, Fung-Leung WP, Ho A, Gray D, Acha-Orbea H, Mak TW. Less mortality but more relapses in experimental allergic encephalomyelitis in CD8−/− mice. Science 1992;256:1210–3.
[10] Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. J Immunol 2002;169:4712–6.
[11] Sun D, Ben-Nun A, Wekerle H. Regulatory circuits in autoimmunity: recruitment of counter-regulatory CD8+ T cells by encephalitogenic CD4+ T line cells. Eur J Immunol 1988;18:1993–9.
[12] Hori S, Haury M, Coutinho A, Demengeot J. Specificity requirements for selection and effector functions of CD25+4+ regulatory T cells in anti-myelin basic protein T cell receptor transgenic mice. Proc Natl Acad Sci U S A 2002;99:8213–8.
[13] Wing K, Lindgren S, Kollberg G, Lundgren A, Harris RA, Rudin A, et al. CD4 T cell activation by myelin oligodendrocyte glycoprotein is suppressed by adult but not cord blood CD25+ T cells. Eur J Immunol 2003;33:579–87.
[14] Kerlero de Rosbo N, Mendel I, Ben-Nun A. Chronic relapsing experimental autoimmune encephalomyelitis with a delayed onset and an atypical clinical course, induced in PL/J mice by myelin oligodendrocyte glycoprotein (MOG)-derived peptide: preliminary analysis of MOG T cell epitopes. Eur J Immunol 1995;25:985–93.
[15] Cobbold SP, Jayasuriya A, Nash A, Prospero TD, Waldmann H. Therapy with monoclonal antibodies by elimination of T-cell subsets in vivo. Nature 1984;312:548–51.
[16] Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. Cancer Res 1999;59:3128–33.
[17] Kerlero de Rosbo N, Milo R, Lees MB, Burger D, Bernard CC, Ben-Nun A. Reactivity to myelin antigens in multiple sclerosis. Peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. J Clin Invest 1993;92:2602–8.
[18] Berger T, Rubner P, Schautzer F, Egg R, Ulmer H, Mayringer I, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. N Engl J Med 2003;349:139–45.
[19] Quintana FJ, Cohen IR. Autoantibody patterns in diabetes-prone NOD mice and in standard C57BL/6 mice. J Autoimmun 2001;17:191–7.
[20] Cohen IR. The cognitive principle challenges clonal selection. Immunol Today 1992;13:441–4.
[21] Cohen IR. Discrimination and dialogue in the immune system. Semin Immunol 2000;12:215–9 [discussion p. 257–344].
[22] Cohen IR. Tending Adam's garden: evolving the cognitive immune self. London: Academic Press; 2000.
[23] Lafaille JJ, Nagashima K, Katsuki M, Tonegawa S. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. Cell 1994;78:399–408.
[24] Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, et al. Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. J Immunol 2001;166:7579–87.
[25] Jiang H, Chess L. The specific regulation of immune responses by CD8+ T cells restricted by the MHC class Ib molecule, Qa-1. Annu Rev Immunol 2000;18:185–216.
[26] Lider O, Reshef T, Beraud E, Ben-Nun A, Cohen IR. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. Science 1988;239:181–3.
[27] Blankenhorn EP, Butterfield RJ, Rigby R, Cort L, Giambrone D, McDermott P, et al. Genetic analysis of the influence of pertussis toxin on experimental allergic encephalomyelitis susceptibility: an environmental agent can override genetic checkpoints. J Immunol 2000;164:3420–5.
[28] Francois Bach J. Regulatory T cells under scrutiny. Nat Rev Immunol 2003;3:189–98.
[29] Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 2001;182:18–32.
[30] Shevach EM. Regulatory T cells in autoimmunity*. Annu Rev Immunol 2000;18:423–49.
[31] Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. Nat Immunol 2001;2:816–22.
[32] Furtado GC, Curotto de Lafaille MA, Kutchukhidze N, Lafaille JJ. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. J Exp Med 2002;196:851–7.
[33] Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. Nat Rev Immunol 2003;3:253–7.
[34] Asseman C, von Herrath M. About CD4pos CD25pos regulatory cells. Autoimmun Rev 2002;1:190–7.
[35] Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol 2002;2:389–400.
[36] Cohen IR, Young DB. Autoimmunity, microbial immunity and the immunological homunculus. Immunol Today 1991;12:105–10.
[37] Cohen IR. The cognitive paradigm and the immunological homunculus. Immunol Today 1992;13:490–4.
[38] Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol 1999;163:5211–8.
[39] Montero E, Amador JF, Perez R, Lage A. Tumor-specific immunotherapy based on dominant models of natural tolerance. Med Hypotheses 2000;54:531–6.
[40] Gonzalez G, Montero E, Leon K, Cohen IR, Lage A. Autoimmunization to epidermal growth factor, a component of the immunological homunculus. Autoimmun Rev 2002;1:89–95.