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ORIGINAL ARTICLE

Elena B. Samoilova · Jennifer L. Horton
Haidi Zhang · Youhai Chen

CD40L blockade prevents autoimmune encephalomyelitis and hampers TH1 but not TH2 pathway of T cell differentiation

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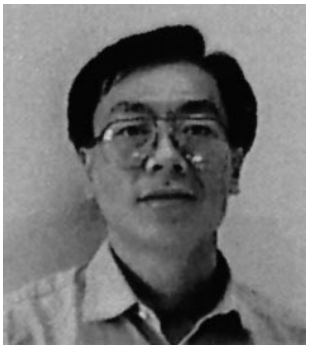
Abstract Although it is well established that CD40 and its ligand (CD40L) play pivotal roles in the development of humoral immunity, their roles in cell-mediated immunity and cell-mediated autoimmune diseases are not well defined. We report here that CD40:CD40L interaction is crucial for the development of experimental autoimmune encephalomyelitis (EAE), a prototype TH1-cell mediated autoimmune disease. Specific blockade of CD40L at the time of immunization markedly suppressed the incidence, mortality, day of onset, and clinical scores of EAE in (PLJ×SJL) F1 mice. Importantly, the disease suppression was not associated with anergy or deletion of autoreactive T cells but was accompanied by a drastic alteration of their cytokine profiles. The production of interferon (IFN)-γ was markedly suppressed while that of interleukin (IL)-4 enhanced. These results suggest that CD40:CD40L interaction plays important roles in the differentiation of autoreactive TH1 versus TH2 cells in vivo, and that CD40L blockade is effective in preventing autoimmune encephalomyelitis.

Key words Costimulation · T lymphocyte · TH1/TH2 · Autoimmune disease · Encephalomyelitis

Abbreviations *EAE* Experimental autoimmune encephalomyelitis · *ELISA* Enzyme-linked immunosorbent assay · *IFN* Interferon · *IL* Interleukin · *mAb* Monoclonal antibody · *MBP* Myelin basic protein

ELENA B. SAMOILOVA received her M.D. degree from the Pavlov Medical Institute, Saint Petersburg, Russia. She is currently a Research Fellow at the Institute for Human Gene Therapy, University of Pennsylvania. Her major scientific interests include autoimmune diseases, costimulation, and immunological tolerance.



YOUHAI CHEN is Assistant Professor of Molecular and Cellular Engineering at the Institute for Human Gene Therapy, University of Pennsylvania School of Medicine. Dr. Chen has long-term interest in pathogenesis of autoimmune diseases, mechanisms of peripheral T cell tolerance, and gene therapy of autoimmune diseases.

E.B. Samoilova · J.L. Horton · H. Zhang · Y. Chen (✉)
Institute for Human Gene Therapy and Department of Molecular and Cellular Engineering,
University of Pennsylvania School of Medicine,
422 Curie Blvd., Philadelphia, PA 19104, USA

Introduction

The interaction between CD40 and CD40L plays pivotal roles in the development of humoral immunity [1]. CD40L deficiency leads to hyper-IgM syndrome in humans, a genetic immunodeficiency disease associated with severely impaired humoral immune responses [2]. Deficiency in CD40L also affects cell-mediated immunity [2–4], and specific blockade of CD40L has recently been shown to hamper the development of T cell responses in a number of systems [5–8]. It appears that CD40:CD40L interaction may affect T cells directly through CD40L-mediated signals [9] or indirectly by upregulating costimulatory molecules (such as B7) on antigen-presenting cells [5, 10, 11]. Whether any of these mechanisms plays a role in cell-mediated autoimmune diseases and in differentiation of different subsets of T cells in vivo is not well understood.

Experimental autoimmune encephalomyelitis (EAE) is a self-contained central nervous system disease which is considered to be a putative animal model for human multiple sclerosis. The disease can be induced in susceptible strains of mice or rat by immunizing animals with myelin antigens such as myelin basic protein (MBP) or

proteolipid protein in complete Freund's adjuvants. The immunization leads to differentiation of myelin-specific precursor T cells into encephalitogenic TH1-type cells secreting interferon (IFN)-γ, tumor necrosis factor, and interleukin (IL)-2. The commitment of myelin-specific precursor T cells (which are nonencephalitogenic and are present in all normal individuals) to the TH1 pathway of T cell differentiation is essential for the development of EAE since myelin-specific TH2 cells are not encephalitogenic and are capable of suppressing EAE [12]. A recent report by Gerritse et al. suggests that CD40L blockade in vivo suppresses EAE in SJL mice immunized with proteolipid protein peptide [13]. The mechanisms of this disease suppression were not investigated, and the effect of CD40L blockade on the development of encephalitogenic TH1 versus TH2 cells was unknown. We report here that CD40L blockade dramatically alters the differentiation of MBP-specific T cells in vivo and suppresses actively induced EAE in (SJL×PLJ) F1 mice.

Materials and Methods

Mice

Female (PLJ×SJL/J) F1 mice, 6–8 weeks of age, were purchased from Jackson Laboratory (Bar Harbor, ME) and were housed in the University of Pennsylvania Animal Care Facilities.

Induction and clinical evaluation of EAE

Each mouse received (a) a subcutaneous injection on flank of 200 μg mouse MBP in 0.1 ml PBS emulsified in an equal volume of complete Freund's adjuvants containing 4 mg/ml mycobacterium tuberculosis H37 RA (Difco, St. Louis, MO), and (b) an intravenous injection of 200 ng pertussis toxin in 0.1 ml PBS. Mice received a second injection of pertussis toxin (200 ng/mouse) 48 h later and were scored for EAE as follows: 0, no disease; 1, tail paralysis; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb plus forelimb paralysis; 5, moribund.

Antigens, antibodies, recombinant cytokines, and ELISA

Mouse MBP was prepared from the brain tissue by a modified method of Deibler et al. [14]; the purity of the MBP preparation was confirmed by gel electrophoresis and amino acid analysis. Hamster anti-mouse CD40L was purchased from TSD Bio (Germantown, NY). The following reagents were purchased from PharMingen (San Diego, CA): purified rat anti-mouse IL-2 (clone JES-1A12), IL-4 (clone BVD4-1D11), and IFN-γ (clone R4-6A2) monoclonal antibody (mAb); biotinylated rat anti-mouse IL-2 (clone JES6-5H4), IL-4 (clone BVD6-24G2), and IFN-γ (clone XMG1.2) mAb; recombinant mouse IL-2, IL-4, IFN-γ. Quantitative enzyme-linked immunosorbent assay (ELISA) for IL-2, IL-4, and IFN-γ were performed using paired mAbs specific for corresponding cytokines per manufacturer's recommendations [15].

Cell culture

Splenocytes or lymph node cells, 1×10^6 each, were cultured in 0.2 ml of serum-free medium (X-vivo 20, Biowhittacker, Walkersville, MD), containing various concentrations of MBP. Culture supernatants were collected 40 h later for cytokine assays. For proliferation assay 1 μCi [^{3}H]thymidine was added to each culture 72 h later. Cells were harvested and radioactivity counted 16 h later using a flatbed beta counter (Wallac, Gaithersburg, MD) [16].

Statistical analysis

Disease severity, day of onset and cytokine concentrations were all analyzed by analysis of variance.

Results

CD40L blockade prevents experimental autoimmune encephalomyelitis

To test the role of CD40L in the development of EAE we studied the effect of CD40L blockade in (PLJ×SJL) F1 mice. As shown in Fig. 1 and Table 1, subcutaneous immunization of mice with MBP in complete Freund's adjuvants induced severe EAE. This was dramatically suppressed by intraperitoneal injection of 100 μg of anti-CD40L mAb three times. Specifically, the incidence of the disease was reduced from 91% in the control to 50%

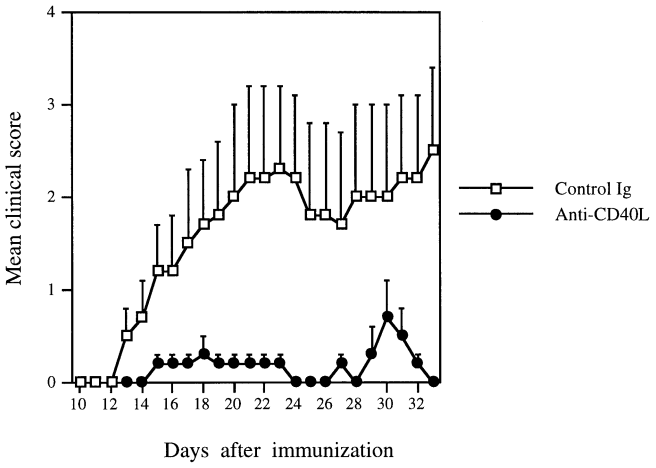


Fig. 1 CD40L blockade prevents EAE. Two groups of (PLJ×SJL) F1 mice, six mice per group, were immunized for EAE as described in the text. Starting from the day of immunization, one group of mice received peritoneal injection of 100 μg hamster anti-CD40L mAb once every other day for a total of three injections. Another group received hamster IgG as control. Mice were monitored for symptoms of EAE as described in the text. The experiments were repeated twice with similar results

Table 1 CD40L blockade prevents experimental autoimmune encephalomyelitis. Mice were treated as in Fig. 1 and monitored for symptoms of EAE; experiments were repeated twice with similar results

Treatment	Incidence	Mortality	Mean day of onset (mean±SE)*	Mean maximal score (mean±SE)**
Control Ig	10/11	6/11	15.6±1.0	2.82±0.71
Anti-CD40L	3/6	0/6	20.7±4.3	0.67±0.49

*P=0.028; **P=0.048

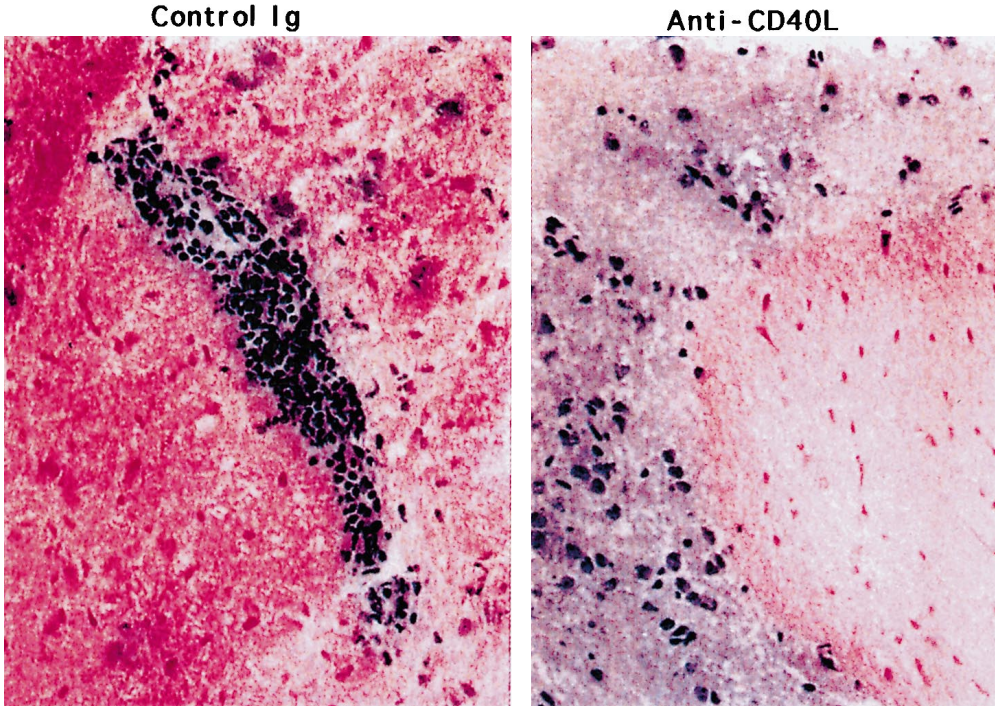


Fig. 2 Histological examination of the brain. Two groups of (SJL×PLJ) F1 mice, three mice per group, were treated as in Fig. 1. Thirty-three days after immunization, mice were sacrificed and their brains harvested. Brain cryosections (6 μm) were stained with hematoxylin and eosin (original magnification ×200)

in anti-CD40L treated group. Mortality was reduced from 55% to 0%. Mean maximal clinical score was reduced from 2.82 to 0.67, and the mean day of onset was delayed from 15.6 to 20.7 days. Consistent with the clinical manifestations, histochemical analysis of the brain samples collected 33 days after the immunization revealed drastic differences between the two groups: severe inflammation and leukocyte infiltration were observed in the control but not CD40L-treated animals (Fig. 2).

CD40L blockade prevents differentiation of autoreactive TH1 but not TH2 cells

To test whether CD40L blockade alters the in vivo differentiation of myelin-specific T cells we examined the cytokine profiles of MBP-specific T cells ex vivo. As shown in Fig. 3, MBP-specific T cells in spleen of immunized mice produced high levels of TH1 cytokine IFN-γ with little IL-4, which is consistent with the generation of encephalitogenic TH1 cells. By contrast, MBP-specific T cells in anti-CD40L-treated mice produced little IFN-γ but markedly enhanced IL-4. The maximum amount of IFN-γ produced by splenocytes of the control group was 1279 pg/ml, and this was reduced to 56 pg/ml in CD40L-treated mice. Surprisingly, more than a sixfold increase in IL-4 production was observed in mice treated with anti-CD40L. These results suggest that CD40L blockade dramatically alters the cytokine profile of MBP-specific T cells in vivo. Of note is that the cytokine ELISA employed here measures the amount of cytokine accumulated in the culture supernatant, not the total amount of the cytokine secreted. Thus, although IL-4

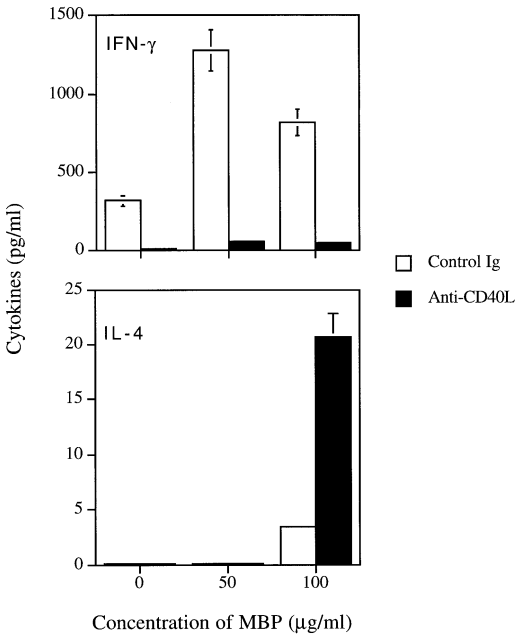


Fig. 3 CD40L blockade prevents activation of TH1 but not TH2 cells. Two groups of (PLJ×SJL) F1 mice, three mice per group, were treated as in Fig. 1. Splenocytes, 1×10^6 cells/well, were cultured in 0.2 ml of serum-free medium with different concentrations of mouse MBP. Culture supernatants were collected 40 h later, and cytokine concentrations were determined by ELISA. The experiments were repeated twice with similar results

might also be produced by the splenocytes in the presence of 50 μg/ml MBP, the amount of IL-4 accumulated in the culture did not reach the detectable level (which was 2 pg/ml for this assay) (Fig. 3). In addition to IFN-γ and IL-4, we also tested IL-10 and transforming growth

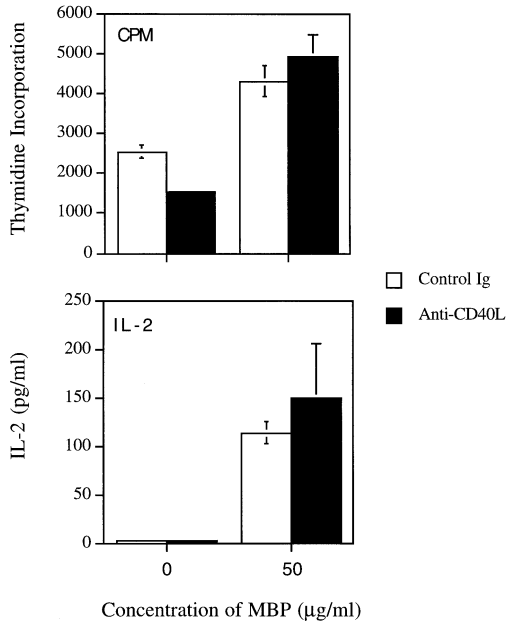


Fig. 4 Effect of CD40L blockade on the proliferation and IL-2 production of MBP-specific splenic T cells. Two groups of (SJL×PLJ) F1 mice, three mice per group, were treated as in Fig. 1. Ten days after immunization mice were killed and their spleens harvested. For proliferation assays splenocytes, 5×10^5 cells/well, were cultured in 0.2 ml of serum-free medium with or without 50 μg mouse MBP. [^{3}H]Thymidine was added 72 h later, and radioactivity was measured an additional 16 h later. For IL-2 production splenocytes, 1×10^6 cells/well, were cultured in 0.2 ml serum-free medium with or without 50 μg of mouse MBP. Culture supernatants were collected 40 h later, and IL-2 concentration was determined by ELISA. The experiments were repeated twice with similar results

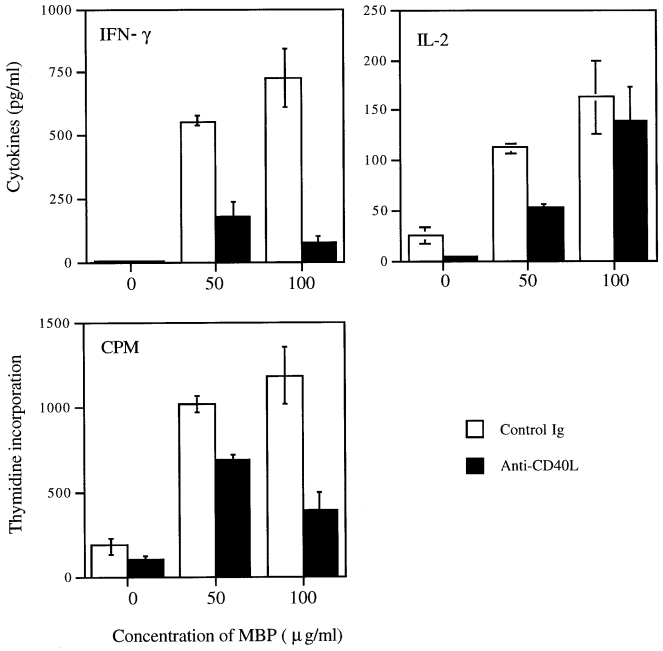


Fig. 5 Effect of CD40L blockade on the proliferation and cytokine production of MBP-specific lymph node T cells. Two groups of (SJL×PLJ) F1 mice, three mice per group, were treated as in Fig. 1. Thirty-three days after immunization mice were sacrificed and their inguinal lymph nodes harvested. For proliferation assays lymph node cells, 5×10^5 cells/well, were cultured in 0.2 ml of serum-free medium with or without mouse MBP. [^{3}H]Thymidine was added 72 h later, and radioactivity was measured an additional 16 h later. For cytokine production lymph node cells, 1×10^6 cells/well, were cultured in 0.2 ml serum- medium with or without various concentrations of mouse MBP. Culture supernatants were collected 40 h later, and cytokine concentration was determined by ELISA

factor-β in the culture supernatants. None of these cytokines reached the detectable levels of our ELISA even in the presence of 100 μg/ml MBP [17] (Samoilova and Chen, unpublished).

CD40L blockade may not induce systemic tolerance of MBP-specific T cells

Anergy and deletion are important mechanisms of peripheral tolerance, which are often characterized by decreased T cell proliferation and IL-2 production [18, 19]. To investigate whether these mechanisms play a role in anti-CD40L-mediated suppression of EAE we tested the proliferative and IL-2 responses of MBP-specific T cells in the spleen. To our initial surprise, anti-CD40L blockade had no effect on the in vitro proliferative activity of splenic T cells specific for MBP, and IL-2 production was not suppressed in anti-CD40L-treated animals (Fig. 4). These results are consistent with previous observations that T cells in CD40L treated animals may not be deleted or anergized [20]. In contrast to T cells in the spleen, MBP-specific cells in the lymph node that drains the site of the immunization exhibited reduced reactivity to MBP. Thus both TH1 cytokine production and thymidine incorporation were reduced in mice treated with anti-CD40L (Fig. 5); TH2 cytokine IL-4 did not reach detectable level in the lymph node cultures (data not shown). These results strongly suggest that anti-CD40L mAb does not induce systemic tolerance of encephalitogenic T cells.

It is to be noted that, unlike IFN-γ and IL-4 which are produced by polarized TH1 and TH2 cells, respectively, IL-2 can be produced by precursor T (THp) cells, TH0 cells as well as TH1 cells. Moreover, IFN-γ and IL-4 act as switching factors for TH1 and TH2 cells, respectively; IL-2, on the other hand, may not regulate T cell commitment to either TH1 or TH2 lineage but is required for the growth of both TH1 and TH2 cells [21, 22]. As both IFN-γ and IL-2 are present in control mice immunized with MBP (Figs. 3, 4), IL-2 may be produced primarily by encephalitogenic TH1 cells in these animals. By contrast, IL-2 production in anti-CD40L-treated mice was not associated with IFN-γ, suggesting that it is produced primarily by THp or TH0 cells (Fig. 6), presumably as a result of specific blockade of TH1 cell differentiation.

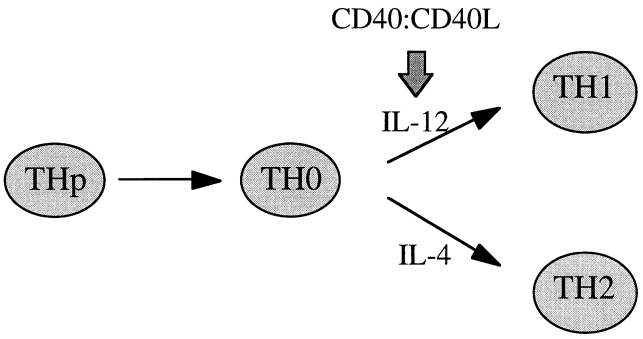


Fig. 6 Effect of CD40L blockade on T cell differentiation. The differentiation of T cells from precursor (THp) to TH0, TH1, and TH2 types involves interactions among various cell surface molecules and soluble mediators. CD40:CD40L interaction may play crucial roles in the commitment of THp cells to TH1 lineage by upregulating IL-12 production (see text)

Discussion

Although initially identified as a costimulatory molecule for humoral immunity, evidence is accumulating that CD40L plays crucial roles in the development of cellular immune responses. Deficiency in CD40L affects not only humoral immunity but T cell immune responses as well [3, 4]. Injection of anti-CD40L mAb suppresses antimicrobial infections [7, 8], allograft rejection [6], TH1 cell-mediated hypersensitivity [23], and collagen-induced arthritis [24]. A recent report suggests that CD40L also plays a role in intrathymic deletion under certain circumstances [25]. Using a similar system, Gerritse et al. recently showed that CD40L blockade prevents the development of EAE in SJL mice immunized with proteolipid protein peptide [13]. The exact mechanisms whereby CD40L mediates these diverse functions in vivo are not well understood.

In this report we show that CD40L blockade may not lead to anergy or deletion of specific cells but to a preferential blockade of TH1 cell differentiation and selective activation of TH2 cells. These findings knit together a number of recent observations concerning the mechanisms of action of anti-CD40L mAb in vivo. On the one hand, unlike B7 blockade which often leads to specific immune tolerance [26], CD40L blockade does not appear to induce tolerance of T cells [20], although CD40:CD40L interaction also upregulates B7 expression [5, 10, 11]. On the other hand, CD40L blockade inhibits IL-12 production, and its in vivo effect can be reversed by IL-12 injection [23, 27, 28]. We thus propose the following theory to explain the role of anti-CD40L in vivo (Fig. 6). CD40:CD40L interaction upregulates IL-12 production by monocytes which in turn enhances TH1 pathway while inhibits TH2 pathway of T cell differentiation; blocking CD40L blocks TH1 pathway and alleviates the inhibition of TH2 pathway. This leads to accumulation of TH0 cells (and possibly activated THp cells as well) and overgrowth of TH2 cells. The end result is reduced IFN-γ production and increased IL-4 secretion. As IL-2 can be produced by non-TH1 cells and as deletion does not appear to occur after CD40L blockade, no suppression of T cell proliferation is evident. As TH1 cells are important for both cellular and humoral immunity, blocking TH1 cell differentiation affects the host's ability to combat infections.

This theory argues against the notion that the effect of anti-CD40L mAb may simply be blocking CD40:CD40L interaction in vivo with no consequences on the subsequent T cell differentiation. To the contrary, it argues that CD40L plays indispensable roles in the commitment of precursor T cells to TH1 lineage in vivo. Our observation that MBP-specific TH2 cells are preferentially activated in anti-CD40L treated animals may have important ramifications for immunotherapy of autoimmune encephalomyelitis through CD40L blockade. Although not encephalitogenic, myelin-specific TH2 cells may serve as negative regulators of encephalitogenic T cells and may help ameliorate encephalomyelitis in an organ-specific, bystander manner [12].

Multiple sclerosis is a chronic inflammatory disease of the central nervous system characterized by focal lymphocyte and macrophage infiltration which leads to demyelination and loss of neurological functions. Although the mechanisms by which this inflammatory process are initiated and regulated are not well understood, T cells recognizing myelin antigens may play an important role [29–31]. In acute multiple sclerosis plaques activated T cells and macrophages secrete various inflammatory cytokines and express various surface markers important for T cell activation. Downregulation of the function of these T cells by CD40L blockade may be effective in preventing or ameliorating the disease. Future studies are therefore needed to test this strategy in multiple sclerosis patients.

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References

1. Hollenbaugh D, Ochs HD, Noelle RJ, Ledbetter JA, Aruffo A (1993) The role of CD40 and its ligand in the regulation of the immune response. Immunol Rev 138:23–37
2. Farrington M, Grosmaire LS, Nonoyama S, Fischer SH, Hollenbaugh D, Ledbetter JA, Noelle RJ, Aruffo A, Ochs HD (1994) CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. Proc Natl Acad Sci USA 91:1099–1103
3. Grewal IS, Xu J, Flavell RA (1995) Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. Nature 378:617–620
4. Renshaw BR, Fanslow WCI, Armitage RJ, Cambell KA, Liggitt D, Wright B, Davison BL, Maliszewski CR (1994) Humoral immune responses in CD40 ligand-deficient mice. J Exp Med 180:1889–1900
5. Buhlmann JE, Foy TM, Aruffo A, Crassi KM, Ledbetter JA, Green WR, Xu JC, Shultz LD, Roopesian D, Flavell RA, Fast L, Noelle R, Durie FH (1995) In the absence of a CD40 signal, B cells are tolerogenic. Immunity 2:645–653

6. Parker DC, Greiner DL, Phillips NE, Appel MC, Steele AW, Durie FH, Noelle RJ, Mordes JP, Rossini AA (1995) Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. Proc Natl Acad Sci USA 92:9560–9564
7. Campbell KA, Ovendale PJ, Kennedy MK, Fanslow WC, Reed SG, Maliszewski CR (1996) CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. Immunity 4:283–289
8. Kamanaka M, Yu P, Yasui T, Yoshida K, Kawabe T, Horii T, Kishimoto T, Kikutani H (1996) Protective role of CD40 in Leishmania major infection at two distinct phases of cell-mediated immunity. Immunity 4:275–281
9. van Essen D, Kikutani H, Gray D (1995) CD40 ligand-transduced co-stimulation of T cells in the development of helper function. Nature 378:620—623
10. Wu Y, Xu J, Shinde S, Grewal I, Henderson T, Flavell RA, Liu Y (1995) Rapid induction of a novel costimulatory activity on B cells by CD40 ligand. Curr Biol 5:1303–1311
11. Grewal IS, Foellmer HG, Grewal KD, Xu J, Hardardottir F, Baron JL, Janeway CA Jr, Flavell RA (1996) Requirement for CD40 ligand in costimulation induction, T cell activation, and experimental allergic encephalomyelitis. Science 273:1864–1867
12. Chen Y, Kuchroo VK, Inobe JI, Hafler DA, Weiner HL (1994) Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 265:1237–1240
13. Gerritse K, Laman JD, Noelle RJ, A A, Ledbetter JA, Boersa WJA, Claassen E (1996) CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. Proc Natl Acad Sci USA 93:2499–2504
14. Higgins P, Weiner HL (1988) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. J Immunol 140:440–445
15. Chen Y, Inobe J-I, Weiner HL (1995) Induction of oral tolerance to MBP in CD8-depleted mice. J Immunol 155:910–916
16. Chen Y, Kuchroo K, Inobe J-I, Baron JL, Janeway CA, Weiner HL (1996) Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. Proc Natl Acad Sci USA 93:388–391
17. Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL (1995) Peripheral deletion of antigen-reactive T cells in oral tolerance. Nature 376:177–180
18. Jenkins MK, Chen CA, Jung G, Mueller DL, Schwartz R (1990) Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immorbilized anti-CD3 monoclonal antibody. J Immunol 144:16–24
19. Marrack P, Hugo P, McCormack J, Kappler J (1993) Death and T cells. Immunol Rev 133:119–129
20. Foy TM, Shepherd DM, Durie FH, Aruffo A, Ledbette RJA, Noelle RJ (1993) In vivo CD40-gp39 interactions are essential for thymus-dependent humoral mmunity. II. Prolonged suppression of the humoral immune response by an antibody to the ligand for CD40, gp39. J Exp Med 178:1567–1575
21. Mosmann TR, Coffman RT (1989) Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 46:111–147
22. Powrie F, Coffman RL (1993) Cytokine regulation of T-cell function. Immunol Today 14:270–274
23. Stuber E, Strober W, Neurath M (1996) Blocking the CD40L-CD40 interaction in vivo specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. J Exp Med 183:693–698
24. Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ (1993) Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. Science 261:1328–1330
25. Foy TM, Page DM, Waldschmidt TJ, Schoneveld A, Laman JD, Masters SR, Tygrett L, Ledbetter JA, AA, Claassen E (1995) An essential role for gp39, the ligand for CD40, in thymic selection. J Exp Med 182:1377–1388
26. Schwartz RH (1992) Costimulation of T Lymphocytes: The Role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell 71:1065–1068
27. Kennedy MK, Picha KS, Fanslow WC, Grabstein KH, Alderson MR, Clifford KN, Chin WA, Mohler KM (1996) CD40/CD40 ligand interactions are required for T cell-dependent production of interleukin-12 by mouse macrophages. Eur J Immunol 26:370–378
28. Shu U, Kiniwa M, Wu CY, Maliszewski C, Vezzio N, Hakimi J, Gately M, Delespesse G (1995) Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. Eur J Immunol 25:1125–1128
29. Chou YK, Buenafe AC, Dedrick R, Morrison WJ, Bourdette DN, Whitham R, Artherton J, Lane J, Spoor E, Hashim GA, Offner H, Vandenbark AA (1994) T cell receptor Vβ gene usage in the recognition of mylelin basic protein by cerebrospinal fluid- and blood-derived T cells from patients with multiple sclerosis. J Neurosci Res 37:169–181
30. Zhang J, Markovic S, Raus J, Lacet B, Weiner HL, Hafler DA (1993) Increased frequency of IL-2 responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 179:973–984
31. Utz U, Brooks JA, McFarland HF, Martin R, Biddison W (1994) Heterogeneity of T-cell receptor a-chain complementarity-determining region 3 in MBP-specific T cells increases with severity of multiple sclerosis. Proc Natl Acad Sci USA 91:5567–5571