This activation was blunted to 12% of cells in CD40L$^{-/-}$ mice (Fig. 3C); activation of CD40 with antibody in the CD40L$^{-/-}$ mice increased B7.2 expression to 32% (Fig. 3D).

We studied the functional importance of B7-mediated costimulation of T cells to vector in wild-type C57BL/6 mice by injecting blocking antibodies to B7.1 and B7.2 at the time of vector administration. T cells from these animals failed to secrete T_H1- and T_H2-specific cytokines in response to antigen in vitro (Fig. 1A), and CTL activity to viral-infected targets was markedly diminished (Fig. 1B). This response was associated with the stabilization of transgene expression, with 82% of hepatocytes expressing *lacZ* at day 24 (Table 1). T cell–dependent B cell responses were also blunted, with diminished formation of germinal centers [20.4 ± 1.5 per section without anti-B7 and 2.5 ± 0.7 per section with anti-B7 (*11*)], less antiviral neutralizing antibody (Table 1), and a notable absence of class switching from IgM to IgG1 and IgG2a (Fig. 2). Another inhibitor of this pathway, CTLA4-Ig, has a similar effect on the cellular responses to vector in liver (*7*).

The well-characterized T and B cell responses to adenoviral vectors were useful in defining the biology of CD40L in T cell activation (*5*). Full immune competence was achieved in the absence of CD40L by activating CD40, thereby ruling out a direct effect of CD40L on the T cell. We show that CD40 signals an up-regulation of B7.2 on the APC that is necessary for T cell activation, presumably through its interaction with CD28. The interdependence of the CD40 and CD28 pathways in this system differs from the situation in models of allograft rejection where redundancies appear to exist (*4*). Our studies suggest that pharmacologic subversion of the CD40 pathway may be effective in abrogating problematic host responses to vectors, a concept that has been recently validated in murine models of liver and lung gene transfer (*8*).

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9. CD40L-deficient (CD40L$^{-/-}$) mice and their control litter mates (CD40L$^{+/+}$) were obtained from Jackson Laboratory (Bar Harbor, ME). Mice (female, 7 to 8 week old) were immunized with the recombinant adenovirus H5.010CMV*lacZ* [2 × 10^9 plaque-forming units (pfu)] through the tail vein and killed 3, 10, and 24 days later. This replication-defective vector is deleted of the early genes *E1a* and *E1b* and expresses *lacZ* from a cytomegalovirus (CMV) promoter. Liver tissues were harvested for morphological analysis, and spleens and blood samples were saved for immunological assays. Some CD40L$^{-/-}$ mice were also treated with a monoclonal antibody to CD40 (anti-CD40) (100 μg/day, Pharmingen, San Diego, CA) for 7 days after immunization, and C57BL/6 mice were treated with monoclonal antibodies to B7.1 and B7.2 (100 μg/day; Pharmingen, San Diego, CA) every 3 days after immunization for the duration of the experiment.
10. Splenocytes harvested from day 10 mice were cultured either for 2 days for analysis of T cell proliferation to antigen or for 5 days in preparation for CTL assays. The cells (6 × 10^6 cells per well) were cultured in 1.6 ml of Dulbecco's minimum essential medium supplemented with 5% fetal bovine serum and 50 μM 2-mercaptoethanol in the presence of *lacZ* virus at a multiplicity of infection of one in 24-well Costar plates. After the 48-hour incubation with antigen, supernatants were analyzed for IL-2, IL-4, IL-10, and IFN-γ by cytokine-specific enzyme-linked immunosorbent assays (ELISAs). A standard 6-hour ^{51}Cr release assay was performed subsequently with different ratios of effector to target cells (C57SV, H-2^b). These target cells were mock-infected or infected with *lacZ* virus for 18 hours and labeled with ^{51}Cr. After incubation for 6 hours, 100-μl samples of supernatant were removed for counting in a gamma counter. The percentage of specific ^{51}Cr release was calculated as follows: [(cpm of sample − cpm of spontaneous release)/(cpm of maximal release − cpm of spontaneous release)] × 100. Spontaneous release was determined by culturing target cells in medium, and maximal release was established by culturing target cells in a 1% solution of SDS. The percentage of specific lysis is expressed as a function of different effector-to-target ratios (6:1, 12:1, 25:1, and 50:1).
11. Spleens from C57BL/6, CD40L$^{-/-}$, and CD40L$^{-/-}$ mice treated with anti-CD40 and C57BL/6 mice treated with anti-B7.1 and anti-B7.2 were harvested 10 days after infusion of H5.010CMV*lacZ* virus, and the frozen sections were analyzed for germinal center formation. Frozen sections were fixed with methanol for 10 min at −20°C, air-dried, and rehydrated with phosphate-buffered saline (PBS). After blocking with 10% goat serum for 20 min, sections were incubated with peanut agglutinin conjugated to fluorescein isothiocyanate (FITC) (Sigma; 20 μg/ml) for 1 hour at room temperature. Sections were washed and mounted with Citiflour (Canterbury, UK). Four spleen sections from two mice (*2*) were stained for the presence of germinal centers (GCs) and analyzed. Data are represented as the number of GCs per section (mean ± SD).
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15. Antiviral antibodies were evaluated with an ELISA assay in which microtiter plates were coated with 200 ng of viral antigen in 100 μl of PBS per well overnight at 4°C, washed three times in PBS, and blocked with 1% bovine serum albumin (BSA) in PBS for 1 hour at 37°C. Serum samples diluted 1:200 were added to antigen-coated plates and incubated for 4 hours at 37°C. Plates were washed three times with 0.05% Tween-20 in PBS and incubated with biotin-labeled goat antibody to mouse IgG1, IgG2a, or IgM (Pharmingen, San Diego, CA) at 1:1000 dilution overnight at 4°C. Plates were washed as above and anti-biotin phosphatase (Sigma) was added to each well at 1:30,000 dilution for 1 hour at 37°C. Wells were washed again as above and *p*-nitrophenyl phosphate substrate was added.

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Requirement for CD40 Ligand in Costimulation Induction, T Cell Activation, and Experimental Allergic Encephalomyelitis

Iqbal S. Grewal, Harald G. Foellmer, Kate D. Grewal, Jianchao Xu, Fridrika Hardardottir, Jody L. Baron, Charles A. Janeway Jr., Richard A. Flavell*

The mechanism of CD40 ligand (CD40L)–mediated in vivo activation of CD4$^+$ T cells was examined by investigation of the development of experimental allergic encephalomyelitis (EAE) in CD40L-deficient mice that carried a transgenic T cell receptor specific for myelin basic protein. These mice failed to develop EAE after priming with antigen, and CD4$^+$ T cells remained quiescent and produced no interferon-γ (IFN-γ). T cells were primed to make IFN-γ and induce EAE by providing these mice with B7.1$^+$ antigen-presenting cells (APCs). Thus, CD40L is required to induce costimulatory activity on APCs for in vivo activation of CD4$^+$ T cells to produce IFN-γ and to evoke autoimmunity.

CD40 ligand is preferentially expressed on the surface of activated CD4$^+$ T cells and is critical for effective humoral immunity (*1*). The receptor for CD40L, CD40, is expressed on various APCs, such as B cells, dendritic cells, and macrophages, and in vitro experimental evidence has shown that CD40-CD40L interaction induces up-regulation of major histocompatibility complex class II, B7, and other molecules that po-

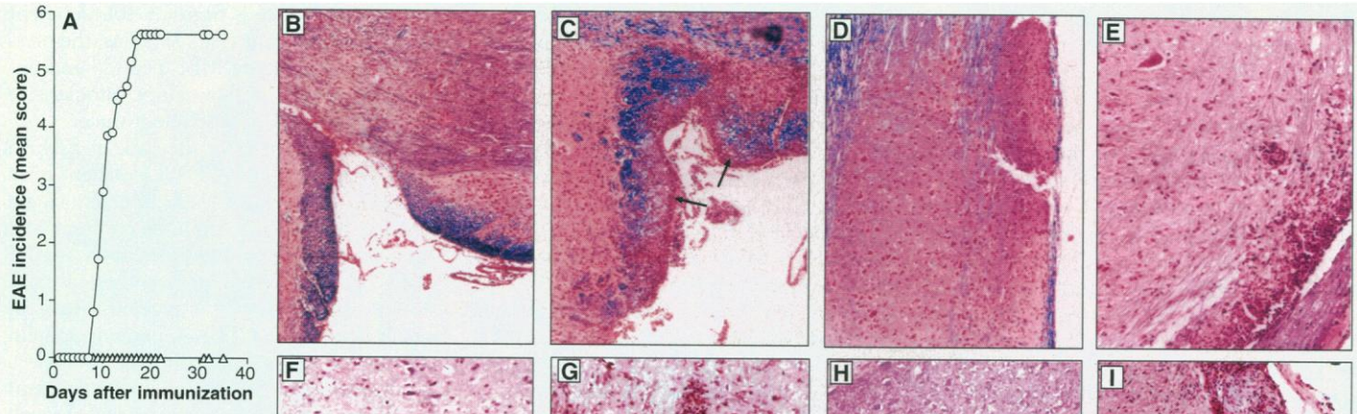

Fig. 1. MBP-TCR transgenic mice lacking CD40L neither develop MBP(Ac1–11)-induced EAE nor show damage to myelin. (**A**) Mean disease score at various days after immunization. (○) Wild-type MBP-TCR transgenic mice (n = 21); (△) CD40L-deficient MBP-TCR transgenic mice (n = 18). (**B**) Paraffin-embedded brain tissue section of MBP-TCR transgenic mice lacking CD40L showing no damage to myelin in CNS. Sections were stained with Luxol fast blue. (**C**) Brain section from wild-type MBP-TCR transgenic mice showing considerable damage to myelin in CNS (arrows). (**D**) Paraffin-embedded spinal cord tissue section of MBP-TCR transgenic mice lacking CD40L showing no damage to myelin. Sections were stained with Luxol fast blue. (**E**) Spinal cord section from wild-type MBP-TCR transgenic mice showing considerable damage to myelin. (**F**) MBP-TCR transgenic mice lacking CD40L showing no infiltration of $CD4^+$ T cells in sections of brain tissue. (**G**) A massive infiltration of $CD4^+$ T cells (arrows) seen in the brain tissue of wild-type MBP-TCR transgenic mice. (**H**) MBP-TCR transgenic mice lacking CD40L showing no infiltration of $CD4^+$ T cells in sections of spinal cord tissue. (**I**) A massive infiltration of CD4 T cells (arrows) seen in the spinal cord tissue of wild-type MBP-TCR transgenic mice.

tentiate the costimulatory activity of APCs (*2*). Our previous studies suggested that in vivo activation and clonal expansion of $CD4^+$ T cells were severely compromised in the absence of CD40L (*3*). The role that CD40L plays in the activation and clonal expansion on T cells is, however, unknown.

To determine the mechanism of enhancement of activation of autoreactive $CD4^+$ T cells by CD40L, we used an in vivo model of a $CD4^+$ T cell–mediated autoimmune disease, experimental allergic encephalomyelitis (EAE) (*4*). CD40L-deficient or wild-type mice that carried a transgenic TCR specific for an NH_2-terminal peptide of myelin basic protein [MBP(Ac1–11)] (MBP-TCR transgenic mice) were examined for the potential role of CD40-CD40L interaction in the development of antigen-induced EAE. The administration of CD40L-specific antibodies to mice has been shown to block EAE, autoimmune oophoritis, and colitis, but the mechanism whereby CD40L mediates this action was not addressed (*5*, *6*). The MBP-TCR transgenic mice described here are well suited to mechanistic studies, because almost all of the $CD4^+$ T cells in these mice carry transgene-encoded TCRs and can be monitored by staining with antibody to V_β8.2 (*4*), and in vivo activation of these T cells by immunization of mice with MBP(Ac1–11) is required to produce EAE. Thus, one can determine the activation requirements of these T cells and the role of CD40L in T cell activation in these mice by inducing EAE with antigen.

CD40L-deficient and wild-type, MBP-TCR transgenic mice were immunized with MBP(Ac1–11) in complete Freund's adjuvant (CFA) and pertussis toxin and were monitored for EAE (*7*). EAE did not develop in CD40L-deficient mice, whereas wild-type mice developed EAE within 7 to 14 days of immunization, indicating that CD40-CD40L interactions affect the onset of EAE (Fig. 1A). To determine whether CD40L-deficient mice had central nervous system (CNS) disease without the appearance of overt symptoms of EAE, we examined the brain and spinal cord from mice for pathology (*8*). Histopathological examination of tissue of wild-type mice indicated substantial damage to myelin in both brain and spinal cord; however, no damage was apparent in tissue sections from CD40L-deficient mice (Fig. 1, B to E). A marked infiltration of $CD4^+$ T cells in the brain and spinal cord was seen in wild-type mice in contrast to CD40L-deficient mice, which exhibited no $CD4^+$ T cells into these tissues (Fig. 1, F to I). Flow cytometric analysis showed that the total number and percentage of CD4- and V_β8.2-expressing T cells was comparable in CD40L-deficient and wild-type mice, which suggested that selection of MBP-TCR transgenic T cells in CD40L-deficient mice was normal (*9*). Because almost all of the $CD4^+$ T cells in these TCR transgenic mice have encephalitogenic potential, we considered that CD40L was probably required for activation or transmigration of encephalitogenic T cells into the CNS.

To determine whether CD40L-deficient MBP-TCR transgenic cells have an intrinsic defect in the ability to respond to MBP-(Ac1–11), we studied the response of naïve T cells to MBP(Ac1–11) in vitro (*10*). The proliferative responses of $CD4^+$ MBP-TCR T cells from CD40L-deficient mice were indistinguishable from those of wild-type $CD4^+$ MBP-TCR T cells (Fig. 2A). Be-

I. S. Grewal, K. D. Grewal, J. Xu, C. A. Janeway Jr., R. A. Flavell, Howard Hughes Medical Institute, and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, USA.
H. G. Foellmer, Yale Neurotransplant Program, Yale University School of Medicine, New Haven, CT 06510, USA.
F. Hardardottir and J. L. Baron, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, USA.

*To whom correspondence should be addressed at Howard Hughes Medical Institute, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, USA.

cause dendritic cells spontaneously up-regulate expression of B7.1 and B7.2 and therefore of costimulatory activity when cultured on plastic (*11*), the in vitro proliferation of MBP-TCR transgenic cells from CD40L-deficient mice in the presence of APCs and antigen was expected. Thus, the frequency of responding T cells and the potential to respond to specific antigen in vitro was preserved in CD40L-deficient mice, and MBP-TCR transgenic T cells that lack CD40L have no gross defects in the potential to be activated.

Despite normal numbers of autoantigen-specific T cells and their ability to proliferate in vitro to encephalitogenic antigen, these MBP-TCR transgenic T cells failed to cause EAE in CD40L-deficient mice. One possible reason for this is that in vivo immunization of CD40L-deficient mice could result in the induction of anergy in MBP-TCR transgenic T cells. We tested this possibility by measuring the in vitro proliferation of draining lymph node (DLN) cells from mice immunized with MBP(Ac1–11) in CFA. DLN cells from both wild-type and CD40L-deficient mice proliferated equally in response to challenge with antigen (Fig. 2B), indicating that the $CD4^+$ T cells in CD40L-deficient mice were not anergized by exposure to antigen. We also tested whether DLN $CD4^+$ T cells from mice immunized with MBP(Ac1–11) in CFA had been primed to produce effector cytokines (*12*). The $CD4^+$ T cells from wild-type TCR transgenic mice produced interferon-γ (IFN-γ), whereas $CD4^+$ T cells from transgenic-positive, CD40L-deficient mice did not produce detectable IFN-γ (Fig. 2C), indicating that CD40L-deficient TCR transgenic T cells were not primed in vivo. Neither T cells from wild-type nor from CD40L-deficient mice produced detectable interleukin-4 (IL-4) (Fig. 2D).

To investigate the lack of development of EAE in CD40L-deficient mice, we examined whether MBP-TCR transgenic T cells devoid of CD40L could be activated in vivo by challenge with antigen. T cell activation is associated with the expression of certain cell surface markers: CD44 expression is increased, whereas CD62L is decreased, and CD25 and CD69 are markers of activation whose surface expression is increased on activated mature T cells. DLN cells from mice immunized with MBP(Ac1–11) in CFA were analyzed by flow cytometry to determine whether CD40L-deficient T cells were activated (*13*). Most MBP-TCR transgenic T cells from wild-type mice were activated, whereas most T cells from CD40L-deficient mice were quiescent (Fig. 3). Thus, the presence of the inducing MBP peptide and encephalitogenic T cells was not sufficient to cause EAE in CD40L-deficient mice.

One explanation for the lack of EAE in MBP-TCR transgenic CD40L-deficient mice could be that CD40-CD40L interactions are required for the induction of costimulatory activity mediated by molecules, such as B7.1 and B7.2, on APCs. This hypothesis is consistent with results of a previous study in which T cells in CTLA-4 immunoglobulin (Ig)-treated mice remained unactivated (*14*). As a consequence, T cells in CD40L-deficient mice may not receive a second signal through CD28 and thus may not be activated. If CD40-CD40L interaction is required for the induction of costimulatory activity, then expression of B7.1 and B7.2 in the DLNs of MBP(Ac1–11)-immunized mice may not be induced. Immunohistological examination of DLNs revealed little B7.1 and B7.2 in T cell areas in the CD40L-deficient mice, whereas wild-type mice showed many cells expressing B7.1 and B7.2 (*15*). Thus, CD40-CD40L interactions were required for the expression of B7.1 and B7.2, which suggested that lack of costimulation through B7.1 and B7.2 may be responsible for protection from EAE in

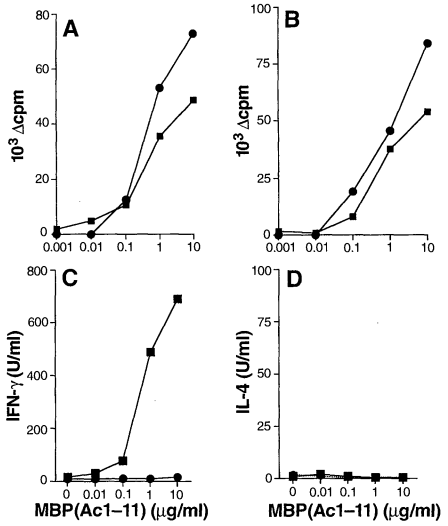

Fig. 2. In vitro responses of $CD4^+$ T cells from MBP-TCR transgenic CD40L-deficient and wild-type mice. (**A**) Proliferative responses of naïve $CD4^+$ MBP-TCR transgenic T cells from wild-type (■) and CD40L-deficient (●) mice to MBP(Ac1–11). (**B**) Proliferative responses of DLN T cells to MBP(Ac1–11) from wild-type (■) and CD40L-deficient (●) MBP-TCR transgenic mice that were immunized with MBP(Ac1–11) and CFA 9 days earlier. ^3H incorporation is shown as counts per minute (cpm), from which values for medium alone were subtracted. (**C**) Production of IFN-γ and (**D**) IL-4 by purified CD4 cells from DLNs of wild-type (■) and CD40L-deficient (●) mice that were immunized with MBP(Ac1–11) in CFA in response to in vitro challenge of MBP(Ac1–11).

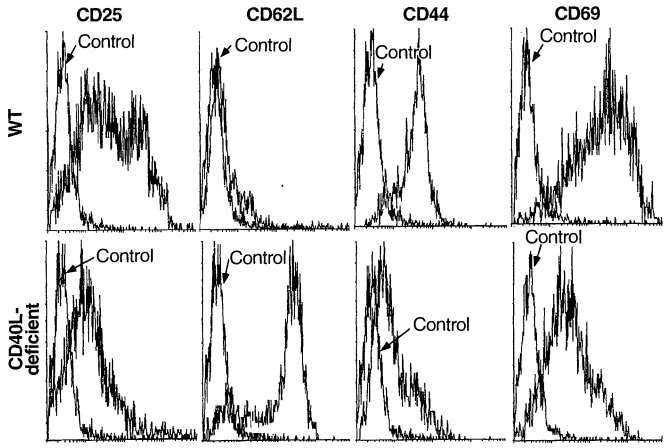

Fig. 3. Flow cytometeric analysis of $CD4^+$ T cells from popliteal lymph nodes, draining the site of MBP(Ac1–11) and CFA immunization. Histograms represent surface staining of cells from DLNs (CD25, CD62L, and CD44) at 5 days or (CD69) 8 days after immunization from wild-type mice (upper panels) and CD40L-deficient (bottom panels). Control indicates nonspecific staining. Samples were gated for $V_\beta 8.2$-expressing cells and were analyzed for the above markers.

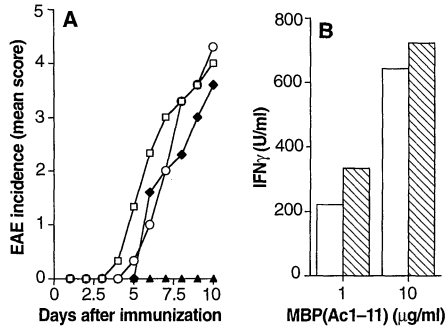

Fig. 4. EAE can be induced and $CD4^+$ T cells can be primed efficiently in MBP-TCR transgenic CD40L-deficient mice by adoptive transfer of B7.1-positive APCs before the immunization with MBP(Ac1–11) in CFA. (**A**) Mean disease score of three mice in each group at various days after immunization. Wild-type MBP-TCR transgenic mice (□), CD40L-deficient MBP-TCR transgenic mice (▲), wild-type MBP-TCR transgenic mice (◆) that received B7.1-positive APCs before induction of EAE, and CD40L-deficient MBP-TCR transgenic mice (○) that received B7.1-positive APCs before induction of EAE. (**B**) Production of IFN-γ by in vivo–primed $CD4^+$ MBP-TCR transgenic T cells from CD40L-deficient mice (open bars) and from wild-type mice (hatched bars). Data are representative of two individual mice from each group.

CD40L-deficient mice.

We then tested whether EAE can develop in CD40L-deficient mice provided with APCs that constitutively express costimulatory molecules (*16*). We injected CD40L-deficient MBP-TCR transgenic mice with APCs expressing B7.1 obtained from B7.1 transgenic mice (*17*) or control nontransgenic APCs before immunizing with MBP(Ac1–11) in CFA. Mice that received B7.1 transgenic APCs developed acute EAE (Fig. 4A), and histopathological examination of CNS tissues showed substantial damage to myelin; control (nontransgenic) APCs did not induce EAE symptoms or damage to myelin in CNS (*18*). We tested whether $CD4^+$ T cells in CD40L-deficient mice were primed in vivo in this adoptive-transfer system (*19*). $CD4^+$ T cells from CD40L-deficient mice that had received $B7.1^+$ APCs before immunization with MBP-(Ac1–11) produced amounts of IFN-γ comparable to those of wild-type mice in response to MBP(Ac1–11), indicating in vivo priming of $CD4^+$ T cells in CD40L-deficient mice (Fig. 4B). The inability of $CD4^+$ T cells lacking CD40L to induce costimulatory activity on APCs in vivo is probably responsible for the failure of T cells to be activated and lack of EAE in CD40L-deficient mice. CD40L undoubtedly plays important roles in subsequent steps in EAE after T cell priming. For example, CD40L may regulate macrophage activation and IL-12 production by both macrophages and dendritic cells (*20*). This observation explains the failure of CD40L-deficient mice to contain *Leishmania* infection (*21*). Moreover, antibodies to CD40L blocked development of a T helper 1–type response in hapten-induced colitis, and administration of IL-12 could reverse this inhibition (*6*). It is likely that many of these functions can be provided by mediators such as adjuvant, for the induction of IL-12, and IFN-γ, for macrophage activation and tissue damage in the CNS.

If CD40L expression is required on T cells for up-regulation of costimulatory activity on APCs, but activation of the T cells is required for expression of CD40L, then how might expression of CD40L on T cells be induced in the absence of costimulation? Up-regulation of CD40L requires only stimulation through the antigenic signal (signal 1), not costimulation (signal 2), as blockade with CTLA-4 Ig, for example, has no effect (*22*). We therefore propose a two-step model for the activation of T cells in the initiation of the immune response. In step 1, T cell sees antigen on the APC, usually a dendritic cell. Dendritic cells constitutively express little B7.2 and generally do not express B7.1 and costimulatory activity (*11*). The T cell receives the antigenic signal (signal 1), which up-regulates CD40L. Engagement of CD40 on the dendritic cell activates the expression of the B7.1 and B7.2 and costimulatory activity, as well as other activators such as IL-12. In step 2 of T cell activation, the costimulatory signal from B7 is received by the T cell through CD28, which drives the cell into proliferation and cytokine production. This model provides an additional regulatory step in the initiation of the immune response—the activation of the APC primed with the cognate antigen by the T cell specific for that antigen. This step may thus provide safeguards against an autoimmune response by not activating bystander cells but only activating T cells specific for a given antigen and the APC carrying that antigen.

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7. Mice were immunized with 10 μg of MBP(Ac1–11) emulsified in CFA (1:1) in hind foot pads. After 24 and 48 hours, 200 μl of Pertussis toxin (0.5 ng/μl; List Biological Laboratory) in phosphate-buffered saline (PBS) were injected intravenously. Mice were monitored daily for the signs of EAE. Disease was scored as described [V. K. Kuchroo *et al.*, *J. Exp. Med.* **179**, 1659 (1994)]: level 1, limp tail; level 2, partial hind limb paralysis; level 3, total hind limb paralysis; level 4, hind limb and 75% of the body paralysis; level 5, moribund; level 6, dead. The signs of EAE did not develop in CD40L-deficient mice, whereas wild-type mice developed EAE within 7 to 14 days of initial immunization. Disease seen in wild-type mice was severe, with 75% of the body paralyzed after 2 to 3 days of the first visual symptoms of EAE; most of these mice either died from EAE or were killed. In some experiments, CD40L-deficient mice were monitored for visual signs of EAE for over 6 months, and no signs of disease were apparent.
8. For histological analysis of CNS tissue, experimental animals were killed together with the appropriate controls at different stages of EAE disease progression, when neurological deficits were apparent (levels 3 to 5). Mice were perfused with 4% paraformaldehyde through the left ventricle. Brain and spinal cord tissues were surgically removed and paraffin-embedded, and 6-mm sections were prepared and stained with either hematoxylin and eosin or Luxol fast blue. For immunohistochemical evaluation, tissue sections were stained with antibody to mouse CD4 (anti-CD4) with the use of an alkaline phosphatase kit (Vector Research).
9. Peripheral lymph node cells and cells from blood samples were stained directly with fluorescein isothiocyanate (FITC)–conjugated anti-V_β8.2 and with anti-CD4 conjugated with Red^{613} as described (*4*) and analyzed with a FACScan (Beckton Dickinson).
10. $CD4^+$ T cells were purified as described [Y. Kamogawa, L. A. Minasi, S. R. Carding, K. Bottomly, R. A. Flavell, *Cell* **75**, 985 (1993)], and proliferative responses were measured by culturing purified $CD4^+$ T cells from naïve mice with irradiated (3000 roentgen) splenic cells from naïve wild-type mice as APCs. T cells (1×10^5) from lymph nodes were cultured in 96-well plates with 5×10^5 APCs as described [K. D. Moudgil and E. E. Sercarz, *J. Exp. Med.* **178**, 2131 (1993)]. For proliferation of in vivo–primed T cells, mice were immunized with 10 μg of MBP(Ac1–11) in a 1:1 emulsion with CFA in the hind foot pads. After 9 days, the popliteal lymph nodes were removed and cell suspensions were prepared, and proliferation of these cells was measured as described above.
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13. DLN cells were stained directly with FITC-conjugated anti-V_β8.2 and with phycoerythrin-conjugated antibodies to CD25, CD62L, and CD69, or anti-CD44 conjugated with Red^{613}, as described (*4*).
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15. Wild-type and CD40L-deficient MBP-TCR transgenic mice were immunized with 10 μg of MBP(Ac1–11) in CFA, and 8 days later their DLNs were examined for expression of B7.1 and B7.2. Briefly, frozen DLN tissue sections were stained with biotin-conjugated anti-B7.1 or anti-B7.2 with the use of an alkaline phosphatase kit (Vector Research). Only small amounts of B7.1 and B7.2 were detected in T cell areas in the CD40L-deficient mice, whereas many cells expressing B7.1 and B7.2 were detected in T cell areas in the wild-type mice. Expression of B7.1 and B7.2 seen in the lymph nodes of unimmunized wild-type mice was low, indicating that most B7.1 and B7.2 expression was induced after immunization with MBP(Ac1–11).
16. For induction of EAE in CD40L-deficient mice, spleen cells from B7.1 transgenic mice (*17*) were prepared devoid of red blood cells and T cells, and 1×10^7 splenic cells were injected into hind foot pads of mice. After 24 hours, mice were immunized with 10 mg of MBP(Ac1–11) emulsified in CFA (1:1) in hind foot pads. Twenty-four and 48 hours after MBP(Ac1–11)-CFA immunization, 200 μl of Pertussis toxin (0.5 ng/μl) in PBS were injected intravenously. Mice were monitored daily for the signs of EAE. Disease was scored as described (*7*). CD40L-deficient MBP-TCR transgenic mice that received B7.1 transgenic APCs developed acute EAE after antigen immunization, and control nontransgenic APCs did not induce EAE in these mice.
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18. CD40L-deficient MBP-TCR transgenic mice that received B7.1 transgenic APCs before induction of EAE were examined for signs of damage to CNS tissues as described (*8*). Damage to myelin in CNS was substantial, whereas no such damage was seen when CD40L-deficient mice received control APCs.
19. Purified $CD4^+$ T cells from DLNs from both CD40L-deficient and wild-type mice that received $B7.1^+$ APCs before immunization with MBP(Ac1–11) were tested for production of IFN-γ in vitro as described (*12*).
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