Role of costimulatory pathways in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis

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Multiple sclerosis is an immune-mediated disorder of the central nervous system. T lymphocytes are thought to play a central role in the initiation and potentially in the propagation of this disease. Two signals are required for T-cell activation. The first signal consists of the interaction of the T-cell receptor with antigen presented by the MHC molecule on antigen-presenting cells. The second signal requires engagement of costimulatory receptors on T cells with their ligands on antigen-presenting cells. Several costimulatory pathways have been shown to play an important role in T-lymphocyte activation. Here we will review the current literature on the contribution of the B7-1/2–CD28/CTLA-4, inducible costimulatory molecule–B7h, programmed death pathway 1–programmed death pathway ligand 1/ligand 2, CD40-CD154, OX40–OX40 ligand, and CD137-CD137 ligand pathways to the pathogenesis of multiple sclerosis and their potential roles as therapeutic targets. (J Allergy Clin Immunol 2003;112:837-49.)

Key words: *B7-1, B7-2, CD28, CD40, CD137 (4-1BB), CD154 (CD40 ligand), costimulation, CTLA-4 (CD152), CTLA4Ig, experimental autoimmune encephalomyelitis, inducible costimulatory molecule, multiple sclerosis, programmed death pathway 1, programmed death pathway ligand 1, programmed death pathway ligand 2, OX40 (CD134), OX40 ligand (CD134L)*

Multiple sclerosis (MS) is an immune-mediated disorder of the central nervous system (CNS) characterized by inflammation, demyelination, and axonal damage.[1] Costimulatory pathways facilitate the activation of certain cell types, predominantly T cells. In addition, there is increasing evidence that some costimulatory pathways affect the function of other inflammatory cells, both in the periphery and in the CNS, and also might modulate neural and glial cell function. Here we will review the literature on the role of costimulatory pathways in the initiation and propagation of MS and its animal models, as well as their potential as therapeutic targets.

Abbreviations used

APC:	Antigen-presenting cell
CNS:	Central nervous system
EAE:	Experimental autoimmune encephalomyelitis
ICOS:	Inducible costimulatory molecule
MBP:	Myelin basic protein
MS:	Multiple sclerosis
OX40L:	OX40 ligand
PD-1:	Programmed death pathway 1
PD-L1:	Programmed death pathway ligand 1
PD-L2:	Programmed death pathway ligand 2
TCR:	T-cell receptor

T-CELL ACTIVATION

T lymphocytes are thought to play a central role in the pathogenesis of MS.[2] Activated myelin-reactive $CD4^+$ T cells have been demonstrated both in the blood and cerebrospinal fluid of patients with MS; in contrast, only nonactivated, myelin-reactive T cells are present in the blood of control subjects.[3] T cells are present in all of the 4 histopathologic subtypes of MS that have been recently described.[4] Both $CD4^+$ and $CD8^+$ T cells have been demonstrated in MS lesions, with $CD8^+$ T cells being more frequent in chronic lesions, and $CD4^+$ T cells being more frequent in more acute lesions.[5]

Two signals are required for T-lymphocyte activation. According to this 2-signal model,[6] signal 1 consists of the interaction of the T-cell receptor (TCR) with antigen presented by the MHC on the surface of antigen-presenting cells (APCs). Signal 2 consists of the engagement of costimulatory receptors on the T cell by ligands present on the surface of APCs.[7,8] Both signals are required for T-cell activation. After contact with specific antigen-MHC complex and adequate costimulatory signals, T cells start to proliferate, differentiate, and deliver a series of signals, enabling effector functions to other cells, such as B cells and natural killer cells. T cells can thereby orchestrate the immune response. Importantly, in the absence of adequate costimulatory signals, T cells die or become anergic in vitro and fail to initiate an effective immune response in vivo. Therefore manipulation of costimulatory signals represents an important mechanism to inhibit immune activation.

Costimulatory molecules may deliver either a stimulatory (positive) or inhibitory (negative) signal for T-cell

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TABLE I. Costimulatory molecules that deliver a positive signal for T-cell activation

Receptor	Location	Ligand	Location
CD28	Naive T cells	B7-1, B7-2	B cells, macrophages, APCs
ICOS	Activated T cells	B7h	APCs, fibroblasts
CD154	Activated T cells	CD40	Macrophages, dendritic cells, astrocytes, endothelial cells
OX40	Activated T cells	OX40L	Activated B cells, dendritic cells, endothelial cells, macrophages, activated T cells
CD137	Activated T cells, monocytes, dendritic cells, B cells	CD137L	Macrophages, B cells, and dendritic cells

TABLE II. Costimulatory molecules that deliver a negative signal for T-cell activation

Receptor	Location	Ligand	Location
CTLA-4	Activated T cells	B7-1, B7-2	B cells, macrophages, APCs
PD-1	Activated T cells	PD-L1	Activated T cells, monocytes, dendritic cells, endothelial cells, syncytiotrophoblasts
		PD-L2	Activated monocytes, dendritic cells

activation.[9] The delicate balance between positive and negative regulatory signals can determine the outcome of a specific immune response. A list of costimulatory molecules that deliver either positive or negative signals for T-cell activation is provided in Tables I and II.

ANIMAL MODELS IN MS RESEARCH

Animal models simulating features of MS provide a powerful tool for investigating the pathogenesis of disease. Experimental autoimmune encephalomyelitis (EAE) is an inflammatory CNS demyelinating disease and can be induced in several animal types through immunization with myelin proteins or peptides, eliciting a $CD4^+$ T-cell response.[10] EAE reproduces many of the clinical and immunologic aspects of MS and has been widely used to study the inflammatory response to myelin components.[11-13] Theiler's murine encephalomyelitis virus is a virally mediated model of CNS inflammatory demyelination with some resemblance to MS. Infection of mice with Theiler's virus results in a virally mediated encephalomyelitis.[14]

The majority of patients with MS initially experience a relapsing-remitting course of disease, followed by a secondary progressive course, whereas a minority experience a primary progressive course.[15] Different animal models mimic different disease courses and might be useful in understanding the pathogenesis of the disease and the response to treatment.

THE B7-1/2–CD28/CTLA-4 PATHWAY

The B7-1/2–CD28/CTLA-4 costimulatory pathway (Fig 1) has been studied in great depth and plays a crucial role in T-cell activation-tolerance. CD28 and CTLA-4 (CD152) are highly homologous costimulatory molecules that are present on the surface of T lymphocytes. CD28 is constitutively expressed on the T-cell surface, whereas CTLA-4 expression is upregulated on T-cell activation.[16] Ligation of CD28 delivers a positive signal for T-cell activation, whereas ligation of CTLA-4 delivers an inhibitory signal for T-cell activation and serves to terminate the immune response.[17]

B7-1 (CD80)[18] and B7-2 (CD86)[19] are members of the Ig superfamily[20,21] and are ligands for both CD28 and CTLA-4.[22] B7-2 is constitutively expressed on most APCs at a low level and is quickly upregulated after cell activation. Meanwhile, B7-1 is only expressed after APC activation. The binding kinetics of B7 molecules to CD28 or CTLA-4 on T cells differ substantially.[23-25] Because CTLA-4 binds B7 molecules with a higher affinity than does CD28, its inhibitory signal eventually predominates, leading to the termination of the immune response.[24]

CD28 synergizes with the TCR signal to promote T-cell activation and likely regulates the threshold for T-cell activation by reducing the number of TCR engagements required.[26] In vitro, the absence of CD28 signaling during T-cell activation leads to cell death[27] or renders the T cell functionally anergic[28] and unable to respond to the presented antigen for several weeks. Signal transduction of CD28 ligation leads to augmented T-cell proliferation,[21] induces IL-2 production,[29,30] and regulates cytokine production.[31,32] Through these and other mechanisms, CD28 ligation prevents the induction of anergy.[33] In addition, ligation of CD28 induces the expression of the antiapoptotic factor Bcl-x_L, which enhances T-cell survival.[34]

CTLA-4 (CD152) is currently viewed as the major negative regulator of T-cell activation. The temporal appearance of CTLA-4 after T-cell activation suggests that one of its major functions is to terminate the ongoing immune response. Engagement of CTLA-4 induces a crucial negative signal through inhibition of TCR- and CD28-mediated IL-2 production, signal transduction,[17,24,35,36] and inhibition of cell-cycle progression.[37] The absence of CTLA-4 in CTLA-4–deficient mice leads to massive lymphoproliferation and fatal multiorgan tis-

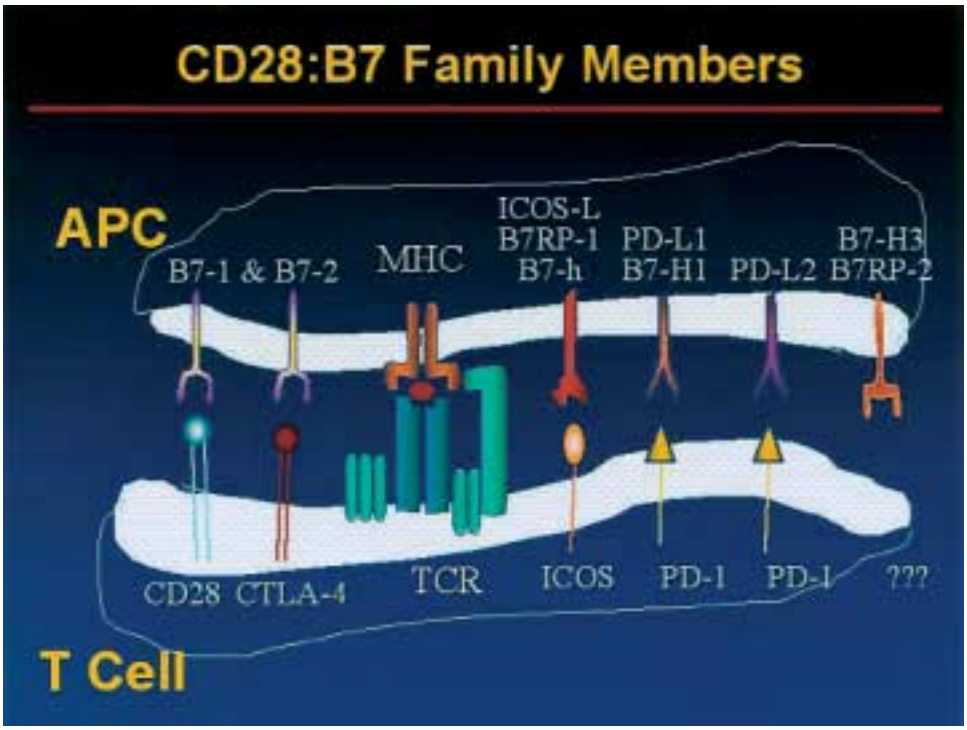

FIG 1. The CD28-B7 family members. The CD28 family members contain a single Ig-V–like domain. CD28 and CTLA-4 molecules bind B7-1 and B7-2 through a MYPPPY motif. Binding of ICOS to its ligand B7h occurs through a FDPPPF motif. PD-1 binds its ligands PD-L1 or PD-L2.

sue destruction.[38,39] Mice having no CTLA-4 and also missing both B7 receptors do not have lymphoproliferative disease,[40] indicating that the unopposed interaction of the B7 receptors with the intact CD28 is the cause of the fatal disorder. Thus the B7-CD28 costimulation pathway delivers a major positive signal for T-cell activation, whereas CTLA-4 costimulation delivers a major negative signal for T-cell activation.

B7-1/2–CD28/CTLA-4 pathway in EAE

Several studies have helped to elucidate the role of the B7-CD28/CTLA-4 pathway in EAE. The expression pattern of these molecules in part reflects their function. During the course of EAE, B7-2 expression in the CNS correlated with clinical disease, whereas B7-1 was exclusively expressed during remissions.[41] Interestingly, B7-1 was demonstrated on neurons during disease remission.[41] CD28 was highly expressed in the CNS and correlated with the clinical signs of EAE. CTLA-4, on the other hand, was expressed by substantially fewer cells during the effector phase of disease and peaked during disease remission.[41]

Studies using anti-CD28 antibodies demonstrated that CD28 blockade during both the initiation of disease and after disease onset ameliorates EAE.[42] In contrast, administration of a blocking anti-CTLA-4 antibody during priming (at the time of immunization) exacerbated EAE, whereas administration after disease onset and during remission induced relapses.[43]

CTLA4Ig is a fusion protein composed of a CTLA-4 surface molecule linked to an Ig tail. It binds B7-1 and B7-2 and thereby blocks their interaction with CD28. CTLA4Ig has been found to profoundly suppress EAE, even when administered after the onset of clinical disease.[44] Disease suppression was associated with inhibition of T_H1 cytokines in the CNS of treated animals but sparing of T_H2 cytokines, suggesting that cytokine shifts are important in CTLA4Ig-induced tolerance.[45] Furthermore, the inhibitory effect of CTLA4Ig could be abrogated by anti-TGF-β–neutralizing antibody.[46] TGF-β has been shown to play a regulatory role in EAE[47,48] and may play a key role in tolerance induction.

Epitope spreading refers to a process whereby epitopes distinct from an inducing epitope become the major targets of an ongoing immune response. Thereby, epitope spreading might induce chronicity of disease.[49] Systemic administration of CTLA4Ig prevents epitope spreading in a relapsing-remitting model of EAE.[50]

The current hypothesis is that the CTLA-4 molecule binds B7 molecules and prevents B7-CD28–induced costimulation of the T cell, thereby preventing disease induction. However, CTLA4Ig might have more diverse effects. APCs treated ex vivo with antigen plus CTLA4Ig have the ability to suppress EAE when transferred to newly immunized recipients.[51] This may be due to the production of regulatory T_H2 cells or through reverse signaling by the B7 receptors to APCs. Recent studies have shown that CTLA4Ig can engage B7 on the surface of

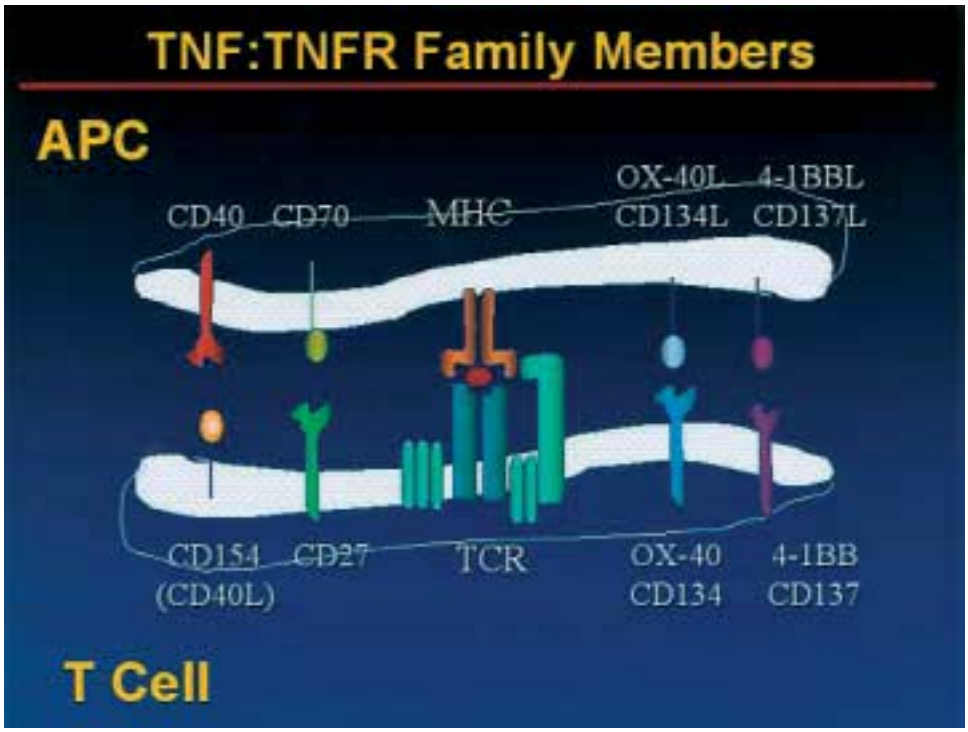

FIG 2. The TNF–TNF receptor family members. Members of the TNF–TNF receptor family include the costimulatory molecules CD154-CD40, CD27-CD70, OX40-OX40L, and CD137-CD137L. CD154, present on activated T cells, binds to its ligand, CD40, on APCs. Similarly, OX40 binds to its ligand, OX40L, initiating costimulatory signals.

dendritic cells, leading to their conditioning and local control of tryptophan catabolism, which results in control of T-cell proliferation and survival.[52] Thus CTLA4Ig is a potentially powerful therapy for autoimmune and immune-mediated conditions and, in addition to EAE, has been used successfully in models of autoimmune lupus[53] and diabetes[54] and a series of animal transplantation models.[55-57]

In the complete absence of the CD28 molecule, mice are resistant to the development of EAE induced through standard immunization techniques.[58-61] Immunization with a higher dose of antigen induced mild disease, suggesting that CD28 costimulation is required to lower the threshold for autoimmune T cells to trigger an immune response.[61] Despite the resistance to disease induction, T cells from CD28-deficient mice were adequately primed to the immunizing myelin antigen.[58,60,61] There appears to be a defect in cell trafficking into the CNS in the absence of CD28 signaling.[58,62]

Absence of both B7-1 and B7-2 rendered C57BL/6 mice resistant to the induction of EAE.[59] However, absence of either B7-1 or B7-2 on the C57BL/6 background did not significantly alter disease course, suggesting overlapping functions of these 2 molecules.[59] In contrast, absence of either B7-1 or B7-2 on the NOD background resulted in disease attenuation.[60] Interestingly, the absence of both B7-1 and B7-2 on SJL mice did not significantly attenuate disease.[63] This suggests that genetic background is a major determinant of the B7 requirement for the induction of autoimmunity.

Selective blockade of B7-1 with CTLA4IgY100F (a mutant form of CTLA4Ig that binds and blocks B7-1 but not B7-2) did not protect from disease or worsened disease in some treated mice.[64] However, other studies have shown that treatment with anti-B7-1 attenuated disease, whereas anti B7-2 antibody worsened disease.[65,66] Interestingly, we have shown that in CD28-deficient mice disease could be induced through administration of anti-B7-1 and anti-CTLA-4 antibodies but not through administration of anti-B7-2 antibody, suggesting that B7-1–CTLA-4 is the dominant pathway for disease suppression.[58]

In summary, the B7-CD28-CTLA-4 pathway plays an important role in the induction of EAE and therefore might play an important role in the pathogenesis of MS.

B7-1/2–CD28/CTLA-4 pathway in MS

Lesions in the CNS of patients with MS were found to be exclusively associated with the expression of B7-1 in perivenular lymphocytes. In contrast, B7-2 was expressed on macrophages in both MS and in other neurologic diseases.[67] Several studies have examined in vitro and ex vivo expression of B7 molecules on microglia and astrocytes in MS and EAE and have attempted to elucidate their role in antigen presentation in the CNS,[68-72] with variable results. Thus the role of the B7 pathway in antigen presentation in the CNS is still under debate.

Blood samples isolated from patients with MS showed increased expression of B7-1 on both $CD4^+$ and $CD8^+$

cells in patients with rapidly progressive disease compared with in those with stable disease or normal control subjects.[73] In a separate study, B7-1 expression was enhanced during MS relapses and localized to B cells.[74] Treatment of patients with IFN-β 1b, a standard treatment for relapsing-remitting MS, reduced the number of B7-1–expressing B cells and increased the number of B7-2 monocytes. In total, these observations suggest that the B7-CD28-CTLA4 pathway is activated in MS and that B7-1 in particular might play an important role in regulating disease activity.

Myelin basic protein (MBP)–reactive T cells have been identified in patients with MS, as well as in healthy control subjects,[3,75] and are thought to play a role in the pathogenesis of disease. In patients with MS but not in healthy control subjects, these cells can be activated in the absence of CD28-B7 costimulation, thus implying that they have been previously activated in vivo.[76] Furthermore, in patients with MS, MBP-reactive T cells isolated from the cerebrospinal fluid have increased expression of the IL-2 receptor,[3] which is consistent with a previously activated or memory phenotype. MBP-reactive T cells from patients with MS were less responsive to CTLA-4 blockade compared with those from healthy control subjects,[77] suggesting that in patients with MS, these cells are not subject to the normal regulatory mechanisms. Although the contribution of MBP-reactive T cells to the pathogenesis of MS is currently unknown, their differential response to costimulatory signals suggests that costimulatory pathways play a role in the immune regulation of this disease.

Genetic polymorphisms might contribute to disease susceptibility. Three *CTLA4* gene polymorphisms were found in patients with MS but not in healthy control subjects[78]; however, no association was found with disease course or severity.[79] In an Olmstead County study 2 polymorphisms were associated with the presence of MS.[80] The Canadian Collaborative Study found no association of *CTLA4* polymorphisms with the disease course of MS.[81] The exon 1 A/G polymorphism was associated with the presence of oligoclonal bands in the cerebrospinal fluid.[82] Thus dysregulation of CTLA-4 signaling might contribute to susceptibility to MS.

In a recent human clinical trial in patients with a T cell–mediated skin disease, psoriasis vulgaris, treatment with CTLA4Ig caused a marked reduction in skin-infiltrating T cells associated with excellent clinical results.[83] No clinically significant adverse effects were observed. Safety and dose-dependent effectiveness were also seen in a rheumatoid arthritis pilot, multicenter, multinational clinical trial.[84] A phase I safety study of CTLA4Ig (Repligen-RG2077), as well as a multicenter study of CTLA4Ig (BMS-188667), for MS are ongoing.

INDUCIBLE COSTIMULATORY MOLECULE/B7h

Inducible costimulatory molecule (ICOS) is a new member of the CD28 subfamily.[85] It shares a 39% similarity with CD28 but lacks the MYPPPY motif that is required for CD28 and CTLA-4 binding to B7-1 and B7-2.[86] ICOS binds to its ligand B7h (also known as B7RP-1, LICOS, GL50, or ICOS-ligand), which is predominantly expressed on APCs (see below).

The *ICOS* gene was mapped to a region of chromosome 1, which also contains genes encoding CD28 and CTLA-4. ICOS is expressed only on activated T cells and on resting memory T cells.[85,87] The inducible expression of ICOS shortly after T-cell activation indicates that ICOS might be particularly important in providing costimulatory signals to activated and memory T cells in contrast to CD28, which is essential in the activation and differentiation of naive T cells.[88] Interestingly, in vitro expression of ICOS can be downregulated by the T_H1 cytokine IL-12,[89,90] suggesting that the ICOS-B7h pathway might be an important costimulator of T_H2, but not T_H1, effector cells. In vivo expression of ICOS is limited to germinal centers, suggesting that this pathway plays a role in promoting differentiation of B cells into memory cells and plasma cells.[85]

B7h or B7RP-1, a murine B7-related protein 1, is a type I transmembrane protein with 20% and 19% amino acid identity to murine B7-1 and B7-2, respectively.[87,91] B7h does not bind to either CD28 or CTLA-4 but binds exclusively to ICOS. B7h is expressed on APCs, constitutively and inducibly, and expression is also inducible on fibroblasts and other cells in response to TNF-α.[88]

ICOS-deficient cells retain CTLA-4 and CD28 expression but, despite this, proliferate poorly. The deficiency in proliferation was rescued in vitro by addition of IL-2.[92] ICOS-deficient cells were able to produce IFN-γ and IL-10 but failed to produce IL-4 when restimulated.[92] ICOS-deficient mice displayed profound deficits in Ig isotype class switching, which was dependent on CD40 stimulation, as well as impaired germinal center formation.[93]

Thus the ICOS-B7h pathway plays important roles in IL-2 regulation, T_H2 cytokine production, and Ig production.

ICOS-B7h pathway in EAE

ICOS-deficient mice on a C57BL/6X129 background had profoundly severe EAE compared with control mice, with massive cellular infiltrates in the CNS.[92] Interestingly, this was not associated with altered expression of IL-4, IL-10, or IFN-γ but was associated with decreased production of IL-13, which may be important for macrophage activation.

Blockade of the ICOS-B7h pathway with a blocking anti-ICOS antibody during the priming stages of EAE (days 0-10) exacerbated disease, whereas blockade during the effector stages of disease (days 9-20) abrogated disease.[94] IFN-γ production and antigen-specific proliferation was increased in animals treated during the priming stage, suggesting that early ICOS blockade promotes T_H1 differentiation. Therefore ICOS-B7h costimulatory signals might favor a T_H2 phenotype, which has generally been found to be protective in EAE.[95]

PROGRAMMED DEATH PATHWAY 1/ PROGRAMMED DEATH PATHWAY LIGAND 1/ PROGRAMMED DEATH PATHWAY LIGAND 2

Recently, a novel negative regulatory molecule and a new member of the B7-CD28 superfamily has been described, termed programmed death pathway 1 (PD-1).[96] Expression was originally thought to be associated with apoptosis but was later shown to be associated with cellular activation and not cell death.[97,98] PD-1 is found on activated $CD4^+$ and $CD8^+$ T cells and binds to 2 known ligands, programmed death pathway ligand 1 (PD-L1) and programmed death pathway ligand 2 (PD-L2), found on APCs, as well as on diverse parenchymal cell types.[96,99-102] Ligation of the PD-1 receptor leads to diminished IL-2 production, and $CD8^+$ T cells appear to be more sensitive to this effect than $CD4^+$ cells.[101]

PD-1–deficient animals have diverse autoimmune conditions, such as autoimmune cardiomyopathy and a lupus-like syndrome with arthralgias and nephritis.[103,104] The precise autoimmune phenotype was dependent on the genetic background of the animal in which the knockout was developed. These autoimmune phenotypes are reminiscent of that of CTLA-4–deficient animals, leading to the suggestion that this pathway might play a central role in the maintenance of peripheral tolerance toward autoantigens.[100]

The PD-1 ligands (PD-L1 and PD-L2) are expressed not only on hemopoietic APCs but also on numerous parenchymal cells, such as cardiac myocytes, renal tubular cells, and microvascular endothelial cells.[100,105-107] This pattern of expression might allow for the termination of an immune response in inflamed tissues, thereby limiting organ damage. Furthermore, the expression of PD ligands and not B7-1 or B7-2 (which interact with CTLA-4) on epithelial or endothelial cells might underlie the findings that such cells, expressing class II MHC molecules after an inflammatory stimulus, can present antigen to T cells, but rather than activating the T cell, instead render them anergic.[108]

The PD-1-PDL1/L2 pathway provides a secondary inhibitory signal for T-cell activation.

PD-1-L1/PD-L2 pathway in EAE

We have recently shown that treatment with a blocking anti-PD-1 antibody during the priming stages of disease exacerbates myelin-oligodendrocyte glycoprotein–induced EAE in wild-type C57BL/6 mice.[109] Treatment during the effector or later stages of disease had no effect. Interestingly, CD28-deficient mice, which are normally resistant to EAE, had a severe form of disease when treated during the priming stages of disease, suggesting that the PD-1 pathway functions independently of the CD28-B7 pathway and might be the dominant inhibitory pathway for T-cell activation, overriding the B7-CTLA-4 pathway. In both anti-PD1–treated wild-type and CD28-deficient mice, more severe disease was associated with increased infiltration of $CD4^+$ cells, macrophages, and, in particular, $CD8^+$ cells in the CNS. In addition, proliferation and production of IFN-γ was enhanced in antigen-specific splenocytes from anti-PD1–treated mice. Treatment with anti-PD-L1 had little effect on wild-type mice, whereas treatment with anti-PD-L2 antibody exacerbated disease, suggesting that PD-1-PD-L2 is the dominant pathway in the regulation of the immune response in EAE.

PD-1 expression in the CNS during the course of EAE began at the onset of disease and peaked with maximal clinical disease and appeared to colocalize with infiltrating T cells.[110] PD-L1 expression followed the same pattern; however, expression was colocalized to astrocytes and in part to microglia, as well as infiltrating cells, suggesting that PD-L1 might play a role in the immune privilege of the CNS. There was little to no expression of PD-L2 in the CNS in naive animals or during EAE, suggesting that PD1-PD-L2–mediated inhibition of T-cell activation in EAE occurs in the periphery and not in the CNS.

CD40-CD154

Recently, there has been great interest in the pathway mediated by CD40 and its ligand, CD154, both members of the TNF receptor family (Fig 2).[111] CD40 is expressed primarily on B cells and APCs, including monocytes-macrophpages and dendritic cells but has been reported on other cells, such as astrocytes, keratinocytes, and endothelial cells. CD154, also known as gp39, is expressed on activated T cells.[111]

CD40 is constitutively expressed on the surface of resting B cells.[111] Expression of CD40 on microglia is upregulated by IFN-γ and TNF-α[112] and is mediated through a signal transducer and activator of transcription 1–dependent mechanism.[113] Binding of CD40 to CD154 has many effects on the APCs, including the induction of B7 expression (particularly B7-1).[114,115] Activation of the CD40-CD154 pathway is crucial for B-cell activation and differentiation, as well as for isotype switching.[116,117] There is also evidence to suggest that engagement of CD154 may provide a direct costimulatory signal to the T cell independent of CD28.[118]

Ligation of CD40 molecules triggers IL-12 production in dendritic cells[119] and microglia.[120] IL-12 is critical for signal transducer and activator of transcription 4–mediated T_H1 cell differentiation and promotes growth and activation of monocytes.[121] Therefore CD40-mediated IL-12 production represents an important control point in the regulation of disease.

CD40-CD154 interactions influence a variety of immune functions, including T_H1 cell differentiation, B-cell activation, and Ig production, as well as B7 molecule expression.

CD40-CD154 in EAE

In EAE, increased expression of both CD40 and CD154 correlated with clinical relapses.[41] In a marmoset model of MS, CD40 expression was localized to macrophages in the CNS.[122]

Treatment of EAE with an anti-CD154 antibody inhibited the induction of disease, dramatically reduced the

severity of established disease,[123,124] and reduced the appearance of further clinical relapses when administered during disease remission.[125] Disease suppression could be reversed by the administration of IL-12.[126] Blockade of EAE with both anti-CD40 ligand antibody and CTLA4Ig had additive effects and resulted in disease suppression with complete absence of CNS inflammatory infiltrates.[127]

CD154-deficient mice transgenic for the MBP TCR,[116] as well as those on a C57BL/6 background,[124] were resistant to the induction of EAE. Ex vivo studies in these mice demonstrated inhibition of T-cell proliferation and IFN-γ production to the immunizing antigen. Tolerance to disease induction was reversed by the addition of B7-1–expressing APCs in vivo,[116] suggesting that the primary function of the CD154 molecule during EAE is the induction of B7 expression, which is necessary for CD28 costimulation.

Activating CD40 antibody–treated cells but not control antibody–treated cells could induce EAE on passive transfer.[128] This was associated with enhanced IL-12 production, suggesting that activation of the CD40-CD154 pathway might be sufficient to overcome normal tolerance mechanisms.

CD40-CD154 in MS

Expression of both CD40 and CD154 was increased in lesions from postmortem brains of patients with MS compared with control brains. CD40 was expressed on macrophages and microglia, whereas CD154 colocalized with the CD4 T-cell marker.[123] Expression of CD154 was found to be higher in peripheral blood monocytes isolated from secondary progressive MS compared with relapsing-remitting MS or healthy control subjects[129,130] and was reduced by IFN-γ treatment.[131]

PBMCs from patients with SPMS produced more IL-12 and IFN-γ when restimulated in vitro compared with those from healthy control subjects.[132] IL-12 production was found to be dependent on CD40-CD154 interactions.[132] Expression of IL-18 in patients with SPMS was also dependent on CD40-CD154 interactions between APCs and activated T cells.[133] Therefore, as has been observed in EAE, the CD40-CD154 pathway is important for the regulation of T_H1 cytokine production in MS.

Clinical trials with an anti-CD40 ligand antibody (Biogen) in autoimmune disease, such as idiopathic thrombocytopenia and lupus, were terminated because of the occurrence of thromboembolic events. Another formulation of the antibody (IDEC pharmaceuticals) is under investigation. A phase I clinical trial in patients with MS was recently performed with good safety data, and therapeutic effects are currently under investigation.

OX40–OX40 LIGAND

OX40 is a member of the TNF receptor superfamily and is expressed on activated T cells.[134] Its ligand, OX40 ligand (OX40L), also a member of the TNF family, is expressed on activated B cells,[134] dendritic cells,[135] and endothelial cells[136] and, under some circumstances, activated T cells.[137] OX40–OX40L interactions can mediate T-cell proliferation and IL-2 production in the absence of CD28.[138] In contrast to CD28-deficient T cells, which have impaired IL-2 production, OX40–deficient T cells have relatively normal IL-2 production, cell division, and expansion.[139] However, OX40–deficient T cells failed to maintain high levels of the antiapoptotic factors Bcl-x_L and Bcl-2 after activation and were highly susceptible to apoptosis, with the converse seen with OX40 signaling.[139] Antigen-specific effector-memory T cells were found to require OX40 signaling for long-term survival.[140,141] Thus the OX40–OX40L pathway might be required for clonal expansion and long-term survival of the memory T-cell pool and may cause preferential survival of T_H2-type cells.[142,143]

CD28-dependent OX40 ligation of CD4 T cells at the time of priming is linked with upregulation of CXCR5 expression and migration of T cells into B-cell areas to support germinal center formation.[144] Activation of OX40L enhances B-cell proliferation and Ig heavy chain production and may be important for the delivery of T-cell help to B cells.[134,145]

$OX40^{-/-}$ mice display defective T-cell proliferation and IFN-γ production.[146] $OX40L^{-/-}$ mice had impaired T-cell priming and a reduction of both T_H1 and T_H2 cytokines, with impaired intrinsic APC function.[147]

Therefore the OX40–OX40L pathway appears to be important for the clonal expansion and long-term survival of the memory T-cell pool, as well as for B-cell activation and Ig production.

OX40-OX40L in EAE

OX40L–deficient mice experienced a milder course of EAE, particularly in the chronic phase of the disease.[148] Ex vivo studies demonstrated decreased antigen-specific T-cell proliferation and diminished IFN-γ, IL-2, and IL-6 production.[148] OX40L–transgenic mice had a greater severity of EAE despite a delayed onset but failed to have disease in the absence of CD28 or CD40.

OX40L expression colocalized to CD11b-positive macrophages-microglia within the CNS during EAE, and expression was associated with clinical relapse.[149] In vitro blockade of OX40L on macrophages-microglia ex vivo inhibited T-cell proliferation, implying that the OX40 pathway can mediate T-cell reactivation within the CNS. In addition, expression was found on endothelial cells,[150] suggesting a role in transmigration.

In vivo blockade with a soluble OX40R-Ig molecule resulted in a milder course of EAE.[149] Treatment with anti-OX40L antibody ameliorated disease in both actively induced and adoptively transferred EAE models.[150] Surprisingly, cells from draining lymph nodes exhibited enhanced antigen-specific T-cell proliferation and IFN-γ production. However, disease attenuation was associated with reduced T-cell and monocytic infiltration into the CNS.[150]

We have shown that resistance to disease induction in CD28-deficient mice can be overcome with the administration of a second dose of antigen 2 weeks after the ini-

tial immunization. Disease, in this case, could be selectively inhibited through the administration of blocking anti-OX40 ligand antibodies, suggesting that in the absence of CD28, the OX40-OX40L pathway provides sufficient costimulatory signals to produce clinical disease.[58] MBP-reactive T cells that receive both CD3 and OX40 stimulation in the absence of CD28 stimulation are able to mediate encephalitogenicity in a passive transfer model of EAE.[151] Thus the OX40-OX40L pathway might provide sufficient costimulatory signals, even in the absence of CD28 to mediate encephalitogenicity. These findings have important implications for the treatment of MS and EAE through costimulatory blockade.

CD137-CD137L

CD137 (4-1BB) is a member of the TNF receptor superfamily and is expressed on activated $CD4^+$ T cells and $CD8^+$ T cells,[152,153] as well as myeloid cells, including monocytes, neutrophils, and dendritic cells.[154-157] Expression has also been reported in the gray matter of the brain.[158] Its ligand, CD137L (4-1BBL) is expressed on activated APCs, including macrophages, B cells, and dendritic cells.[159] Stimulation of T cells with agonistic anti-4-1BB antibodies increased TCR-induced proliferation and cytokine production[160] and enhanced $CD8^+$ T-cell survival through increased expression of the antiapoptotic genes encoding Bcl-x_L and Bfl-1.[161] There appears to be a preferential role for CD137 in the costimulation of $CD8^+$ T cells,[162,163] although $CD4^+$ T-cell costimulation has also been reported.[164,165] CD137 can provide CD28-independent costimulation; however, it is most effective in the presence of a strong TCR signal.[166] Signaling through the CD137 pathway can induce the preferential expansion of $CD8^+$ cytotoxic T lymphocytes that in turn can enhance tumor rejection.[167]

CD137-CD137L in EAE

Paradoxically, administration of an agonistic anti-4-1BB (CD137) mAb resulted in profound suppression of EAE; however, treatment of donor cells did not ameliorate passive transfer of disease.[168] EAE attenuation was associated with a suppression of T_H1 cytokine production in antigen-stimulated cells and delayed-type hypersensitivity responses. Interestingly, the initial expansion of $CD4^+$ cells was not inhibited, but deletion of activated $CD4^+$ T cells was enhanced. Taken with previous data, this suggests that CD137 signaling induces preferential survival of $CD8^+$ T cells and preferential deletion of $CD4^+$ T cells. Further studies are required to elucidate the role of CD137 signaling in EAE and MS.

CONCLUSIONS

Costimulatory signals are necessary for the activation, differentiation, and survival of several immune cell types, particularly T cells. T cells may play an important role in the pathogenesis of MS and have proven to be critical in the induction of EAE. Costimulatory signals, such as CD28, ICOS, CD154, and OX40, deliver a positive signal for T-cell activation, whereas CTLA-4 and PD-1 deliver inhibitory signals. Several costimulatory pathways play overlapping roles, and one pathway might replace another in its absence. In vitro studies have largely shown that absence of positive signals results in anergy or cell death, whereas the absence of negative signals results in lymphoproliferation. However, in vivo, as has been demonstrated in the EAE animal model, the roles of these costimulatory pathways might be more complex, mediating not only cell activation and survival but also T_H1/T_H2 differentiation, production of factors necessary for cell trafficking into the CNS, Ig production, and APC functions. Within the CNS, costimulatory pathways potentially have effects on the activation, differentiation, and survival of several other cell types, including myeloid cells and glial cells. Thus costimulatory signals potentially play a role in the induction of disease in the periphery and in the propagation of disease in the CNS (Fig 3).

The CD28-B7-CTLA-4 pathway has been extensively studied in MS. There is evidence that this, as well as other costimulatory pathways, play an important role in the regulation of disease. Thus targeting costimulatory pathways, in particular the CD28-B7-CTLA-4 pathway with CTLA4Ig might be a viable therapeutic option for the treatment of MS.

Further studies on the mechanisms of costimulation in the immune regulation of MS are required.

Key concepts

- Costimulatory signals are necessary for T-cell activation, differentiation, and survival.
- Costimulatory receptors on T cells are activated by their ligands present on antigen-presenting cells.
- Costimulatory signals such as CD28, ICOS, CD154, OX40, and CD137 deliver a positive signal for T-cell activation.
- Costimulatory signals such as CTLA-4 and PD-1 deliver a negative signal for T-cell activation.
- Absence or blockade of the CD28-B7 pathway reduces the severity of EAE.
- CTLA4Ig can block activation of the CD28-B7 pathway and can regulate the function of antigen-presenting cells.
- CTLA4Ig is an effective therapy in EAE, an animal model of MS.
- Blockade of the CD154-CD40 pathway attenuates EAE.
- Manipulation of costimulatory signals is a viable therapeutic option for the treatment of MS.

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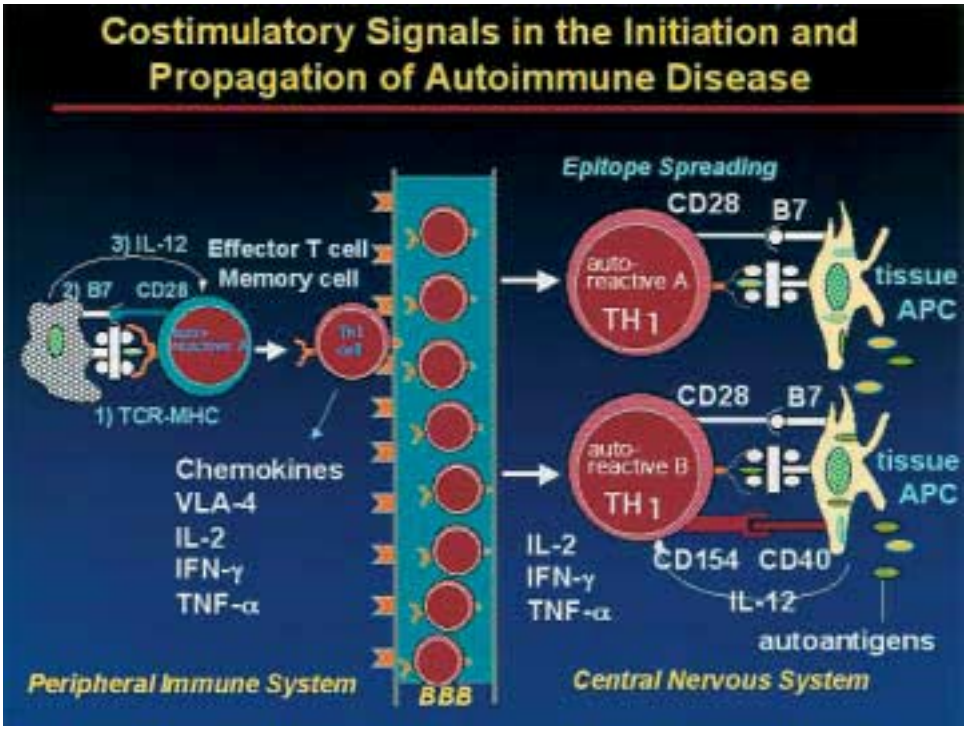

FIG 3. Costimulatory signals in the initiation and propagation of autoimmune disease. Antigen presented to T cells in the presence of costimulatory signals results in T-cell activation. These activated T cells traverse the blood-brain barrier *(BBB)* and reach the CNS tissue. Within the CNS, T cells are reactivated by presentation of myelin antigens by local APCs. The inflammatory environment can induce upregulation of additional costimulatory molecules and can facilitate presentation of antigens to T cells by CNS APCs. Reactivation of T cells induces production of cytokines, in particular TNF-α, as well as recruitment of macrophages into the CNS, which facilitates tissue damage. This results in a release of additional tissue antigens, which can be taken up by potential APCs in the CNS, such as macrophages-microglia, and astrocytes, thus inciting further T-cell activation and tissue damage, thereby propagating the cycle.

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