Immunology and Cell Biology (1999) **77**, 304–311

Review Article

The role of B7 costimulation in T-cell immunity

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Summary $CD4^+$ T cells are considered to be the major controlling element of the adaptive immune response. They recognize foreign peptides by interaction of the T cell receptor (TCR) with peptide complexed to major histocompatibility complex (MHC) class II molecules on the surface of antigen presenting cells (APC). Once activated, $CD4^+$ T cells orchestrate the various phases of the immune response. They are responsible for the production of numerous cytokines, which activate specific immune effector cell populations including B cells, eosinophils, mast cells and macrophages. Not surprisingly, the activation of $CD4^+$ T cells needs to be tightly regulated and is subject to finely tuned control mechanisms. The requirement for a second or 'costimulatory' signal, in addition to the antigenic signal, provides a key element for the exquisite control of T cell activation. One of the major signalling pathways responsible for delivery of this costimulatory signal is induced by interaction of CD28 on T cells with B7 molecules found only on APC. The present review outlines our current understanding of the physiological role of B7 costimulatory signals in regulating $CD4^+$ T cell responses.

Key words: B7-1, B7-2, CD28, costimulation, effector cell, humoral immunity, memory cell, Th1/Th2 cell.

Introduction

Interaction of antigen (Ag) with the TCR results in either clonal expansion or unresponsiveness (anergy). This can be explained by view that T cells require two signals for activation. The first signal is delivered via TCR occupancy, which alone fails to induce complete T cell activation, whereas a TCR signal plus a second or 'costimulatory' signal derived from APC causes activation. The best accepted and most rigorously characterized costimulatory molecules are now considered to be the family of B7 proteins (B7-1 and B7-2). B7 proteins are found on APC[1,2] and their receptor CD28 is expressed on T cells.[3–5] B7 costimulation in the presence of a TCR signal results in up-regulation of the IL-2 receptor (IL-2R) α, β, and γ chains,[6–9] cytokine transcription,[10–13] T cell proliferation[14–16] and expression of *Bcl-*x_L.[17] A diagrammatic illustration of the two-signal model of T cell activation is shown in Fig. 1.

A second receptor on T cells for B7 has now been described.[18] This molecule is called cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) and is thought to negatively regulate T cell responses.[19] The CTLA-4 molecule is not found on the surface of resting T cells but is up-regulated following T cell activation.[11,18,20] Indeed, CD28 signalling can lead to increased levels of CTLA-4 mRNA.[20]

The role of B7 costimulatory signals in T cell activation has been intensely studied, both *in vitro* and *in vivo*. A commonly used B7 antagonist used for these studies is CTLA4-Ig. The CTLA4-Ig fusion protein consists of the extracellular portion of CTLA-4 and the Fc portion of human IgG. Similarly, antibodies against B7-1 and/or B7-2 can be used to block B7 costimulation. Alternatively, the generation of CTLA4-Ig transgenic mice and CD28-, B7-1-, B7-2-, and B7-1/B7-2-deficient mice has provided a direct means to investigate the function of these molecules both *in vivo* and *in vitro*. Lastly, anti-CD28 mAb has been found to induce a costimulatory signal *in vitro* and has been used extensively to deliver optimal CD28 signals to cultured T cells receiving suboptimal TCR stimulation.

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Received 24 December 1998; accepted 11 March 1999.

Structure and expression of the CD28 and B7 molecules

CD28 and CTLA-4

The CD28 glycoprotein is expressed on virtually 100% of murine T cells, all human $CD4^+$ T cells and about 50% of human $CD8^+$ T cells.[5,21] Although CD28 is expressed constitutively, its levels increase after T cell activation.[22] CD28 is also highly expressed on developing thymocytes,[23] although its role in thymocyte selection is not well understood. The CTLA-4 molecule is expressed on the surface of activated $CD4^+$ and $CD8^+$ T cells. However, unlike CD28, CTLA-4 is not expressed on the surface of resting T cells. Moreover, CTLA-4 expression on activated T cells is only 2–3% of the levels of CD28.[24] Both CD28 and CTLA-4 exist as disulphide-linked homodimeric glycoproteins.[5,22,24,25] However, a few studies have suggested that these molecules can also exist in monomeric form.[26,27] The ligands for CD28 and CTLA-4 are B7-1 (CD80) and B7-2 (CD86), which are expressed on a variety of APC.

CD28 and CTLA-4 have single extracellular Ig-like domains containing CDR1, CDR2 and CDR3 loops. The highest concentration of conserved residues in CD28 and CTLA-4 is the hexapeptide MYPPPY motif in the CDR3-like loop.[28] Mutagenesis studies have shown that these residues

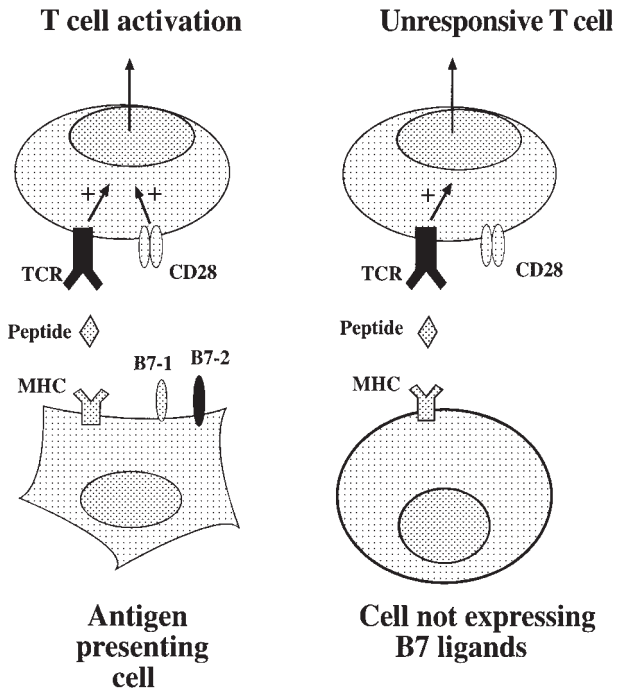

Figure 1 Two-signal model of T cell activation. T cell activation requires two signals. The first signal is provided by interaction of the TCR with antigenic peptide presented on MHC molecules. The second 'costimulatory' signal can only be delivered by APC and is required for IL-2 production. In the absence of a costimulatory signal, T cells fail to make IL-2 and may become unresponsive to further stimulation with antigen. The best-characterized costimulatory signal involves the interaction of B7-1/B7-2 on the APC with CD28 on the T cell.

are important for binding to B7-1 and B7-2.[28] Numerous studies have shown that CD28 has a lower avidity than CTLA-4 for B7-1 and B7-2.[29,30] B7-1 and B7-2 bind to CTLA4-Ig with similar avidities, but they differ slightly in their dissociation rates. The half-maximal dissociation time for ^{125}I-CTLA4-Ig binding to B7-2 Chinese Hamster Ovary cells (CHO) was 2.5–6 min, while the half-maximal dissociation time for B7-1 CHO was 19–28 min.[29] B7-1 and B7-2 must use different binding determinants upon interaction with the CDR3 region of CTLA4-Ig, as predicted by the >200 fold difference in the ability of B7-1 and B7-2 to bind a CTLA4-Ig mutant (Y100A-Ig) that carries a mutation in the MYPPPY motif.[29]

B7-1 and B7-2: Ligands for CD28 and CTLA-4

The two distinct CD28 ligands, B7-1 and B7-2, appear to be only distantly related, with an amino acid sequence identity of 26%.[31] Lipopolysaccharide-dextran sulphate-stimulated splenocytes from mice deficient for both B7-1 and B7-2 lack any detectable binding to mCTLA4-Ig, indicating that there are no further ligands for CTLA-4.[32] B7-1 and B7-2 exist as monomers and have an extracellular V-like and a C-type domain. Conserved residues in B7-1 and B7-2 found in these V-and C-like domains have been found to be critical for the binding activity of B7-1 to CD28 and CTLA-4.[33]

The most marked differences between B7-1 and B7-2 are found in the cytoplasmic tail. B7-1 has a very short cytoplasmic tail, while the cytoplasmic tail of B7-2 is much longer and contains three potential sites for phosphorylation by protein kinase C (PKC).[34] However, much of the homology between human and murine B7-1 is concentrated in the Ig-like extracellular domains and not in the cytoplasmic tail. This indicates that B7-2 may not have an important signalling role, but may rather act primarily as a ligand.[25]

B7-2 is constitutively expressed on resting monocytes and dendritic cells (DC) *in vitro* and is up-regulated rapidly after activation of monocytes, DC, Langerhans cells (LC), B cells and T cells.[35–38] In contrast, B7-1 shows very little constitutive expression on any cell type. However, B7-1 is up-regulated on monocytes, DC, LC, B cells and T cells following activation *in vitro*.[35,39–42] B7-2 is generally expressed more rapidly and to a higher level than B7-1. For example, B7-2 is more rapidly expressed than B7-1 following B-cell activation.[43,44] In addition, Inaba *et al*.[37] noted that B7-2 was the main (> 90%) CTLA4-Ig ligand on murine DC and was largely responsible for the ability of DC to activate T cells.

Many factors have been found to regulate the expression of B7-1 and B7-2, including mitogens,[44] MHC class II ligation,[41] surface Ig cross-linking,[35,38,45] ligation of CD40[46,47] and a variety of cytokines. Interestingly, IFN-γ has been shown to increase B7-2 expression and decrease B7-1 expression on LC[35,40] and on peritoneal macrophages.[35] Therefore, B7-1 and B7-2 expression can be independently regulated by the same stimulus.

Role of B7 costimulation in primary T cell activation

In vitro studies have indicated that CD28 signalling prevents T cell anergy.[48,49] For example, murine and human IL-2-producing T cell clones are unresponsive to interaction with peptide-pulsed metabolically inactive APC that cannot up-regulate B7 molecules.[25] These T cells are anergic, because they are unable to produce IL-2 when rechallenged with costimulatory APC and Ag. However, the anergic state can be reversed by provision of exogenous IL-2.[48] *In vivo* treatment of mice with CD28 antagonists has not been demonstrated to induce anergy, but can suppress humoral responses,[50] inhibit the progression of autoimmune disease and prevent graft rejection.[51–54]

Paradoxically, CD28-deficient mice can clear infection with lymphocytic choriomeningitis virus (LCMV) and reject some skin grafts.[55–57] Therefore, in certain circumstances naïve T cells can become activated in the absence of CD28. Our own studies have shown that while B7 costimulation is required for T cell-dependent eosinophilia in a model of ovalbumin (OVA)-induced airway inflammation, Th2 cells are able to develop in CTLA4-Ig treated mice.[58] In a second study, we have found that CTLA4-Ig treatment has little effect on Th2 cell development or eosinophilia in *Nippostrongylus brasiliensis*-infected mice, although Ab responses are severely reduced.[59] We have concluded from these studies that the requirement for B7 costimulation during activation of naïve Th2 cells *in vivo* is determined by the type of Ag encountered. The properties of a pathogen or protein Ag that lower the requirement for B7 costimulation may include: (i) high dosage; (ii) complexity; or (iii) ability to induce

pro-inflammatory cytokines, such as IL-1, TNF, IL-6, IL-12, IFN-α and IFN-β.

There are several possible explanations for the observation that B7 costimulation is not always required for *in vivo* T cell activation. First, other costimulatory molecules may be able to substitute, or complement, CD28–B7 interactions. Second, B7-independent T-cell activation may occur under conditions of supraoptimal antigen presentation or TCR occupancy. In this regard, it has been shown that B7 costimulation causes T cells to become more sensitive to antigenic stimulation by lowering the number of TCR that need to be triggered before activation occurs.[60,61] Last, CD28 signals may not be absolutely required for the initiation of T cell activation, but may instead have a role in sustaining T cell expansion,[62] perhaps via the induction of T cell survival genes.

Role of B7 costimulation in the development and activation of effector and memory T cells

The activation requirements of naïve, effector and memory T cells have been well studied using *in vitro* assays. Naïve T cells were found to respond poorly to TCR stimulation alone and required B7 costimulation in order to become fully activated.[63] In contrast, B7 costimulation is not necessary for effector T cell activation, although these cells do require B7 costimulation for optimal cytokine production.[64] Interestingly, the requirement for B7 costimulation appears to differ when individual cytokines are studied. For example, in the study by Dubey *et al.*[64] IL-4 production was B7 independent and IFN-γ production was slightly B7 dependent, while IL-2 and IL-5 production were strongly dependent on B7 costimulation. Activation requirements of memory T cells have been studied by isolating TCR transgenic T cells from adults that express memory cell surface markers. Alternatively, memory T cells are generated by producing effector T cells *in vitro* and 'parking' these cells in thymectomized, bone marrow reconstituted adult mice until they return to a resting state.[65] Memory T cell activation differs from naïve T cell activation, in that memory T cell responses to anti-CD3 can be elicited in the absence of B7 costimulation. However, B7 costimulation enhances memory T cell activation in this system.[65] Therefore, B7 costimulatory signals are not necessary for memory T cell activation, but they do optimize the responses of these cells.

Differences in the costimulatory requirements of naïve, effector and memory T cells are reflected in the range of APC that can activate these cells. For example, naïve T cells only respond well to DC that express high levels of B7.[65,66] However, effector T cells can be stimulated by DC, macrophages or B cells.[65,67] Memory T cells respond best to DC, but have the ability to respond to other APC, albeit less well than effector T cells.[65,68]

Differential activation requirements of naïve, effector and/or memory T cell populations are likely to have important consequences during *in vivo* immune responses. As discussed, numerous studies have investigated the role of B7 costimulation in the activation of naïve T cells *in vivo*. However, only a few studies have been made regarding the role of B7 costimulation during *in vivo* activation of effector and memory T cells. One study has addressed the role of B7 costimulation in effector T cell activation by treating mice with CTLA4-Ig after the onset of murine lupus.[53] The CTLA4-Ig-treated mice show a reduction in autoimmune lupus. However, it was not clear whether effector T cell responses or activation of newly recruited naïve T cells were blocked. A later study by Via *et al.*[69] investigated the effect of CTLA4-Ig treatment during a model of graft-versus-host disease and found that disease progression is blocked, thus demonstrating more convincingly that CTLA4-Ig treatment can inhibit effector T cell responses *in vivo*.

A study addressing the role of B7 costimulation during memory T cell activation has shown that CTLA4-Ig inhibits primary but not memory T cell responses to a gastrointestinal nematode *Heligmosomoides polygyrus*.[70] Conversely, CTLA4-Ig administered prior to intranasal challenge inhibited airway eosinophilia in a study of Ag-induced lung inflammation by Keane-Myers *et al.*[71] and in a similar study carried out in our own laboratory (Harris, unpubl. obs., 1997). Recently, we have observed that mice treated with CTLA4-Ig during primary and secondary infection with *N. brasiliensis* exhibit intact memory T cell responses, although IgE responses are decreased. We have concluded from these data that the requirement for B7 costimulatory signals during memory T cell responses *in vivo* is determined by the type of Ag encountered, in a similar fashion to primary T cell responses.

Role of B7 costimulation in Th1/Th2 cell development and effector function

Mosmann *et al*.[72] have demonstrated that mouse $CD4^+$ T cell clones can be classified into distinct populations, based on their patterns of cytokine production. They have termed these populations Th1 and Th2. Generally, Th1 clones produce IL-2, IL-3, IFN-γ, TNF-β and granulocyte–macrophage colony stimulating factor (GM-CSF) and Th2 clones produce IL-4, IL-5, IL-9, IL-10 and IL-13. A number of *in vitro* studies have shown that B7 costimulatory signals promote Th2 cytokine production over Th1 responses. For example, King *et al.*[73] stimulated human $CD4^+$ T cells in the presence of anti-CD28 mAb and found IL-4, IL-5, IL-2 and IFN-γ production. Strikingly, IL-4 and IL-5 were progressively enhanced and IL-2 and IFN-γ diminished after consecutive cycles of CD28 ligation. Similarly, Rulifson *et al.*[74] have found that increasing CD28 ligation during primary culture of $CD4^+$ TCR transgenic T cells increases production of IL-4 and IL-5, but not IFN-γ, upon restimulation of these cells with immobilized anti-CD3 mAb. The data obtained in this study by Rulifson and colleagues were not the result of an outgrowth of Th2 cells by increased IL-2 production, because the addition of exogenous IL-2 could not compensate for CD28 ligation.

In support of the *in vitro* studies, several *in vivo* studies have noted that blockade of B7 costimulation preferentially inhibits Th2 cell responses, while leaving Th1 responses intact. For example, infection with *Leishmania major* normally causes susceptible BALB/c mice to develop a deleterious Th2 response, while resistant C57BL/6 mice develop a protective Th1 response. Administration of CTLA4-Ig during *Leishmania* infection protects BALB/c mice from disease but has no effect on the immune response in C57BL/6 mice.[75] Similarly, CTLA4-Ig treatment resulted in a diminution of

clinical disease in mice adoptively transferred with experimental allergic encephalomyelitis (EAE)-inducing T cell clones.[54] Disease prevention was associated with a reduced production of IL-2 and IL-4 but not IFN-γ. Recently, Lenschow *et al.*[76] bred the non-obese diabetic (NOD) mouse to mCTLA4-Hγ1-transgenic or CD28-deficient backgrounds and found a dramatic increase in disease incidence and severity. Increased disease incidence correlated with enhanced IL-2 and IFN-γ and diminished IL-4.

The role of B7 costimulation during Th1 and Th2 cell differentiation has also been studied using models of more polarized Th1 and Th2 responses. T cell responses to LCMV (which classically induces a strong Th1 response) are intact in CD28-deficient mice.[57,77] Conversely, CTLA4-Ig treatment abrogates Th2 responses during a model of airway inflammation[71] and after infection with *Schistosoma mansoni*[78] or *H. polygyrus*.[79]

Collectively, the data discussed support the hypothesis that Th2 responses are more B7 costimulation dependent than Th1 responses. However, some Th1 responses have been shown to be highly B7 costimulation dependent. These include T cell responses to vesicular stomatitis virus (VSV) infection,[77] mouse mammary tumour viruses (MMTV),[80] alloantigens,[51,52,81] soluble Ag[50,56] and tumours.[82] In addition, we have shown that Th2 responses to certain Ag, such as *N. brasiliensis,* can occur in the absence of B7 costimulation.[59]

In contrast to the priming of naïve T cells, *in vitro* activation of effector Th2 cells appears to be less dependent on B7 costimulation than effector Th1 cell activation. Effector Th2 cells only require IL-4 as their growth factor, whereas naïve T cells and effector Th1 cells require IL-2. Most established Th1 clones continue to require CD28 signalling to produce IL-2,[63] whereas the production of IL-4 by Th2 effector cells is relatively B7 costimulation independent.[83] However, Th2 clones may require B7 costimulation to respond to IL-4.[84]

In summary, numerous studies have indicated that *in vitro* and *in vivo* Th2 cell priming is more dependent on B7 costimulation than Th1 cell development. Conversely, activation of Th2 effector cells is less sensitive to B7 costimulation than Th1 effector cell activation. Collectively, these data indicate that the outcome of CTLA4-Ig-induced blockade of B7 costimulation on an immune response will be complicated by the nature of immune response (Th1 or Th2) and the timing of the treatment.

Role of B7 costimulation in humoral immune responses

T cell-dependent B cell responses require that T helper cells provide the necessary signals for B cell growth and Ig class switching. These signals are provided in the form of soluble cytokines and membrane-bound CD40 ligand (CD40L) following T cell recognition of Ag presented by MHC class II molecules on B cells. Numerous studies have reported an essential role for B7 costimulation in T cell-dependent humoral responses. For example, compared with normal mice, levels of total serum Ig are decreased in unimmunized mCTLA4-Hγ1 transgenic mice. This is true for all Ig isotypes, except IgA. Interestingly, gut-associated lymphoid tissue from mCTLA4-Hγ1 transgenic mice has normal germinal centres and is probably the source of serum IgA.[85] CD28-deficient mice have also been found to exhibit decreased levels of total serum Ig. However, only IgG1 and IgG2b have been found to be decreased, while IgG2a levels remain high.[57] This observation supports the proposal that there is a greater requirement for B7 costimulation during Th2 responses than for Th1 responses.

B7 costimulatory signals appear to be essential for Ag-induced Ab production. For example, primary T-dependent Ab responses to sheep red blood cells (SRBC) or key-hole limpet haemocyanin (KLH) are inhibited in a dose-dependent fashion by CTLA4-Ig.[50] In addition, mCTLA4-Hγ1 transgenic mice exhibit reduced primary T-dependent Ab responses[85,86] and lack germinal centre formation[85] following immunization with soluble Ag. Normal Ab levels are not achieved in mCTLA4-Hγ1 transgenic mice, even at long intervals after Ag immunization or after multiple immunizations.[85] Examination of Ig isotypes in Ag immunized mCTLA4-Hγ1 transgenic mice has found that all isotypes are lowered. CD28-deficient mice also exhibit decreased T-dependent Ab responses.[57]

Remarkably, mCTLA4-Hγ1 transgenic mice that exhibit lowered Ag-induced Ab production show enhanced expansion of Ag-specific T cells, indicating that B7 costimulation is more stringently required for B cell responses than for T cell priming.[86] Blockade of B7 costimulation does not alter the ability of mice to generate normal cytotoxic T cell responses to LCMV infection.[57,87] However, production of LCMV-specific IgG is decreased 3–20 fold in mCTLA4-Hγ1 transgenic mice. In our own studies, we have found that B7 costimulation is required for optimal Ab production in response to OVA immunization and airway challenge or to *N. brasiliensis* infection.[58,59] Interestingly, Th2 cell cytokine production and blood and airway eosinophilia were only mildly effected in *N. brasiliensis*-infected mice, while Ab responses were severely decreased. Collectively, these data indicate that humoral responses exhibit a greater requirement for B7 costimulation than T cell priming, differentiation, cytotoxicity or cytokine production.

Differential roles for B7-1 and B7-2 in T cell immune responses

It is unknown, at present, whether costimulatory signals provided by B7-1 and B7-2 are functionally distinct or overlapping. *In vitro* studies have found that either B7-1 or B7-2 is sufficient, in the absence of the other, to provide some costimulatory function.[88,89] Nevertheless, other studies have provided evidence for a preferential role for B7-1 or B7-2 during T cell immune responses, even when both are expressed by the stimulatory APC. For example, Razi-Wolf *et al.*[90] have shown that T cell proliferation to concanavalin A (Con A) or alloantigen is solely dependent on B7-1. In addition, Fleischer *et al.*[39] have demonstrated that T cell activation by human monocytes is inhibited by anti-B7-1 but not anti-B7-2, despite higher expression of B7-2 relative to B7-1. Conversely, Hathcock *et al.*[35] have found that T cell proliferation to allogeneic APC or soluble anti-CD3 presented by B cells is highly B7-2 dependent, with little (if any) dependence on B7-1.

More recently, B7-1- and B7-2-deficient mice have been generated. Mice deficient for both B7-1 and B7-2 fail to generate Ag-specific IgG1 and IgG2a responses. However, mice

lacking only B7-1 or B7-2 mount high titre Ag-specific IgG1 and IgG2a responses when immunized in Complete Freund's Adjuvant (CFA). Therefore, B7-1 and B7-2 can have overlapping compensatory functions for IgG production. However, when immunized intravenously with Ag in PBS, B7-2-deficient mice failed to produce Ab, whereas B7-1-deficient mice gave normal Ab responses. These data indicate that although B7-2 and B7-1 have compensatory roles, B7-2 is more dominant.[32]

B7-1 and B7-2 may differentially affect Th1/Th2 cell development

A number of *in vitro* studies have indicated an important role for B7-1 and B7-2 in the differentiation of Th1 and Th2 cells. For example, Freeman *et al.* have found that repetitive costimulation of naïve T cells, with CHO cells expressing B7-2, results in moderate levels of IL-4 and IL-2 production.[7] In contrast, repetitive costimulation with CHO cells expressing B7-1 results in high levels of IL-2 and low levels of IL-4 production. Similarly, Kuchroo *et al.* have found that when naïve myelin basic protein (MBP)-specific TCR transgenic T cells are activated *in vitro* in the presence of anti-B7-1 or anti-B7-2 mAb, Th2 or Th1 cytokine profiles, respectively, are produced.[91] These two studies indicate that B7-1 signals preferentially promote development of Th1 cells, while B7-2 signals promote Th2 cell development.

Other *in vitro* studies have failed to identify any difference between B7-1 and B7-2 in driving Th1 or Th2 differentiation. For example, Lanier *et al.* have found that both B7-1 and B7-2 transfectants can stimulate resting human peripheral blood T cells to secrete IL-2 and IFN-γ or to differentiate into cytotoxic T lymphocytes.[92] However, neither B7-1 nor B7-2 promote the development of IL-4-secreting T cells. Moreover, Anderson *et al.* have recently demonstrated that both B7-1 and B7-2 CHO transfectants increase proliferation and cytokine secretion following activation of a Th2 clone.[93] Only B7-1 can provide the costimulatory signals required for activation of the Th2 clone by a weak peptide agonist, whereas both B7-1 and B7-2 induce activation to a strong peptide agonist. Based on this data, Anderson and colleagues have suggested that it is necessary to decrease the strength of the TCR signal to see differences in B7-1 and B7-2 costimulation.

In vivo studies have also indicated differential roles for B7-1 and B7-2 in the differentiation of Th1 and Th2 cells. As discussed earlier, Kuchroo *et al.*[91] have proposed a role for B7-1 and B7-2 during *in vitro* Th1 and Th2 cell differentiation and, using a model of experimental autoimmune encephalomyelitis, have suggested that B7-1 and B7-2 promote *in vivo* Th1 and Th2 cell responses, respectively. In this system, treatment with anti-B7-1 mAb reduces the incidence of disease by promoting Th2 cell development. Conversely, treatment with anti-B7-2 mAb increases disease severity by promoting the development of Th1 cells.

A study by Lenschow *et al.*[94] has investigated the roles of B7-1 and B7-2 costimulation using an autoimmune model of diabetes, mediated by Th1 cells. These authors have shown that blockade of B7-1 costimulation increases disease incidence, presumably by increasing development of Th1 cells. Blockade of B7-2 costimulation prevents the onset of diabetes by down-regulating Th1 cell responses. This study indicates that B7-1 and B7-2 promote Th2 and Th1 cell responses, respectively, and is in direct contrast to the data obtained in the EAE model. However, regardless of whether B7-1 and B7-2 costimulation directly modulates Th1/Th2 differentiation, the Kuchroo and Lenschow studies establish that B7-1 and B7-2 can have differential effects on the T cell immune response.[91,94]

Potential mechanisms for differential regulation of T cell immune responses by B7-1 and B7-2

There are a number of mechanisms by which B7-1 and B7-2 costimulatory ligands may have a differential effect on T cell immunity. For example, B7-1 and B7-2 may engage CD28 in distinct ways, resulting in differences in CD28 signal transduction. Two studies that have examined the effects of B7-1 and B7-2 costimulation have failed to find any significant differences in the activation of T cell second messengers or cytokine production.[92,95] Alternatively, engagement of B7-1 or B7-2 could lead to alterations in APC function. Indeed, the possibility that B7-2 may function as a signalling molecule is supported by the finding that B7-2 is phosphorylated following B-cell activation.[34] Based on the relative timing of their expression, B7-2 may be the physiological ligand for CD28 and B7-1 the primary ligand for CTLA-4. However, Linsley *et al.*[29] have failed to find any evidence for preferential binding of CD28 and CTLA-4 to B7-1 or B7-2.

B7-1 and B7-2 may differentially alter the immune response simply due to variation in their expression. In support of this hypothesis, Miller *et al.* have noted preferential up-regulation of B7-1 during relapsing EAE and a selective increase in its functional costimulatory activity relative to B7-2.[96] Similarly, Windhagen *et al.* have reported a selective up-regulation of B7-1 in patients with multiple sclerosis.[97] Our own studies have indicated that B7-1 costimulation is required for the induction of optimal airway eosinophil inflammatory responses in OVA-immunized and airway-challenged mice, while it is not necessary for OVA-specific Ab production or blood eosinophilia.[98] These data may indicate that B7-1 costimulation is more stringently required for activation in the peripheral tissues, such as lung, than in the lymphoid tissues. However, this is not due to selective expression of B7-1 by lung APC. Alternatively, there may be a requirement for more potent T-cell activation in the lymphoid tissues before peripheral inflammation can occur or a selective role for B7-1 costimulation in allowing T-cell migration into the periphery.

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