Cell, Vol. 81, 979–982, June 30, 1995, Copyright © 1995 by Cell Press

Distinct Roles for the Costimulatory Ligands B7-1 and B7-2 in T Helper Cell Differentiation?

Minireview

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Introduction

Signal transduction through CD28 plays an important role in regulating the initial response of a T cell to antigen. Two distinct CD28 ligands, B7-1 (CD80) and B7-2 (CD86), have been identified. These ligands also bind to CTLA-4, a receptor closely related to CD28 that is expressed on activated T cells. Recent studies designed to examine the individual roles of B7-1 and B7-2 in the regulation of an in vivo immune response have demonstrated that costimulation through CD28, CTLA-4, or both is more complex than previously believed. In vivo, the costimulatory ligands B7-1 and B7-2 appear to differ in their ability to potentiate the differentiation of T helper (Th) cells into either type 1 (Th1) cells, which direct cell-mediated immunity, or type 2 (Th2) cells, which support a humoral immune response. These results have important implications for our understanding of in vivo immune responses as well as for strategies of immunotherapy involving the CD28 costimulatory pathway.

The Role of Costimulatory Receptors in the Initiation of an Immune Response

An antigen-specific T cell immune response is initiated as a result of interaction between a T cell receptor (TCR) and antigen–major histocompatibility complex (MHC) complexes expressed on the surface of an antigen-presenting cell (APC). However, while TCR signal transduction is necessary for the activation of a naive T cell, TCR ligation alone is not sufficient to initiate an immune response under most circumstances (Figure 1). For optimal activation of a naive T cell, additional or costimulatory signals are needed. Activation through the TCR in the presence of such costimulatory signals results in T cell clonal expansion and in the induction of effector functions such as lymphokine production. Interaction of naive T cells with antigen in the absence of a costimulatory signal can result in T cell unresponsiveness or death. In this model, T cell costimulatory signals play a critical role in dictating the subsequent fate of a T cell that initiates a response to antigen.

The B7/CD28 Activation Pathway Transmits a Costimulatory Signal

Work over the last several years has demonstrated that CD28 is one of the major costimulatory receptors on the surface of a resting T cell (for review see Allison, 1994). Signal transduction through CD28 synergizes with TCR signal transduction to augment both interleukin-2 (IL-2) production and proliferation of naive T cells. Blockade of the CD28 signal transduction pathway by noncross-linking monovalent antibody fragments can render T cells hyporesponsive to subsequent challenge with antigen. Several years ago, the B cell activation antigen B7-1 (CD80) was found to be a ligand for CD28. B7-1 was subsequently found to be expressed as an activation antigen on a variety of additional APCs, including dendritic cells and monocytes.

In addition to CD28, B7-1 can bind to the T cell activation antigen CTLA-4. CTLA-4 binds to B7-1 with ~20-fold higher avidity than CD28. In contrast with CD28, CTLA-4 is not expressed on quiescent T cells, but CTLA-4 expression is detectable following TCR ligation and can be further augmented by CD28 costimulation. At present, the role of CTLA-4 in costimulation is uncertain. Depending on the circumstances, CTLA-4 has been reported to act as an additional costimulatory signal or to act as a negative signal to down-modulate an immune response either by terminating a proliferative response or directly inducing apoptosis. A soluble recombinant form of CTLA-4, CTLA4Ig, has been used as a competitive inhibitor of CD28 activation. In vivo, CTLA4Ig treatment can suppress the ability to mount T cell–dependent antibody production as well as suppress the ability to mount a cell-mediated immune response against tissue grafts.

The demonstration that CTLA4Ig could inhibit T cell–dependent immune responses that were not inhibited by B7-1 antibodies, as well as the recognition that mice deficient in B7-1 could still induce CD28 costimulation, resulted in the discovery of a second ligand for the CD28 receptor, B7-2. Although B7-2 shares only 26% amino acid identity with B7-1, B7-2 is also a member of the immunoglobulin gene superfamily and appears to be closely linked

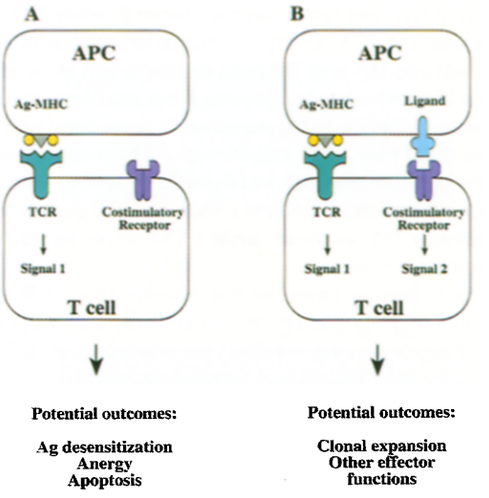

Figure 1. Potential Model for the Role of Costimulation in the Response of a Naive T Cell to TCR Engagement by Antigen–MHC Complexes on the Surface of an APC

Ag, antigen.

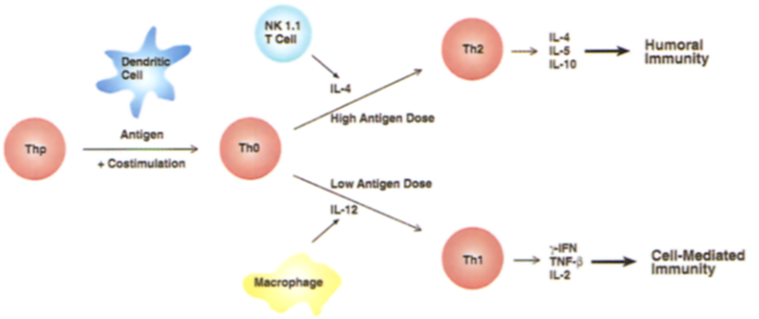

Figure 2. Schematic Model for the Activation and Differentiation of Th Cell Precursors into Th1 and Th2 Effector Cells

See text for details. Thp, Th cell precursor; TNF, tumor necrosis factor.

in the genome to B7-1. B7-2 displays a different pattern of expression from that of B7-1. B7-2 is rapidly induced on the surface of activated B cells or monocytes and is expressed at high levels on dendritic cells. Its kinetics of induction suggest that B7-2 is the major CD28 ligand expressed early during an immune response.

Activated Th Cells Can Differentiate into Distinct Types of Effector Cells

One unexplained aspect of the B7/CD28 activation pathway is the reason there is redundancy of both the receptors and the ligands in the B7/CD28 pathway(s). Experiments designed to examine the differential roles of the individual ligands are now being reported, and the early reports are startling. Kuchroo et al. (1995) and Lenschow et al. (1995) have reported evidence that isolated blockade of B7-1 or B7-2 in vivo does not inhibit the ability to initiate an immune response but rather affects the type of immune response that develops by altering the differentiation of Th cells.

$CD4^+$ Th cells can differentiate into either of two distinct types of effector cells: Th1 cells, which regulate cell-mediated immunity, and Th2 cells, which regulate humoral immune responses (for review see Paul and Seder, 1994). Each of these two Th cell subtypes produces a distinct set of regulatory cytokines. Th1 cells produce IL-2, γ-interferon (IFNγ), and tumor necrosis factor β, while Th2 cells produce IL-4, IL-5, and IL-10. The differentiation of naive Th cells into Th1 and Th2 cells has important implications in the immune response. In several models of autoimmune disease, a Th2 immune response is associated with disease resistance while induction of a Th1 response leads to progressive disease. In contrast, protective immunity from intracellular pathogens such as Leishmania major requires a Th1 response, while a Th2 response results in progressive disease.

The molecular bases for the differentiation of Th cells are just beginning to be delineated (Figure 2). Activation of precursor Th cells results in the production of IL-2 with relatively little IFNγ or IL-4 being produced (Th0). Subsequent events appear to bias the cell toward differentiation into a Th1 or Th2 phenotype. The most effective inducers of such differentiation appear to be IL-12 and IL-4. Under the influence of IL-4, precursor cells differentiate preferentially down the Th2 pathway, while in the presence of IL-12, cells differentiate down the Th1 pathway. Subsequent activation of these differentiating cells by antigen reinforces these differentiation events since Th2 cells preferentially produce IL-4 and Th1 cells produce IFNγ. These lymphokines in turn help perpetuate humoral and cellular immune responses, respectively.

Several types of immune cells have been shown to be able to produce IL-4 during an immune response, including the NK1.1 subset of T cells and mast cells. IL-12 appears to be produced primarily by monocytes, but several other cell types, including dendritic cells and keratinocytes, can produce IL-12. The provision of either IL-12 or IL-4 by the APC plays a strong role in biasing Th cell differentiation. However, even in the absence of exogenous lymphokines, T cells can be directed to either Th1 or Th2 differentiation (Kalinski et al., 1995). High doses of antigen appear to favor differentiation down the Th2 pathway, while low antigen doses appear to favor a Th1 response (Bretscher et al., 1992). The route of antigen administration also appears to play an important role, perhaps through the recruitment of distinct populations of APCs (Paul and Seder, 1994).

Costimulation by B7-1 and B7-2 Can Differentially Regulate Th Cell Differentiation

Kuchroo et al. (1995) have now presented evidence that B7-1 and B7-2 may play distinct roles in the differentiation of Th cells. These investigators studied experimental allergic encephalomylitis induced by immunization with proteolipid protein in animals simultaneously treated with either anti-B7-1 or anti-B7-2. Treatment with anti-B7-1 resulted in the generation of effector T cells that had a Th2 phenotype. These Th2 cells could both prevent the induction of experimental allergic encephalomylitis and abrogate established disease. In contrast, treatment with anti-B7-2 resulted in the production of effector cells of a Th1 phenotype, resulting in increased disease severity. These data suggest that not only is costimulation important during the initial activation of uncommitted Th cells, but that costimulation may also play a crucial role in regulating the subsequent differentiation of Th0 cells along the Th1 or Th2 developmental pathways.

Other groups have also begun to report that differential blockade of B7-1 or B7-2 can have distinct effects on in vivo immune responses. Lenschow et al. (1995) have studied the role of B7-1 and B7-2 antibodies on the development of autoimmune diabetes in the NOD mouse strain. Anti-B7-2 prevented the onset of diabetes, while treatment with anti-B7-1 resulted in an increased incidence and an accelerated course of diabetes.

Although the findings by Kuchroo et al. (1995) and Lenschow et al. (1995) may initially seem contradictory,

both studies suggest that costimulation plays a role in the differentiation of Th cells. Further support for this hypothesis can be drawn from observations that Th1 and Th2 responses display differences in their dependence on costimulation (Lu et al., 1994; McKnight et al., 1994; King et al., 1995). For example, Corry et al. (1994) have studied the effect of CTLA4Ig treatment on the response of different mouse strains to L. major infection. In mice that are genetically predisposed to produce the Th1 response to L. major, induction of the Th1 response is not affected by CTLA4Ig treatment. This suggests that this response can occur under conditions of either low or no costimulation. In contrast, the ability of susceptible mouse strains to mount a Th2 response can be blocked by CTLA4Ig.

Potential Mechanisms for the Differential Regulation of Th1/Th2 Development by B7-1 and B7-2

There are several mechanisms by which the two costimulatory ligands may have a differential effect on Th cell differentiation. Based on the relative timing of their expression, B7-2 may be the physiologic ligand for CD28, while B7-1 is primarily a ligand for CTLA-4. However, Linsley et al. (1994) failed to find evidence for differential pairing of the proteins. They found that B7-1 and B7-2 display similar avidity for CD28. Both B7-1 and B7-2 display higher avidity for CTLA-4, although B7-2 binding to CTLA-4 displays more rapid dissociation kinetics than B7-1. An alternative possibility suggested by Kuchroo et al. (1995) is that B7-1 and B7-2 engage CD28 in distinct ways that result in differences in receptor signal transduction. Nunès et al. (1994) have shown that differential signal transduction through CD28 can occur. Antibodies that cross-link CD28 induce activation of Ras, while costimulation by B7-1-transfected cells does not appear to induce Ras activation. As suggested by Lenschow et al. (1995), differences in B7-1- and B7-2-induced signal transduction could also be due to differential signaling through CTLA-4. Studies involving animals with a germline deficiency in CTLA-4 may help to resolve this issue.

There is some evidence against a role for differential signal transduction in explaining the differences between B7-1 and B7-2 costimulation. Two groups that have examined the effects of signal transduction using B7-1 and B7-2 transfectants have failed to find any significant differences in the activation of second messengers or lymphokine production (Lanier et al., 1995; Levine et al., 1995). While Freeman et al. (1995) have demonstrated that multiple rounds of antigen priming by B7-2-transfected cells can lead to a Th2 phenotype, Seder et al. (1994) have used B7-1-expressing cells to induce the development of Th2 clones. These data suggest that there is no absolute difference in B7-1- and B7-2-initiated signaling.

There are several additional possibilities for the differential ability of B7-1 and B7-2 to affect Th cell differentiation. There are clear differences both in the timing of the expression of B7-1 and B7-2 during an immune response as well as differences in their expression on different APCs (Hathcock et al., 1994). Antibody blocking experiments suggested that B7-2 is the major CD28 ligand that initiates CD28-dependent costimulation in unprimed lymph node cells. In contrast, B7-1 costimulation appears to be up-regulated later during activation of an immune response, and has been proposed to be the major costimulatory ligand present during persistent infections. Distinct roles for B7-1 and B7-2 may also result from differences in their relative expression on the APCs involved in T cell differentiation (Figure 2).

B7-1 and B7-2 could also serve as counterreceptors that transduce distinct signals to the APC upon engagement by CD28 or CTLA-4. The intracellular domains of B7-1 and B7-2 are quite distinct and could mediate differential signal transduction. Such signaling could alter the APC's ability to function as an effector cell. Delineation of these possibilities may come from characterization of mice with germline deletions of the B7-1 gene, the B7-2 gene, or both.

Th1 and Th2 Cells Differ in their Requirements for Costimulation

Experiments to define the parameters that control Th2 development have suggested that Th2 immune responses are dependent on high initial antigen doses. Th2 immune responses also appear to be dependent on CD28 costimulation during their initiation, but thereafter appear to be relatively less dependent on costimulation for their maintenance (McKnight et al., 1994). A potential mechanism for this is the observation that CD28 costimulation is necessary for the induction of T cell sensitivity to IL-4 (McArthur and Raulet, 1993). Thus, CD28 costimulation is essential for initiating a state of responsiveness to induction of Th2 differentiation by IL-4, but is not necessary for the maintenance of the Th2 state. In contrast, the initiation of Th1 responses appears to be less dependent on CD28 costimulation. Th1 responses can be generated in CD28-deficient animals or in animals in which CD28 costimulation is blocked by CTLA4Ig (Shahinian et al., 1993; Green et al., 1994). However, Th1 immune responses, even when established, appear to be sensitive to modulation by CD28 costimulation. Most established Th1 clones continue to require CD28 costimulation for activation (Allison, 1994).

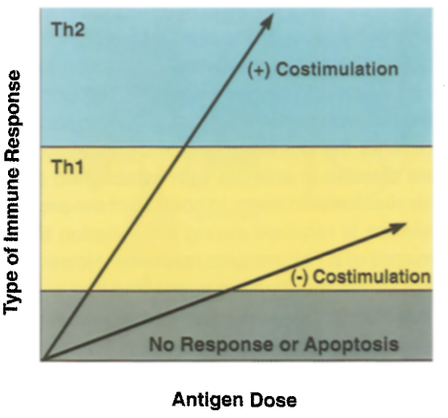

Figure 3. The Differentiation of Th Cells May Be Influenced by the Strength of the Activation Signal

In this model, the strength of the activation signal is determined by both the dose of antigen as well as the presence or absence of costimulation. The slope of the dose response curve may also vary with the magnitude of the costimulatory signal.

These observations suggest that B7-2, by being the primary costimulatory ligand present in unprimed animals, may play a more critical role in the ability to initiate a Th2 response, while B7-1, with its later kinetics of induction, may be more important in the maintenance of Th1 responses. Consistent with this hypothesis, Sayegh et al. (1995) have shown that delaying CTLA4Ig treatment until 48 hr after renal allografting selectively spares a protective Th2 response while still inhibiting a Th1 response.

A possible model for how CD28 costimulation could affect the initial commitment of a naive Th cell is one in which Th1/Th2 differentiation is determined by the strength of the activation signal (Figure 3). Depending on the initial antigen dose, CD28 costimulation could shift the response to antigenic challenge from no response to a Th1 response or from a Th1 response to a Th2 response. Depending on when a naive T cell is recruited into an ongoing immune response and the type of APC involved, the major costimulatory ligand could shift from B7-2 to B7-1. Such a model has the potential to explain some of the seemingly paradoxical responses observed in response to blocking B7-1 and B7-2 in vivo. For example, a destructive Th1 response could either be prevented at low antigen doses by blocking CD28 costimulation or be shifted to a protective Th2 response at high antigen doses by augmenting costimulation. In addition, a protective Th2 response that is dependent on costimulation could be shifted to either a pathogenic Th1 response or no response by blocking costimulation.

B7/CD28 Costimulation in the Maintenance of Established Immune Responses

While the activation of naive T cells is critically dependent on costimulatory signals, memory T cells are less dependent on costimulation for activation (Croft et al., 1994). These data have been interpreted to suggest that blocking B7-1/B7-2 costimulation would be relatively ineffective at blocking established or recall immune responses. One recent set of data that appears difficult to reconcile with the above observations is the ability of CTLA4Ig to block established autoimmune disease in NZB/NZW mice. These mice are studied as a murine model of systemic lupus erythematosus, a disease that results from the production of autoantibodies. B cell production of autoantibodies is believed to be dependent on Th2 cells. Finck et al. (1994) have demonstrated that CTLA4Ig treatment not only prevents the development of disease but can also abrogate disease in animals with established autoimmunity. This result would seem to contradict the proposal that costimulation is required during the initiation but not the maintenance of a Th2 immune response. However, recent studies of autoimmunity have suggested that autoimmune disease results in the sequential activation of autoreactive clones with differing specificities, a process referred to as epitope spreading. Thus, some autoimmune diseases may be perpetuated through the sequential activation of naive T cells responding to distinct autoantigens exposed during ongoing immune destruction. If this is the case, CTLA4Ig may block established disease by inhibiting the ongoing differentiation of cells into the Th2 lineage rather than by inhibiting established Th2 clones.

In summary, the B7/CD28 costimulatory pathway plays a complex role in regulating in vivo immune responses. In addition to augmenting the lymphokine production and initial proliferation of naive T cells, the B7/CD28 costimulatory pathway also appears to play an important role in regulating the differentiation of Th cells into Th1 or Th2 cells. A clearer understanding of these additional roles for the CD28/CTLA-4 receptors and the differential ways in which B7-1 and B7-2 affect these aspects of immune activation will have important implications for immunotherapy.

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