Review

T Lymphocyte Co-Signaling Pathways of the B7-CD28 Family

Shengdian Wang[1] and Lieping Chen[1, 2]

The past years have witnessed significant advance in our understanding of critical roles of T cell co-signals in B7-CD28 family molecules in regulating T cell activation and tolerance. New co-signaling molecules have been identified and their functions have been delineated. These co-signaling pathways play overlapping and distinct regulatory roles at various stages of a T cell response. By expressing in appropriate time and location, these pathways have different regulatory functions through independent receptors or on different subsets of lymphocytes. Precise understanding of these pathways will allow the development of novel approaches to treatment of human diseases such as cancer, viral infection, autoimmune diseases and transplantation rejection. ***Cellular & Molecular Immunology*****. 2004;1(1):37-42.**

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Introduction

The T cell co-signaling pathways within the B7-CD28 family are crucial for the regulation of activation and tolerance of T cell immunity. These pathways not only provide critical positive second signals that initiate, augment and sustain T cell responses, but also contribute key negative signals that limit, terminate and/or attenuate T cell responses. Appropriate manipulation of these co-signaling pathways may promote immune responses against viral infection and cancer, and reduce graft rejection and autoimmune diseases (1). In the past five years, much excitement has been centered in rapid identification of novel pathways and characterization of their functions. The B7-CD28 family is rapidly expending in recent years (Figure 1). This review will focus on discussion and synthesis of newly discovered pathways including B7-H1/B7-DC/PD-1, B7-H2/ICOS, B7-H3, B7-H4 and BTLA, as well as their functional implications in the context of pathogenesis and potential applications for the treatment of human diseases.

New insights into the B7-1/B7-2/CD28/CTLA-4 pathway

This pathway consists of two B7 family members, B7-1 (CD80) and B7-2 (CD86), which bind to the same two receptors, CD28 and CTLA-4 (CD152). CD28 is constitutively expressed on the surface of T cells whereas CTLA-4 expression is rapidly upregulated following T cell activation. CTLA-4 exhibits an affinity for B7-1/B7-2 that is 10 to 100 times higher than CD28. The kinetics of expression of B7-1 and B7-2 also differ: B7-2 is constitutively expressed at low levels and rapidly upregulated, whereas B7-1 is inducibly expressed later than B7-2 on antigen-presenting cells (APCs). Engagement of CD28 on naïve T cells by either B7-1 or B7-2 ligands on APCs provides a potent costimulatory signal to T cells activated through their T cell receptor (TCR), which results in induction of IL-2 transcription, expression of CD25, and entry into the cell cycle. CD28 engagement also confers critical survival signals to activated T cells through the Bcl-xL pathway. However, engagement of CTLA-4 on activated T cells might deliver a negative signal that inhibits TCR- and CD28- mediated signal transduction and terminates T cell response (1, 2).

Because of its dominant role in modulating T cell activity, CTLA-4 has received considerable attention as a therapeutic target. The CTLA-4-immunoglubulin (CTLA-4-Ig) fusion protein acts as an inhibitor of B7-CD28 costimulation and has specific inhibitory effects in animal models of autoimmune diseases, transplantation rejection, asthma and allergy. In contrast to the strategies that interfere with the B7-CD28 association, reagents that interfere with the B7/CTLA-4 interaction could intensify specific T cell responses. For example, blocking antibodies against CTLA-4 could enhance rejection of pre-established tumors (1, 2).

The roles of the B7-CD28 pathway on self-tolerance

The signal through CD28 is required for initiating response of naïve T cells. Several recent studies, however, suggested that the B7-CD28 pathway is also involved in the maintenance of self-tolerance. CD28-deficient or B7-1/B7-2 double-deficient non-obese diabetic (NOD) mice develop more severe and accelerated diabetes than wild-type NOD mice (3). These mice also show a marked reduction in the $CD4^+CD25^+$ regulatory T cells, which are best known to

[1]The Sidney Kimmel Comprehensive Cancer Center, Department of Dermatology, Department of Oncology and Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland,.

[2]Corresponding to: Dr. Lieping Chen, Jefferson Bldg. 1-121, Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Baltimore, MD21287, USA. E-mail: lchen42@jhmi.edu.

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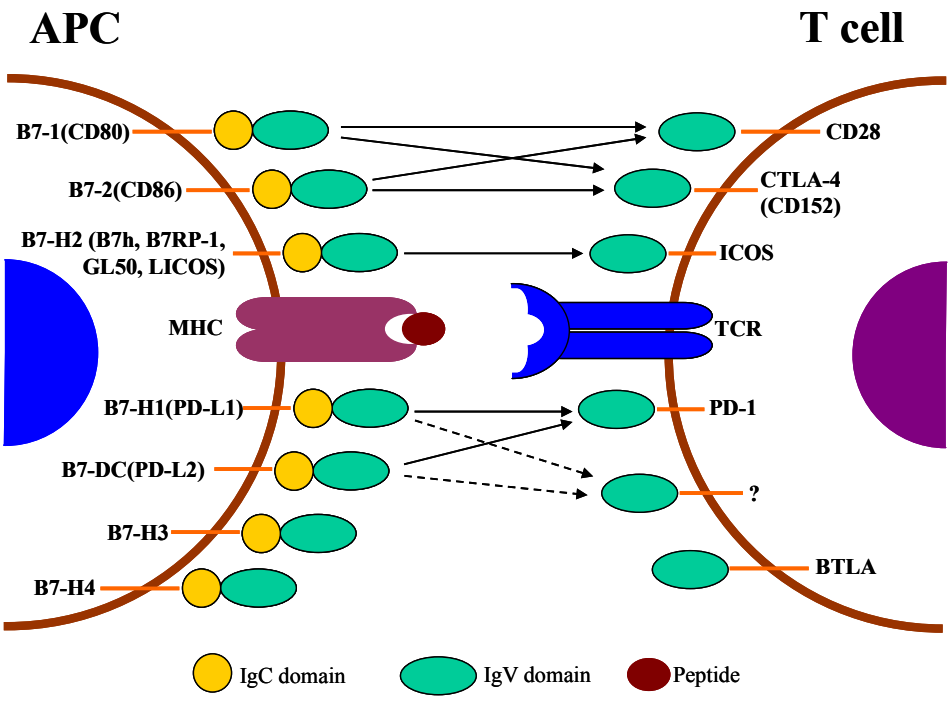

Figure 1. Summary of B7-CD28 superfamily members. The name of receptors and ligands are indicated. The ligands of B7-CD28 family are Ig superfamily members with an IgV-like and an IgC-like domains in the extracellular portion, whereas the receptors are Ig superfamily members with a single IgV-like domain in the extracellular portion. CD28 and CTLA-4 have a MYPPPY motif in the IgV domain that is essential for binding B7-1 and B7-2. ICOS has a FDPPPY motif in the IgV domain that is involved in binding B7-H2. B7-H1 and B7-DC bind to PD-1 and other, as yet unknown, receptors. B7-H3 and B7-H4 are two orphan ligands, whereas BTLA is an orphan receptor.

control pathologic immune responses against a wide variety of self-antigens. CD28-deficient regulatory T cells retain regulatory function *in vivo* and *in vitro*, suggesting that CD28 is not required for the effector function of regulatory T cells. More recent studies showed an essential role of the B7-CD28 interaction in the development and maintenance of regulatory T cells (4). When wild-type T cells are transferred into recipient mice lacking B7-1 and B7-2 and primed with antigen-pulsed wild-type dendritic cells, the T cells show much greater responses than do the cells primed in normal recipients. This effect is not mediated by CTLA-4, because the production of effector cytokines in B7-1/B7-2 double-deficient mice is enhanced even after CTLA-4 blockade. But transfer of antigen-specific $CD4^+CD25^+$ cells significantly reduces the exaggerated responses. Thus, endogenously expressed B7s could function to suppress T cell activation and maintain self-tolerance, principally by sustaining regulatory T cells. An emerging concept thus is that basal or constitutive expression of B7 is required for maintaining a stable population of regulatory T cells, and thus, for preventing immune responses against self-antigens. Upon exposure to inflammatory stimuli, APCs express high level of B7, which engages CD28 and initiates naïve T cell activation in conjunction with TCR signal. Later, engagement of CTLA-4 expressed on activated T cells with B7 delivers negative signals that terminate the immune responses and prevent autoimmune diseases.

Modulation of tryptophan catabolism by CTLA-4/B7 pathway

Indoleamine 2, 3-dioxygenase (IDO) degrades tryptophan and initiates the production of immune regulatory metabolites, collectively known as kynurenines. By locally depleting tryptophan and increasing kynurenines, which inhibit T cell proliferation and induce T cell apoptosis respectively, the expression of IDO activity by different types of APCs, including DCs, could have a broader immunological role in tolerance and immunoregulation (5).

A widely accepted explanation for the inhibitory activity of CTLA-4 is that it functions through a combination of inhibitory T cell signaling and blockade of the B7s-CD28 costimulatory pathway. However, using an experimental system of allogeneic islet transplant tolerance, Grohmann et al. (6) showed that engagement of B7s by CTLA-4-Ig conditions the DCs to produce IFN-γ, which acts in an autocrine or paracrine manner to promote IDO induction in the DCs, thereby initiating the degradation of tryptophan to kynurenines in the local tissue microenvironment. This may reduce T cell function and/or viability and enable long-term transplant survival and tolerance. Likewise, membrane-bound CTLA-4 on regulatory T cells also have similar effect (7). $CD4^+CD25^+$ regulatory T cells, either resting or induced to overexpress CTLA-4 by treatment with anti-CD3 antibody, induce IFN-γ and kynurenine production by DCs through a CTLA-4-dependent mechanism (7). Therefore, reverse signaling of CTLA-4 through B7s by induction of IDO in DCs could be an important mechanism by which regulatory T cells mediate their suppressive effect.

Thus, the interaction of CTLA-4 and B7s may play two complementary roles: first, at the level of T cells where CTLA-4 as a negative receptor regulates TCR and CD28 signal transduction; second, at the level of the APCs where CTLA-4 as a ligand signals to the DCs to induce IDO. In this regard, recent studies (8) showed that the proliferation of T cells from CD28 and CTLA-4 double knockout mice is inhibited by CTLA-4-Ig treatment, and CTLA-4-Ig prolongs survival of cardiac allografts in these double knockout mice. These data were originally interpreted as evidence for the third receptor for B7-1/B7-2, distinct from CD28 and CTLA-4, but may now be interpreted as direct signaling of CTLA-4-Ig to the DCs.

Costimulation through B7-H2/ICOS pathway

ICOS was identified as an inducible T-cell costimulator homologous to CD28 (9). In contrast to CD28, ICOS is expressed on activated, but not resting T cells. Its ligand, B7-H2 (also known as B7h, B7RP-1, GL50, LICOS), is expressed on B cells, monocytes, DCs and non-professional APCs, such as fibroblasts, endothelial cells etc (10-14). The expression of B7-H2 on B cells, monocytes, fibroblasts and endothelial cells could be upregulated by TNF-α or LPS stimulation. Engagement of ICOS on T cells that have been stimulated through the TCR results in augmented proliferative responses and cytokine production. In comparison with CD28, costimulation of $CD4^+$ T cells through ICOS does not produce sustained proliferative responses due to limiting IL-2 production (15) whereas the production of IL-10 is more profound (9, 10, 16). Thus, in contrast to CD28 mediated costimulation, B7-H2/ICOS pathway preferentially regulates effector function of T cells.

Regulation of Th1 and Th2 effector responses

In a culture system of Th1 and Th2 polarization, ICOS is expressed at a high level on both Th1 and Th2 cells after primary stimulation, but remains high level of expression only on Th2 cell line after repeated activation steps (16, 17). Costimulation through ICOS can augment induction of both Th1 and Th2 cytokines, including IL-4, IL-10 and INF-γ, but $CD4^+$ T cells produce more IFN-γ and less IL-4 and IL-10 when the B7-H2-ICOS interaction is blocked (16). The production of Th2 cytokines such as IL-4 and IL-10 by primed T cells in B7-H2-deficient mice are reduced (18-20). ICOS-deficient T cells primed *in vivo* and restimulated *in vitro* with specific antigen also produce low level of IL-4, but remain fully competent to produce IFN-γ (21-23). Löhning M et al. (24) examined ICOS-expressing T cells in the second lymphoid organs of mice at the single cell level. $ICOS^{low}$ cells were found to be loosely associated with the early cytokines IL-2, IL-3, IL-6 and IFN-γ. $ICOS^{medium}$ cells, the large majority of $ICOS^+$ T cells *in vivo*, are very tightly associated with the synthesis of the Th2 cytokines IL-4, IL-5 and IL-13, and these cells exhibit potent inflammatory effects *in vivo*. In contrast, $ICOS^{high}$ cells are highly and selectively linked to IL-10. Thus, it appears that Th2 cytokines production will generally be more dependent on ICOS stimulation, whereas the dependence on ICOS for Th1 cytokines will be determined by the precise conditions under which that Th1 response is elicited. Recent studies in a B and T cells double-adoptive transfer system suggested that ICOS signaling is involved in the initial clonal expansion of naive and primed Th1 and Th2 cells in response to immunization (25). Co-culture of MHC class II^+ endothelial cells with resting memory $CD4^+$ T cells in the presence of superantigen leads to a marked upregulation of ICOS on T cells and to the production of Th1 and Th2 cytokines, including IL-2, IFN-γ, IL-4, IL-10 and IL-14. Blockade of interaction of B7-H2 and ICOS reduced secretion of all cytokines (26). Therefore, ICOS appears to control both Th1 and Th2 functions. The expension and function of T cells may be independently regulated by ICOS co-signaling during the effector phase of Th1 and Th2 responses.

Roles in T cell-B cell collaboration

B7-H2/ICOS pathway appears to play a large role in humoral immune responses. ICOS could be found on T cells in germinal center while splenic B cells express B7-H2. Transgenic mice expressing secreted form of B7-H2-Ig develop lymphoid hyperplasia and high serum levels of IgG (12). ICOS- and B7-H2-deficient mice exhibit profound deficits in immunoglobulin isotype class switching and germinal center formation after immunization with model protein antigens (18-23). The impaired class switching could not be rescued by secondary immunization and appears to be due to a lack of T cell help, as isotype switching to T cell-independent antigens is intact (23). Human ICOS deficiency was found in common variable immunodeficiency (CVID) patients (27). Due to a homozygous deletion of ICOS and impaired T cell help for B cells, the ICOS-deficient patients develop an adult-onset immunodeficiency characterized by decreased numbers and lack of memory of B cells as well as low serum immunoglobulins. However, the T cells from these patients are normal with regard to subset distribution, activation, cytokine production and proliferation. The phenotype of human ICOS deficiency, which differ in key aspects from that of ICOS-deficient mice, suggests a critical involvement of ICOS in T cells help for late B cell differentiation, class switching and memory B cell generation. It is worth noting that mice deficient in CD28 (28), CD40L (29), or both B7-1 and B7-2 (30) are also defective in isotype switching and germinal center formation. It will be interesting to determine precisely how these three pathways interact in directing the humoral immune response.

B7-H1/B7-DC/PD-1 pathway

B7-H1/B7-DC/PD-1 pathway has been discovered and characterized in recent five years. Current experimental results support that this pathway has both positive and negative regulatory functions in T cell responses, a feature similar to the classic B7-CD28 pathway. However, this pathway is independent and non-redundant from the B7-CD28 pathway and plays critical roles in the control of T cell responses. B7-H1 and B7-DC are two B7 homologous molecules, which were initially identified as T cell costimulatory molecules that augment T cell proliferation and cytokine production in the presence of either anti-CD3 or alloantigens (31, 32). Recent studies demonstrate that these two molecules have also co-inhibitory functions. Both B7-H1 and B7-DC bind to programmed cell death-1 (PD-1), an Ig superfamily member with limited structural and functional homology to CTLA-4 (32, 33). PD-1 is expressed by activated T cells, B cells and myeloid cells, and current data indicate that PD-1 is an inhibitory receptor which mediates inhibitory function of B7-H1 and B7-DC while another yet unidentified receptor is responsible for their costimulatory functions.

Expression of B7-H1 and B7-DC

The overall expression of B7-H1 and B7-DC transcripts was similarly found in various lymphoid and nonlymphoid tissues (31, 33-35), whereas the expression profiles of their proteins are quite distinct. Although resting T cells, B cells and monocytes do not express B7-H1, they express high

level of B7-H1 on cell surface following activation. DCs and some endothelial cells constitutively express B7-H1 on cell surface, and *in vitro* treatment with IFN-γ results in its rapid upregulation (31, 35). In contrast, expression of B7-DC was only detected on DCs and monocytes (32).

Functions of B7-H1 and B7-DC

Both immortalized B7-H1-Ig and B7-DC-Ig costimulate T cell proliferation in the presence of anti-CD3 mAb as a mimic antigenic signal (31, 32). B7-H1 costimulation *in vitro* strongly upregulates IL-10 production and modestly upregulates IFN-γ and GM-CSF production, but has little effect on IL-2 and IL-4 production (31, 35), while B7-DC costimulation strongly enhances IFN-γ production, but not IL-2, IL-4 and IL-10 (32). However, some studies showed that *in vitro* stimulation of T cells with B7-H1 or B7-DC inhibits TCR-mediated proliferation and cytokine (IL-2, IL-4, IL-10 and IFN-γ) production, and results in cell cycle arrest (33, 34). In addition, B7-DC displays potent synergy with B7-1 and B7-2 for T cell proliferation and IL-2, IFN-γ production (36). DCs from B7-DC-deficient mice are diminished in their ability to activate $CD4^+$ T cells (36). These seemly contradictory data, however, could be best interpreted by expression of additional costimulatory receptor on T cells other than PD-1. Our recent studies using structural biology and site-directed mutagenesis approach have led to characterization of B7-H1 and B7-DC mutants with abolishing PD-1 binding capacity (37). Interestingly several such mutants are still able to costimulate proliferation and cytokine production of T cells from normal or PD-1-deficient mice in a comparable level to the wild type of B7-H1 and B7-DC. The costimulation with B7-DC alone or in conjunction with B7-H1 for cytokine production is PD-1 independent (36). These studies strongly suggest that B7-H1 and B7-DC costimulate T cell growth through a receptor other than PD-1.

Distinct roles of B7-DC and B7-H1 in inducing antitumor immunity

Immunohistochemical analysis showed that many human tumors express B7-H1, whereas the corresponding normal tissues do not. The presence of B7-H1 on tumor cells can actively inhibit immune response by promoting the apoptosis of effector CTLs, partially mediated by Fas and IL-10 pathways (38). In contrast to low level expression of B7-H1 on myeloid dendritic cells (MDCs) in normal individuals, the percentage of B7-H1-positive MDCs and the intensity of B7-H1 expression are significantly higher on MDCs isolated from tissues or draining lymph nodes of ovarian carcinomas. Activation of allogeneic human T cells with tumor MDCs induces significant IL-10 production. Treatment of tumor MDCs with a specific anti-B7-H1 monoclonal antibody (mAb) significantly decreases IL-10 and increases IL-2 and IFN-γ production from T cells (39). Expression of B7-H1 on P815 tumor could reverse the effect of B7-1 costimulation and render them less susceptible to the cytotoxic activity of CTLs and markedly enhance its tumorigenesis and invasiveness *in vivo*. These effects could be reversed by treatment of anti-B7-H1 antibody (38, 40). Therefore, the presence of B7-H1 in the tumor microenvironment down-regulates T cell immunity.

The expression of B7-DC on plasmocytoma J558 cells caused rapid rejection of the tumors in syngeneic mice and development of immunity to subsequent tumor challenge, suggesting that unlike B7-H1, B7-DC promotes, rather than inhibits, antitumor immunity. This costimulatory function of B7-DC is achieved by enhanced both T cell priming and effector function (41).

Two orphan ligands: B7-H3 and B7-H4

B7-H3 pathway

B7-H3 was identified as a costimulatory molecule for T cell responses through a distinct receptor form CD28, CTLA-4, ICOS and PD-1 (42). Northern blot analysis revealed that transcripts of human and mouse B7-H3 are widely expressed in lymphoid and non-lymphoid tissues. Interestingly, B7-H3 mRNA cannot be detected in human peripheral blood leukocytes (PBLs). B7-H3 protein is not constitutively expressed, but could be induced on monocytes by GM-CSF and on DCs by IFN-γ. A B7-H3-Ig fusion protein binds to activated but not resting T cells, indicating the expression of a receptor on activated T cells (42). In addition to a typical IgV-IgC like structure in both mouse and human B7-H3, a non-typical IgV-IgC-IgV-IgC structure has been found in human B7-H3 genomic DNA. It is unclear whether this gene of two IgV and two IgC encodes a protein with costimulatory functions (43, 44).

In the presence of anti-CD3 mAb, B7-H3-Ig fusion protein costimulates human T cell proliferation and selectively induces IFN-γ production. Expression of B7-H3 on human melanoma cell line results in increased lytic activity of allogeneic CTL (41). Injection of mouse B7-H3 expression plasmid directly into established mouse EL-4 lymphoma induces partially regression of tumors (45). However, one group (46) recently reported that the costimulation of B7-H3 inhibits T cell proliferation, IL-2 and IFN-γ production medicated by anti-CD3 mAb or allogeneic APCs. Lung infiltration of inflammatory cells increased after challenge with a model antigen in B7-H3 deficient mice whereas there is virtually no effect in the induction of experimental autoimmune encephalitis and CTL to LCMV. Therefore, the functions of B7-H3 remain to be clarified and identification of its receptor will facilitate this study.

B7-H4 pathway

B7-H4 (also called B7S1 and B7x) is a recent addition to B7 superfamily (47-49). It has about 25% amino acid homology in the extracellular portion with other B7 family members. The mouse and human B7-H4 amino acid sequences share about 87% identity. B7-H4 mRNA is expressed in lymphoid and nonlymphoid tissues, but immunohistochemistry analysis did not reveal positive staining in any human organs from healthy individuals (47). B7-H4 expression can be induced on human T cells, B cells, macrophages and DCs following activation (47, 48). Some tumor cells also express B7-H4 (49, 50). In mice B7-H4 was found to be constitutively expressed on $B220^+$ B cells in spleen and down-regulated after activation (48). A B7-H4-Ig fusion protein binds to activated but not naïve T cells, indicating a putative receptor for B7-H4 on activated T cells (47, 48).

Immobilized B7-H4-Ig fusion protein or cell-associated

B7-H4 has a profound inhibitory effect on the proliferation of T cells activated through TCR signaling, and significantly reduce cytokine production (47-49). Including CD28 costimulation cannot reverse B7-H4-induced inhibition (47, 48). B7-H4 inhibits T cell proliferation by cell cycle arrest at G0/G1 phase (47). Administration of B7-H4-Ig protein can inhibit T cell proliferation *in vivo* (47, 48). In a GVHD model, treatment of recipient mice with B7-H4-Ig significantly reduces CTL activity and also extends the survival of the mice (47). Blockade endogenous B7-H4 on host cells by specific mAb promotes T cell responses and exacerbates EAE *in vivo* (48). Thus, together with a broad and inducible expression pattern, B7-H4 may be involved negative regulation of immune response in peripheral tissues.

One orphan receptor: BTLA

BTLA (B and T lymphocyte attenuator) was identified as an anonymous Th1 specific expressed sequence tag (51). Mouse and human BTLA share 48% amino acid identity. Although BTLA shares only 9-13% amino acid identity with other receptors of B7 family, it is structurally similar to CTLA-4 and PD-1. The presence of two ITIM motifs in its cytoplasmic region suggests that BTLA may function as an inhibitory receptor. BTLA mRNA is expressed strongly in spleen and lymph node but not in thymus, liver, kidney, lung, heart, brain and muscle. BTLA mRNA is also found on splenic B cells, but not naïve T cells and it can be induced on T cells during activation and remains expressed more strongly on polarized Th1, not Th2 cells. The protein expression pattern of BTLA, however, is not yet known.

Fully polarized BTLA-deficient Th1 cells show a roughly twofold increase in proliferation in response to specific antigen. BTLA-deficient T cells also show a heightened response to the stimulation with anti-CD3. Immunization of BTLA-deficient mice with nitrophenol-conjugated keyhole limpet hemocyanin (NP-KLH) results in a threefold increase in the mount of NP-KLH-specific IgG1, IgG2a and IgG2b antibodies. These results suggest that BTLA has an inhibitory effect on T cell responses. Consistent with this, BTLA-deficient mice show enhanced disease incidence and severity in an EAE model.

B7-H4-Ig binds to activated wild-type, but not BTLA-deficient T cells in flow cytometry analysis (49, 51). Thus, it has been proposed that BTLA is the receptor of B7-H4 (49, 51). However, it remains possible that BTLA down-regulates the expression of B7-H4 receptor on activated T cells. Therefore, the receptor for B7-H4 remains to be determined.

Perspectives

The discovery of new functions for the classical T cell costimulatory pathway and identification of novel ligands and receptors of the B7-CD28 family have shed the light on the functions of co-signaling molecule interactions occurring at multiple stages of the immune responses. It is tempting to speculate that these molecules form a delicate network to regulate and control initiation, expansion, development of effector and memory T cell responses as well as T cell homeostasis. Several fundamental questions remain to be answered in the future regarding the identity of their receptors and ligands, control of the expression in normal and disease status, as well as intracellular biochemical signals. Precise understanding of these pathways should provide insights into the mechanisms of T cell activation and tolerance, and the development of new therapeutic approaches to immunological diseases.

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