The expanding world of co-stimulation: the two-signal model revisited

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The crucial role for CD28, its homolog CTLA-4 and their binding partners B7-1 and B7-2 in the generation of effective T-cell responses has been well documented. Recently, two new pairs of the CD28/B7 families were identified. The ability of these molecules to regulate T-cell expansion and effector function and the dynamic integration of the co-stimulatory and T-cell receptor signals are just beginning to be explored. Understanding these processes will be crucial for designing clinically relevant approaches to manipulate the adaptive immune system.

Optimal T-cell activation requires T-cell receptor (TCR) signaling upon binding to the peptide–MHC on the antigen-presenting cell (APC), and a second antigen-independent signal called co-stimulation. CD28 is the prototypic T-cell co-stimulatory molecule (reviewed in Ref. 1). Other members of the CD28 family have been identified, and their role in the 'two-signal' model of T-cell activation is just beginning to be appreciated. The known CD28 family members and their B7 ligands are listed in Table 1. These molecules share several common structural and functional features. All of the CD28-like receptors and their ligands are type I transmembrane glycoproteins and are members of the immunoglobulin (Ig) superfamily. The members of the CD28 family possess a single Ig-V-like extracellular domain necessary for ligand binding[2]. Their cytoplasmic tails contain putative SH2- and SH3-binding domains thought to be involved in signal transduction. The ligands for the CD28 family are members of the B7 family[1], and possess one Ig-V-like and one Ig-C-like extracellular domain (reviewed in Refs 3,4). The cytoplasmic tails of these molecules are generally short and the ability to transduce signals is largely unknown. The primary sequence similarity of the CD28 (Fig. 1) and B7 family members is generally low (within the range of 20–40%), but orthologs are more similar (>60%), indicating the conserved function of these molecules. With the exception of CD28, the expression of the CD28/B7 family members is markedly induced/enhanced following activation of the respective cell type. This provides an additional mechanism for regulating receptor–ligand interactions, and facilitates the generation of the appropriate effector function in response to specific stimuli during an immune response. The common and unique features of the co-stimulatory molecules and their roles in the regulation of lymphocyte homeostasis and immune responses are discussed below.

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CD28 – the classic co-stimulatory molecule

CD28-mediated co-stimulation influences multiple aspects of T-cell physiology, resulting in an enhanced T-cell response (reviewed in Refs 1,5). CD28 ligation enhances the magnitude and duration of T-cell responses; induces the anti-apoptotic gene *BCLXL*; increases cytokine secretion, particularly interleukin 2 (IL-2); enhances cell adhesion; facilitates reorganization of the T-cell plasma membrane upon binding to an APC; prevents anergy induction; and supports germinal center formation[1,6]. Generally, CD28 engagement does not have a physiological effect in the absence of TCR signaling.

The importance of the role of CD28-mediated co-stimulation has been demonstrated in a variety of model systems *in vitro* and *in vivo* (reviewed in Refs 1,7,8). Most dramatically, the absence of CD28 function, either by blockade, CD28-deficiency or deficiency of CD28 ligands, results in a greatly reduced ability to respond to protein antigens, parasites and some viruses, and to generate germinal centers and mediate B-cell help. Graft rejection, and systemic and tissue-specific autoimmune disease can be prevented by blocking CD28-mediated co-stimulation. However, some responses appear to be co-stimulation independent, for example, $CD8^+$ T-cell responses to strong antigenic stimulation such as lymphocytic choriomeningitis virus (LCMV) infection[9,10], and some effector functions of $CD4^+$ T helper (Th) cells[11]. The question remains whether these responses are truly co-stimulation-independent or simply CD28-mediated co-stimulation independent.

Inducible costimulator

Structure

Inducible co-stimulator (ICOS) was first identified in a screen for unique molecules expressed on human peripheral blood T cells following activation[12]. Rodent ICOS was identified soon after from a variety of sources including intestinal epithelial lymphocytes (IELs), Th2-cell clones and activated peripheral T cells[13–16]. ICOS shares approximately 30–40% sequence similarity with CD28 and CTLA-4 (Fig. 1). The *ICOS* gene maps to the *CD28/CTLA-4* locus, suggesting that these arose by gene duplication[14,16]. ICOS contains several conserved motifs found in CD28, including the extracellular Ig-V-like domain

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Table 1. The CD28 family of co-stimulatory molecules and their ligands

Receptor	Ligand(s)	Biological consequence	Signal transduction
CD28	B7-1 and B7-2	Activation	YXXM motif, PI3K, ITK, Grb2, PP2A, PP6, SHP-2 (?), raft or DIG formation
CTLA-4	B7-1 and B7-2	Inhibition	YXXM motif, PI3K, SHP-2, PP2A, PP6, AP-50-intracellular localization
ICOS	B7h/B7RP-1/ LICOS/B7H2	Activation	YXXM motif, PI3K
PD-1	B7H1/PD-L1	Inhibition	ITIM motif, B cell: SHP-2 (?)

and the YXXM motif in the cytoplasmic tail[12,13]. However, ICOS does not have a conserved MYPPPY motif, which is necessary for CD28 and CTLA-4 binding to B7-1/2 ligands[17].

Expression pattern

ICOS has a distinct expression pattern compared with that of CD28. ICOS is not constitutively expressed on naive T cells but is induced on $CD4^+$ and $CD8^+$ T cells following cell activation[12–16,18]. Increased ICOS mRNA levels can be detected as early as 1 h following TCR crosslinking, and cell surface expression is detected 12 h after activation, reaching maximum levels at 48 h, followed by a slow decline[14,16]. Although the induction of ICOS expression is enhanced by CD28-mediated co-stimulation[19], ICOS can be induced in the absence of CD28 and biologically relevant levels of ICOS are reportedly expressed on T cells in response to submitogenic anti-CD3 antibody (Ab)[13,19,20]. Resting, previously activated T cells ($CD69^-CD44^+CD45RB^{lo}$) and Th2 clones (of those tested) constitutively express ICOS, the levels of which are further upregulated following activation[12–14]. Interestingly, the level of ICOS expression decreased during the differentiation of activated Th0 ($ICOS^{hi}$) cells to Th1 (Refs 14,19). Further, ICOS is expressed on murine but not human thymocytes[12,16,19], and on activated rat B cells[15].

Function

Based on the homology with CD28, it was proposed that ICOS provides co-stimulatory signals to T cells. Much progress has been made in a short time and, although many questions remain, it is clear that ICOS ligation can enhance T-cell proliferation and influence T-cell effector function. Antibody- or ligand-mediated ICOS crosslinking on human and mouse T cells *ex vivo* supports co-stimulation of the T cells in the presence of mitogenic and sub-mitogenic anti-CD3 Ab crosslinking[12,13,21,22], albeit less potently than CD28-mediated co-stimulation. Conversely, the proliferative response of D0.11.10 TCR transgenic (tg^+) T cells to peptide-pulsed APCs is not inhibited by ICOS–Ig fusion protein[14,19]. Instead, under these stimulation conditions, ICOS engagement appeared to be important for the cytokine responses of recently activated T cells. These results suggest that, although ICOS engagement might support activation of naive T cells, it could be more important for enhancing ongoing and/or recall proliferative responses.

CD28-mediated co-stimulation induces cytokine production, primarily IL-2, following activation of naive T cells[1]. IL-2 functions as a T-cell growth factor, supporting expansion and differentiation of the antigen-specific T cells. The demonstration that ICOS-mediated co-stimulation does not (or suboptimally) induce IL-2 production but increases secretion of IL-4, IL-5, IL-10, interferon γ(IFN-γ), and tumor necrosis factor α (TNF-α)[12–14] suggests that ICOS functions primarily to induce T-cell effector function. The upregulation of CD40 ligand (CD40L) expression[11,23] on human T cells following ICOS crosslinking supported the notion that ICOS functions to enhance T-cell-dependent B-cell help[12]. This is further supported by the dramatic lymphoid hyperplasia and plasmacytosis observed in transgenic mice overexpressing a soluble ICOS ligand that is presumably crosslinked *in vivo* (Ref. 13). Also, Th2 $CD4^+$ T-cell responses are inhibited when ICOS–ligand interaction is blocked *in vivo*[14,24]. Finally, the $CD4^+$ T cell but not the $CD8^+$ response is significantly affected by the blockade of ICOS during parasite or viral infection[24]. Collectively, these results suggest that ICOS performs a distinct and essential role in the generation of T-cell dependent B-cell help.

Recently, ICOS-deficient mice have been generated and the results support the above proposal for ICOS function. $ICOS^{-/-}$ mice are healthy and appear to have normal T-cell development and lymphoid compartments[25–27]. The peripheral T cells are normal in number and phenotype, as is the case in $CD28^{-/-}$ mice[9]. Although there appears to be a CD28-independent component of the T-cell proliferative response *in vitro*, impaired T-cell proliferation by $ICOS^{-/-}$ T cells was observed only at the highest anti-CD3 concentrations[25,26], supporting the notion that CD28 is the primary co-stimulatory molecule for naive T-cell activation. $ICOS^{-/-}$ T cells display a striking defect in the ability to secrete Th2 cytokines IL-4 and IL-13, resulting in a defect in Ig isotype switching, particularly IgG1 and IgE, and germinal center formation upon immunization with T-cell-dependent antigens[25–27]. In addition, the $ICOS^{-/-}$ mice fail to regulate models of Th1-mediated inflammation, namely myelin oligodendrocyte glycoprotein (MOG)-induced experimental allergic encephalomyelitis (EAE) and a model for asthma[25]. Anti-CD40 Ab injection *in vivo* rescued the inability to provide B-cell help[26], suggesting that ICOS functions, at least in part, by inducing the expression of members from other families that are known to support T-cell co-stimulation[11,23]. Interestingly, the defect in Ig isotype switch could be overcome when Freund's complete adjuvant was used, suggesting that, given

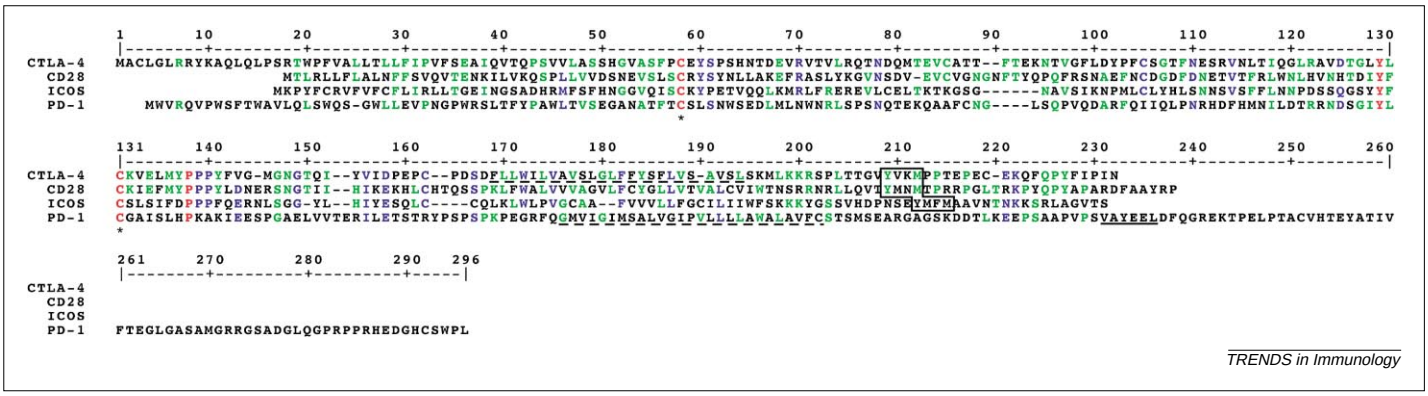

Fig. 1. Comparison of the primary amino acid sequences of the mouse CD28 family members. The primary protein sequence homologies of mouse CD28 family members derived from standard computer-generated alignment [performed using MULTALIN program; http://www.toulouse.inra.fr/multalin/cgi-bin/multalin.pl] are shown to highlight key areas of homology. The sequences are available from GenBank. Green letter, conserved/identical amino acids in two of the four sequences; blue letters, conserved in three or more; red letter, identical in all four sequences; boxes indicate conserved YxxM sequence; - -, transmembrane region: —, ITIM; *, conserved cysteines required for Ig-V-like domain formation.

enough inflammation and B7-1/2 upregulation, CD28-mediated co-stimulation provides sufficient T-cell help, consistent with previous observations demonstrating a role for CD28 in providing B-cell help[1,8,9,28,29]. Based on the ability of ICOS to stimulate cytokine production, the induction of ICOS expression upon cell activation and the unique expression pattern of its ligand (see below), it appears that ICOS performs a distinct co-stimulatory function compared with CD28, and primarily functions to induce specific effector functions.

Cytotoxic T lymphocyte antigen-4

There is a growing appreciation for the concept that lymphocyte responses are regulated by inhibitory as well as activating signals. CTLA-4 (CD152) mediates such an inhibitory signal (reviewed in Refs 5,30,31). Although CTLA-4 displays the common features of the CD28 family members, it is unique in several important ways. First, CTLA-4 has a markedly higher affinity for shared ligands B7-1 and B7-2 compared with CD28 (K_d 0.2–0.4 μM versus 4.0 μM), and a 40–100-fold higher avidity[32,33]. Second, CTLA-4 has a unique expression pattern. Unlike CD28, CTLA-4 is not expressed constitutively on the cell surface of naive T cells at detectable levels, although physiologically significant CTLA-4 levels on naive T cells have been reported[34]. It is quickly upregulated upon TCR–CD28 engagement, with the biological effects of CTLA-4 crosslinking often being observed before the detection of protein[31,35]. This appears to be because the majority of CTLA-4 is sequestered inside the cell; it traffics to the site of T-cell–APC interaction and is quickly endocytosed into clathrin-coated pits (reviewed in Ref. 31). Detectable basal levels of intracellular CTLA-4 are maintained in resting, previously activated T cells, a subset of immunoregulatory T cells, and Th-cell clones[36–40].

Inhibition of T-cell responses by CTLA-4 ligation has been demonstrated *in vitro* and *in vivo* (reviewed in Ref. 31). CTLA-4–B7 interaction leads to a decrease in T-cell activation and progression through the cell cycle, and inhibits IL-2 synthesis. Conversely, CTLA-4 blockade *in vivo* enhances antigen-specific and anti-parasite responses, tumor rejection and autoimmune disease, and exacerbates graft rejection (reviewed in Refs 7,30,31). Further, animals deficient in CTLA-4 develop a fatal lymphoproliferative disorder, owing to CD28-dependent polyclonal T-cell activation, and die within one month after birth (Table 2)[31]. Recently, it has been shown that CTLA-4$^{-/-}$ T cells can be regulated non-autonomously in mixed radiation chimeric animals reconstituted with CTLA-4$^{-/-}$ bone marrow (BM) and CTLA-4 wild-type BM (Ref. 41). This, together with the observation that a subset of immunoregulatory T cells are CTLA-4^{+} (Refs 40,42), led to the suggestion that the absence of regulatory T cells is responsible for the lymphoproliferation. However, this remains controversial, as this subset of T cells displays immunosuppressive function in the absence of CTLA-4 (Ref. 42), and it is inconsistent with a large body of data indicating that peripheral CTLA-4$^{-/-}$ T cells have an intrinsic defect[31]. Regardless, it is clear that CTLA-4 is an important inhibitory CD28 family member, and that it functions to inhibit TCR- and CD28-mediated signal transduction at various stages of the T-cell response. A number of mechanisms have been proposed to account for CTLA-4 function, including competition with CD28 for ligand, induction of immunosuppressive cytokine secretion, sequestration of signaling proteins involved in T-cell activation, and reduction of phosphorylation levels by phosphatase recruitment via the YXXM cytoplasmic motif[31].

Programmed death 1

Programmed death 1 (PD-1) was cloned initially from T-cell hybridoma 2B4.11 and a lymphoid/myeloid cell line undergoing apoptosis[43]. PD-1 is unique among the CD28 family members in that it is widely expressed on hematopoietic-derived tissues. PD-1 is constitutively expressed on a subset of CD4^{-}CD8^{-} thymocytes, immature B cells and some peripheral T cells, and is expressed on T cells,

Table 2. Comparison of phenotypes of CTLA-4 and PD-1 knockout mice[a]

Phenotypic feature	CTLA-4$^{-/-}$	PD-1$^{-/-}$
Phenotype dependent on genetic background	No	Yes
Lethality	3–4 weeks of age	B6 : No Balb/c: 5–30 weeks
Onset of phenotype	5–6 days post-partum	B6 : ≥24 weeks Balb/c: <5 weeks
Complete penetrance	Yes	No
Defect in thymocyte development	No	Yes
Lymphoproliferation	Severe	Mild
Polyclonal T-cell activation	Yes	Yes
Polyclonal B-cell activation	Yes: secondary to T-cell activation	Yes: primary defect
Elevated serum Ig levels	All Ig isotypes	B6: primarily IgG3 Balb/c: IgG1
Glomerulitis	No	B6: Yes Balb/c: NT
Arthritis	Periodic synovitis	Yes
Dilated cardiomyopathy	No	B6 : No Balb/c : Yes
Lymphocyte infiltration into non-lymphoid tissues	Yes	Yes
Phenotype recapitulated in 100% bone marrow chimeras	No	NT

[a]Abbreviation: NT, not tested.

B cells, monocytes and myeloid cells following activation[43–47]. Although it shares a number of common structural features with the CD28 family (23% sequence identity to CD28) (Fig. 1), PD-1 does not contain the MYPPPY motif, and possesses an immunoreceptor tyrosine-based inhibitory motif (ITIM) motif rather than a YXXM motif in the cytoplasmic tail. ITIMs have recently been characterized in several molecules known to inhibit lymphocyte responses, including killer cell inhibitory receptors (KIRs), Ly49A, FcγRII and CD22 (Ref. 48). Preliminary results suggest that tyrosine phosphatase SHP-2 binds to the ITIM motif of PD-1 in a B-cell line[49], but the mechanism of action is not yet known. In addition, there is no conserved cysteine juxtaposed to the transmembrane region necessary for the interchain disulfide bond found in CD28, CTLA-4 and ICOS (Fig. 1), suggesting that PD-1 is expressed as a monomer[44]. Finally, PD-1 does not map to the same locus as the genes encoding CD28/CTLA-4/ICOS (Refs 50,51).

PD-1, like CTLA-4, appears to mediate an inhibitory signal. However, the exact role of PD-1 in thymocyte development, T-cell responses and B-cell responses is just beginning to be elucidated. Initial reports found that antibody-mediated PD-1 crosslinking does not enhance or inhibit anti-CD3 or PMA/ionomycin-induced T-cell responses[44]. Conversely, Dong *et al.* reported an IL-2-dependent increase in IL-10 secretion as a result of PD-1 crosslinking in conjunction with anti-CD3 or in mixed lymphocyte reactions (MLRs)[52]. Most recently, it was reported that crosslinking PD-1 with soluble ligand inhibits proliferation of human and murine T-cell-enriched lymphocytes, albeit within a narrow range of anti-CD3 or anti-CD3 plus CD28-mediated proliferation[49]. Under these conditions, PD-1 crosslinking inhibited IFN-γ, IL-10 and IL-2 secretion[49]. Unlike CTLA-4, PD-1 reportedly influences positive and negative thymocyte selection[43–45,53,54], however the exact role requires further clarification. Also, PD-1 appears to transmit inhibitory signals in B cells[46,49].

The phenotype displayed by PD-1$^{-/-}$ mice is consistent with the interpretation that PD-1–PD-1 ligand (PD-L1) interaction transduces an inhibitory signal essential for lymphocyte homeostasis[54]. The PD-1$^{-/-}$ mice become ill owing to the inappropriate activation of lymphocytes, but they display a markedly different phenotype compared with CTLA-4$^{-/-}$ mice (Table 1). The predominant characteristic of the phenotype observed in the PD-1$^{-/-}$ mice on the C57Bl/6 genetic background is enhanced IgG3 serum levels, leading to glomerulitis and a lupus-like disorder[54]. However, severe autoimmune symptoms are observed only in approximately half of the animals, even at 14 months of age. This is in marked contrast to CTLA-4$^{-/-}$ mice, which all die within 3–4 weeks after birth. Previously activated 2C TCR tg^{+} PD-1-deficient T cells have increased proliferative responses to antigen at the highest antigen concentrations, albeit less striking than CTLA-4-deficient T cells[54,55], suggesting that with high TCR occupancy PD-1 can attenuate T-cell responses. Interestingly, the phenotype of the BALB/c PD-1$^{-/-}$ mice is distinct[56]. These mice develop dilated cardiomyopathy and 70% of the animals die by 7 months of age. This disease is mediated by IgG1 antibodies specific to a heart-specific antigen.

The long delay in the onset of the phenotype in the PD-1$^{-/-}$ mice suggests PD-1 is not the primary inhibitory signal for T cells. Moreover, it is obvious that the presence of PD-1 does not protect the CTLA-4$^{-/-}$ mice from developing severe lymphoproliferation. The converse is also true, but the disease in PD-1$^{-/-}$ mice appears to arise owing to a predominant defect in B cells. Both CTLA-4 and PD-1 might regulate T-cell responses once initiated, although PD-1 appears to be much less potent compared with CTLA-4. It will be interesting to determine if and how these two inhibitory signals are integrated in T cells. Finally, PD-1 appears to influence B-cell homeostasis *in vivo* and the role of PD-1 in the regulation of the responses by non-T cells requires further characterization.

B7 family members

There is an ever growing number of members of the B7 family (reviewed in Refs 1,3). B7-1 and B7-2 were the first members of the B7 family to be identified. Subsequently, additional family members encoded in

the MHC locus have been identified, namely butyrophilin (BT), MOG and the chicken B-G antigen[3,4]. In addition, there are subfamilies, such as three BT subfamilies, of which each has multiple genes. Although some of these B7 family members have been associated with immunological phenomena, such as B-G-mediated activation of APCs, and BT association with the regulation of superoxides[3], the exact function and number of these molecules is not known. Recently, additional molecules with the highest similarity to the original B7-1/2 have been identified, namely B7-homolog (B7h) and PD-L1. These molecules share only approximately 20–25% sequence identity with each other, although there is conservation of the IgV and C domains[21,49]. There is often a second, shorter mRNA B7 species detected, and the possible role of secreted forms of these proteins is beginning to be examined[57]. Importantly, expression of the members of the B7-1/2 family can be induced upon inflammation or infection[1].

B7-1 and B7-2

B7-1 and B7-2 are the ligands for both CD28 and CTLA-4 (reviewed in Refs 1,8). Their expression is almost exclusively restricted to lymphoid tissues, primarily on professional APCs, including dendritic cells (DCs), monocytes and activated B cells. Both B7-1 and B7-2 expression is induced upon cell activation but with strikingly different kinetics and in response to a variety of stimuli. B7-2 expression is modulated rapidly, whereas B7-1 is induced slowly and expressed for a longer duration (maximal expression at 48 h versus 4–5 days, respectively). In addition, B7-1 and B7-2 have different receptor-binding properties, with CD28 and CTLA-4 binding to B7-2 at a slightly lower affinity and faster off-rate compared with B7-1 (Refs 32,33,58). Despite these differences in ligand binding and expression kinetics, these molecules appear to support T-cell activation equivalently[8].

B7 homolog

Within the past year, several laboratories have identified a new member of the B7 family, namely B7h [B7-related protein 1 (B7RP-1); GL50; ligand for ICOS (LICOS); ICOS ligand (ICOSL); B7-homolog 2 (B7-H2)][13,16,21,22,59,60]. B7h was identified as being a member of the B7 family based on the common structural features. However, it does not contain the SQDXXXELY domain possessed by B7-1/2 and does not bind to CD28 or CTLA-4 and has been identified as the ligand for ICOS (Refs 13,16,21,22,59–61).

B7h was cloned from several sources, including TNF-α-activated fibroblasts[21], IELs (Ref. 13), brain tissue[60], a B-cell line, lymph node and thymic tissues[59], and DCs (Ref. 22). It has a unique expression pattern compared with B7-1 and B7-2, in that it is differentially induced on lymphoid tissues and is expressed on non-lymphoid tissues *in vitro* and *in vivo*. B7h is highly expressed on murine but not human resting B cells, and splenic and peritoneal macrophages, and it appears to be expressed on some but not all DCs (Refs 13,20,22,61). B7h is expressed strongly in the B-cell area of the lymph node in normal mice, and in primary and secondary follicles of the spleen, Peyers's patches and the medulla of the thymus[13]. Expression is upregulated upon activation of B cells, and on monocytes and some dendritic cells by IFN-γ (Refs 13,20). Conversely, IFN-γ appears to inhibit TNF-α-mediated B7h induction on embryonic fibroblasts[21]. B7h is expressed on lung and heart, and is rapidly upregulated (within 6 h) by lipopolysaccharide (LPS) on non-lymphoid tissues such as fibroblasts. The physiological significance of B7h expression on non-lymphoid tissues is unclear. There are conflicting reports concerning the induction of B7h on B cells by LPS (Refs 20–22). Further, Ig or CD40 crosslinking does not upregulate B7h expression on human B cells[20], unlike B7-1/2 (Ref. 1). Comprehensive characterization of the expression patterns and the agents responsible for the induction will be necessary to appreciate fully the potential for ICOS/B7h-mediated co-stimulation.

Programmed death 1 ligand/B7-homolog 1

Although PD-1 was cloned almost a decade ago, the human and mouse ligands have only been identified recently [B7-homolog 1 (B7H1) and programmed death 1 ligand (PD-L1), respectively][49,52]. PD-L1 shares 20% sequence similarity with B7-1 and B7-2 in the Ig-V- and Ig-C-like extracellular domains but does not contain the SQDXXXELY domain possessed by B7-1 and differs markedly in its expression pattern. PD-L1 is constitutively expressed on a wide range of non-lymphoid cells such as placenta, heart, lung, kidney and skeletal muscle[49,52]. It is constitutively expressed on some hematopoietic tissues, including a subset of $CD14^+$ macrophages and T cells[52]. PD-L1 is upregulated by IFN-γ but not TNF-α on monocytes, by LPS and IFN-γ on DCs, by anti-IgM crosslinking on B cells, and on a subset of activated T cells, with kinetics similar to B7-2 induction on APCs (Refs 49,52). In addition, PD-L1 expression can be upregulated on non-lymphoid tissues such as keratinocytes[49]. The expression pattern of PD-L1 suggests that PD-1 might function to inhibit lymphocyte responses in non-lymphoid tissues, consistent with the phenotype observed in the BALB/c PD-$1^{-/-}$ mice[56].

CD28–B7 family member interactions

The crystal structures of B7-1 and CTLA-4 have recently been solved[33,62]. Based on the results, it has been proposed that CTLA-4 might bind its ligand in a rather unique orientation, with each molecule in one CTLA-4 homodimer interacting with a B7 molecule of different B7 homodimers. This arrangement would allow one CTLA-4 homodimer to bring together two

B7 homodimers, thereby forming a lattice-like structure[33,62]. This model for CTLA-4–B7 interaction suggests that, if it is allowed to occur readily at high levels, it could alter the ability of the T-cell membrane to reorganize itself and thus interrupt signal transduction. Conversely, one CD28 homodimer is presumed to bind to one B7 homodimer, analogous to grabbing an object with forceps. The configuration of the ICOS–B7h interaction has yet to be examined, but the affinity of interaction appears to be similar to CD28–B7 engagement (K_d 4 μM)[32,60]. PD-1 is predicted to exist as a monomer, and thus likely to have a lower avidity for ligand compared with the CD28 family members that exist as homodimers on the cell surface.

Reassessing the two-signal model of T-cell activation

The spirit of the two-signal model of T-cell activation remains intact, however it seems that regulation of T-cell activation by co-stimulation is more complex than originally envisioned. It appears that, although there is some overlap, each member of the CD28/B7 family has distinct functions. Moreover, it is clear that the influence of the signals mediated via CD28, ICOS, CTLA-4 and PD-1 will differ depending on the nature of the stimuli and the antigenic history of the lymphocytes, presumably to fashion the appropriate and optimal effector response. A major challenge will be to elucidate how these molecules act in concert to orchestrate immune responses. Based on the results outlined above, it appears that CD28-mediated co-stimulation in conjunction with TCR engagement by MHC–peptide complexes on the surface of an APC would initiate T-cell activation, thereby inducing the expression of ICOS and, indirectly, the B7 family members. ICOS-mediated co-stimulation could then act to further expand the T-cell population and to influence effector function differentiation. In addition, T-cell stimulation would lead to the upregulation/translocation of CTLA-4 to the cell surface, with the potential to inhibit T-cell activation early in the response owing to its competitive advantage for ligand over CD28 (Ref. 31). CTLA-4 and PD-1 levels would continue to increase for several days following activation. The inhibitory signals mediated by these molecules could then function to limit, terminate and/or attenuate the nature of the adaptive immune response. The spatial and temporal regulation of expression of the ligands for the CD28 family members on lymphoid and non-lymphoid tissues lends another level of regulation. Finally, this scenario might differ for previously activated T cells because basal levels of CTLA-4, ICOS and PD-1 are reportedly expressed on these cells, and the activational requirements for proliferation, effector differentiation and cytokine release differ in previously activated compared to naive T cells[14,63,64].

Tolerance

The requirement for a second, co-stimulatory signal for T-cell activation has important implications for peripheral T-cell tolerance, as has been demonstrated in many model systems. Several mechanisms have been proposed for the role of co-stimulation in tolerance, including anergy, ignorance, immune deviation of Th cells and CTLA-4-mediated inhibition[1,31,65]. Because CD28 is constitutively expressed on most $CD4^+$ and $CD8^+$ T cells, and CD28-mediated co-stimulation seems to be the primary co-stimulatory signal for naive T cells, it is important to limit the availability of the ligand. Thus, in the absence of sufficient B7-1 and B7-2, T cells do not become activated to tissue-specific antigens. The constitutive expression of co-stimulatory ligands on non-lymphoid cells is an added complexity to understanding peripheral tolerance. ICOS expression on activated T cells provides the potential for co-stimulation to be provided by non-lymphoid tissues, for example, at the site of inflammation. Alternatively, $PD\text{-}1^+$ T cells could receive inhibitory signals upon PD-L1 interaction on non-lymphoid tissues. Finally, co-stimulation via non-lymphoid tissues will require the T cells to migrate into these tissues. Limiting T-cell access to these tissues by regulating cell adhesion and migrations, for example by chemokine receptors[14], might be an important means of preventing aberrant T-cell activation by constitutively expressed co-stimulation ligands. One potential mechanism of tolerance shown to be mediated by ICOS co-stimulation is the skewing of cytokine secretion or immune deviation[25]. Clearly there are many interesting possibilities yet to be explored.

Conclusions

Although substantial progress in the field of co-stimulation has been made, understanding how the various co-stimulatory pathways regulate T-cell activation and function is just beginning to be elucidated. The unique expression patterns and functions of the most recently discovered members of the CD28/B7 families require further characterization to answer a number of outstanding questions: (1) What is the role of localized co-stimulation in non-lymphoid tissue? (2) What are the roles of co-stimulation during localized inflammation and infection? (3) Do the signals from the various co-stimulatory molecules differ quantitatively and/or qualitatively and how are they integrated in the T cell? (4) How are signals via other co-stimulatory family members, such as the TNF receptor (TNFR) family[11], integrated with those transduced by the CD28 family members? Resolution of these questions will be paramount to elucidating the role of co-stimulation in the regulation of lymphocyte homeostasis and adaptive immune responses. Further, these results will have important implications for understanding the dysregulation of T-cell activation in autoimmune and allergic diseases, and for modulating transplant and tumor immunity.

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