

T cells: A proliferation of costimulatory molecules

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The identification and characterization of a newly extended family of molecules related to the T-cell costimulatory proteins CD28 and B7 suggests that a distinct form of costimulatory signals could be important for effector T-cell responses outside of lymphoid tissues.

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A T cell becomes aware of the presence of a pathogen only when two molecular events occur simultaneously: firstly, its antigen receptor complex detects the presence of a pathogen-derived peptide antigen displayed on the surface of an antigen-presenting cell (APC); and secondly, a costimulatory receptor co-expressed on the T cell finds a ligand on the surface of the APC. The coordinated triggering of these two independent receptor systems on the T cell ensures the correct interpretation of T-cell receptor occupancy as a true consequence of a dangerous infection. Costimulation ultimately induces the efficient production of the autocrine growth factor interleukin 2 (IL-2), leading to the generation of an expanded population of effector T cells. T-cell receptor occupancy in the absence of costimulatory signals, however, is not interpreted by the T cell as an inherently dangerous situation. Such occupancy does not lead to the development of autoimmunity, but instead leads to a sustained loss of antigen-responsiveness, called clonal anergy [1]. The identification of molecules with costimulatory activities in T cells is therefore of keen interest. Several recent papers, including one in this issue of *Current Biology* [2], have revealed that, in addition to the long-standing costimulatory proteins CD28 and B7, there are a whole host of related molecules in the family.

CD28 and B7

By all accounts, the CD28 molecule is the most effective T-cell costimulatory receptor discovered to date [3]. A member of the immunoglobulin (Ig) superfamily, it has a single Ig variable-like extracellular domain in addition to its transmembrane and cytoplasmic domains. Expressed as a disulfide-linked homodimer, it is present on most CD4⁺ and many CD8⁺ T cells, and binds to the APC costimulatory ligands B7.1 and B7.2 (Figure 1). B7.1 and B7.2 are also Ig superfamily members, with two Ig-like extracellular domains (one variable and one constant), in addition to transmembrane and short cytoplasmic domains. Both of these proteins are expressed as monomers on professional

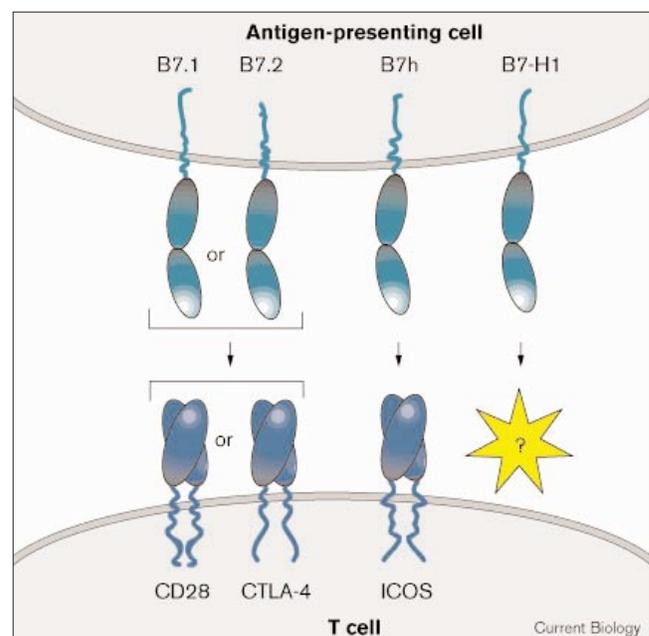
APCs — dendritic cells, activated B cells, and macrophages — in the setting of infection. B7.1 is structurally similar to B7.2, with ~26% sequence identity between their extracellular domains (Figure 2a).

Numerous experiments performed in tissue culture and *in vivo* have indicated that CD28–B7 interactions are both necessary and sufficient for the effective costimulation of antigen-specific T-cell growth and the avoidance of clonal anergy. Nevertheless, T cells can develop in *CD28*^{-/-} mice and are capable of mounting a significant, albeit suboptimal, growth response following antigen stimulation. This observation raises the possibility that other costimulatory receptors are present in the immune system and, under certain circumstances, play an important role in the response to pathogens.

CTLA-4

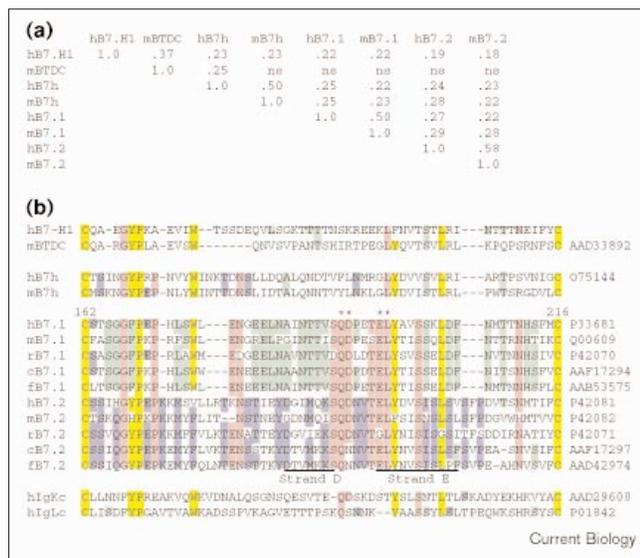
The cloning of the gene for cytotoxic T lymphocyte-associated protein-4 (CTLA-4, CD152) revealed a homology with CD28 of ~30% (Figure 3a), initially suggesting a role for this molecule as an alternative costimulatory signal receptor. Like CD28, CTLA-4 is an Ig superfamily protein with a single extracellular variable-like domain expressed as a disulfide-linked homodimer that is capable

Figure 1



Model of CD28 and B7 family receptor–ligand pairs. A receptor for B7-H1 has not yet been determined.

Figure 2



Amino acid sequence sharing among B7 family members. **(a)** Extracellular domain sequence identities between select human and murine B7 family members. Putative signal, transmembrane region, and cytoplasmic domain sequences were excluded from the analysis: 'ns' indicates no sequence alignment was possible due to low homology. **(b)** Ig constant-like domain sequence alignment of putative B7 family members. Sequence numbers correspond to hB7.1. The asterisks refer to CD28/CTLA-4-binding residues of B7.1 and B7.2 adjacent to strand D, and within the DE-loop and strand E sequences, according to Peach *et al.* [12]. Human Ig kappa (hlgKc) and lambda (hlgLc) chain constant domain sequences are also shown for reference. Colored blocks indicate sequence sharing: yellow, common to Ig superfamily members; pink, shared between B7.1 and B7.2; green, B7.1 characteristic; blue, B7.2 characteristic. Proteins are identified at the left edge of the panels (h, human; m, murine; r, rabbit; c, canine; f, feline) and accession numbers are at the right. Percentage sequence sharing was determined with the Basic Local Alignment Search Tool (BLAST) blastp algorithm, the non-redundant (nr) sequence database, and the PAM-250 matrix using the WWW server at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>).

of binding B7.1 and B7.2 molecules, but with an avidity more than 500 times that of the CD28 homodimer (Figure 1). The solution structure of the CTLA-4 extracellular domain has been determined by nuclear magnetic resonance spectroscopy [4] and reveals a patch of amino acid residues that is highly conserved between CTLA-4 and CD28 (Figure 3b). Site-directed mutagenesis has confirmed this patch to contain the B7.1- and B7.2-binding site for both CTLA-4 and CD28.

Despite the structural similarities to CD28, CTLA-4 is expressed on T cells only after a period of activation. In addition, CTLA-4 triggering has been shown to inhibit T-cell activation and antagonize the costimulatory effects of B7 molecules on T-cell clonal expansion. Consistent with this notion of CTLA-4 being a negative regulator, the absence of this molecule on T cells in *CTLA-4^{-/-}* mice

Figure 3



Amino acid sequence sharing among CD28 family members. **(a)** Sequence identities between human, murine, and rat CD28 family members. **(b)** Partial Ig variable-like domain sequence alignment of putative CD28 family members. Sequence numbers correspond to hCD28. The series of asterisks refer to B7.1/B7.2-binding residues of CD28 and CTLA-4 within strand F and the FG-loop, and adjacent to strand G, according to Metzler *et al.* [4]. Colored blocks indicate sequence sharing: pink, shared between CD28 and CTLA-4; green, CD28 characteristic; blue, CTLA-4 characteristic; yellow, ICOS characteristic. Proteins are identified at the left edge of the panels (h, human; m, murine; r, rat) and accession numbers are at the right. Percentage sequence sharing was determined as described in Figure 2.

leads to the development of autoimmunity and lethal lymphoproliferative disease [5].

Another CD28-like costimulatory receptor

Recently, Hutloff *et al.* [6] identified a third member of the CD28 family — called inducible co-stimulator (ICOS) — using a monoclonal antibody raised against activated human T cells. This antibody recognizes a disulfide-linked homodimer present on the surface of activated and memory T cells that are enriched in the germinal centers of the human thymus, spleen, and tonsils. The ICOS gene was then obtained and found to have 27% and 18% sequence identity with human CD28 and CTLA-4, respectively (Figure 3a). Triggering of ICOS does significantly costimulate the proliferation of T cells but fails to substitute for CD28 ligation in the induction of IL-2 secretion. ICOS costimulation is equivalent to that mediated by CD28 for the production of the cytokines IL-4, IL-5, interferon γ , and tumor necrosis factor α , but shows a greater induction of IL-10. ICOS costimulation also enhances CD40 ligand expression, and this can lead to increased polyclonal antibody secretion in T- and B-cell co-cultures.

Tamatani *et al.* [7] have also produced monoclonal antibodies against ICOS (which, in their studies, is called AILIM, for activation-inducible lymphocyte immunomodulatory molecule) and have independently cloned the rat,

mouse, and human ICOS cDNA orthologs. Like Hutloff *et al.* [6], they observe the expression of ICOS on activated mature T cells, but also note its expression on unstimulated thymocytes. Interestingly, their work suggests that ICOS may regulate the capacity of activated T cells to adhere to and interact with potential target cells, as well as influence their capacity to proliferate and differentiate into effector cells that resemble those of the T helper 2 subtype, capable of secreting IL-4, IL-5, and IL-10.

The discovery of a ligand for ICOS

ICOS lacks several highly conserved amino acid residues that are known to be necessary for B7 binding [4] therefore suggesting that this molecule will not bind to B7.1 or B7.2 (Figure 3b). In support of this, *three* groups have now independently discovered a third B7-like molecule that acts as the ICOS ligand. Using a subtraction screening approach, Swallow *et al.* [8] identified mRNA for B7 homologous protein (B7h) in TNF- α -treated mouse fibroblasts. The cloned cDNA for B7h encodes a new member of the Ig superfamily with both variable- and constant-like extracellular Ig domains, as well as transmembrane and short cytoplasmic domains. The predicted amino acid sequence for the extracellular domain of murine B7h is 23% and 22% identical to that of murine B7.1 and B7.2, respectively, and all three share significant structural features (Figure 2a,b).

Consistent with a role for B7h as a costimulatory ligand, T-cell proliferation in response to antigen presentation by B7h-transfected fibroblasts is significantly higher than that with untransfected fibroblasts, and similar to that observed with B7.2-transfected cells. B7h costimulation of T cells, however, does not depend on either CD28 or CTLA-4 molecules, because antagonism of B7-CD28 and B7-CTLA-4 interactions had no effect on costimulation by fibroblasts expressing B7h.

Yoshinaga *et al.* [9] have also independently cloned a pair of mouse genes, one identical to B7h, and encoding a protein they call B7-related protein-1, or B7RP-1, and a second encoding a protein called CD28-related protein-1 (CRP-1) and likely to be the murine ortholog of ICOS, given that it shows 71% identity with human ICOS (Figure 3a). Using transfected fibroblasts and recombinant fusion proteins, this group has definitively shown that B7h binds only to ICOS and not to CD28, and that ICOS binds to B7h but not B7.2. A gene originally cloned from a brain cDNA library and called human hypothetical protein KIAA0653 (accession number O75144) has been shown by Brodie *et al.* [2] to be the human ortholog of B7h. Human B7h, which they refer to as ligand of ICOS (LICOS), shares 50% of its extracellular domain sequences with murine B7h (Figure 2) and can specifically bind human ICOS *in vitro*.

In mice, B7h mRNA expression is high in lymph node follicles and in the marginal zone of the spleen in both normal

and antigen-sensitized mice. Consistent with this, ICOS binds a ligand on both resting splenic B cells and macrophages. Following exposure to endotoxin in mice, however, B7h mRNA expression is induced in testes, kidney, and peritoneal tissues. Interestingly, *in vitro* differentiated dendritic cells do not express B7h, even though they express B7.1. ICOS mRNA is only expressed in the T-cell-rich paracortex of immunized lymph nodes, but not in resting tissues in mice. As one additional functional test of the importance of B7h-ICOS interactions in the growth and differentiation of mature T cells, transgenic mice have been engineered to secrete a soluble form of the B7h molecule into the bloodstream [9]. These animals develop a lymphoid hyperplasia by 12 weeks of age involving both the T-cell and B-cell compartments. Although it is difficult to ascertain whether this soluble B7h molecule is acting as an agonist or an antagonist for ICOS in this system, the results do support a role for B7h-ICOS interactions in the regulation of T-cell homeostasis.

A fourth B7 family member

One additional B7-like molecule has recently been identified on the basis of a predicted amino acid sequence similarity between B7.1 and B7.2 and a previously uncharacterized cDNA expressed sequence tag. Using this tag as a probe, Dong *et al.* [10] have cloned the B7 homolog-1 (B7-H1) gene from a human placental cDNA library. B7-H1 is predicted to have a similar structure to other B7 family members. B7-H1 mRNA is abundant in heart, skeletal muscle, placenta, and lung, but weakly expressed in thymus and spleen, and absent in peripheral blood mononuclear cells. An antiserum specific for B7-H1 fails to detect significant B7-H1 protein expression on the surface of activated T and B cells; however, activation of macrophages upregulates the expression of this molecule. B7-H1 is not a ligand for either CD28, CTLA-4, or ICOS. Although the receptor for B7-H1 has not yet been identified, the molecule can act as a T-cell costimulatory ligand during suboptimal T-cell receptor stimulation. As with B7h, both proliferation and IL-10 secretion are costimulated in the presence of immobilized B7-H1 or B7-H1-transfected COS cells, whereas IL-2 production is only minimally enhanced.

An extended B7 family

It is remarkable that the amount of sequence sharing between B7-H1 and other B7 family members is no greater than that observed between B7-H1 and a protein called butyrophilin. The human butyrophilin gene is located within the major histocompatibility complex among numerous other Ig superfamily members and has been suggested to be an immunological molecule and a member of an extended B7 family [11]. In fact, a BLAST search using the human B7-H1 sequence identifies its nearest relative to be the murine butyrophilin-like molecule of dendritic cells (BTDC, accession number AAD33892), with an

extracellular sequence identity of 37% (Figure 2a). Nevertheless, these molecules may not be functional homologs, as their pattern of tissue expression is very different. Unlike B7-H1, BTDC is highly and specifically expressed in activated dendritic cells, whereas no expression is detected in activated macrophages (D. Pardoll, personal communication). Sequence similarity between B7-H1, BTDC, and other butyrophilin-like proteins may indicate a common receptor for these molecules on T cells. A role for BTDC and other butyrophilin-like proteins in the regulation of immunity has not yet been determined, however.

These recent reports therefore document a qualitatively distinct form of T-cell costimulation that can occur as a result of ICOS–B7h interactions, and through the triggering of an as yet unidentified receptor by B7-H1. The B7h and B7-H1 molecules are not ligands for either CD28 or CTLA-4, and they do not regulate IL-2 gene expression. Rather, costimulation of activated T cells through these receptor–ligand pairs enhances the secretion of effector cytokines, particularly IL-10, in addition to promoting the proliferation and/or survival of the cells. The relatively broad tissue distribution of B7h and B7-H1 expected in the setting of infection or inflammation, and the preferred migration of recently activated T effector cells into non-lymphoid compartments, suggest a biological role for these new costimulators that is quite different from that of CD28–B7 interactions on naive T cells.

Antigen recognition within an infected tissue is necessary for the targeting of lymphokine secretion and cytolytic granule release. The presence of these alternative costimulatory ligands on target cells may increase the efficiency of such recognition — without stimulating the counter-regulatory receptor CTLA-4 — as well as influence the effector phenotype of the responder T-cell population. The expression of B7h or B7-H1 on non-lymphoid target cells expressing their own unique self-peptides would not necessarily pose a threat to immune self-tolerance, because aggressive (and protective) effector T-cell responses only initiate within a lymphoid organ when a naive T cell recognizes antigen on a professional APC that bears B7.1 and/or B7.2. By leaving the regulation of IL-2 gene expression and, perhaps, clonal anergy to B7.1 and B7.2, the ICOS, B7h, and B7-H1 molecules may instead assume the equally important task of controlling the intensity and duration of an effector immune response at the site of infection.

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