
The evolution of the immunobiology of co-stimulatory pathways: clinical implications

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ABSTRACT

Co-stimulatory pathways are a prerequisite for the regulation of T-cell activation and tolerance. After the engagement of the T-cell receptor (TCR) to the major histocompatibility complex (MHC)/peptide complex, the co-stimulatory signals are critical to decide the outcome of the immune response. While positive co-stimulatory signals promote T-cell activation, negative signals delivered through inducible co-inhibitory receptors limit immune response, thereby regulating tolerance and autoimmunity. This review is intended not only to give an overview of the immunobiology of co-stimulatory pathways and their role in autoimmune diseases, but will also highlight the complexities and relevant issues one may have to think through to translate the targeting of co-stimulatory pathways into successful therapeutic interventions.

Introduction

The ability to discriminate self from non-self is perhaps one of the most crucial factors in immune regulation (1, 2). While recognition of foreign pathogens is vital to fight infections, aberrant immune responses to self-antigens can lead to autoimmune diseases. T cells are key mediators in the initiation and regulation of the adaptive immune response to both foreign and self-antigen. Full activation of T cells requires two signals. The first signal, which confers antigen specificity to the immune response, is provided by the T-cell receptor (TCR) after interacting with the major histocompatibility complex (MHC)/antigenic peptide complex. The second, or 'co-stimulatory signal' is provided by interactions between specific receptors on the T cell and their respective ligands on antigen-presenting cells (APCs) (3). Experimental data suggest that TCR engagement in the

absence of effective co-stimulation often results in T-cell anergy and/or apoptosis (4).

Classically, positive co-stimulatory pathways promote T-cell activation – the prototype being the CD28:B7 pathway. Over the past decade this concept has further evolved to include negative or co-inhibitory pathways that terminate the T-cell response. An intricate balance between the positive and negative co-stimulatory signals ultimately determines the fate of immune responses (5, 6). Emerging data have suggested that the novel co-stimulatory molecules are expressed not only on T cells and APCs but also on non-hematopoietic tissues (Fig. 1). The expression of co-stimulatory molecules on parenchymal tissue has been shown to be important in regulating the immune response in the target organ (7). Recent work also suggests a role of the CD28: B7 family in controlling the homeostasis and function of T-regulatory cells (Tregs) in mice. The expression of cytotoxic T-lymphocyte-associated antigen (CTLA)-4, inducible co-stimulator (ICOS), programmed death (PD)-1 and PD ligand 1 (PDL1) by Tregs has been observed, but the exact role of these molecules still needs to be dissected (8). This could provide further insight into the immunoregulation and control of autoimmune disease.

In this review, the co-stimulatory molecules are grouped broadly into the CD28:B7 family and the tumor necrosis factor (TNF):TNF receptor (TNFR) family, based on their structural characteristics. The role of the new T-cell immunoglobulin and mucin-domain (TIM) family in regulating T-helper (Th)1/Th2 responses, and their potential therapeutic role in autoimmunity also are discussed. This review also reflects on the clinical implications of each pathway and how they could be utilized as therapeutic targets.

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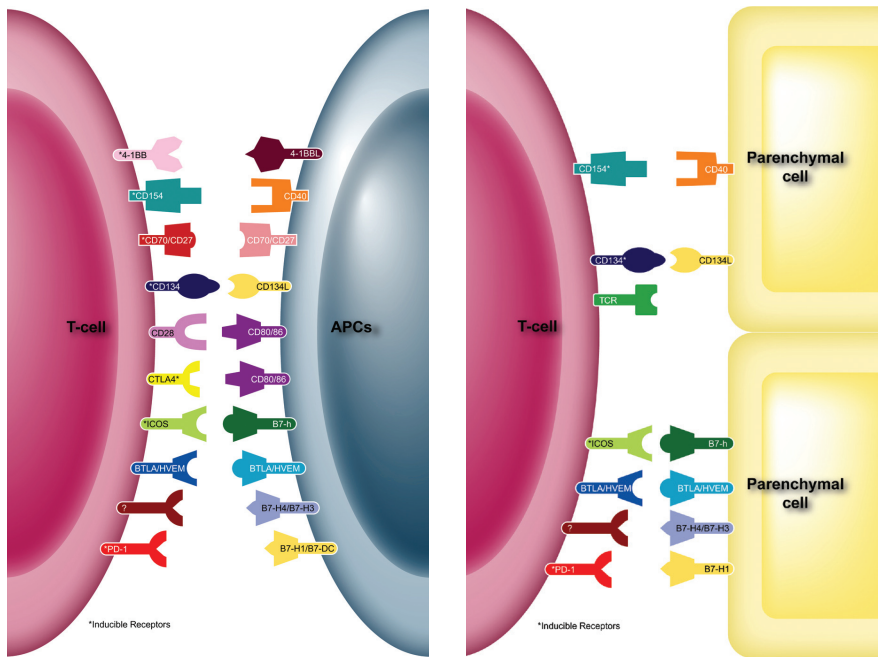


Fig. 1. Expression of co-stimulatory molecules on (A) T cells and antigen-presenting cells and (B) non-hematopoietic tissues. TCR: T-cell receptor; ICOS: inducible co-stimulator.

The CD28:B7 family

CD28/CTLA-4:B7 pathway

The CD28:B7 pathway is one of the best described positive co-stimulatory pathways. Stimulation of CD28 with TCR signaling augments interleukin (IL)-2 production (9), a critical T-cell growth factor, and facilitates T-cell survival by inducing the upregulation of anti-apoptotic bcl-xL genes (10, 11). Its ligands, B7-1 (CD80) and B7-2 (CD86), are primarily expressed on activated APCs such as dendritic cells (DCs), macrophages and B cells. B7-1 is expressed in lower levels on resting cells and is upregulated with prolonged T-cell stimulation, while B7-2 is constitutively expressed and rapidly up-regulated on APCs with an antigen-specific signal. Hence, these distinct differences in expression indicate that B7-2 is chiefly involved in mediating initial T-cell activation, while B7-1 may play a significant role in propagating the immune response (12). After activation, T cells express CTLA-4 (CD152), which has a higher affinity for CD80 and CD86 compared with CD28; its engagement delivers a negative signal into the T cell, inhibiting/terminating T-cell responses. Hence CTLA-4 may function as a master switch for peripheral T-cell tolerance (13), and the outcome of an

immune response depends on the equilibrium created between CD28-mediated T-cell activation and CTLA-4 mediated inhibition.

CD28:B7 interactions are vital for the expansion and maintenance of CD4⁺CD25⁺ Tregs (14). Both CD28^{-/-} and B7-1/B7-2^{-/-}NOD mice have a strikingly low number of CD4⁺CD25⁺ Tregs, although the function of these regulatory cells is intact (15). Reverse signaling into B7-expressing APCs may occur after engagement of CTLA-4 via the induction of indoleamine 2,3 dioxygenase (IDO), an enzyme that breaks down tryptophan into byproducts which inhibit T-cell proliferation and promote tolerance (16, 17).

Experiments on NOD mice (a mouse model of human type I diabetes) showed that early blockade of co-stimulatory signals with anti-B7-2 or CTLA-4 immunoglobulin (Ig) prevented diabetes; however, these antibodies were ineffective when administered after the progression of insulinitis (18). Collagen-induced arthritis (CIA), a murine model of RA, has been previously reported to be dependent on the presence of the CD28 pathway. CD28-deficient DBA/1 mice are resistant to CIA, and combined treatment with anti-B7-1 and anti-B7-2 monoclonal antibody (mAb) ameliorated the disease,

pointing to a critical role of CD28 co-stimulation in the development and perpetuation of CIA in DBA/1 mice (19, 20). However, in a recent study in CD4-dependent DQ8 transgenic, CD28-deficient mice it was shown that CD28 is not an absolute requirement for the initiation of CIA (21). CD28-deficient mice developed a milder disease, with delayed onset and lower incidence. This illustrates the point that other co-stimulatory molecules can lead to the development of disease in the absence of CD28 signaling due to the late production of Th1 and Th2 cytokines. In experimental allergic (autoimmune) encephalomyelitis (EAE), the mouse model for multiple sclerosis, CD28:B7 co-stimulation blockade at the onset of the disease resulted in the reduction of EAE due to limited T-cell expansion *in vivo* (22, 23). Studies in the MRL-lpr/lpr mouse (a murine model for human systemic lupus erythematosus [SLE]) have shown that anti-B7.2 Ab therapy inhibited anti-dsDNA autoantibodies, whereas anti-B7.1 Ab had no effect, suggesting that each B7 co-stimulatory signal may control unique pathological events in the development of murine lupus that may not be apparent by measuring autoantibody titers alone (24, 25). The crucial role of CTLA-4 in regulating T-cell tolerance is highlighted by the observation that CTLA-4^{-/-} mice develop a fatal lymphoproliferative disorder with multi-organ autoimmune disease (26, 27). CTLA-4 is found to have an important role in modulating autoimmune T-cell responses and in the maintenance of tolerance. Polymorphisms of the CTLA-4 gene have been associated with a susceptibility to autoimmune diseases such as diabetes and thyroiditis (28, 29). Abatacept, or CTLA-4Ig, is a fusion protein of the extra-cellular binding domain of human CTLA-4 with the modified Fc region of human IgG1. Abatacept specifically binds to B7-1/B7-2, thereby blocking the engagement of CD28 on T cells and downregulating T-cell proliferation (30). Initial results when used in patients with psoriasis vulgaris revealed the efficacy and immunosuppressive effects of CTLA-4Ig in a T-cell mediated autoimmune disease.

In Phase II and III trials of abatacept in combination with methotrexate in rheumatoid arthritis (RA) patients with inadequate response to methotrexate, statistically significant improvements were noticed clinically, with reduced levels of inflammatory mediators and reduced progression of joint damage when compared with the methotrexate group (31–33). Abatacept has now been approved for the treatment of adult patients with moderate to severe RA. It has been shown to be useful in patients with an inadequate response to methotrexate or anti-TNF therapy. Clinical trials are underway to examine the efficacy and safety of CTLA-4Ig in patients with multiple sclerosis and SLE. Belatacept (LEA29Y) (34), which was rationally designed from abatacept, binds more avidly to CD86, hence providing more potent immunosuppression, and is currently in Phase III trials in renal transplantation.

Recently, novel 'designer' approaches have been used to target CTLA-4 on activated T cells via genetically engineered B cells, to prevent autoimmune diabetes in the NOD mouse. The mechanism of regulation is thought to be the prevention of autoreactive T-cell expansion and the development of effector function in the pancreatic lymph nodes (35, 36). This is a new immunomodulation strategy designed to prevent or cure autoimmune diseases. Another approach to up-regulate CTLA-4 would be to target CD45RB, thus increasing the natural expression of CTLA-4 in addition to other mechanisms that promote tolerance (37). Anti-CD45RB mAb has been shown to prolong renal allografts in cynomolgous monkeys, and is currently in preparation for a Phase I trial with human renal transplant recipients (36, 38).

ICOS:ICOSL pathway

ICOS is distinct from CD28 in that it is not constitutively expressed on naïve T cells; however, it is rapidly up-regulated on T-cell activation, and is found on effector and memory T cells. ICOS lacks the MYPPPY motif, and hence does not bind to B7-1 and B7-2 ligands (39, 40). ICOSL (also called B7h, B7RP-1) is present on certain APCs (such as B

cells, DCs and macrophages), and can be induced on non-lymphoid cells (such as renal tubular epithelial cells, prostate epithelial cells and brain tissue) by inflammatory signals (41). The role of ICOS on initial T-cell proliferation and IL-2 production is minimal, as it does not possess an SH3-kinase binding site (PYAP) (42, 43). While the CD28 pathway is involved in these functions, ICOS signaling is vital for regulating effector Th2 responses, especially IL-4 expression through a c-Maf (transcription factor)-dependent mechanism. Blockade of ICOS during initial stimulation of naïve T cells enhances Th1 differentiation. ICOS has an important task in enhancing T-cell dependent B-cell function. This is evidenced by defects in immunoglobulin isotype class switching and germinal center formation in the spleens of ICOS-deficient mice (44–46). This shortcoming can be overcome by CD40 co-stimulation, thus suggesting critical interactions with the CD40:CD40L pathway (47).

The timing of ICOS blockade makes a significant difference to the course of an autoimmune disease. In the EAE model using SJL mice with proteolipid protein, ICOS blockade during the efferent immune response (9–20 days after immunization) abrogated disease, while early blockade during antigen priming (1–10 days after immunization) exacerbated disease (48). It has been further shown that ICOS blockade during antigen priming leads to excessive PLP-specific splenocyte proliferation and interferon (IFN)- γ production, resulting in polarization to a Th1 response (49). Recent evidence from the BDC2.5 transgenic mouse, which expresses a TCR derived from a diabetogenic CD4⁺ T-cell clone, suggest that ICOS is expressed by IL-10 producing CD4⁺CD25⁺ Tregs in the pancreas. This study showed that the loss of immunoregulatory capacity by CD4⁺CD25⁺ Tregs is actually the mechanism by which ICOS blockade functions early on to accelerate autoimmunity (50). In stark contrast to EAE, which is exacerbated in ICOS-deficient mice, it has been shown that ICOS-deficient mice on DBA/1 background are completely resistant to CIA (51). These mice

exhibited reduced B-cell responses in anti-collagen II antibody production. In addition, ICOS regulates IL-17 expression, which contributes to the absence of joint inflammation in the knockout animals (52, 53). Therefore, anti-ICOS approaches may provide novel means of treating patients with RA (54). However, the differential roles of ICOS in EAE and CIA have not yet been elucidated.

In SLE models, ICOS is one of the forces driving the formation of memory B cells and plasma cells (55). It has been shown that the administration of anti-ICOSL mAb after the onset of proteinuria in murine lupus nephritis prevented disease progression and improved renal pathology by effectively inhibiting IgG autoantibody production (56). Though interactions with ICOS and its ligand are not absolutely essential in the development of colitis, in collaboration with CD28 Th1 inflammatory cells could be generated. Treatment with anti-ICOS mAb has been shown to prevent and reverse intestinal inflammation by inducing apoptosis of ICOS-expressing T lymphocytes (57, 58). Thus, harnessing the ICOS pathway, especially in combination therapies, could prove to be a promising therapeutic target in various autoimmune diseases.

PD:PDL1 pathway

PD-1 is a transmembrane protein of the Ig superfamily that possesses two tyrosine residues within its cytoplasmic domain. Though the presence of the immunoreceptor tyrosine-based inhibition motif (ITIM) indicates that it should serve to inhibit immune responses, mutagenesis studies suggest that the tyrosine within the immunoreceptor tyrosine-based switch motif (ITSM) is required for the inhibitory activity of PD-1 (59–61). PD-1 also lacks the MYPPPY motif required for B7-1 and B7-1 binding.

PD-1 is expressed during thymic development on double-negative (CD4[−]CD8[−]) thymocytes. Peripherally, PD-1 is upregulated on activated CD4⁺ and CD8⁺ T cells, B cells and monocytes. PD-1 engages with PDL1 and PDL2 ligands, which have distinct patterns of expression. PDL1 is expressed on

resting, activated T cells, B cells and DCs, and on non-lymphoid organs such as endothelium, heart, placenta, pancreas and lungs. The presence of PDL1 within non-lymphoid tissues suggests that PDL1 regulates self-reactive T or B cells in peripheral tissues, and may regulate inflammatory responses in the target organs (7). In contrast, PDL2 is induced by cytokine only on macrophages and DCs (62). PD-1 functions as a co-inhibitory receptor; depending on their genetic background, strains of the knockout mice exhibit various autoimmune disorders (63). PD-1 deficient mice on a C57BL/6 background develop lupus-like arthritis and glomerulonephritis, while on the BALB/c background they show fatal dilated cardiomyopathy (41, 64).

In the spontaneous autoimmune diabetes model (NOD mice), PD-1 and PDL1 blockade (but not PDL2 blockade) strikingly accelerated the time-of-onset of disease (65). PDL1 expression was found on the inflamed islets and more recently, with our collaborators we have demonstrated that it is the PDL1 expression on parenchymal cells rather than hematopoietic cells that protect mice against diabetes (7). In this model, our experiments also indicated that CTLA-4 regulation is restricted to the initial phase of the disease, while the PD-1:PDL1 pathway plays a major role in both the initiation and the progression of disease. In keeping with these findings, PDL1 knockout mice on the NOD background developed diabetes rapidly at 4–6 weeks, as opposed to wild-type NOD mice, which typically show diabetes at 12–14 weeks of age (7, 66). Interestingly, PDL2 but not PDL1 blockade augmented EAE in C57BL/6 mice, with minimal and delayed expression of PDL2 in the central nervous system (CNS) (67). Recently, there has been evidence from our group that PDL1 and PDL2 differentially regulate the susceptibility and chronic progression of EAE in a strain-specific manner; as in the case for BALB/c mice immunized with MOG peptide, PDL1 but not PDL2 blockade significantly increased the incidence of EAE (68). It has also been shown that in mice with EAE, PDL1 is upregulated

on endothelium in the brain (69). Recent studies of patients with SLE and type 1 diabetes have revealed the association of an intronic single nucleotide polymorphism of PD-1 with increased risk of the disease (70, 71).

B7-H3 (B7RP-2)

B7-H3, a new member of the B7 family, is broadly expressed in lymphoid and non-lymphoid organs (72). Though B7-H3 mRNA is not detectable in human peripheral blood mononuclear cells, its expression can be induced by inflammatory cytokines on T cells, B cells, natural killer (NK) cells, monocytes and DCs (73, 74). Soluble B7-H3 protein binds a putative, as yet unidentified receptor on activated T cells, which is distinct from known CD28 family members.

There is still uncertainty regarding the stimulatory/inhibiting function of B7-H3. Initial studies have shown that human B7-H3Ig fusion protein co-stimulated CD4⁺ and CD8⁺ T-cell proliferation and enhanced IFN- γ production and the cytotoxic activity of CD8⁺ cells (72). Recent studies on B7-H3^{-/-} mice have supported a co-inhibitory function, as these mice have an earlier onset of EAE and developed anti-DNA autoantibodies (75). B7-H3^{-/-} mice, under Th1- but not Th2-polarizing conditions, developed severe airway inflammation as opposed to wild-type control mice, and showed increased T-cell responses. Overall, these studies indicate that B7-H3 negatively regulates the T-cell responses that occur under Th1-polarizing conditions. Further studies are needed to resolve this controversy, but such inhibitory and stimulatory functions of B7-H3 could be explained by the existence of two receptors with opposing functions, such as CD28 and CTLA. On the other hand, this discrepancy could also be a result of B7-H3 mediated signals in different cell types.

B7-H4 (B7x/B7S1)

B7-H4, a novel addition to the B7 family, is anchored to the cell membrane via a glycosyl phosphatidylinositol (GPI) linkage and works as a negative regulator of CD4⁺, CD8⁺ T cells,

and B cells (76). It can be induced on human T and B cells, DCs and monocytes after *in vitro* stimulation (77, 78). Immunohistochemical staining shows that B7-H4 is highly expressed in lung and ovarian tumors, suggesting its potential role in the evasion of tumor immunity (77). Its receptor, which is likely to be on activated T cells, has not yet been identified. B7-H4 ligation of T cells has a profound inhibitory effect on T-cell proliferation, cytokine secretion and the development of cytotoxicity. Functional studies with B7-H4 Ig demonstrate arrest in the G0/G1 phase of the cell cycle with inhibition of IL-2 production, while anti-B7-H4 blocking mAb has opposing effects (79). Anti-B7-H4 blocking mAb markedly accelerated the onset and severity of EAE and increased CD4⁺, CD8⁺ T cells and CD11b⁺ macrophages in the brains of the treated mice (41). Recent studies have shown that ectopic expression of B7-H4 can render macrophages suppressive (80, 81). IL-6 and IL-10 are found in high concentrations in the tumor microenvironment, and stimulate macrophage B7-H4 expression, while IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) strongly suppress it. These tumor macrophages strongly express B7-H4, and inhibit tumor-associated antigen (TAA)-specific T-cell immunity. This group also showed that Tregs trigger high levels of IL-10 production by APCs, stimulate APC B7-H4 expression, and render APCs immunosuppressive (80). Therefore, one can expect that targeting B7-H4 may be a novel strategy to inhibit Treg-mediated suppression *in vivo*.

BTLA

B- and T-lymphocyte attenuator (BTLA) is induced on T cells upon activation and remains predominantly expressed on Th1 cells, in contrast to ICOS, which is upregulated on Th2 cells. BTLA is also expressed on resting and activated B cells, macrophages and bone marrow-derived myeloid DCs (82). Recently, herpes virus entry mediator (HVEM) has been demonstrated to be the ligand for BTLA (83, 84). This interaction reveals the existence of cross-

talk between BTLA (which belongs to the CD28 family) and HVEM (which is from the TNFR family) that culminates in the termination of T-cell responses (85-87). HVEM is detected on resting T cells and immature DCs. BTLA-deficient T cells proliferate more than wild-type T cells when stimulated by anti-CD3 mAb. BTLA-deficient mice have an increased susceptibility to MOG peptide-induced EAE, which further illustrates its inhibitory nature. BTLA may specifically downregulate Th1-mediated inflammatory responses (82). Of the three inhibitory receptors (CTLA-4, PD-1, and BTLA) CTLA-4 deficient mice show the most striking lymphoproliferative phenotype, while BTLA deficiency shows a subtle phenotype characterized by an increased predisposition to autoimmunity. PD-1 deficiency, depending on the genetic background of the mouse, results in organ-specific autoimmunity. These data suggest a non-redundant hierarchical regulation of autoimmune responses by T-cell co-inhibitory pathways.

The TNF:TNFR family

CD40:CD40L (CD154) pathway

The CD40:CD154 pathway represents a prototype within the TNF:TNFR family of co-stimulatory molecules. CD40 is expressed on APCs and binds to its ligand CD154, which is upregulated on T cells upon antigen recognition. CD40 plays an important role in the activation and maturation of B cells and DCs (88, 89). CD40:CD154 interactions enhance the ability of APCs to present antigen and activate the T cell. Hence, in the absence of this interaction there is impairment of CD4⁺ effector cells, a lack of Ig switching, and inhibition of IL-12 and Th1 cytokine expression (90). Presently it is believed that CD40L engagement of CD40 on APCs increases the expression of B7 molecules on APCs, thereby amplifying the T-cell response (91).

Blocking CD40L has been shown to prevent autoimmune diseases in several experimental models (92). In the CIA model, anti-CD154 blocks the development of joint inflammation, serum antibody titers to collagen, and the infiltration of inflammatory cells into

the sub-synovial tissue. Subsequently, it was demonstrated that there is enhanced expression of CD40L on lymphoid cells within human rheumatoid joints and synovial fibroblasts, which stimulates the production of TNF- α to induce articular erosion (93). Anti-CD40L mAb, which blocks the CD40:CD154 pathway, was found to delay murine lupus nephritis when given early (94). However, when CTLA-4Ig was combined with blocking anti-CD40L mAb, there was long-lasting inhibition of autoantibody production and renal disease (95).

In the autoimmune diabetes (NOD) mouse model, anti-CD40L was found to be effective only when given early in the development of insulinitis (96). However, the recurrence of autoimmunity (in transplanted isografts) was diminished after treatment with anti-CD154 antibody, with the effect being more pronounced in rats than in mice (97, 98). There is a reduced frequency of inflammatory, encephalitogenic T cells in the absence of CD154 in the EAE model (99). There is also *in vivo* evidence that CD40 expression in CNS endogenous cells is responsible for the migration and retention of effector T cells in the CNS of mice during EAE. Despite promising results using anti-CD154 mAb (hu5C8) in rodent models of autoimmunity and transplantation, therapy with humanized anti-CD154 mAb in humans produced thromboembolic complications. It has been shown that platelets express CD154 both on the surface and in a soluble form, which on activation plays a potential role in thrombus formation and the stabilization of arterial thrombi (100, 101). Anti-CD40 mAb, which may not show these complications, are currently in preclinical development (102).

OX40 (CD134):OX40L (CD134L) pathway

Expression of OX40 is typically restricted to activated T cells, largely on CD4⁺ T cells, but upon strong antigenic stimulation OX40 can also be induced on CD8⁺ T cells (103, 104). CD28 augments OX40 expression on T cells, which typically peaks 48 hours after *in vitro* activation and wanes after 72-96 hours (105). OX40L is expressed on

activated B cells, DCs and vascular endothelial cells. OX40 and its ligand have also been detected in the inflamed tissues of autoimmune disorders such as EAE, ulcerative colitis and RA (106). The engagement of OX40 to its ligand signals the T cells to proliferate and enhance cytokine production, and increases the formation of memory T cells (107, 108). It improves T-cell survival by sustaining the gene expression of anti-apoptotic Bcl-2 family members (109). The OX40 and OX40L interaction is needed for the induction of EAE in mice; blocking this interaction reduces the disease, especially in CD28-deficient mice or when combined with CTLA-4Ig (110, 111). Blockade of the OX40:OX40L interaction also reduces the severity of CIA and models of colitis (112, 113). OX40L-deficient mice on a NOD background did not develop diabetes (114). This study found that the OX40:OX40L axis may be instrumental in orchestrating the late phases of autoimmune infiltration, allowing for the long-term establishment of an insulinitic lesion.

Recent studies have shown the expression of OX40 on naïve and activated CD4⁺CD25⁺ Tregs; stimulation of OX40 on CD4⁺CD25⁺ T cells using an agonistic antibody abrogated the suppressive function of these cells (115). OX40L strongly inhibited the production and functioning of IL-10 producing Tr1 cells induced by ICOSL and immature DCs (116). Cumulatively, these findings suggest that in addition to controlling effector/memory T-cell numbers, OX40 directly controls Treg-mediated suppression of immune responses. Thus, timing and dosing needs to be carefully considered when planning future clinical trials with human OX40L mAb and human OX40 Ig fusion protein.

4-1BB:4-1BBL pathway

4-1BB is an inducible member of the TNFR superfamily, and is chiefly expressed on activated T cells, NK cells and DCs. Its ligand, 4-1BBL, has been detected on activated T and B cells, macrophages and mature DCs (103). This pathway, similar to ICOS and

OX40, is important for the maintenance of T-cell responses rather than for initiating T-cell expansion, especially for CD8⁺ T cells. 4-1BB ligand knockout mice have deficient CD8 T-cell responses to viral infections, suggesting that this pathway is required for optimal CD8 T-cell responses and survival (117). 4-1BB:4-1BBL interactions have been noted to control the survival of CD8 effector memory cells through IL-15. Indeed, IL-15, a cytokine involved in the regulation of CD8⁺ memory T-cell survival, induces the expression of 4-1BB on memory CD8 cells (118). Anti-4-1BB therapy in a mouse lupus model has been found to inhibit dsDNA antibody and consequently to block immune complex formation in kidneys. These data also indicated that 4-1BB engagement in lupus-prone mice alters DC function, leading to anergy of CD4⁺ T cells that, in turn, reduce autoantibody production. These mice, with a few injections of the antibody, remained lupus free for about 1 year post-treatment (119).

Administration of agonistic anti-4-1BB antibodies is known to enhance tumor immunity and allergenic responses. In contrast, when administered in EAE, these antibodies remarkably reduce the incidence and severity of the disease (120). This result has been explained by the observation that anti-4-1BB treatment initially promotes CD4⁺ T-cell proliferation, but subsequently accelerates their activation-induced cell death (AICD) (121). Therefore, the engagement of 4-1BB by an agonistic Ab may provide a novel approach to effectively deplete autoreactive T cells, especially CD8⁺ T cells.

CD27:CD70 pathway

CD27 is present on NK cells, B cells and naïve T cells. CD27 expression on activated T cells is lost after many rounds of cell division, and this loss of CD27 correlates with high levels of effector function. Its ligand, CD70, is found on activated T and B cells and DCs (5). CD27 interacts with its ligand to induce TNF- α production and cytotoxic T-lymphocyte effectors, and enhance T-cell survival. While CD28 promotes cell division, CD27 helps

T-cell expansion by allowing the T cells to survive through successive divisions. Transgenic mice expressing CD70 show elevated numbers of CD4 and CD8 effector T cells, enhanced CD8 T-cell responses to influenza virus, and increased IFN- γ secretion by T cells (123). CD27-deficient mice have demonstrated defective memory T-cell responses, as reflected in an impaired response to influenza virus (122). Treatment with anti-CD70 antibody prevents EAE in SJL/J mice, mainly by suppressing the generation of TNF- α (124). In transplant models, CD70 prevented CD8⁺ T-cell mediated rejection by inhibiting the proliferation, activation and expansion of CD8⁺ effector/memory cells. Hence, like the 4-1BB pathway, CD27:CD70 may be a potential target for the development of improved strategies to overcome resistance to tolerance mediated via CD8 T cells.

CD30 /CD30L pathway

Although CD30 was originally found on Reed-Sternberg cells in Hodgkin's lymphoma, it is also expressed on activated T cells, B cells and NK cells, as well as eosinophils (125). CD30L is found on resting B cells, activated T cells, eosinophils, neutrophils and inflamed tissues (103). *In vitro* studies have shown that engagement of CD30 by its ligand enhances T-cell proliferation and cytokine production. CD30 has been implicated as a possible candidate for *Idd9.2*, a gene controlling diabetes progression (126, 127). Anti-CD30L mAb markedly inhibited the development of spontaneous diabetes in NOD mice, and prevented islet-specific T-cell proliferation (128). Further studies are required to scrutinize the defects in CD30 knockout mice, and to plan the development of therapy with antibodies in humans.

The T-cell immunoglobulin and mucin-domain family

The TIM family of genes, which consists of TIM 1–8 on mouse chromosome 11B1.1 and TIM 1, 3 and 4 on human chromosome 5q33.2, is a group of cell-surface proteins that have recently been shown to play an important role in regulating immune-mediated diseases,

especially autoimmunity and asthma/allergy (129, 130). TIM-3, the first identified molecule of the TIM family, is expressed on terminally differentiated Th1 cells (131) and its ligation with galectin-9 serves to dampen Th1 immunity by eliminating Th1 effector cells (132, 133). Initial studies with anti-TIM-3 antibody treatment exacerbated EAE (131). Interestingly, Th-17 cells (IL-17 expressing pathogenic cells) express low levels of TIM-3, and are thought to be less susceptible to the negative regulation of the TIM-3–galectin-9 pathway (134). Recent data, which await further exploration, have proposed the TIM-3 dependent upregulation of B7-1 on APCs and CTLA-4 on T cells (135). In contrast to TIM-3, murine TIM-2, an ortholog of human TIM-1, is preferentially expressed on differentiated Th2 cells and regulates Th2 responses during autoimmune inflammation (136). Human TIM-1 originally was identified as a hepatitis A viral cellular receptor, and epidemiologic studies have linked protection against atopy and asthma to hepatitis A virus infection (137). TIM-4, which is predominantly expressed on mature DCs, is the natural ligand for TIM-1 (138). Studies with TIM-4.Ig have shown that the TIM-4:TIM-1 interaction could potentially co-stimulate T-cell proliferation, especially Th2 (129). Thus, close scrutiny of the TIM pathway, along with further understanding of the mechanisms by which they regulate Th1 and Th2 effector functions, and possibly Tregs, could indicate a potential therapeutic target in chronic autoimmune diseases (130).

Conclusions

The last decade has witnessed tremendous progress in the discovery and characterization of novel T-cell co-stimulatory pathways, in addition to the increased understanding of conventional pathways. Inhibition of the CD28 co-stimulatory pathway has emerged as a rational therapeutic strategy for selectively modulating full T-cell activation. Innovative technology in protein engineering has enabled the creation of abatacept (CTLA-4Ig), which has now been approved for the treatment of moderate to severe RA, and could have

a potential role in the treatment of other T-cell mediated autoimmune diseases. However, complex but balanced interactions between positive (co-stimulatory) and negative (co-inhibitory) pathways are required for the maintenance of self tolerance. This issue becomes further complicated by the parenchymal expression of several co-stimulatory molecules and the intricate interactions between different pathways in the inflammation of target organs. While the CD28/CTLA-4 pathway appears to be dominant, the question of redundancy versus hierarchy among the many other co-stimulatory pathways needs to be further clarified. Although emerging data suggest that a pecking order does exist for the different pathways at various stages of the immune response and at distinct tissue sites, these points need to further explored.

Despite these complexities, the precise tailoring of agents to block the positive co-stimulatory pathway while harnessing the negative pathways at appropriate time points in the disease process may provide unique opportunities for the treatment of autoimmune disease. Further studies are needed to pin down the key players at the crucial time points of pathogenesis in order to translate these agents into more successful clinical therapies.

Key points box

- T cells require both antigen-specific and co-stimulatory signals for their full activation
- The CD28/CTLA-4:B7 pathway is one of the best characterized and most critical pathways for T-cell activation. Manipulation of this pathway using CTLA-4Ig (abatacept) has already been shown to be effective in rheumatoid arthritis
- Members of the TNF/TNFR family – CD40:CD40L being the classic prototype – are emerging as key mediators of survival signaling in T cells after initial CD28-dependent T-cell activation

- The role of co-inhibitory pathways such as PD-1, B7H3, B7H4 and BTLA have been shown to attenuate T-cell responses and promote T-cell tolerance. Their parenchymal expression may regulate key immune responses in peripheral target tissues
- A complex but intricate balance between the co-stimulatory and co-inhibitory pathways is required to maintain self tolerance
- The precise tailoring of agents to block the positive co-stimulatory signal while exploiting the co-inhibitory pathway may prove useful in treating autoimmune diseases

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