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# The Role of T-Cell Costimulatory Pathways in Regulation of Autoimmune Diabetes

**I-Fang Lee[1], Dawei Ou[1], Daniel L. Metzger[2] and Garth L. Warnock[1]***

[1]Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada
[2]Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

## Abstract

Many factors contribute to the pathogenesis of autoimmune diabetes. Targets for treating this debilitating disease will become more apparent by understanding the nature of immune activation. This review examines the possibility of targeting costimulation and discusses the molecules found on the T cell and the antigen-presenting cell (APC) that participate in T-cell activation. The B7-1/B7-2:CD28/cytotoxic T lymphocyte antigen-4 (CTLA-4) pathway has been shown to be crucial in regulating T-cell activation and tolerance. Novel members of the B7-CD28 superfamily have recently been discovered, and they appear to be particularly important for regulating the responses of previously activated T cells. Superimposition of inhibitory signals, such as those delivered by CTLA-4 and the programmed death (PD)-1-PD-1 ligand (PD-L1) pathway, leads to a complex network of positive and negative costimulatory signals, the integration of which modulates immune responses. Furthermore, expression of the B7 homolog B7-H4 on cells in peripheral tissues indicates new mechanisms for regulating T-cell responses. This review focuses on our current understanding of the members of the B7:CD28 superfamily and discusses their therapeutic potential in autoimmune diabetes.

**Keywords:** T cell; Costimulatory pathway; Autoimmune diabetes

## Introduction

Type 1 diabetes mellitus (T1DM), also known as autoimmune diabetes, most commonly presents in patients in mid- and late childhood, and is believed to be caused by T cell–mediated destruction of pancreatic islet β-cells, occurring over a 3–5 year period of time [1,2]. Islet autoreactivity is an acquired phenomenon and not simply a developmental event. Therefore, the signals that regulate T-cell activation and modify their responsiveness are centrally involved in the pathogenesis of this disease. As such, these signals may represent appropriate targets for immunotherapy.

T-cell activation occurs following complex signaling pathways among different cell types. With specific T-cell receptors (TCR), T cells recognize antigen peptides which are presented to them, along with major histocompatibility complex (MHC) molecules, by APCs. Interaction between TCR and a co-receptor (CD4 or CD8) with the antigen/MHC complex on APCs provides the first signal of T-cell activation and determines its specificity [3]. This signal alone is not able to induce full T-cell activation, but leads to T-cell anergy (loss of T-cell immune competence) or apoptosis. In order to create full T-cell activation, a second, non-specific signal is mandatory. This second signal, known as the costimulatory signal, is provided through the interaction of one or multiple T-cell surface receptors with corresponding ligands on APCs. According to the resulting T-cell response, these signals can be divided into 'positive' (activating) or 'negative' (inhibitory) costimulatory signals. Positive costimulation leads to T-cell activation, which includes clonal expansion of T cells, further differentiation into memory or effector cells, cytokine production, and inhibition of T-cell anergy and apoptosis [4,5]. Negative costimulatory signals regulate T-cell responses by inhibiting T-cell proliferation and cytokine production, inducing anergy, limiting T-cell survival and increasing T-cell apoptosis. Costimulatory signals are not only restricted to the interaction between T cells and APCs, but also participate in the dialog between T cells themselves, between T and B cells, and between T cells and non-hematopoietic (endothelial or parenchymal) cells [6].

Costimulatory pathways mediated by the B7:CD28 family has been shown to play key roles in regulating T-cell activation and are promising therapeutic targets (B7 receptors and ligands are summarized in Table 1 and Table 2) [7-13]. These pathways not only provide critical positive second signals that promote and sustain T-cell responses, but they also contribute critical negative second signals that downregulate T-cell responses. These negative signals function to limit, terminate, and/or attenuate T-cell responses and they appear to be especially important for regulating T-cell tolerance and autoimmunity. The B7-1/B7-2:CD28/CTLA-4 pathway is the best characterized T-cell costimulatory pathway, but it is complex because of the dual specificity of B7-1 (CD80) and B7-2 (CD86) for the stimulatory receptor CD28 and the inhibitory receptor CTLA-4 (CD152). CD28 delivers signals important for T-cell activation and survival, whereas CTLA-4 inhibits T-cell responses and regulates peripheral T-cell tolerance [7-9,12]. Researchers have found two new pathways in the B7:CD28 superfamily: one involves ICOS (inducible costimulator) [14] and ICOS ligand [15-19]; the other involves the PD-1 (programmed death-1) receptor [20] and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) [21]. Two additional B7 homologs, B7-H3 [22] and B7-H4 (B7x, B7S1) [23], also have been identified, indicating that there are still additional pathways within the B7:CD28 superfamily to be characterized.

This article will review the current understanding of T-cell costimulatory pathways in autoimmune diabetes. In particular, we will

***Corresponding author:** Garth L. Warnock, M.D., Department of Surgery, 3100, 910 West 10th Avenue, Vancouver, BC, Canada, V5Z 4E3. Tel: 1-604-875-4136; Fax: 1-604-875-4376; E-mail: Garth.Warnock@vch.ca

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| Name | % identity to CD28 | Ligand binding motif | Expression cell type |
|---|---|---|---|
| **CD28** | 100% | MYPPPY | T |
| **CTLA-4** | 30% | MYPPPY | T |
| **ICOS** | 24% | FDPPPF | T, NK |
| **PD-1** | 21% | ? | T, B, MΦ |

Abbreviations: T = T cells; B = B cells; NK = natural killer cells; MΦ = macrophages

**Table 1:** CD28 family receptors.

| Ligand name (alternative names) | %identity of extracellular domain | Protein expression pattern in lymphoid cells | mRNA expression in non-lymphoid tissue or cells | Receptor | Function of ligand-receptor interaction |
|---|---|---|---|---|---|
| **B7-1 (CD80)** | 100% | Induced in T, B DCs and monocytes | Rare | CD28<br>CTLA-4 | Costimulation<br>Coinhibition |
| **B7-2 (CD86)** | 27% | Constitutive and upregulated upon activation in B, DCs, and monocytes; induced in T | Rare | CD28<br>CTLA-4 | Costimulation<br>Coinhibition |
| **ICOSL (B7h; B7RP-1; B7-H2; GL-50)** | 27% | Constitutive in B, DCs, macrophages, and a subset of T | Lung, liver, kidney, and testes | ICOS | Costimulation |
| **PD-L1 (B7-H1)** | 25% | Constitutive and upregulated upon activation in B, DCs, and monocytes; induced T | Placenta, heart, pancreas, lung, liver, and tumor cells (carcinomas and melanomas) | PD-1 | Coinhibition |
| **PD-L2 (B7-DC)** | 23% | Induced in DCs and monocytes | Heart, placenta, lung, liver keratinocytes, and epithelial cells | PD-1 | Coinhibition |
| **B7-(B7S1; B7x)** | 25% | Induced in T, B, DCs and monocytes | Heart, kidney, testes, lung, liver, pancreas, prostate, colon, osteoblasts, and tumor cells | Unknown | Coinhibition |

Abbreviations: B = B cells; DCs = dendritic cells; T = T cells

**Table 2:** Structure, expression patterns and receptors of B7 ligands.

focus on B7-CD28/CTLA-4 as well as the novel members of the B7 and CD28 superfamilies, especially our findings with B7-H4, and on how strategies to regulate these pathways may be used for this disease.

## B7–CD28/CTLA-4

Many T-cell molecules may serve as receptors for costimulatory signals, and CD28 molecule is the best characterized of these molecules [24-26]. According to recent gene expression studies, CD28 costimulation can lead to significant augmentation of the expression of genes induced by TCR signaling alone. These findings are consistent with a model of costimulation in which CD28 signaling lowers TCR thresholds for activation of cells [27,28]. CD28 has two known ligands; B7-1 (CD80) and B7-2 (CD86), both of which are expressed primarily on activated bone marrow–derived professional APCs. T cells also express CTLA-4, a molecule that is highly homologous to CD28, which also binds to CD80 and CD86 [29]. However, unlike CD28, CTLA-4 transmits a negative signal that serves to terminate the immune response [30,31]. While CD28 is expressed on both resting and activated T cells, CTLA-4 is expressed only on activated T cells [32]. Since CTLA-4 binds the B7 molecule with higher affinity than does CD28, its inhibitory interaction eventually predominates, leading to the termination of the immune response [33]. The expression of the B7 antigens, CD80 and CD86, is tightly regulated [34]. On resting B lymphocytes and other APCs, CD86 is expressed at low density and CD80 is not often expressed. Thus, most APCs require stimuli for the induction of CD80 expression and, significantly, CD86 expression is induced more rapidly and to a greater degree than CD80. This led to the hypothesis that CD86 may participate in initiating an immune response, whereas CD80 may serve to amplify or regulate an immune response. Therefore, distinct expression patterns, timing and affinity of CD80 and CD86 for CD28 and CTLA-4 may influence whether B7–CD28 or B7–CTLA-4 interactions predominate at distinct stages of an immune response.

Ligation of CD28 by B7 on the naive T cell provides the costimulatory signal needed to trigger T-cell activation (Figure 1). Specifically, CD28 ligation in the presence of a TCR signal results in T-cell proliferation and expansion, increased production of IL-2 and upregulation of IL-2 receptors, as well as expression of Bcl-xL [35-38]. Furthermore, the importance of CD28 as a regulatory T-cell costimulatory molecule has also been reported in the non-obese diabetic (NOD) mouse, an animal model of autoimmune diabetes with a breakdown in the balance between autoreactivity and immune regulation in the periphery. $CD4^+CD25^+$ regulatory T cells (Tregs) play a fundamental role in maintaining immune homeostasis and limiting autoimmune responses in the periphery, and CD28 engagement has been shown to be essential for maintaining a functional Treg compartment. NOD mice deficient either for CD28 or for its ligands B7-1/B7-2 develop an aggressive T1D, due to a profound decrease in Treg cells, demonstrating the role of CD28/B7 pathway in the maintenance of Treg cells homeostasis in the periphery [39]. The experiments using anti-B7 monoclonal antibody (mAb) or $B7^{-/-}$ recipients demonstrated that CD28 plays a critical role in Treg cells expansion and blockade of CD28/B7 interaction completely disrupts Treg cells proliferation *in vivo* [40]. Adoptive transfer of Treg cells into $CD28^{-/-}$ NOD mice prevents T1D development demonstrating the importance for costimulation in Treg cell fitness [39,40]. Treg cells from $CD28^{-/-}$, B7-1/B7-$2^{-/-}$ mice retain their regulatory activity *in vitro* and *in vivo*, indicating that CD28 does not influence the suppressive effector function of Treg cells,

but is important for their homeostasis [41-43]. One of the important functions of CD28 costimulation is the induction of IL-2 production by T effector (Teff) cells [44]. It has been established that IL-2 is a critical cytokine for Treg cell functional stability; although Treg cells them self cannot produce it [45-48]. Tang et al. reported that steady state levels of IL-2 mRNA are significantly decreased in the T effector cell–deficient $CD28^{-/-}$NOD mice relative to wildtype (WT) NOD mice, and that low IL-2 levels in these mice contributed to defective homeostasis of Treg cells [40]. This is further confirmed by the study where in adoptively transferred WT Treg cells numbers results in rapid decline in $CD28^{-/-}$ NOD mice [40]. Overall, CD28 maintains a stable pool of peripheral Treg cells by supporting their cycling and their survival *in vivo* [40].

CTLA-4 as a negative regulatory T cell costimulatory molecule has also been highlighted in the physiologic termination of autoimmune diabetes. Animal studies have demonstrated that blockage of CTLA-4 signaling results in exacerbation of the disease if administrated when the animals first exhibit insulitis [49,50], suggesting a critical role of CTLA-4 in controlling the autoreactive response. Because CTLA-4 has a higher affinity for CD80 and CD86 than CD28, the soluble fusion protein CTLA-4–immunoglobulin (CTLA-4.Ig, a recombinant fusion protein consisting of the extracellular domain of CTLA-4 linked to the constant region of IgG1) [29] offers a potential therapy to terminate T-cell responses. In the model of islet transplantation, local delivery of CTLA-4.Ig, either by co-transplantation of CTLA-4.Ig-secreting muscle cells [51] or by gene-gun biolistic delivery of naked DNA coding for CTLA-4.Ig [52], has been shown to enhance considerably islet allograft survival in diabetic mice. In diabetes, previous reports have shown that injection of CTLA-4.Ig to NOD mice is able to block the development of diabetes when administrated in mice 2–4 weeks of age [53].

The mechanisms of CTLA-4–mediated inhibition of T-cell responses have been studied. Ligation of CTLA-4 is found to induce T-cell proliferation, cell cycle progression, and IL-2 production (Figure 1) [30,33]. Additionally, CTLA-4 signals influence $CD4^+$ T-cell differentiation. In CTLA-4–deficient mice, T cells were shown to be strongly skewed towards a Th2 phenotype, even in the absence of the Th2 lineage transcription factor signal transducer and activator of transcription-6 (Stat-6) [54,55]. CTLA-4 has also been shown to influence Th17 responses, as blockade of CTLA-4 with anti–CTLA-4 mAb or using CTLA-4.Ig and CD28 knockout (KO) T cells results in increased Th17 differentiation and IL-17 production *in vitro* and *in vivo* [56]. Furthermore, it has been implicated that the influence of CTLA-4 in the activation of Treg cells contributes to tolerance induction. Treg cells express high levels of CTLA-4 [39,43,57] and antagonist CTLA-4–specific antibody abrogates Treg cell functions [43,57]. CTLA-4 blockade alters the suppression of CTLA-4–deficient effector cells by WT Tregs, suggesting a direct functional role of CTLA-4 on Tregs. However, despite a loss of Treg function following CTLA-4 blockade, Bluestone's and Powrie's laboratories [58,59] observed that CTLA-4-deficient Tregs may suppress effector responses through increasing level of the immunoregulatory cytokines TGF-β and IL-10 [58,59], suggesting that CTLA-4 seems important for Treg function, and that Tregs developing in a CTLA-4–deficient environment may be able to overcome the need for CTLA-4 through compensatory mechanisms

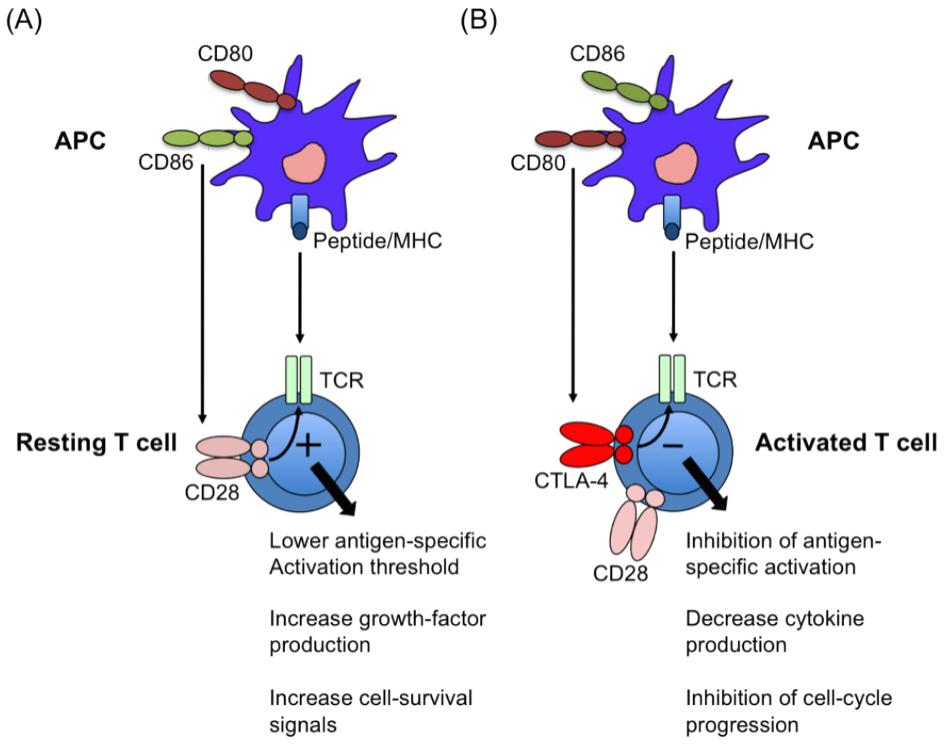


**Figure 1: Costimulation and coinhibition.** The binding of CD28 or CTLA-4 receptors on T cells by CD80 and CD86 ligands on antigen-presenting cells (APCs) can lead to either costimulation or coinhibition depending upon the precise expression patterns of the receptors and ligands and on the state of activation of the two cells. (a) CD28 is expressed on resting T cells and can be engaged by either CD80 or CD86 on APCs, leading to activation of resting T cells. This costimulation leads in the T cells to increase production of growth factor, such as IL-2 and increase cell-survival signals, such as Bcl-xL. (b) CD28 and CTLA-4 are both expressed on activated T cells. Engagement of CTLA-4 on CD80 or CD86 on APCs decreases T cell proliferation, IL-2 production, and cell cycle progression.

of suppression. Altogether, strategies that promote CTLA-4–mediated negative signaling may be useful in the therapy of autoimmune diabetes.

## ICOS/ICOSL

The function and structure of ICOS is similar to that of CD28. ICOS associates with its ligand B7h (also described as ICOSL, B7RP-1, B7H-2 and GL-50), which is strongly expressed in B cells, macrophages and dendritic cells (DCs) [17] and is present in a number of non-lymphoid tissues, such as lung and heart [60].

Structurally, ICOS shares ~30–40% sequence similarity with CD28 and CTLA-4. It also maps to the CD28/CTLA-4 locus, suggesting that it arose by gene duplication. However, unlike CD28, ICOS is up-regulated following T cell activation and through interactions with ICOSL on antigen presenting cells (APC), promotes T cell proliferation and T helper 2 (Th2) differentiations [61,62]. Recent studies have further expanded this view to show that ICOS ligation can also promote Treg [63,64], Th17 [65], Th1 [66], B cells [67], and T follicular helper responses [67] depending on the context of the inflammatory response [61,67]. CD28 and ICOS synergize to promote the activation of T cells; while CD28 plays a predominant role during an initial T cell activation, ICOS promotes activation for antigen-experienced T cells [17]. ICOS signaling enhances T cell proliferation, survival and cytokine production and is important for T–B cell interactions [67].

In a model of islet transplantation utilizing a chemically induced diabetic non-autoimmune strain of mice, Nanji et al. have previously reported increased ICOS expression in rejected islet allografts, and using the combination therapy of anti-ICOS mAb and sirolimus were able to prolong graft survival [68]. In addition, a later publication from the same group has also showed that combined ICOS and CD40-ligand blockade led to a significant prolongation of graft survival after islet transplantation [69]. In autoimmune diabetes, previous studies have demonstrated that ICOS blockage also significantly reduces the onset of primary autoimmune diabetes in NOD mice, indicating that the effectiveness of blocking ICOS signaling is not limited to controlling alloimmune responses [69,70]. Moreover, administration of anti-ICOS mAb disrupts Treg/Teff cell balance and results in exacerbated pancreatic lesions suggesting that ICOS may be important in controlling Treg-cell function in pre-diabetic islets and that Treg cells may suppress T1D development in an ICOS-dependent manner [63,64].

## PD-1/PD-L

A new member of the CD28 superfamily to be described is PD-1. Like CD28 and CTLA-4, it is a transmembrane protein of the Ig superfamily and, like CTLA-4; it possesses only a single V-like domain and an immunoreceptor tyrosine-based inhibiting motif (ITIM) within its cytoplasmic tail. It shares 23% homology with CTLA-4 but lacks the MYPPY motif required for CD80 and CD86 binding [21].

PD-1 is expressed by activated, but not by unstimulated, $CD4^+$ and $CD8^+$ T cells, B cells and myeloid cells [71], in contrast to the more restricted expression of CD28 and CTLA-4. It binds two known ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), found on professional APCs such as DCs and monocytes, but also found constitutively on certain parenchymal cells (heart, lung and kidney), as well as on a subpopulation of T and B cells [72]. In an analogous manner to CTLA-4, engagement of PD-1 by its ligands results in a regulatory effect, with inhibition of downstream cellular signaling events, diminished cellular proliferation and cytokine production (Figure 2) [21,72]. Furthermore, mice deficient in PD-1 expression develop autoimmune disorders characterized by production of high titers of autoantibodies [73], indicating an important role in the regulation of autoreactive B cells.

Previous studies in autoimmunity and tolerance have revealed that PD-1/PD-L interactions not only are important in the initial phase of activation and expansion of autoreactive T cells, but also influence autoreactive T-cell effector function upon antigen reencounter. In NOD mice, PD-L1 is upregulated in the pancreatic islet cells [74], and loss or blockade of PD-1 or PD-L1 accelerates diabetes in pre-diabetic NOD mice, which is correlated with increased pro-inflammatory cytokine production by autoreactive T cells [75,76]. In a model of antigen-specific therapy in which administration of antigen-coupled fixed splenocytes induces tolerance and reverses diabetes in NOD mice, Fife et al. has shown that similar to CTLA-4, PD-1 plays an important role in the induction of peripheral tolerance [77]. This study also demonstrated that PD-1, but not CTLA-4, is critical for the long-term maintenance of tolerance in this model. PD-1/PD-L1 interactions are required for both the induction and maintenance of $CD4^+$ T cell tolerance [77]. Notably, blockade of PD-1 or PD-L1 reverses anergy in islet-antigen–specific T cells, whereas CTLA-4 blockade does not break tolerance, indicating a unique function for PD-1/PD-L1 interactions in maintaining T-cell anergy. Bone marrow chimera experiments have demonstrated that PD-L1 expression on non–bone marrow–derived cells, including islet cells, inhibits T-cell effector function in tissues [75,78,79]. These studies also suggest a key role for the PD-1/PD-L1 pathway in limiting immune-mediated tissue damage caused by pathogenic T cells upon antigen reencounter in the periphery. Collectively, these findings demonstrate that PD-1/PD-L1 interactions regulate both the initiation and progress of autoimmune diabetes in NOD mice and identify PD-1/PD-L1 interactions as key mediators of T-cell tolerance in tissues.

Another important mechanism of peripheral tolerance also involves Treg cells. It has been established that PD-L1 can promote Treg development and function. Francisco et al. demonstrated that PD-L1-Ig–mediated blockade promotes the TGF-β–induced *de novo* generation of induced $CD4^+Foxp3^+$ Treg (iTreg) cells [80]. Consistent with this, adoptive transfer of T cells in mice lacking PD-L1 and PD-L2 abrogates Treg-cell induction, and mice quickly develope inflammatory disease [80]. Moreover, T-cell activation in the presence of PD-L1–Ig enhances Foxp3 expression and suppressive function of iTreg cells [80]. Under these circumstances, PD-L1 promotes the differentiation iTreg cells from naive T cells by blocking the Akt-mTOR signaling pathway [80]. These findings establish PD-1 as an attractive therapeutic target in autoimmune diseases and tolerance induction. Overall, PD-L1 can induce and maintain expression of Foxp3 in iTreg cells, suggesting that PD-L1 may function to stabilize and sustain Treg-cell function in the periphery, as has been recently documented for IL-10 and TGF-β [81]. Therefore, the role of PD-1-PD-L1 in control of peripheral tolerance results not only from inhibition of activation and expansion of autoreactive T cells, but also from restriction of their effector function while promoting *de novo* generation of Treg cells.

## B7-H4

B7-H4 was first identified by DNA sequence homology with other molecules of the B7 family in 2003 by three laboratories, which designated three different names to the same molecule (i.e. B7S1, B7-H4, and B7x, respectively) [23,82,83], but now the term B7-H4 has been most widely used. B7-H4 is a type I transmembrane protein and has about 25% homology in the extracellular portion with other

B7 family members. The mouse and human amino acid sequences of B7-H4 share approximately 87% amino acid identity [23]. B7-H4 mRNA is widely expressed in both lymphoid and non-lymphoid tissues, including brain, lung, heart, stomach, intestine, liver, pancreas, kidney, skin, muscle, uterus, prostate, testis, placenta, ovary, and spleen [23,82,83]. However, its protein expression on tissues appears to be limited [23,84]. B7-H4 has been found to be expressed by a minor population of cells in the thymus [83]. B7-H4 is not found in freshly isolated splenic $CD4^+$, $CD8^+$ or $CD11c^+$ cells, but is constitutively expressed on B cells. B7-H4 expression can be detected on activated macrophages and bone marrow-derived DCs (BMDCs). LPS treatment of peritoneal macrophage or BMDC does not significantly alter the B7-H4 expression. On the other hand, LPS, IL-4, anti-IgM and anti-CD40 treatment all resulted in B7-H4 down-regulation in splenic B cells. This analysis indicates that B7-H4 is expressed by a variety of professional APCs and may possess a regulatory role on T cells. In addition to the expression of B7-H4 in lymphoid tissues and cells, it has been shown that B7-H4 is also expressed in non-lymphoid tissues, including pancreatic islet cells [85], suggesting a role of B7-H4 in maintaining peripheral tolerance. According to flow cytometric analysis of the B7-H4-transfected 293 cell line, B7-H4 binds to a receptor on activated T cells but not to CTLA-4, ICOS, or PD-1. B7-H4 is expressed on WT but not on B and T lymphocyte attenuator– (BTLA-) deficient cells, suggesting that BTLA might be a receptor for B7-H4 [82]. However, a recent study has indicated that BTLA does not bind to B7-H4 directly, and that herpes virus entry mediator (HVEM) may be the unique BTLA ligand [86]. To date, the receptor for B7-H4 is still unclear.

Several reports have been suggested a role of B7-H4 in immunoregulation. In adaptive immunity, B7-H4 has been shown to inhibit the activation, proliferation, and clonal expansion of $CD4^+$ and $CD8^+$ T cells, thus suppressing the production of cytokines (IL-2 and IFN-γ), and generation of alloreactive cytotoxic T lymphocytes (CTLs) by arresting the cell cycle, in an *in vitro* T-cell activation assay (Figure 3) [23,83,87]. B7-H4 expressed on the surface of artificial APCs (EL4 cells transfected with the mB7-H4 cDNA plasmid to establish a line that stably expresses B7-H4) also inhibits the proliferation of T cells [23,87]. *In vivo* blockade of endogenous B7-H4 by a specific mAb promotes a T-cell response, indicating that B7-H4 plays an inhibitory role in T-cell activation [23]. B7-H4–deficient Balb/c mice mounted mildly augmented Th1 responses and displayed slightly lower parasite burdens upon *Leishmania major* infection, compared to WT mice, indicating that B7-H4 can inhibit Th1 responses against infection [88]. However, the lack of B7-H4 did not affect hypersensitive inflammatory responses in the airway or skin that are induced by either Th1 or Th2 cells. Likewise, B7-H4–deficient mice developed normal CTL reaction against viral infection [88]. The inhibitory effect of B7-H4 has also been found to be used by $CD4^+CD25^+$ regulatory T cell–mediated suppression, in which Treg cells conveyed suppressive activity of APC through stimulating the expression of B7-H4 on APC in an IL-10–dependent manner [89]. Together, these results suggest that B7-H4 may be one of multiple negative co-signaling molecules that collectively provide a fine-tuning mechanism for T-cellmediated immune responses. Other than in adaptive immunity, B7-H4 has also been shown to have an inhibitory effect on innate immunity through controlling the growth of neutrophils [90]. B7-H4 knockout mice were more resistant to infection by *Listeria monocytogenes* than their littermates, suggesting that B7- H4 plays an inhibitory role on innate immunity. Further studies have shown that more neutrophils were observed in peripheral organs of B7-H4 knockout mice than their littermates, but their bactericidal functions remained unchanged. *In vitro,* B7-H4 inhibited the growth of bone marrow–derived neutrophil progenitors, suggesting an inhibitory function of B7-H4 on neutrophil expansion. As augmented innate resistance is completely dependent on neutrophils, even in the absence of adaptive immunity, the results indicate that B7-H4 serves as a negative regulator of the neutrophil response to infection and provides a new target for manipulation of innate immunity.

We have investigated the role of B7-H4 in the regulation of islet transplantation. In an allotransplant mouse model, we showed that local expression of B7-H4 by recombinant adenovirus significantly prolongs islet allograft survival, and that this survival is associated with up-regulation of $Foxp3^+$ cells in the allograft [91]. Moreover, using a

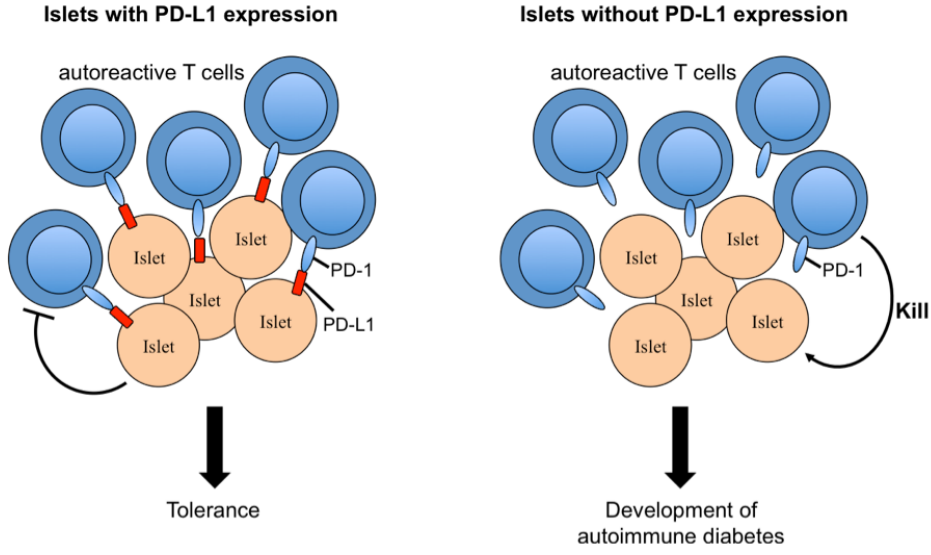


**Figure 2: The PD-1/PD-L1 pathway.** PD-1 mediates protection from autoimmune T effector cells in pancreas. Islets protect themselves from autoreactive T cells by expressing PD-L1 on b-cells. Absence of PD-L1 leads to infiltration of autoreactive T cells and destruction of islets leading to development in autoimmune diabetes.

secondary transplantation model, our data demonstrated that B7-H4 not only promotes allograft survival, but also induces donor-specific tolerance [91,92].

Using a model of transferring disease-inducing BDC2.5 T cells into B7-H4-deficient mice, Wei et al. has recently shown that these mice results in a more aggressive form of diabetes than in WT animals. This exacerbation of disease correlates with higher frequencies of islet-infiltrating Th1 and Th17 cells. Conversely, local B7-H4 over-expression inhibits the development of autoimmunity, as crossing diabetes-susceptible BDC2.5/B6$^{g7}$ mice to animals over-expressing B7-H4 in pancreatic islets abrogates disease induction. This protection is caused by the inhibition of IFN-γ production by CD4$^{+}$ T cells and not by a skewing or expansion of Th2 or Treg cells [85]. Consistently, our recently published data also support the role of B7-H4 in regulation of autoimmune diabetes. In this study, we injected soluble B7-H4.Ig fusion protein into prediabetic NOD mice and found that this treatment significantly reduces the incidence of spontaneous autoimmune disease [93]. In addition, our data show that B7-H4–treated mice have increased Treg cells in the pancreatic lymph nodes (PLNs), reduced infiltrating Teff cells and IFN-γ production, suggesting that B7-H4. Ig has the local effect of Treg in the islets to limit the Th1 response, proliferation and/or cytokine production by the pathogenic T cells, and therefore control the progression from insulitis to overt diabetes in prediabetic mice. Together, the data exhibit a potential role of B7-H4 in inhibiting the development of autoimmune diabetes and thus provides a potential target for therapeutic intervention in the clinic.

## Conclusions

Autoimmune diabetes is a chronic disorder in which pancreatic β cells are selectively destroyed by autoreactive T cells. This persistent, targeted destruction may go undetected for many years. By the time the first clinical symptoms become apparent, nearly 80% of β cells in patients have been destroyed, rendering the individual dependent on insulin injections for their survival. Although the administration of insulin maintains tight glycemic control, it does not stop the persistent autoimmune response or long-term complications in patients. These severe degrees of morbidity, coupled with the increasing incidence of the disease and a current lack of tried and tested alternative therapeutic approaches, bring an urgency to the search for tolerance-inducing therapies that could halt disease progression following diagnosis, or prevent the disease altogether.

Immune-based intervention in autoimmune diabetes has been attempted at two main stages of the disease process: (1) prior to clinical onset but after the appearance of islet autoantibodies (secondary prevention); and (2) immediately after diagnosis (intervention). Immunotherapy at the stage when there is still some residual β-cell mass (about 15–20%), aims to provide evidence of safety and efficacy in prolonging or enhancing endogenous β-cell function, leading to improved blood glucose homeostasis. The Diabetes Control and Complications Trial (DCCT) [94] and the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study [95] have shown that maintaining tight blood glucose control after onset can prevent or delay the long-term complications of diabetes. Thus, maintaining and enhancing endogenous β-cell function as a result of immune intervention after diagnosis may have a significant impact on the long-term disease outcome, as well as allowing for the testing of potential agents that might be effective for secondary prevention.

Decades of intense investigations of animal models of autoimmune diabetes, especially the NOD mouse, have led to the evaluation of a large number of potential interventions [96], many of which have shown promising results. However, translating these findings successfully to humans is proving to be significantly more challenging– both at the prevention and intervention stages. A number of therapeutic candidates have shown promise in animal models, although mainly at the early stages of disease progression, i.e. during the preclinical phase. Unfortunately, this is also the stage at which the accuracy of disease prediction is the lowest, making it difficult to justify ethically the application of many early treatments to individuals who may never develop autoimmune diabetes and thus face unwarranted potential side effects [97]. At the stage at which the disease can be predicted

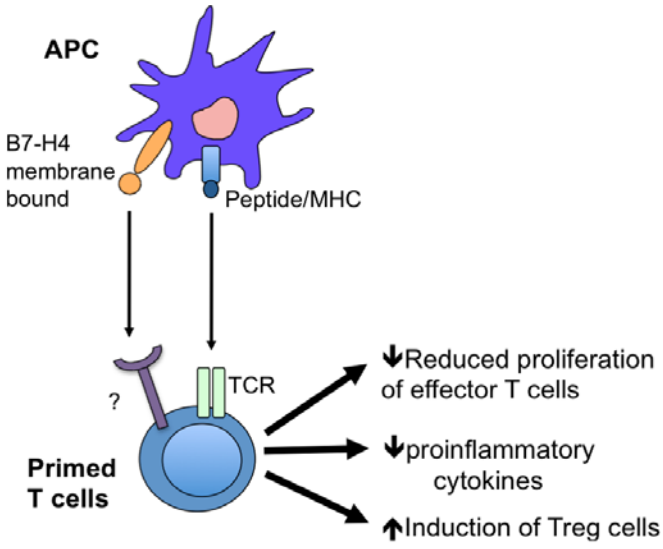


**Figure 3: Function of B7-H4.** Interaction of B7-H4 and its unknown receptors on primed T cells results reduction of effector T cell proliferation, decrease Th1 cytokine secretion (IL-2 and IFN-g) and induction of Treg cells.

accurately or is already clinically manifest, the loss of β-cell mass is substantial, making potential therapies less effective in reversing or halting the autoimmune assault. Thus, as ever, the optimal therapeutic approach will be one that strikes the best risk–benefit balance between side effects and efficacy.

Several additional factors underlie the chance of success of autoimmune diabetes clinical trials aimed at preventing or halting the disease. These include: the accurate translation of the dosing and scheduling regimen from animal models to human prevention/intervention trials; the notion that animal models are sufficiently good indicators of therapeutic success in a patient; and that the course of the autoimmune process is still amenable to modulation, especially after overt disease has been established. These factors add significant complexity to the quest for developing a successful prevention/intervention therapy.

Immunotherapeutic approaches for preventing or halting autoimmune diabetes have involved both antigen-specific and antigen non-specific approaches. Because autoimmune diabetes results from a failure to maintain immune tolerance to islet autoantigens, targeting these autoantigens should provide not only an effective means of controlling the autoimmune response but should also avoid the harmful effects associated with non-specific immunosuppression. Thus, antigen-specific approaches have been favored over globally immunosuppressive therapies.

Costimulatory signals are critical for the regulation of T cell responses and modulation of costimulatory signals represents a potential therapeutic target for the prevention of autoimmune disorders. Targeting has the potential to be far more advantageous than current immunosuppressive therapies in autoimmunity. One important advantage that these therapies could provide is a costimulatory molecules reduction in the number of dangerous side effects, or the infection risk caused by non-specific immunosuppression. Of even greater importance to the treatment of diabetes is the ability to target the population of autoreactive T cells and induction of peripheral tolerance. Moreover, due to the specificity of these costimulatory molecules for a particular receptor, a therapy combining any one of the costimulatory treatments could prove synergistic and therefore improve treatment outcome of treatment.

Current treatments for autoimmune diabetes do not effectively target an important player in disease pathogenesis, the T cell. Instead, they have relied on general suppression of all immune cells. Through the understanding of T cell morphology and function, certain therapies are on the forefront of targeting the autoreactive T cell. Given time and further research, costimulatory therapy may prove to be important in the treatment of human autoimmune diabetes.

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