Published in final edited form as:

Am J Reprod Immunol. 2010 June ; 63(6): 506–519. doi:10.1111/j.1600-0897.2010.00841.x.

B7 Family Molecules as Regulators of the Maternal Immune System in Pregnancy

Margaret G. Petroff and Antoine Perchellet

Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, USA

Abstract

Placental and fetal growth and development are associated with chronic exposure of the maternal immune system to fetally derived, paternally inherited antigens. Because maternal lymphocytes are aware of fetal antigens, active tolerance mechanisms are required to ensure unperturbed progression of pregnancy and delivery of a healthy newborn. These mechanisms of tolerance may include deletion, receptor downregulation, and anergy of fetal antigen-specific cells in lymphoid tissues, as well as regulation at the maternal–fetal interface by a variety of locally expressed immunoregulatory molecules. The B7 family of costimulatory molecules comprises one group of immunoregulatory molecules present in the decidua and placenta. B7 family members mediate both inhibitory and stimulatory effects on T-cell activation and effector functions and may play a critical role in maintaining tolerance to the fetus. Here, we review the known functions of the B7 family proteins in pregnancy.

Keywords

Fetus; maternal–fetal interface; placenta; tolerance

Introduction

Placental and fetal growth and development are associated with chronic exposure of fetally-derived, paternally inherited antigens to the maternal immune system. Based on studies in mice, this exposure to paternal antigens is thought to occur as early as insemination, wanes until establishment of the fully mature placenta, and again becomes robust when the uterine blood supply to the placenta is established.^{1–3} Once this occurs, the placenta is inundated with maternal blood, and antigen efflux from the fetus persists for the last 1/2 of pregnancy in mice, and 2/3 of pregnancy in women. In women, the continuous shedding of trophoblast cells and other soluble fetal products is thought to be a major source of antigen to maternal immune cells.³ The trafficking of fetal cells into the mother, which results in fetal microchimerism, is also thought to provide the maternal immune system with fetal antigen.⁴ Together, insemination, trophoblast shedding, and fetal microchimerism lead to a robust, antigen-specific tolerance in maternal T cells to fetal products that ensures unperturbed progression of pregnancy and delivery of a healthy newborn. Persistence of this tolerance is furthermore needed during pregnancies faced with infection to avoid antigen-specific immunity to the fetus.

Much research has focused on mechanisms by which the fetus and placenta establish tolerance in the maternal immune system, including non-specific suppression of activated T cells by cell surface-associated and soluble products produced locally at the maternal–fetal interface. Increasing understanding of the properties of T cells that tolerate specific fetal antigens is also being gained, facilitated by the use of animal models that enable tracking of maternal lymphocytes targeted to defined fetal antigens. Although tolerance to fetal antigens is very robust, little is known about the mechanisms that establish this tolerance. Recent gains have indicated an important role for members of the B7 family of immunomodulators.

The Two Signal Model of T-Cell Activation

The response of T cells to their cognate antigens is governed principally by two distinct molecular signals that are provided to T cells upon their interaction with antigen presenting cells (APCs). The first signal (signal 1) results from ligation of the T-cell receptor (TCR) by antigen associated with major histocompatibility complex (MHC) molecules. A costimulatory signal (signal 2) occurs through the CD28 molecule, which is recruited to the immunological synapse following TCR ligation and is provided by B7-1 or B7-2. Like the MHC, the B7 proteins are expressed by APCs. The costimulatory signal serves to induce T-cell production of interleukin (IL) -2, drive their proliferation, and protect them from apoptosis and anergy. IL-2 acts in an autocrine/paracrine fashion on the T cells and is obligatory for their survival and differentiation into effector cells. Without the costimulatory signal, signal 1 from the TCR by itself induces T cells to become tolerant to their cognate antigen instead of activated.^{5–7} Both the TCR and CD28 are constitutively expressed on most naïve T cells, such that the T cell is ready to respond to antigen as presented by an MHC-expressing APC.

Cytotoxic T lymphocyte antigen 4 (CTLA-4) is a second, inhibitory receptor of the B7-1/-2 ligands, and its surface expression is upregulated on T cells following their activation. The precise mechanism of action of CTLA-4 is not completely understood, but because its affinity for B7-1/-2 is higher than that of CD28, it is thought to control the T-cell response by competing for binding and blocking the costimulatory signal.^{8,9} CTLA-4 also induces intracellular signal transduction distinct from that of CD28, and thus may also actively induce inhibitors of CD28 signaling.^{10,11} Altogether, its effect is to block further progression of the cell cycle and prevent IL-2 production. In addition, CTLA-4 seems to be critical for function of regulatory T cells (T_{Regs}), which are powerful suppressors of T cells.^{12–14} Finally, CTLA-4 appears to play an indispensable role in regulating homeostatic T-cell proliferation. The regulatory functions of CTLA-4 are illustrated in CTLA-4-deficient mice in which rapid, polyclonal expansion of T cells occurs, which is ultimately fatal to the animals.¹⁵ The functions of CTLA-4 are thus critical in controlling immune responses to both foreign and self-antigens.

While other molecules support T-cell activation, CD28-B7 signaling seems to be the sole mechanism that acts directly to promote IL-2 production, proliferation, and thereby prevent tolerance to naïve T cells.¹⁶ Because of this decisive role in determining the outcome of T-cell recognition of foreign antigen, there has been a concerted effort over the last 10 years to identify proteins related to B7, CD28, and CTLA-4. The B7 family has now grown to eight members; however, identification of their receptors has proved more difficult, and only four receptors that are ligated by the B7s are currently known (Table I).

The identification of these novel proteins and their fundamental importance in determining both immunity and tolerance has prompted investigation into their potential role in maternal acceptance to the fetal semi-allograft. Our laboratory and others have mapped the expression of the B7 family proteins at the maternal–fetal interface (Fig. 1). In short, both APCs and non-APCs, that is, trophoblast cells, express B7 family proteins in abundance. In the

following paragraphs, we review the known functions of B7 family proteins in pregnancy, with particular attention to the cell types that express them at the maternal–fetal interface.

B7-1 and B7-2 and their receptors in pregnancy

B7-1/-2 on Maternal and Placental Cells

B7-1 and B7-2 were first cloned in the early 1990s, and their central role in the immune response was shortly realized. The requirement for costimulation delivered by APCs for productive T-cell activation raised the question of whether cells at the maternal–fetal interface express B7 proteins and might serve as APCs. Olivares and colleagues first reported that cells within the decidua can express B7-1 and B7-2 and have the ability to stimulate a mixed lymphocyte reaction.¹⁷ Since then, studies have further characterized decidual APCs, including macrophages and dendritic cells (DCs), and taken together, these studies suggest that there are subsets of APCs that serve a range of physiological functions. For example, Miyazaki et al.¹⁸ investigated a subpopulation of decidual DCs characterized by high expression of HLA-DR, B7-1, and B7-2 relative to peripheral blood DCs. *In vitro* culture of these cells suggested that they promote a Th2 phenotype in responding T cells. On the other hand, other studies in humans and non-human primates have reported low-to-absent expression of B7-2 along with higher expression of markers of immaturity on decidual DCs.^{19–22} The authors of these studies speculated that these represent immature, tolerogenic DCs. An immature phenotype of uterine DCs in normal pregnancy would in fact be expected, because DCs possess this phenotype prior to encounter with pathogen.²³ Blois et al.²¹ hypothesized that an advanced maturation state of the DC could induce immunity rather than tolerance to paternally derived antigens and play a role in the etiology of spontaneous abortion. Experimental evidence for these hypotheses is limited, and further investigation is needed to confirm a role for decidual APCs in inducing fetal tolerance or anti-fetal immunity. In addition, the migratory capabilities of human decidual DCs to reach draining lymph nodes must be taken into consideration in light of the recent report that murine decidual DCs remain entrapped within the uterus during pregnancy.²⁴

In the placenta, the resident macrophages, or Hofbauer cells, constitute another source of B7 ligands. Although B7-1 is absent, B7-2 is expressed by placental macrophages.²⁵ This observation, along with their expression of class I and class II MHC,²⁶ supports a role for these cells in immunological reactions. Although T cells are normally absent from placental villi, villitis of unknown etiology (VUE) is associated with maternal CD4⁺ and CD8⁺ T-cell infiltration into the chorionic villi.^{27–29} The molecular pathogenesis leading to this phenomenon is unknown, but it has been proposed that VUE could be a reflection of maternal reaction to fetal antigen, possibly being presented by the macrophages. Infectious villitis is also characterized by maternal CD4⁺ and CD8⁺ T-cell infiltration, and it is conceivable that Hofbauer cells could present pathogen-derived antigens in this situation, leading to local immune responses.^{30,31}

A paucity of B7-1/-2 on immature DCs implies a passive role for these proteins in inducing tolerance. However, B7-1/-2 may also actively promote T-cell tolerance via back signaling into the APC. Reverse signaling through B7-1/-2 after ligation by a soluble form of CTLA-4 was shown to upregulate the tryptophan catabolic enzyme, indoleamine-2,3-dioxygenase (IDO).³² The potent immunosuppressive activity of IDO was first identified in pregnancy, in which chemical inhibition of IDO activity abolished allogenic pregnancy.³³ Although genetic deletion of IDO did not recapitulate the effect of enzyme inhibition,³⁴ other evidence supports a role for this protein in maternal–fetal immunotolerance. For example, human decidual monocytes and DCs upregulate IDO dramatically in response to either interferon (IFN)- γ or a CTLA-4/Fc fusion protein.³⁵ Higher B7-2 expression on decidual monocytes and DCs correlated with increased IDO production. This finding supports a potential

protective role for decidual DCs with a ‘mature’ phenotype, as suggested previously with their Th2 skewing ability.¹⁸ Indeed, levels of both IDO and B7 in this study correlated with pregnancy success, as both are decreased in cases of spontaneous abortion. It is speculated by these authors that *in vivo*, CTLA-4 could originate from T_{Regs} to stimulate IDO from decidual DCs. The protective role for CTLA-4 in pregnancy is supported by the fact that both the number of T_{Regs} and the level of CTLA-4 on T_{Regs} are lower in the decidua in cases of spontaneous abortion.³⁶ CTLA-4 expression by placental fibroblasts has also been reported,³⁷ providing another potential source of ligand to mediate reverse signaling at the maternal–fetal interface. IDO production following reverse signaling may also occur in placental macrophages. Hofbauer cells express both IDO²² and B7-2,²⁵ suggesting that CTLA-4 ligation of B7-2 on placental macrophages may induce IDO from these cells in the same manner as in decidual DCs.³⁵

Function of B7 Proteins in Murine Pregnancy

The role of B7-1 and B7-2 in pregnancy has been investigated in the ‘abortion-prone’ CBA × DBA mouse model using blocking antibodies administered at approximately the time of implantation. It was reported that blocking the signaling pathway of both B7-1 and B7-2 or B7-2 alone improved viability of fetuses³⁸. This was accompanied by an increase in the percentage of CD4⁺ CD25⁺ Treg cells expressing CTLA-4 as well as skewing toward a Th2 response. These authors also found that unfractionated T cells transferred from anti-B7-1/-2-treated mice into subsequent CBA × DBA matings were protective, suggesting that an anti-B7 antibody-induced population of T_{Regs} was able to suppress endogenous maternal immune reactivity to the fetus.³⁹ However, these results were not supported in another abortion-prone model of allogeneic pregnancy, where blocking B7-2 did not improve fetal viability in CBA × B6 breedings.⁴⁰

B7-H1, B7-DC, and their shared receptor, PD-1

B7-H1 was identified 10 years ago through searches of the expressed sequence tag database for molecules containing homology to B7-1 and B7-2.^{41,42} It shares approximately 20% amino acid sequence identity with B7-1 and B7-2. Such low levels of sequence homology are commonly seen among members of the B7 family, which instead share high levels of similarity in their secondary and tertiary structures.⁴³ B7-H1 mRNA has been found to be broadly distributed among many tissues, but its protein distribution is more restricted, suggesting that post-transcriptional mechanisms may have an important role in controlling B7-H1 expression, an idea that has received experimental support from our laboratory and others.^{44,45} However, its expression can also be induced in parenchymal cells of most organs under inflammatory conditions. Constitutive B7-H1 expression occurs on only a few cells: APCs, lymphocytes, cardiac endothelial cells, and, notably, trophoblast cells.

The cellular expression on both APCs and parenchymal cells reflects the ability of B7-H1 to interfere with T-cell activation at both the priming and effector stages of the immune response within lymphoid organs and peripheral tissues, respectively.⁴⁶ B7-H1 possesses both costimulatory and inhibitory actions on T cells. The basis of its stimulatory function is not well understood but is thought to occur through an as yet unidentified receptor.⁴³ In contrast, many of the studies published to date have focused on its inhibitory function, which occurs by signaling through programmed death-1 (PD-1). B7-H1 shares this receptor with the related B7 family member B7-DC. B7-DC appears to have higher affinity for PD-1 than B7-H1,⁴⁷ but its expression is much more limited than B7-H1, and is found predominantly on macrophages and DCs following cytokine induction.⁴⁸ Like B7-H1, B7-DC exhibits dual inhibitory and stimulatory functions, but its restricted expression to APCs suggests that it primarily affects the priming stage of immune responses.^{49,50} PD-1 is expressed on activated T cells, B cells, and cells of the myeloid lineage and contains two

cytoplasmic signaling domains consisting of an intracellular tyrosine inhibitory motif (ITIM) and an intracellular tyrosine switch motif (ITSM).⁵¹ *In vitro* studies have suggested that the ITSM on PD-1 is critical for its inhibitory activity and acts by recruiting SHP-1 and/or SHP-2 phosphatases which then interfere with CD28 signaling by preventing activation of phosphoinositide 3-kinase (PI3K) activation – a critical enzyme in CD28 signaling.^{52–54}

The ultimate effect of PD-1 ligation on self-reactive T cells can be apoptosis or anergy. This regulatory pathway appears essential, as peripheral tolerance to some MHC class I-restricted self-antigens requires PD-1.^{55,56} In addition, genetic deletion of PD-1 results in severe autoimmunity because of the loss of peripheral tolerance of self-reactive T cells.^{57,58} Blocking PD-1 accelerated the onset and worsened the severity of both spontaneous and induced autoimmune disease.^{59,60} Similarly, accumulation of self-reactive T cells occurs when B7-H1 and B7-DC are depleted, resulting in increased susceptibility to induced autoimmune disease.^{46,61} T-cell exhaustion, a state of gradually acquired unresponsiveness to antigen, can also occur when PD-1 is chronically ligated by B7-H1, although this phenomenon has only been implicated in the failure to clear infection, and it is not certain whether this occurs in tolerance to self-antigen.⁶² Finally, B7-H1 has also recently been recognized to have a novel role in inducing differentiation of Tregs from naïve CD4⁺ T cells.^{63,64}

There is also evidence that binding of PD-1 to B7-H1 or B7-DC can induce signaling through their intracellular domains, back into the APC; although the biological roles of this reverse signaling are less clear. Tumor cells receiving this signal become resistant to CTL-induced cytotoxicity, without the requirement for PD-1 signaling into the T cell.⁶⁵ The signaling mechanism for this remains enigmatic but does require the approximately 30 amino acid, evolutionarily conserved cytoplasmic domain of B7-H1. Reverse signaling appears to occur through B7-DC as well. Antibody cross-linking of B7-DC on DCs was found to enhance antigen presentation and secretion of the Th1 cytokine IL-12.⁶⁶ In contrast, soluble PD-1 binding to B7-H1 and/or B7-DC on DCs inhibited their maturation and induced IL-10 production, thus promoting a more suppressive phenotype.⁶⁷ Together, these results demonstrate that signals delivered through B7-H1/-DC affect APCs; however, the downstream effect of these signals vary, and it is unclear what dictates the outcome of reverse signals because these effects have been studied with isolated cell populations *in vitro*.

B7-H1, B7-DC, and PD-1 at the Human Maternal–Fetal Interface

Decidual stromal cells express class I MHC and can express class II MHC *in vitro* after treatment with proinflammatory cytokines, therefore raising the possibility that they can present antigen. These cells lack the costimulatory molecules B7-1 and B7-2 but express B7-H1 and B7-DC.^{25,68} *In vitro*, it was found that decidual stromal cells could stimulate an allogeneic reaction from unrelated CD4⁺ T cells, a response that was kept in check by B7-H1 and B7-DC.⁶⁸ The potential role of these cells in controlling an immune response during pregnancy poses an interesting question that warrants further investigation. For example, they might play a role in local control of T-cell responses to infection by presenting foreign antigen, with the inhibitory B7s tailoring cytokine production to an appropriate balance for the maternal–fetal environment. Whether or not these cells could present fetal antigen and play a role in tolerance to the fetus has not yet been investigated.

In the human placenta, B7-H1 is expressed by all trophoblast populations.^{25,69} Its expression is increased during placental development, possibly attributed to the increases in oxygen levels from the first to the second trimester concurrent with the influx of maternal blood to the placenta.⁷⁰ In addition, syncytiotrophoblast expresses more B7-H1 than does cytotrophoblast, its immediate precursor. Treatment with epidermal growth factor *in vitro*,

which promotes syncytialization, recapitulates this effect by the post-transcriptional mechanism of shifting mRNA to the polysomes.⁴⁴ This mechanism of regulation is in line with B7-H1 expression being regulated post-transcriptionally, which is likely, as mentioned previously, based on the broad distribution of its mRNA compared with the more restricted expression of B7-H1 protein.

Although B7-H1 is occasionally expressed by placental macrophages (M. Petroff, unpublished observations), the syncytiotrophoblast and extravillous trophoblast cells are the major sources of the protein at the human maternal–fetal interface. Our laboratory has identified PD-1 expression on CD4⁺ and CD8⁺ T cells, as well as CD4⁺ CD25⁺ FoxP3⁺ T_{Regs} isolated from the decidua. Using a human choriocarcinoma cell line transfected with B7-H1, we showed that B7-H1 promotes Th2 but suppresses Th1 cytokine production by decidual lymphocytes.⁷¹ These results highlight an interesting conundrum regarding B7-H1 function in trophoblast cells. Others have shown that both costimulatory and co-inhibitory signals provided by B7 family proteins are required to act in *cis* with the MHC – that is, they are required to arise from the same cellular surface, probably to ensure that PD-1 signaling effectors are brought into sufficiently close proximity to affect CD28/TCR signaling.⁷² The situation may differ at the maternal–fetal interface, however, because of the unique patterning of MHC molecules in placental cells. Syncytiotrophoblast, which abundantly expresses B7-H1, represses virtually all MHC expression, effectively ruling out the possibility that in *cis* signaling to the T cell with MHC would occur from these cells. Our data suggest that these cells can in fact suppress TCR-mediated events on T cells in *trans*.⁷¹ Other trophoblast cells express B7-H1, including extravillous trophoblast cells, that express a restricted array of MHC. Although most investigators do not consider these cells to function as APCs, which possibility has not been formally ruled out. B7-H1 and HLA-G, for example, are co-expressed on the surface of invading cytotrophoblast cells and those found in the chorion membrane (Fig. 2). Another possibility is that reverse-signaling through B7-H1 can occur, transmitting a signal not to the lymphocyte, but to the syncytiotrophoblast and/or cytotrophoblast itself.

Functions of B7-H1 in Murine Pregnancy

In the mouse, it is not entirely clear as yet whether the trophoblast, decidua, or both express B7-H1.^{40,48} Nonetheless, given its suppressive role in controlling self-reactive T cells and autoimmunity, we and others tested whether maternal B7-H1 or PD-1 is mandatory for successful allogeneic pregnancy. Guleria and colleagues reported that systemic blockade of B7-H1 but not B7-DC disrupted allogeneic, but not syngeneic, pregnancy in mice.⁴⁰ Fetal resorption was also observed in allogeneic pregnancies using B7-H1-deficient mice. This group also found that B7-H1 may influence the local cytokine milieu at the maternal–fetal interface, as IFN- γ and IL-17 were increased, whereas IL-4 and IL-5 were reduced in the placenta of B7-H1-deficient mice.⁷³ These authors additionally provide evidence to propose that the requirement for B7-H1 in allogeneic pregnancy lies in its utilization by maternal T_{Regs} to control maternal anti-fetal T cells.⁷³ On the other hand, we have shown in several models of pregnancy that genetic deletion or blockade of PD-1 has no obvious detrimental effect on pregnancy (Fig. 3).⁷⁴ Similarly, in our hands, dams lacking B7-H1 carry allogeneic pups to term unimpeded.⁷⁴ We carried these studies a step further to discern whether PD-1 on maternal T cells play any role in the maternal response to fetal antigen. Adopting a model of a defined fetal alloantigen, ovalbumin, combined with maternal anti-ovalbumin T cells, we showed that PD-1 prevents over-accumulation of fetal antigen-specific T cells in maternal lymphoid organs, possibly via a mechanism involving apoptosis.⁷⁴ These results suggest that PD-1 is involved in maternal tolerance to fetal alloantigen insofar as it controls their abundance, but our fertility data do not argue for a critical role for PD-1 in suppressing cytotoxic maternal T-cell responses to the fetus. It remains to be determined whether these

results reflect a redundancy of functions of the B7-H1/PD-1 pathway with other immunomodulatory proteins and their receptors, including other members of the B7 and CD28 families.

Other members of the B7 family: B7-H2, B7-H3, B7-H4, and B7-H6

B7-H2 was identified independently by several laboratories and, like B7-H1, is broadly expressed at the mRNA level. B7-H2 protein is more restricted and is primarily found on B cells, macrophages, and DCs but can also be detected on fibroblasts, endothelial cells, and epithelial cells. B7-H2 serves as the ligand for inducible costimulator of T cells (ICOS), another CD28 family molecule present on T cells, and provides a positive stimulatory effect that promotes T-cell activation, differentiation, and effector responses^{75,76} In addition, B7-H2 plays a critical role in T-cell-dependent B-cell responses, as demonstrated by defects in germinal center formation and antibody class switching in B7-H2-deficient and ICOS-deficient mice.^{77,78} ICOS is not present on naïve T cells but is rapidly induced upon activation and remains expressed on memory T cells.^{76,78} Although ICOS stimulates IFN- γ , IL-4, and IL-10 production by T cells, it most effectively induces IL-10^{79,80} Notably, ICOS does not induce IL-2 production, which distinguishes its costimulatory function from that of CD28. ICOS also stabilizes IL-10R expression on T cells, rendering them sensitive to IL-10.⁸¹ Evidence suggests that ICOS directs T cells toward Th2 effector functions, as its expression is elevated on Th2 cells compared to Th1 cells, and because blockade of ICOS *in vitro* polarizes T cells toward Th1 cytokine production.⁸⁰ Additional functions for B7-H2 have been identified in other immune processes. B7-H2 on DCs has been demonstrated to be involved in the development of T_{Regs} that secrete IL-10⁸² Likewise, in humans, ICOS has been implicated in the induction of anergic, IL-10-producing CD4⁺ T_{Regs} following their interaction with tolerogenic DCs.^{81,83} In natural killer cells, ICOS can be upregulated by IL-2, IL-12, and IL-15 and was shown to enhance their cytotoxicity and promote IFN- γ production.⁸⁴ The function for B7-H2 in pregnancy has not been assessed; however, B7-H2 is present at the maternal-fetal interface and thus may play a role in regulating local immune responses. B7-H2 mRNA was identified in the embryonic yolk sac by Ling et al.,⁸⁵ and we have found that B7-H2 is highly expressed on extravillous trophoblast cells.⁸⁶ Given the reported importance of B7-H2 in Th2 effector function, it will be interesting to learn the role of this protein in pregnancy.

Like many of the other B7 family proteins, B7-H3 has been implicated in both inhibitory and stimulatory actions on T cells, affecting both proliferation and cytokine production.^{87,88} B7-H3 is structurally organized similarly to other B7 proteins in the mouse, but it is of note that the predominant form of B7-H3 in humans contains tandemly duplicated IgV-IgC extracellular domains.^{89,90} Like other B7 family members, B7-H3 mRNA is broadly expressed, but protein expression is restricted. B7-H3 protein can be detected on human myeloid DCs but can only be detected following induction with inflammatory stimuli in other leukocyte populations in both humans and mice.^{87,91,92} The triggering receptor expressed on myeloid cells (TREM)-like transcript 2 (TLT-2) has been identified as a stimulatory counter receptor for B7-H3 on T cells, although this finding is controversial.^{93,94} Studies with B7-H3-deficient mice support an inhibitory function for B7-H3, displaying elevated T-cell responses in several experimental settings.⁹¹ B7-H3 also appears to have an important function outside the immune system, as B7-H3-deficient mice exhibit reduced bone strength because of impaired osteoblast differentiation.⁹⁵ In relation to pregnancy, B7-H3 expression is observed in the villous placenta and changes with advancing gestation, starting within the mesenchymal cells of villi early, and shifting to the syncytiotrophoblast by term.⁸⁶ The role of B7-H3 in pregnancy is unknown.

B7-H4 is another B7 family protein that has been shown to exhibit negative costimulatory activity on T cells, including inhibiting proliferation and cytokine production.^{96,97} As with the other B7 family members, B7-H4 mRNA is widely distributed, including in human placenta.⁹⁶ B7-H4 protein expression appears to be restricted to activated hematopoietic cells in humans, but murine B cells constitutively express B7-H4.^{96,97} Although the CD28 family member B and T lymphocyte attenuator (BTLA) was initially proposed as a counter-receptor for B7-H4, this no longer seems likely as herpes virus entry mediator (HVEM) is now considered the unique ligand for BTLA.⁹⁸ T cells express the unknown receptor for B7-H4 following activation.^{96,97} Studies using B7-H4-deficient mice suggest that B7-H4 suppresses Th1 immune responses and also inhibits expansion of neutrophils from their progenitors.^{99,100} Reverse signaling through B7-H4 has also been reported in EBV-transformed B cells, resulting in upregulation of FasL and subsequent apoptosis.¹⁰¹ The role of B7-H4 in pregnancy has not been addressed; however, B7-H4 has been detected on decidual macrophages from term decidua basalis by flow cytometry¹⁰² and may therefore potentially affect pregnancy in some manner.

B7-H6 is the newest member to the B7 family. It is an activating ligand for the NK receptor, NKp30, and appears to be involved in inducing NK lysis of tumor targets.¹⁰³ Expression of B7-H6 appears to be highly restricted to tumor cells. In contrast to other B7 family members, B7-H6 mRNA was not detected in any normal tissues, and surface protein expression was absent on both freshly isolated and activated PBMCs.¹⁰³ Although further examination for B7-H6 is still required, given its function in tumor cell lysis and its paucity of expression, it seems unlikely to play a significant role in pregnancy.

Conclusions and future directions

The unique regulation and patterning of B7 family molecules in the placenta, together with emerging empirical data, suggests that these proteins may play an important role in shaping the milieu of the local maternal–fetal environment. In addition, the nature of the costimulatory and co-inhibitory signals B7 family members provide will also influence the outcome of the interaction of maternal lymphocytes with fetal antigen in lymphoid tissues. From the experimental data in humans, we can infer that B7 family proteins could function in at least three distinct capacities (Fig. 4). First, the B7 expressing cells in pregnancy that could function as APCs, i.e., those that express both B7 molecules and MHC, may directly influence T-cell activation and effector functions by delivering a positive or negative costimulatory signal in conjunction with TCR stimulation. Second, trophoblast cells that repress MHC might affect lymphocytes through B7/CD28 family molecules in *trans*. Finally, B7 molecules on either decidual APCs or trophoblast cells may backsignal toward the B7-expressing cell and influence the local immune environment through induced expression of immunosuppressive factors independently of their effects on T cells. Thus, in determining the functions of these key regulators of the immune system, there is a need to think ‘outside the box’ when considering B7 family molecules during pregnancy.

Acknowledgments

The authors thank Sarika Kshirsagar and Joseph Juscus for their technical contributions and Stanton Fernald (University of Kansas Interdisciplinary Center for Male Contraceptive Research & Drug Development Imaging Core) for assistance with images. A.L.P. is supported by NIH training grant T32HD007455. This work is supported by NIH grants R01 HD045611, P01 HD049480, and P20 RR16475.

References

1. Erlebacher A, Vencato D, Price KA, Zhang D, Glimcher LH. Constraints in antigen presentation severely restrict T cell recognition of the allogeneic fetus. *J Clin Invest.* 2007; 117:1399–1411. [PubMed: 17446933]
2. Moldenhauer LM, Hayball JD, Robertson SA. Conceptus antigens activate the maternal immune response in pregnancy utilising maternal antigen presenting cells. *J Immunol.* 2009; 71:148.
3. Taglauer ES, Waldorf KM, Adams, Petroff MG. The hidden maternal–fetal interface: events involving the lymphoid organs in maternal–fetal tolerance. *Int J Dev Biol.* 2010; 54:421–430. [PubMed: 19876825]
4. Waldorf, KM, Adams; Yan, Z.; Stevens, AM.; Nelson, JL. The changing maternal “self” hypothesis: a mechanism for maternal tolerance of the fetus. *Placenta.* 2007; 28:378–382. [PubMed: 16934327]
5. Jenkins MK, Pardoll DM, Mizuguchi J, Quill H, Schwartz RH. T-cell unresponsiveness in vivo and in vitro: fine specificity of induction and molecular characterization of the unresponsive state. *Immunol Rev.* 1987; 95:113–135. [PubMed: 2437012]
6. Gimmi C, Freeman G, Gribben J, Sugita K, Freedman A, Morimoto C, Nadler L. B-cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. *Proc Natl Acad Sci U S A.* 1991; 88:6575–6579. [PubMed: 1650475]
7. Gimmi CD, Freeman GJ, Gribben JG, Gray G, Nadler LM. Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci U S A.* 1993; 90:6586–6590. [PubMed: 7688125]
8. van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. *J Exp Med.* 1997; 185:393–403. [PubMed: 9053440]
9. Ostrov DA, Shi W, Schwartz JC, Almo SC, Nathenson SG. Structure of murine CTLA-4 and its role in modulating T cell responsiveness. *Science.* 2000; 290:816–819. [PubMed: 11052947]
10. Walunas TL, Bakker CY, Bluestone JA. CTLA-4 ligation blocks CD28-dependent T cell activation. *J Exp Med.* 1996; 183:2541–2550. [PubMed: 8676075]
11. Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. *J Exp Med.* 1996; 183:2533–2540. [PubMed: 8676074]
12. Bachmann MF, Kohler G, Ecabert B, Mak TW, Kopf M. Cutting edge: lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous. *J Immunol.* 1999; 163:1128–1131. [PubMed: 10415006]
13. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, Appleby M, Der SD, Kang J, Chambers CA. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J Exp Med.* 2009; 206:421–434. [PubMed: 19188497]
14. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science.* 2008; 322:271–275. [PubMed: 18845758]
15. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunol Rev.* 2008; 224:166–182. [PubMed: 18759926]
16. Powell J, Ragheb J, Kitagawa-Sakakida S, Schwartz R. Molecular regulation of interleukin-2 expression by CD28 co-stimulation and anergy. *Immunol Rev.* 1998; 165:287–300. [PubMed: 9850868]
17. Olivares EG, Montes MJ, Oliver C, Galindo JA, Ruiz C. Cultured human decidual stromal cells express B7-1 (CD80) and B7-2 (CD86) and stimulate allogeneic T cells. *Biol Reprod.* 1997; 57:609–615. [PubMed: 9282998]
18. Miyazaki S, Tsuda H, Sakai M, Hori S, Sasaki Y, Futatani T, Miyawaki T, Saito S. Predominance of Th2-promoting dendritic cells in early human pregnancy decidua. *J Leukoc Biol.* 2003; 74:514–522. [PubMed: 12960246]
19. Breburda EE, Dambaeva SV, Slukvin II, Golos TG. Selective distribution and pregnancy-specific expression of DC-SIGN at the maternal–fetal interface in the rhesus macaque: DC-SIGN is a putative marker of the recognition of pregnancy. *Placenta.* 2006; 27:11–21. [PubMed: 16310033]

20. Gardner L, Moffett A. Dendritic cells in the human decidua. *Biol Reprod.* 2003; 69:1438–1446. [PubMed: 12826583]
21. Blois SM, Kammerer U, Soto CA, Tometten MC, Shaikly V, Barrientos G, Jurd R, Rukavina D, Thomson AW, Klapp BF, Fernandez N, Arck PC. Dendritic cells: key to fetal tolerance? *Biol Reprod.* 2007; 77:590–598. [PubMed: 17596562]
22. Heikkinen J, Mottonen M, Komi J, Alanen A, Lassila O. Phenotypic characterization of human decidual macrophages. *Clin Exp Immunol.* 2003; 131:498–505. [PubMed: 12605704]
23. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol.* 2002; 2:151–161. [PubMed: 11913066]
24. Collins MK, Tay CS, Erlebacher A. Dendritic cell entrapment within the pregnant uterus inhibits immune surveillance of the maternal/fetal interface in mice. *J Clin Invest.* 2009; 119:2062–2073. [PubMed: 19546507]
25. Petroff MG, Chen L, Phillips TA, Azzola D, Sedlmayr P, Hunt JS. B7 family molecules are favorably positioned at the human maternal–fetal interface. *Biol Reprod.* 2003; 68:1496–1504. [PubMed: 12606489]
26. Sutton L, Gadd M, Mason DY, Redman CW. Cells bearing class II MHC antigens in the human placenta. *Immunology.* 1986; 58:23–29. [PubMed: 3086220]
27. Labarrere CA, Faulk WP. Maternal cells in chorionic villi from placentae of normal and abnormal human pregnancies. *Am J Reprod Immunol.* 1995; 33:54–59. [PubMed: 7619234]
28. Redline RW, Patterson P. Villitis of unknown etiology is associated with major infiltration of fetal tissue by maternal inflammatory cells. *Am J Pathol.* 1993; 143:473–479. [PubMed: 8342596]
29. Kim J-S, Romero R, Kim MR, Kim YM, Friel L, Espinoza J, Kim CJ. Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. *Histopathology.* 2008; 52:457–464. [PubMed: 18315598]
30. Kapur P, Rakheja D, Gomez AM, Sheffield J, Sanchez P, Rogers BB. Characterization of inflammation in syphilitic villitis and in villitis of unknown etiology. *Pediatr Dev Pathol.* 2004; 7:453–458. [PubMed: 15547769]
31. Brito H, Juliano P, Altemani C, Altemani A. Is the immunohistochemical study of the inflammatory infiltrate helpful in distinguishing villitis of unknown etiology from non-specific infection villitis? *Placenta.* 2005; 26:839–841. [PubMed: 16169075]
32. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, Puccetti P. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol.* 2002; 3:1097–1101. [PubMed: 12368911]
33. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science.* 1998; 281:1191–1193. [PubMed: 9712583]
34. Baban B, Chandler P, McCool D, Marshall B, Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase expression is restricted to fetal trophoblast giant cells during murine gestation and is maternal genome specific. *J Reprod Immunol.* 2004; 61:67–77. [PubMed: 15063630]
35. Miwa N, Hayakawa S, Miyazaki S, Myojo S, Sasaki Y, Sakai M, Takikawa O, Saito S. IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon- γ increase in normal pregnancy but decrease in spontaneous abortion. *Mol Hum Reprod.* 2005; 11:865–870. [PubMed: 16421220]
36. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+ CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol Hum Reprod.* 2004; 10:347–353. [PubMed: 14997000]
37. Kaufman KA, Bowen JA, Tsai AF, Bluestone JA, Hunt JS, Ober C. The CTLA-4 gene is expressed in placental fibroblasts. *Mol Hum Reprod.* 1999; 5:84–87. [PubMed: 10050667]
38. Jin L-P, Zhou Y-H, Wang M-Y, Zhu X-Y, Li D-J. Blockade of CD80 and CD86 at the time of implantation inhibits maternal rejection to the allogeneic fetus in abortion-prone matings. *J Reprod Immunol.* 2005; 65:133–146. [PubMed: 15811518]
39. Jin L-P, Li D-J, Zhang J-P, Wang M-Y, Zhu X-Y, Zhu Y, Meng Y, Yuan M-M. Adoptive transfer of paternal antigen-hyporesponsive T cells induces maternal tolerance to the allogeneic fetus in abortion-prone matings. *J Immunol.* 2004; 173:3612–3619. [PubMed: 15356105]

40. Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, Noelle RJ, Coyle A, Mellor AL, Khoury SJ, Sayegh MH. A critical role for the programmed death ligand 1 in fetomaternal tolerance. *J Exp Med.* 2005; 202:231–237. [PubMed: 16027236]
41. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T cell proliferation and interleukin 10 secretion. *Nat Med.* 1999; 5:1365–1369. [PubMed: 10581077]
42. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation. *J Exp Med.* 2000; 192:1027–1034. [PubMed: 11015443]
43. Wang S, Bajorath J, Flies DB, Dong H, Honjo T, Chen L. Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. *J Exp Med.* 2003; 197:1083–1091. [PubMed: 12719480]
44. Holets LM, Carletti MZ, Kshirsagar SK, Christenson LK, Petroff MG. Differentiation-induced post-transcriptional control of B7-H1 in human trophoblast cells. *Placenta.* 2009; 30:48–55. [PubMed: 19010538]
45. Gong A-Y, Zhou R, Hu G, Li X, Splinter PL, O'Hara SP, LaRusso NF, Soukup GA, Dong H, Chen X-M. MicroRNA-513 regulates B7-H1 translation and is involved in IFN- γ -induced B7-H1 expression in cholangiocytes. *J Immunol.* 2009; 182:1325–1333. [PubMed: 19155478]
46. Dong H, Zhu G, Tamada K, Flies DB, van Deursen JMA, Chen L. B7-H1 determines accumulation and deletion of intrahepatic CD8⁺ T lymphocytes. *Immunity.* 2004; 20:327–336. [PubMed: 15030776]
47. Youngnak P, Kozono Y, Kozono H, Iwai H, Otsuki N, Jin H, Omura K, Yagita H, Pardoll DM, Chen L, Azuma M. Differential binding properties of B7-H1 and B7-DC to programmed death-1. *Biochem Biophys Res Commun.* 2003; 307:672–677. [PubMed: 12893276]
48. Liang SC, Latchman YE, Buhlmann JE, Tomczak MF, Horwitz BH, Freeman GJ, Sharpe AH. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. *Eur J Immunol.* 2003; 33:2706–2716. [PubMed: 14515254]
49. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, Greenfield EA, Bourque K, Boussiotis VA, Carter LL, Carreno BM, Malenkovich N, Nishimura H, Okazaki T, Honjo T, Sharpe AH, Freeman GJ. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol.* 2001; 2:261–268. [PubMed: 11224527]
50. Tseng S-Y, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, Shalabi A, Shin T, Pardoll DM, Tsuchiya H. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med.* 2001; 193:839–846. [PubMed: 11283156]
51. Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, Shin T, Tsuchiya H, Pardoll DM, Okumura K, Azuma M, Yagita H. Expression of programmed death 1 ligands by murine T cells and APC. *J Immunol.* 2002; 169:5538–5545. [PubMed: 12421930]
52. Okazaki T. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. *Proc Natl Acad Sci USA.* 2001; 98:13866–13871. [PubMed: 11698646]
53. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J Immunol.* 2004; 173:945–954. [PubMed: 15240681]
54. Riley JL. PD-1 signaling in primary T cells. *Immunol Rev.* 2009; 229:114–125. [PubMed: 19426218]
55. Probst HC, McCoy K, Okazaki T, Honjo T, van den Broek M. Resting dendritic cells induce peripheral CD8⁺ T cell tolerance through PD-1 and CTLA-4. *Nat Immunol.* 2005; 6:280–286. [PubMed: 15685176]
56. Keir ME, Freeman GJ, Sharpe AH. PD-1 regulates self-reactive CD8⁺ T cell responses to antigen in lymph nodes and tissues. *J Immunol.* 2007; 179:5064–5070. [PubMed: 17911591]

57. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, Honjo T. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science*. 2001; 291:319–322. [PubMed: 11209085]
58. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*. 1999; 11:141–151. [PubMed: 10485649]
59. Ansari MJ, Salama AD, Chitnis T, Smith RN, Yagita H, Akiba H, Yamazaki T, Azuma M, Iwai H, Khoury SJ, Auchincloss H Jr, Sayegh MH. The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med*. 2003; 198:63–69. [PubMed: 12847137]
60. Salama AD, Chitnis T, Imitola J, Ansari MJ, Akiba H, Tushima F, Azuma M, Yagita H, Sayegh MH, Khoury SJ. Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. *J Exp Med*. 2003; 198:71–78. [PubMed: 12847138]
61. Latchman YE, Liang SC, Wu Y, Chernova T, Sobel RA, Klemm M, Kuchroo VK, Freeman GJ, Sharpe AH. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. *PNAS*. 2004; 101:10691–10696. [PubMed: 15249675]
62. Shin H, Wherry EJ. CD8 T cell dysfunction during chronic viral infection. *Curr Opin Immunol*. 2007; 19:408–415. [PubMed: 17656078]
63. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med*. 2009; 206:3015–3029. [PubMed: 20008522]
64. Wang L, Pino-Lagos K, de Vries VC, Guleria I, Sayegh MH, Noelle RJ. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. *Proc Natl Acad Sci U S A*. 2008; 105:9331–9336.
65. Azuma T, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood*. 2008; 111:3635–3643. [PubMed: 18223165]
66. Nguyen LT, Radhakrishnan S, Ciric B, Tamada K, Shin T, Pardoll DM, Chen L, Rodriguez M, Pease LR. Cross-linking the B7 family molecule B7-DC directly activates immune functions of dendritic cells. *J Exp Med*. 2002; 196:1393–1398. [PubMed: 12438431]
67. Kuipers H, Muskens F, Willart M, Hijdra D, Friso BJ, Assema, Anthony J Coyle, Henk C Hoogsteden, Bart N Lambrecht. Contribution of the PD-1 ligands/PD-1 signaling pathway to dendritic cell-mediated CD4⁺ T cell activation. *Eur J Immunol*. 2006; 36:2472–2482. [PubMed: 16917960]
68. Nagamatsu T, Schust DJ, Sugimoto J, Barrier BF. Human decidual stromal cells suppress cytokine secretion by allogeneic CD4⁺ T cells via PD-1 ligand interactions. *Hum Reprod*. 2009; 24:3160–3171. [PubMed: 19729380]
69. Petroff MG, Chen L, Phillips TA, Hunt JS. B7 family molecules: novel immunomodulators at the maternal–fetal interface. *Placenta*. 2002; 23(Suppl. A):S95–S101. [PubMed: 11978065]
70. Holets LM, Hunt JS, Petroff MG. Trophoblast CD274 (B7-H1) is differentially expressed across gestation: influence of oxygen concentration. *Biol Reprod*. 2006; 74:352–358. [PubMed: 16251499]
71. Taglauer ES, Trikhacheva AS, Slusser JG, Petroff MG. Expression and function of PDCD1 at the human maternal–fetal interface. *Biol Reprod*. 2008; 79:562–569. [PubMed: 18550794]
72. Bennett F, Luxenberg D, Ling V, Wang IM, Marquette K, Lowe D, Khan N, Veldman G, Jacobs KA, Valge-Archer VE, Collins M, Carreno BM. Program death-1 engagement upon TCR activation has distinct effects on costimulation and cytokine-driven proliferation: attenuation of ICOS, IL-4, and IL-21, but not CD28, IL-7, and IL-15 responses. *J Immunol*. 2003; 170:711–718. [PubMed: 12517932]
73. Habicht A, Dada S, Jurewicz M, Fife BT, Yagita H, Azuma M, Sayegh MH, Guleria I. A link between PDL1 and T regulatory cells in fetomaternal tolerance. *J Immunol*. 2007; 179:5211–5219. [PubMed: 17911606]
74. Taglauer ES, Yankee TM, Petroff MG. Maternal PD-1 regulates accumulation of fetal antigen-specific CD8⁺ T cells in pregnancy. *J Reprod Immunol*. 2009; 80:12–21. [PubMed: 19368976]

75. Yoshinaga SK, Whoriskey JS, Khare SD, Sarmiento U, Guo J, Horan T, Shih G, Zhang M, Coccia MA, Kohno T, Tafuri-Bladt A, Brankow D, Campbell P, Chang D, Chiu L, Dai T, Duncan G, Elliott GS, Hui A, McCabe SM, Scully S, Shahinian A, Shaklee CL, Van G, Mak TW, Senaldi G. T-cell co-stimulation through B7RP-1 and ICOS. *Nature*. 1999; 402:827–832. [PubMed: 10617205]
76. Coyle AJ, Lehar S, Lloyd C, Tian J, Delaney T, Manning S, Nguyen T, Burwell T, Schneider H, Gonzalo JA, Gosselin M, Owen LR, Rudd CE, Gutierrez-Ramos JC. The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity*. 2000; 13:95–105. [PubMed: 10933398]
77. McAdam AJ, Greenwald RJ, Levin MA, Chernova T, Malenkovich N, Ling V, Freeman GJ, Sharpe AH. ICOS is critical for CD40-mediated antibody class switching. *Nature*. 2001; 409:102–105. [PubMed: 11343122]
78. Tafuri A, Shahinian A, Bladt F, Yoshinaga SK, Jordana M, Wakeham A, Boucher LM, Bouchard D, Chan VS, Duncan G, Odermatt B, Ho A, Itie A, Horan T, Whoriskey JS, Pawson T, Penninger JM, Ohashi PS, Mak TW. ICOS is essential for effective T-helper-cell responses. *Nature*. 2001; 409:105–109. [PubMed: 11343123]
79. Hutloff A, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, Anagnostopoulos I, Kroczeck RA. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature*. 1999; 397:263–266. [PubMed: 9930702]
80. McAdam AJ, Chang TT, Lumelsky AE, Greenfield EA, Boussiotis VA, Duke-Cohan JS, Chernova T, Malenkovich N, Jabs C, Kuchroo VK, Ling V, Collins M, Sharpe AH, Freeman GJ. Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4+ T cells. *J Immunol*. 2000; 165:5035–5040. [PubMed: 11046032]
81. Tuettenberg A, Huter E, Hubo M, Horn J, Knop J, Grimbacher B, Kroczeck RA, Stoll S, Jonuleit H. The role of ICOS in directing T cell responses: ICOS-dependent induction of T cell anergy by tolerogenic dendritic cells. *J Immunol*. 2009; 182:3349–3356. [PubMed: 19265111]
82. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med*. 2002; 8:1024–1032. [PubMed: 12145647]
83. Vermeiren J, Ceuppens JL, Van Ghelue M, Witters P, Bullens D, Mages HW, Kroczeck RA, Van Gool SW. Human T cell activation by costimulatory signal-deficient allogeneic cells induces inducible costimulator-expressing anergic T cells with regulatory cell activity. *J Immunol*. 2004; 172:5371–5378. [PubMed: 15100277]
84. Ogasawara K, Yoshinaga SK, Lanier LL. Inducible costimulator costimulates cytotoxic activity and IFN-gamma production in activated murine NK cells. *J Immunol*. 2002; 169:3676–3685. [PubMed: 12244160]
85. Ling V, Wu PW, Finnerty HF, Bean KM, Spaulding V, Fouser LA, Leonard JP, Hunter SE, Zollner R, Thomas JL, Miyashiro JS, Jacobs KA, Collins M. Cutting Edge: identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor. *J Immunol*. 2000; 164:1653–1657. [PubMed: 10657606]
86. Petroff MG, Kharatyan E, Torry DS, Holets L. The immunomodulatory proteins B7-DC, B7-H2, and B7-H3 are differentially expressed across gestation in the human placenta. *Am J Pathol*. 2005; 167:465–473. [PubMed: 16049332]
87. Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, Dong H, Sica GL, Zhu G, Tamada K, Chen L. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. *Nat Immunol*. 2001; 2:269–274. [PubMed: 11224528]
88. Prasad DV, Nguyen T, Li Z, Yang Y, Duong J, Wang Y, Dong C. Murine B7-H3 is a negative regulator of T cells. *J Immunol*. 2004; 173:2500–2506. [PubMed: 15294965]
89. Sun M, Richards S, Prasad DV, Mai XM, Rudensky A, Dong C. Characterization of mouse and human B7-H3 genes. *J Immunol*. 2002; 168:6294–6297. [PubMed: 12055244]
90. Ling V, Wu PW, Spaulding V, Kieleczawa J, Luxenberg D, Carreno BM, Collins M. Duplication of primate and rodent B7-H3 immunoglobulin V- and C-like domains: divergent history of functional redundancy and exon loss. *Genomics*. 2003; 82:365–377. [PubMed: 12906861]

91. Suh WK, Gajewska BU, Okada H, Gronski MA, Bertram EM, Dawicki W, Duncan GS, Bukczynski J, Plyte S, Elia A, Wakeham A, Itie A, Chung S, Da Costa J, Arya S, Horan T, Campbell P, Gaida K, Ohashi PS, Watts TH, Yoshinaga SK, Bray MR, Jordana M, Mak TW. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol.* 2003; 4:899–906. [PubMed: 12925852]
92. Steinberger P, Majdic O, Derdak SV, Pfistershammer K, Kirchberger S, Klauser C, Zlabinger G, Pickl WF, Stockl J, Knapp W. Molecular characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. *J Immunol.* 2004; 172:2352–2359. [PubMed: 14764704]
93. Hashiguchi M, Kobori H, Ritprajak P, Kamimura Y, Kozono H, Azuma M. Triggering receptor expressed on myeloid cell-like transcript 2 (TLT-2) is a counter-receptor for B7-H3 and enhances T cell responses. *Proc Natl Acad Sci U S A.* 2008; 105:10495–10500. [PubMed: 18650384]
94. Leitner J, Klauser C, Pickl WF, Stöckl J, Majdic O, Bardet AF, Kreil DP, Dong C, Yamazaki T, Zlabinger G, Pfistershammer K, Steinberger P. B7-H3 is a potent inhibitor of human T-cell activation: no evidence for B7-H3 and TREML2 interaction. *Eur J Immunol.* 2009; 39:1754–1764. [PubMed: 19544488]
95. Suh WK, Wang SX, Jheon AH, Moreno L, Yoshinaga SK, Ganss B, Sodek J, Grynepas MD, Mak TW. The immune regulatory protein B7-H3 promotes osteoblast differentiation and bone mineralization. *Proc Natl Acad Sci U S A.* 2004; 101:12969–12973. [PubMed: 15317945]
96. Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, Chapoval AI, Flies DB, Bajorath J, Chen L. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity.* 2003; 18:849–861. [PubMed: 12818165]
97. Prasad DV, Richards S, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. *Immunity.* 2003; 18:863–873. [PubMed: 12818166]
98. Sedy JR, Gavrieli M, Potter KG, Hurchla MA, Lindsley RC, Hildner K, Scheu S, Pfeffer K, Ware CF, Murphy TL, Murphy KM. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat Immunol.* 2005; 6:90–98. [PubMed: 15568026]
99. Suh WK, Wang S, Duncan GS, Miyazaki Y, Cates E, Walker T, Gajewska BU, Deenick E, Dawicki W, Okada H, Wakeham A, Itie A, Watts TH, Ohashi PS, Jordana M, Yoshida H, Mak TW. Generation and characterization of B7-H4/B7S1/B7x-deficient mice. *Mol Cell Biol.* 2006; 26:6403–6411. [PubMed: 16914726]
100. Zhu G, Augustine MM, Azuma T, Luo L, Yao S, Anand S, Rietz AC, Huang J, Xu H, Flies AS, Flies SJ, Tamada K, Colonna M, van Deursen JM, Chen L. B7-H4-deficient mice display augmented neutrophil-mediated innate immunity. *Blood.* 2009; 113:1759–1767. [PubMed: 19109567]
101. Song H, Park G, Kim YS, Hur I, Kim H, Ryu JW, Lee HK, Cho DH, Choi IH, Lee WJ, Hur DY. B7-H4 reverse signaling induces the apoptosis of EBV-transformed B cells through Fas ligand upregulation. *Cancer Lett.* 2008; 266:227–237. [PubMed: 18417276]
102. Galazka K, Wicherek L, Pitynski K, Kijowski J, Zajac K, Bednarek W, Dutsch-Wicherek M, Rytlewski K, Kalinka J, Basta A, Majka M. Changes in the subpopulation of CD25+CD4+ and FOXP3+ regulatory T cells in decidua with respect to the progression of labor at term and the lack of analogical changes in the subpopulation of suppressive B7-H4+ macrophages – a preliminary report. *Am J Reprod Immunol.* 2009; 61:136–146. [PubMed: 19143677]
103. Brandt CS, Baratin M, Yi EC, Kennedy J, Gao Z, Fox B, Haldeman B, Ostrander CD, Kaifu T, Chabannon C, Moretta A, West R, Xu W, Vivier E, Levin SD. The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. *J Exp Med.* 2009; 206:1495–1503. [PubMed: 19528259]

Fig. 1. Overview of expression patterns of B7 family proteins at the human maternal–fetal interface. Expression of the B7 proteins is indicated by +; variable expression of the protein is indicated by -/+; and absence of the protein is indicated by -. cTB, cytotrophoblast; evTB, extravillous cytotrophoblast; Mφ, macrophages; sTB, syncytiotrophoblast. Reprinted from Am J Pathol 2005, 167:465–473 with permission from the American Society for Investigative Pathology.

Fig. 2.

Extravillous trophoblast cells express an unusual combination of HLA and B7 proteins. Term basal plate placenta from a normal pregnancy was subjected to dual color immunofluorescence using antibodies targeting HLA-G (clone MEM-G/9; alexa-fluor 488 secondary antibody) and B7-H1 (clone MIH1). Near complete colocalization was observed in three separate basal plate placentas. Trophoblast cells of the term chorion membrane as well as invading trophoblast cells from first-trimester placentas exhibited similar colocalization. Isotype-matched controls yielded negligible fluorescence.

Fig. 3. Lack of effect of PD-1 depletion on viability of mid-gestation fetuses. Wild-type (WT) or PD-1-deficient female mice ($n = 13$ for both), both on the CBA genetic background, were allogeneically bred to C57Bl/6 males, and killed on gestation day (gd) 13.5 (day of copulation, gd 0.5). Viable and non-viable fetuses were counted.

Fig. 4.

Trophoblast-derived B7 proteins may function in pregnancy in several different capacities. (a) B7s may regulate the outcome of interaction between trophoblast cells directly with T cells and other immune cells that express their counter-receptors. The implications for this are two-fold. (1) Trophoblast cells could provide both the first signal (MHC/antigen to TCR) and the second signal (B7 ligation of counter-receptor). This scenario is presumably only possible for those trophoblast cells that possess cell surface MHC – i.e. extravillous trophoblast cells that express HLA-C, -E, -F, and/or G. (2) A second possibility exists for trophoblast that lacks surface-associated MHC: the syncytiotrophoblast and possibly the villous cytotrophoblasts. The presence of copious quantities of B7 proteins on these cells together with the deficiency of MHC suggests that B7 ligates the counter-receptor on blood-borne lymphocytes and functions on its own, without concomitant MHC signaling. (b) B7s may also regulate the outcome of interactions between decidual APC with maternal lymphocytes. In this case, both signal 1 and signal 2 could be provided by the APC. With all of these scenarios, reverse-signaling can occur, transmitting a signal not to the lymphocyte, but rather to the syncytiotrophoblast, cytotrophoblast or decidual APC itself.

Table 1

Properties and Placental Expression of B7 Family Proteins and their Receptors

Ligand	Alternative names	Effects on T cells	Stimulatory Receptor	Inhibitory Receptor	Placental Expression	References
B7-1	CD80	Positive or negative	CD28	CTLA-4 (CD152)	None detected	25
B7-2	CD86	Positive or negative	CD28	CTLA-4	RNA, protein	25
B7-H1	CD274, PD-L1,	Positive or negative	Not determined	PD-1 (CD279)	RNA, protein	25, 69
B7-DC	CD273, PD-L2	Positive or negative	Not determined	PD-1	RNA, protein	86
B7-H2	CD275, B7h, B7RP-1, ICOSL hGL50	Positive	ICOS (CD278)	None	RNA, protein	86
B7-H3	CD276, B7RP-2	Positive or negative	Not determined	Not determined	RNA, protein	86
B7-H4	B7x, B7-S1	Negative	None	Not determined	RNA, no protein	Petroff unpublished
B7-H6		Not determined	NKp30	Not determined	None detected	103