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Inhibitory costimulation and anti-tumor immunity

Natalia Martin-Orozco^a and **Chen Dong^a**

^a Department of Immunology, The University of Texas M. D. Anderson Cancer Center

Abstract

Costimulation was originally shown to be important in T-cell activation and effector differentiation. Recent characterization of B7/butrophilin and members of the CD28 superfamily has revealed a large number of negative costimulatory molecules that dampen T-cell activation and regulate immune tolerance. Some of these molecules have been shown to be upregulated in the tumor microenvironment and may serve as potential targets for augmenting anti-tumor immunity. In this article, we summarize recent developments in the field of inhibitory costimulation and discuss the future direction of therapeutic manipulation of inhibitory costimulation in tumor immunotherapy.

Keywords

Costimulation; B7; anti-tumor immunity; tolerance

Introduction

Stimulation of T cells by peptides presented on MHC molecules is accompanied by an array of cell-surface costimulatory molecules that are present on antigen-presenting cells (APC), which engage their corresponding receptors on T cells. For many years, costimulation has underscored the “two-signal” theory. This theory states that: 1) to obtain optimal T-cell activation, costimulation would complement the signal provided by MHC-peptide to ensure productive T-cell activation leading to the effector function; and conversely, 2) the lack of costimulation would result in T-cell tolerance or “anergy” [1]. CD28 on naïve T cells is by far the most important costimulatory receptor [2]. Naïve T cells costimulated with anti-CD28 have been shown to greatly enhance proliferation and interleukin (IL)-2 production. Consistently, mice deficient in CD28 or both of its ligands (B7.1 and B7.2, hereafter referred to as B7-deficient mice) have been shown to be severely impaired in CD4⁺ T-cell proliferation [3,4]. The expression of ICOS, a member of the CD28 family, on T cells has also been demonstrated [5,6]. Analysis of mice deficient in ICOS or its ligand, B7h, revealed that this pathway, although not globally required for CD4⁺ T-cell activation and effector differentiation, regulates their selective effector function [7-10]. CD28 and ICOS pathways have a synergistic yet redundant function. In recent studies, deficiencies in both pathways led to complete T-cell tolerance *in vitro* and *in vivo* [11,12].

Accompanying genomic and cDNA sequencing projects, a number of novel B7-like molecules have been discovered and characterized. It has become clear that numerous inhibitory pathways exist to dampen T-cell function (Table 1). In this review, we will first summarize reports in

Department of Immunology, Unit 906, 7455 Fannin St., Houston, TX 77030, USA. Phone No.: 1-713-563-0406. Fax No.: 713 563 0406.
E-mail: nmorozco@mdanderson.org, cdong@mdanderson.org

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the current literature about the biology of these pathways and their presence in tumors and then discuss their potentials as targets for cancer therapy.

1. Negative costimulatory molecules

1.1. Cytotoxic T-lymphocyte-associated antigen 4—Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a homologue of CD28 that binds to B7.1 (CD80) and B7.2 (CD86) with 10-fold higher affinity than CD28 [13]. Unlike CD28, which is expressed on the surface of naïve T cells, CTLA-4 is not found in naïve T cells but is strongly induced on activated T cells. The role of CTLA-4 as a negative regulator was clearly shown in CTLA-4-deficient mice, which display polyclonal T-cell activation and lymphoproliferative disorder that results in neonatal lethality [13]. In addition, a number of studies have shown a critical role of CTLA-4 in induction of peripheral tolerance [14]. Some recent additional studies have implicated T-cell suppressor function mediated by CTLA-4 expressed by CD4⁺CD25⁺ regulatory T (Treg) cells. Blockade of CTLA-4 with an antagonistic antibody abrogates Treg function [15]. Another possible way whereby CTLA-4 inhibits T-cell function is through reverse signaling of B7.1 and B7.2 in dendritic cells (DC). This unique pathway was reported to induce tryptophan catabolism by upregulating the expression of indoleamine 2,3-dioxygenase (IDO) in DC, which subsequently inhibits T-cell proliferation [16,17].

1.2. PD-1 and its ligands—Programmed cell death-1 (PD-1) is another transmembrane glycoprotein belonging to the CD28 superfamily [18]. PD-1 is expressed on activated T cells, B cells, and monocytes [19,20] and at low levels in natural killer (NK) T cells [21]. The extracellular region of PD-1 consists of a single immunoglobulin (Ig)V domain with 23% identity to the equivalent domain in CTLA-4 [22]. Originally isolated as an apoptosis-associated gene [18], it has become clear that PD-1 provides a crucial negative costimulatory signal to T and B cells. PD-1 regulation of peripheral tolerance was firmly demonstrated in PD-1 deficient mice, which develops autoimmune diseases. Interestingly, the genetic background influences the autoimmune phenotype. For example, knockout of *Pdcd-1* gene on the C57BL/6 background leads to arthritis and lupus-like glomerulonephritis [23], whereas in Balb/c mice knockout of *Pdcd-1* yields dilated cardiomyopathy with the presence of elevated titers of anticardiac troponin I auto-antibodies [24,25].

PD-1 has two ligands belonging to the B7 superfamily: PD-L1 (B7-H1) and PD-L2 (B7-DC) [26-29]. PD-L1 mRNA, broadly expressed in different human and mouse tissues, such as heart, placenta, muscle, fetal liver, spleen, lymph nodes, and thymus for both species as well as liver, lung, and kidney in mouse only. In humans, PD-L1 protein expression has been found in human endothelial cells [30-32], myocardium [33], syncytiotrophoblasts [33,34], resident macrophages of some tissues, or in macrophages that have been activated with interferon (IFN)- γ or tumor necrosis factor (TNF)- α [28], and in tumors [35]. In the mouse, PD-L1 protein expression is found in heart endothelium, islets cells of the pancreas, small intestines, and placenta [36]. In mouse hematopoietic cells, PD-L1 is expressed constitutively on T cells, B cells, macrophages, and DCs and can be upregulated upon activation [20]. In contrast to PD-L1, PD-L2 mRNA and protein expression do not correlate so well. PDL-2 mRNA is expressed in heart, placenta, lung, liver, muscle, pancreas, spleen lymph nodes, and thymus of both humans and mice and in brain and kidney of mouse only [28]. PD-L2 protein expression is only found in macrophages and DC and can be upregulated upon activation with IFN- γ , granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4 [20]. Macrophages are interesting in that Th1 cytokines regulate the expression of PD-L1 expression, whereas Th2 cytokines regulate PD-L2 expression[37].

The function of PD-L1 and PD-L2 has been controversial. Some studies suggest that their function is to provide a positive costimulation, whereas others suggest that they are negative

costimulators. Opposite results have been reported using blocking antibodies and knockout mice [38,39]. The possible role of an unknown positive receptor for these two ligands remains an open question. However, recent evidence supports the roles of PD-L1 and PD-L2 in downregulating T-cell responses *in vivo* and maintaining T-cell tolerance to tissue and oral antigens [38,40,41].

1.3. B7-H3—B7-H3 was first identified in human DCs activated by inflammatory cytokines [42]. Mouse B7-H3 appears to be more broadly expressed in lymphoid organs and other tissues, and its expression on DCs has been found to be upregulated by lipopolysaccharide (LPS) [43, 44]. Both human and mouse B7-H3 recombinant proteins have been reported to bind to an unidentified receptor expressed on activated but not naïve T cells [42,43]. Human B7-H3 was initially reported to augment the proliferation of both CD4⁺ and CD8⁺ T cells and to selectively enhance IFN- γ production in the presence of T-cell receptor signaling [42]. However, this function was not reproducible in murine systems. Mouse B7-H3 moderately reduced proliferation of T cells and IL-2 production [44]. Mice deficient in B7-H3 or treated with a blocking antibody against B7-H3 exhibited enhanced experimental autoimmune encephalomyelitis (EAE) characterized by excessive inflammatory infiltrates in the central nervous system [44,45]. Interestingly, deficiency in B7-H3 did not affect Th2 responses or eosinophilia in an asthma model [45]. The late onset of autoantibody production in B7-H3-deficient animals suggests a modest role of this pathway in maintaining immune tolerance to self-antigens.

1.4. B7S1—B7S1, also called B7-H4 and B7x, was discovered simultaneously by three groups [46-48]. Mouse B7S1 shares greater similarity with B7-H3 in amino acid sequences than any other member of the B7 family. B7S1 is also broadly expressed in tissues and appears to be upregulated in certain tumors [46,49-51]. Like B7-H3, B7-S1 engages an unidentified receptor on activated T cells. *In vitro* studies consistently indicate that B7S1 potently inhibits T-cell proliferation and IL-2 production [46]. In a recent study, a blocking antibody to B7S1 enhanced T-cell activation *in vitro* and the same antibody administered *in vivo* greatly exacerbated induced EAE [46]. However, a role of B7-H4 in peripheral tolerance has not been documented. Recently, Nurieva et al reported that PD-1, B7S1, and B7-H3 participate in development of T-cell tolerance when activated in the absence of CD28 and ICOS, suggesting that the T-cell activation/tolerance decision is determined by the combinatorial positive and inhibitory co-stimulatory signals [11].

1.5 B- and T-lymphocyte attenuator—The B- and T-lymphocyte attenuator (BTLA), another member of the CD28 family, is expressed at very low levels on resting T cells and is upregulated on activated T cells [52]. BTLA intracellular domain has two immunoreceptor tyrosine-based inhibitory motifs (ITIM) that recruit phosphatases SHP1 and SHP2 [53], which mediate the inhibitory function of BTLA. The permanent expression of BTLA on Th1 cells and absence on Th2 cells when they undergo secondary activation strongly suggest that BTLA may regulate Th1 function. In contrast to other CD28 molecules whose ligands are B7 family members, BTLA interacts with a ligand in the TNF receptor (TNFR) family called Herpes virus enter mediator (HVEM) [54], which is the receptor of Herpes simplex virus type-1 (HSV-1) [55]. HVEM binds to two other TNF molecules, LIGTH and lymphotoxin alpha (LT- α). Structural studies have suggested that HVEM interacts with LIGTH to form a homo-trimer or a hetero-trimer LIGTH-BTLA [56]. HVEM is expressed on resting T cells, B cells, macrophages, and immature DCs, and its expression is downregulated on activated T cells [57]. BTLA-deficient T cells show an increased response to T-cell receptor stimulation; moreover mice deficient in BTLA exhibit higher incidence, earlier onset and more aggressive EAE [52]. BTLA-deficient mice also show enhanced antibody response to haptens and their B cells exhibit modestly increased responses to anti-IgM but not LPS activation. This indicates

that BTLA signaling intercepts with that B cell receptor while have no role in Toll like receptor (TLR) pathways.

1.6. Butyrophilin-like 2 protein—Butyrophilin is a milk glycoprotein associated with the milk-fat globule membrane important in regulating the secretion of milk droplets [58,59]. Interestingly, all novel B7 family members share considerable homology to the butyrophilin family proteins, which typically have an extracellular IgV-like domain followed by an IgC-like domain and a heptad repeat, a seven amino acid sequence [60-63]. Mouse butyrophilin-like 2 (BTLN2) protein was recently characterized, its gene is located in the MHC class II locus between I-Ea and Notch on mouse chromosome 17 [64]. Unlike most B7 and butyrophilin molecules with two Ig domains, the BTLN2 protein has four extracellular Ig domains, two IgV-IgC pairs (IgVa-IgCa and IgVb-IgCb). Similarly to other butyrophilin members, BTLN2 has a heptad sequence located between the two pairs of IgV-IgC domains, but it does not have a B30.2 domain in the intracellular region [63]. BTLN2 mRNA is found abundantly in intestine and at low levels in lung and stomach. It is also expressed in the lymphoid organs, spleen, lymph nodes, and thymus. B cells, T cells, and macrophages express BTLN2 mRNA. Its putative receptor is constitutively expressed on resting B cells, upregulated in B cells activated with LPS, and expressed only on activated CD4⁺ and CD8⁺ T cells. BTLN2's function on B cells is unknown; it does not influence proliferation of B cells that undergo activation through either LPS or the combination of anti-IgM and anti-CD40. In T cells, however, BTLN2 does reduce T-cell proliferation when activated by mitogen *in vitro* [64]. The importance of BTLN2 has been highlighted by recent reports that associate BTLN2 gene mutation with human sarcoidosis and myositis diseases [65,66].

1.7. V-set and Ig domain containing-4—V-set and Ig domain containing-4 (VSIG4), also known as complement receptor of the Ig superfamily (CRIG) is a relative of the B7 family. Mouse VSIG4 contains one complete IgV-type domain and a truncated IgC-type domain [67]. Its human ortholog, called Z39Ig, contains two IgV-type domains. Human and mouse molecules contain a cytoplasmic consensus adaptor protein-2 (AP-2) internalization motif, YARL and DSQALI, respectively [68]. VSIG4 is expressed on resting tissue macrophages, DC, neutrophils, and in liver and also at low levels in lung, heart, spleen, and lymph nodes [67]. VSIG4 recycles to the membrane from a pool of intracellular vacuoles in a ligand-independent fashion [69]. However, the expression of VSIG4 is downregulated in activated macrophages. The VSIG4 recombinant protein reduces T-cell proliferation and IL-2 production during activation with anti-CD3 or anti-CD3/CD28 *in vitro*. Administration of VSIG4-Ig to mice immunized with virus-like particles carrying P33 protein of lymphocytic choriomeningitis virus (LCMV) glycoprotein leads to reduced CTL response against P33 and reduced IgG titer when compared to non-treated mice [67]. Thus, VSIG4 is likely another negative regulator of T-cell activation expressed on macrophages. The specific receptor for VSIG4 is unknown, but VSIG4 binds to complement C3 fragment (more specifically C3b and iC3b) and can bind IgM-bound sheep erythrocytes in the presence of C3. As a complement receptor, VSIG4 is essential for clearance of bacterial infections [69]. It remains unclear how the VSIG interaction with T cells and the complement system function in immune response and how VSIG mediates the crosstalk between the innate system and T-cells response; however, its expression in tissue macrophages suggests regulation in tissue tolerance.

1.8. B7S3—B7S3 is the newest member of the B7 family. It has two differentially spliced isoforms expressed in lymphoid and non-lymphoid tissues [70]. A soluble B7S3-Ig protein binds to resting and activated APC constitutively but not to naïve T cells. B7S3-Ig treatment greatly inhibits T-cell proliferation and IL-2 production. B7S3-Ig also reduces cytokine production by effector T cells. Interestingly, whereas the human genome appears to contain a

single-copy B7S3 homologue, the mouse B7S3 gene has 10 relatives within a 2-Mb region, which constitute the B7S3 gene family of molecules.

2. Expression of negative costimulatory molecules in tumors and their regulation of anti-tumor immune response

The regulation of peripheral tolerance mediated by inhibitory B7 molecules and the finding of their expression in tumors suggests a mechanism of immune evasion by tumors [35]. It is well established that tumor-specific T cells can be found in the tumor mass [tumor infiltrating lymphocytes (TILs)] but their effector activity (cytokine production and CTL activity) is dampened by multiple mechanisms, such as prostaglandins (PGE₂) [71,72], transforming growth factor (TGF)- β [73], IL-10 [71,72], inhibitory tumor-associated macrophages (TAMs) [74], tolerizing DCs [16], and regulatory T cells [75,76], to mention a few [76].

2.1. PD-L1 and tumor-T-cell interaction—The inhibitory costimulatory molecules may have an important function during the direct interaction between tumor cells and tumor-specific effector CD8⁺ T cells. The best studied B7 molecule in this case has been PD-L1, which has been found to be expressed in numerous freshly isolated tumors (Table 2) [77]. PD-L1 expression in tumors has been shown to induce apoptosis in CTLs specific against the tumors [77]. Moreover, tumor cell lines expressing PD-L1 are more resistant to specific lysis by CTLs than those that do not express PD-L1. The idea of immune evasion has been further promoted by the fact that PD-L1 expression can be induced by IFN- γ treatment in tumor cell lines that do not express PD-L1, indicating that tumor cells can sense the presence of an active immune response and can counteract by upregulating PD-L1 to form a protective shelf [77,78]. Furthermore, mouse tumors expressing PD-L1 are more aggressive than those without PD-L1. Consistent with this idea, one study of human renal cell carcinoma have shown a direct correlation between tumor expression of PD-L1, tumor aggressiveness, and high risk of death [79].

2.2. B7S1 and T cell-macrophage interaction—The inhibitory activity of B7 molecules can also occur between TIL and TAMs, which are major components of the stroma of virtually all types of solid malignancy. Recently, Kryczek et al described the cell-surface expression of B7S1 in TAMs from tumor ascitis and in human ovarian epithelial carcinoma cells [80]. However, B7S1 expression in the actual ovarian tumor cells and tumor mass was found to be intracellular, indicating that the subcellular localization of B7S1 protein is regulated, albeit by an unknown mechanism. Interestingly, IL-6 and IL-10 present in the ascites can induce expression of B7S1 on normal blood monocytes. However, tumor ascites had no direct effect on intracellular or cell surface expression of B7S1 in the tumor cells. In contrast, IL-4 and GM-CSF suppressed IL-10-induced B7S1 expression, which suggests that downregulation of B7S1 may contribute to the potent adjuvant effects of GM-CSF in cancer immunotherapy [80].

In the study by Kryczek and associates, expression of B7S1 by TAMs inhibited the *in vitro* proliferation and effector function of CD8⁺ T cells specific for the tumor antigen Her-2/neu [80]. Macrophages treated with oligonucleotides to block B7S1 expression reduced the growth of tumors implanted in lymphocyte-deficient mice that received T cells specific for tumor antigens. In contrast, macrophages treated with control oligonucleotides did not influence the tumor growth [80]. This further indicates that B7S1 is the critical immunosuppressive molecule expressed by TAMs.

A possible mechanism of B7S1 regulation is that ovarian cancer cells secrete IL-6 and IL-10 to induce the expression of B7S1 on infiltrating macrophages, which would then inhibit the proliferation of and cytokine production by infiltrating T cells via B7S1. In addition, IL-10 produced by macrophages could also directly suppress T-cell function [81]. It remains unclear

what type of T cells (for example, Th cells, regulatory T cells, or cytotoxic T cells) are targeted by the suppressive macrophages. Moreover, the B7S1 receptor on T cells is not known. It will be important to identify this receptor, determine which cells express it, and investigate its regulatory activity. Similarly to PD-L1, B7S1 is expressed by multiple tumors except melanoma (Table 2), and interestingly, its expression in breast and ovarian cancer does not directly correlate with tumor grade, stage, or patient survival [49,50,82]. However, in renal cell carcinoma, B7S1 expression correlates with pathologic tumor growth and reduced patient survival [51].

2.3. B7-H3 function—Another B7 molecule present in several tumors is B7-H3 (Table 2). Whether this molecule function as a modulator of anti-tumor immune response in the tumor microenvironment is controversial. In the mouse model of EL-4 lymphoma, the injection of a plasmid encoding for B7-H3–induced regression of 50% of the tumors, and this effect was mediated by CD8⁺ T cells and NK cells [83] (Table 3). However, in this study, the effect of B7-H3 in the TILs was not defined. In a similar study, where P815 cells were transfected with a plasmid containing full-length mouse B7-H3 cDNA and inoculated into DBA/2 mice, 44% tumor regression and slow tumor growth were observed [84] (Table 3). The researchers reported that the effect of B7-H3 on T cells was to induce clonal expansion of tumor-specific CD8⁺ T cells and increased cytolytic activity. These results corroborate the finding that gastric carcinoma patients that have tumors expressing higher levels of B7-H3 have higher survival rates than patients with less expression (Table 2)[85]. In contrast, in a neuroblastoma study, the presence of B7-H3 in neuroblastoma cells had a protective effect against NK-mediated cell lysis [86]. Because the receptor, or receptors, of B7-H3 on T cells and NK cells is unknown, the role of B7-H3 as a costimulatory molecule remains questionable. It is possible that both a positive receptor and a negative receptor modulate B7-H3 and that the expression of these receptors could be influenced by the tumor microenvironment.

3. Unleash inhibition by negative costimulation in cancer

The use of antibodies to block negative costimulatory molecules for cancer therapy has been an attractive solution in boosting anti-tumor immune responses in the immunosuppressive tumor microenvironment. This field was pioneered by Jim Allison and associates using CTLA-4 as a target (Table 3). The regimen of anti-CTLA-4 has been effective in some mouse tumor models used as a monotherapy or in conjunction with tumor vaccination, GM-CSF, and peptide vaccination or when depleting Treg cells (Table 3). Although tumor reduction has been observed accompanied with CD8⁺ T-cell response against tumor antigens [87-89], the precise action of CTLA-4 blockade is not fully understood. After CTLA-4 blockade, multiple CD4⁺ and CD8⁺ self-reactive T-cell clones are activated, and only a few that react against the tumor have been found after the treatment [89]. In fact, mouse pmel TcR transgenic CD8⁺ T cells specific against gp100 peptide from B16 melanoma are not affected by CTLA-4 blockade [90].

Another possible target for anti-CTLA-4 treatment is the Treg cell, which expresses CTLA-4. Although, the results of several studies agree that CTLA-4 blockade reduces Treg function [15], it is still controversial whether the treatment can deplete this population [91-93]. It was recently reported that anti-CTLA-4 therapy alters the balance of Tregs/CD8⁺ effector T cells inside the tumor, promoting the presence of effector cells in the tumor [93]. Thus, CTLA-4 blockade seems to help reduce Treg effect on tumor-specific CD8⁺ T cells in the tumor and at the same time increase tumor-specific immunity.

Although anti-CTLA-4 treatment seems effective for immunotherapy of cancer, the main concern is the simultaneous development of autoimmune manifestations [94]. This effect in terms of therapy highlights the need for a tumor-specific immune response induced by multi-

tumor peptide vaccination, DC vaccination with multiple peptides, or adoptive T-cell therapy [87-89]. Immunomodulators, like glucocorticoids, administered following anti-CTLA-4 blockade might be useful in downplaying unwanted autoreactivities elicited by anti-CTLA-4 treatment, but the low-degree of anti-tumor response limits the feasibility of this option.

Another molecule that has been explored is PD-1 and its ligand PD-L1. Blockade of either of these molecules have been effective in reducing tumor growth, and it seems to be a more promising approach due to the specific elevated expression of PD-L1 in the tumors and PD-1 on activated T cells (Table 3). In fact, in most of the studies where PD-1 or PD-L1 were blocked, an increase in effector CD8⁺ T cells at the tumor site was observed [78,95]. Albeit these findings present strong evidence for the use of PD-L1 blockade to boost anti-tumor immune response, there are some reports showing PD-L1 and PD-L2 as positive costimulators that are required in anti-tumor immunity (Table 3) [39].

The main challenge for anti-tumor immunotherapy is the generation of effector CD8⁺ T cells resilient to tumor immunosuppression. These cells need to be capable of killing substantial amounts of tumor cells and in the long-term produce memory CD8⁺ T cells to prevent any further tumor relapse. In this case, a similar challenge is chronic viral infections where impaired anti-viral CD8 T cells are found in hosts with persistent viremia. PD-1 expression was found in anti-viral CD8 T cells from chronically infected mice with LCMV and humans infected with HIV [96-98]. PD-L1 blockade was shown to revitalize “exhausted” antiviral CD8⁺ T cells [96-98]. These results strongly indicate the potential of PD-1/PDL1 blockade in reversing the unresponsive status of CD8⁺ T cells.

4. Conclusion and Perspectives

The battle by the immune system in controlling tumors is not easy to win. Although tumor-specific T cells have been found in patients with cancer and sometimes are activated after vaccination, tumors arising from normal tissue have the capacity to suppress T-cell function. The recent discovery of the novel inhibitory costimulatory molecules indicates the complex mechanisms by which the host prevents immune over-activation. These molecules have been shown to be important in maintaining self-tolerance. Elevated expression of these molecules in the tumor microenvironment also suggests their participation in tumor evasion of immune surveillance. The use of agents to block the function of inhibitory costimulatory molecules is promising, judging from the limited human trials on anti-CTLA-4 therapy.

Much more needs to be learned about the basic biology of the expanding number of negative costimulatory molecules in regulation of tumor immunity. First, we need to elucidate the context in which each pathway functions and whether there is specific regulation on the expression of each B7 family member. Secondly, the redundancy of various costimulatory pathways must be examined. Lastly, how T cells, including TILs and Treg cells, respond to costimulation and identification of the signal transduction and transcriptional regulation that account for the biologic effect of costimulation regulation need to be addressed.

With the progress in the above fields, we may come up with smarter and better strategies targeting various B7 molecules for different types of tumors. However, based on the severe side effects observed with the use of anti-CTLA-4 alone, we should focus our efforts in a therapy that generates specific tumor-antigens immune responses. A number of tumor antigens have been discovered and are now being tested for immunogenicity. We believe costimulation blockade could perhaps better serve as an adjuvant in combination with peptide vaccination, DC therapy, and adoptive T-cell therapy in boosting the priming of tumor-specific T cells or in alleviating the suppression from tumor microenvironment on TILs. In this way, the therapeutic effect would be more specific and safer.

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Table 1

B7 family members

Ligand	Expression	Receptor	Function
B7.1/B7.2 (CD80/CD86)	Activated APC	CD28/CTLA-4	T-cell activation and tolerance
B7h (B7RP1, ICOS-L, B7-H2)	B cells, macrophages and non lymphoid tissues	ICOS	T-cell activation
B7-H1 (PD-L1)/B7DC (PD-L2)	APC and nonlymphoid tissues	PD-1	Inhibition of T-cell activation and tolerance
B7-H3	Lymphoid and nonlymphoid tissues	Unknown	Inhibition of T-cell activation
B7S1 (B7-H4, B7x, VTCN1)	Lymphoid and nonlymphoid tissues	Unknown	Inhibition of T-cell activation
HVEM	Lymphoid and nonlymphoid tissues	BTLA	Inhibition of T-cell and B-cell activation
BTNL2	Lymphoid and nonlymphoid tissues	Unknown	Inhibition of T-cell activation
VSIG4	Macrophages and nonlymphoid tissues	Unidentified receptor, Complement C3b/iC3b	Inhibition of T-cell activation
B7S3	Lymphoid and nonlymphoid tissues	Unknown	Inhibition of T-cell activation

Abbreviations: antigen-presenting cells, APC; cytotoxic T-lymphocyte-associated antigen 4, CTLA-4; inducible T cell costimulator, ICOS; programmed cell death, PD; Herpes virus enter mediator, HVEM; B- and T-lymphocyte attenuator, BTLA; V-set and Ig domain containing-4 (VSIG4).

Table 2

Expression pattern of B7 molecules in tumors

Molecule	Tumor	Expression Pattern	Reference
PD-L1	Lung carcinomas	> 95% of the tissues examined	[77]
	Ovarian carcinomas	87 % of the tissues examined	[77]
	Melanomas	100% of the tissues examined	[77]
	Colon carcinomas	52% of the tissues examined	[77]
	Renal cell carcinoma	Expression found in more than 10% of the tumor mass in 37.2% of patients examined and correlates with higher risk of death.	[79]
	Non-small cell lung cancer	Expression found in 59% of patients and does not correlate with tumor pathology or survival.	[99]
	Squamous cell carcinomas of the head and neck (SCCHN)	Expression found in 66% of samples analyzed.	[100]
	Esophageal cancer	Expression found in 44% of samples analyzed and it correlates with poor prognosis.	[101]
	Esophageal cancer	Expression found in 56% of samples analyzed and it inversely correlates with CD8+ infiltrating lymphocytes.	[101]
	Neuroblastoma	Expression of B7-H3 inhibits NK cell mediated lysis	[86]
B7-H3	Gastric Carcinoma	High intratumoral expression of B7-H3 correlates with survival time of cancer patients	[85]
	Ovarian and lung cancer	Expression found in 85% of ovarian samples and 31% in lung cancer, higher in squamous cell carcinoma. Absent in melanoma.	[82]
	Breast Cancer	Expression found in primary and metastatic breast cancer. Higher levels in lobular and ductal carcinomas. No relation with pathology or prognosis.	[49]
	Ovarian cancer	Expression found in 100% of serous cystadenocarcinomas, endometroid adenocarcinomas, clear cell carcinomas, and tumor metastases. Less than 10% expression in mucinous tumors. No correlation with tumor grade, stage or survival.	[50]
	Renal cell carcinoma	Expression found in 59.1% of sampled patients and correlates with pathological tumors and reduced patient survival.	[51]

Table 3

Blocking B7/CD28 family of molecules for tumor therapy

Molecule	Tumor model	Intervention	Result	Ref
CTLA-4	Mouse colon tumor (S1BLim10)	Antibody blockade of CTLA-4	Tumor regression	[102]
	Mouse prostate tumor (TRAMP transgenic mice)	Antibody blockade of CTLA-4	Reduce tumor growth	[103]
		Tumor resection and antibody blockade of CTLA-4	Reduced metastatic relapse	[103]
		Vaccination with irradiated tumor cells and antibody blockade of CTLA-4	Reduce tumor incidence	[104]
		Vaccination with irradiated tumor cells expressing GMCSF and antibody blockade of CTLA-4	Reduce tumor incidence	[104]
	Mouse mammary carcinoma (SM1) ^b	Vaccination with irradiated tumor cells expressing GMCSF and antibody blockade of CTLA-4	Reduce tumor growth and regression	[105]
	Mouse melanoma (B16) ^c	Vaccination with irradiated tumor cells expressing GMCSF and antibody blockade of CTLA-4	Reduce tumor growth	[88]
		Depletion of CD25+ cells and vaccination with irradiated tumor cells expressing GMCSF and antibody blockade of CTLA-4	Reduce tumor growth	[106]
	Mouse sarcoma (methA) ^d	Vaccination with vaccinia virus expressing p53 (rMVAp53) and antibody blockade of CTLA-4	Tumor regression and increased survival	[107]
	Human metastatic melanoma	gp100 peptide vaccination and multiple rounds of antibody blockade of CTLA-4 ^e	4/29 patients respond (2 complete response and 2 partial response) 3/14 patients respond and autoimmune disease developed in 6/14 patients	[108], [109]
PD1		Multiple-peptide vaccination and multiple rounds of antibody blockade of CTLA-4**	7/19, no relapse	[110]
		Vaccination with irradiated tumor cells expressing GMCSF and multiple rounds of antibody blockade of CTLA-4	Tumor necrosis in 3/3 patients	[111]
	Human metastatic Ovarian carcinoma	Previous vaccinated with DC engineered to express gp100 and MART-1; gp100, peptide plus IL-2 underwent multiple rounds of antibody blockade of CTLA-4	No tumor necrosis in 4/4 patients, 2 patients showed CD8+ infiltration	[111]
		Vaccination with irradiated tumor cells expressing GMCSF and multiple rounds of antibody blockade of CTLA-4	Stabilization of CA125 in 2/2 patients.	[111]
	Mouse mastocytoma ^f	Antibody blockade of PD1	Enhance antitumor immunity induced by vaccination in combination with T-cell therapy.	[78]
	Mouse subcutaneous and Hematogenous melanoma (B16)	Inoculation of B16 in PD-1 deficient mice and PD-1 transgenic mice	Decreased tumor growth Reduced tumor foci in the liver, increased CD8+ cells at the tumor site and enhanced effector function.	[112]
	Mouse colon cancer (CT26) ^g	Intravenous inoculation of CT26 in Balb/c PD-1 deficient mice	Decreased tumor growth.	[112]
	Mouse hepatoma (H22) ^h	Vaccination with plasmid encoding secondary lymphoid tissue chemokine (SLC) CCL21 and plasmid encoding the extracellular domain of PD-1	Reduced tumor size and increase survival.	[113]
	Mouse mastocytoma ^f	-Transfectant expression of PD-L1 in P815. -Transfectant expression of PD-L1 in P815 expressing B71.	-Increased tumor resistance to anti CD137 (41BB) or CD8 specific killing or transfer therapy. -Increase tumor growth.	[77,78, 114]
	Autologous human ovarian cancer grown in NOD-SCID mice	Antibody blockade of PD-L1	Enhanced MDC-mediated ⁱ T-cell activation with upregulation of IL-2 and IFN- γ and reduced production of IL-10	[115]
PD-L1	Mouse SCC (A14) ^j	-Transfectant expression of PD-L1 in A14. -T cell therapy with activated and non activated SCVII T cell clones and antibody blockade of PD-L1	Equal tumor growth. Increased survival only when activated SCVII T cell clones were used	[100]
	Mouse hematogenous melanoma (B16) ^c	Inoculation of B16 in the spleen of in B6 mice and treatment with anti PD-L1	Decreased tumor growth.	[112]
	Mouse colon cancer (CT26) ^g	Inoculation of CT26 in Balb/c mice and treatment with anti PD-L1	Decreased tumor growth.	[112]

Molecule	Tumor model	Intervention	Result	Ref
B7-DC	Mouse colon cancer (CT26) ^g	Inoculation of CT26 in B7-DC deficient mice.	Reduced survival and increased foci in the liver. Decreased anti-tumor CD8 response.	[39]
B7-H3	Mouse lymphoma (EL-4) ^k	Intratumoral injection of a B7-H3 pcDNA3 expression plasmid.	Enhanced antitumor immunity mediated by CD8 and NK cells.	[83]
	Mouse mastocytoma (P815) ^f	Expression of B7-H3 in transfectant P815.	Enhanced antitumor CD8 response, slowed tumor growth and increased survival.	[84]

^aTRAM transgenic mice express SV40 T antigen transgene under the control of rat probasin promoter, which directs expression to prostatic epithelium in an androgen-regulated manner. TRAMP mice develop pathogenesis of neoplasia that mirrors that in man.)

^bSM1: BALB/C-derived mammary carcinoma

^cB16: C57BL/6-derived melanoma

^dMethylcholanthrene-induced sarcoma

^eThere are other studies not mentioned here in which anti-CTLA-4 therapy was used alone and only once with less success [111]

^fP815: Balb/c-derived mastocytoma.

^gCT26: Balb/c-derived colon cancer cell line.

^hH22 murine hepatoma, Balb/c derived

ⁱMDC: monocyte-derived dendritic cells

^jA14: C3H/HeN mice derived SCC.

^kEL-4: C57BL/6-derived thymoma