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The complex role of B7 molecules in tumor immunology

Barbara Seliger¹, Francesco M. Marincola², Soldano Ferrone³, and Hinrich Abken^{4,5}

¹ Martin Luther University, Institute of Medical Immunology, 06112 Halle, Germany

² National Institute of Health, Department of Transfusion Medicine & Immunogenetics, Bethesda, MD 20862, USA

³ University of Pittsburgh Cancer Institute, Departments of Surgery, Immunology and Pathology, Pittsburgh, PA 15213, USA

⁴ University of Cologne, Center for Molecular Medicine, 50931 Cologne, Germany

⁵ University of Cologne, Tumor Genetics, Clinic I Internal Medicine, 50391 Cologne, Germany

Abstract

T-cell activation requires the interaction of the T-cell receptor with a cognate major histocompatibility complex (MHC)–peptide complex. Initiated by antigen engagement, the adaptive immune response is orchestrated by a complex balance between stimulatory and inhibitory signals that are predominantly controlled by members of the B7 family. Here, we review the current knowledge on B7 family members concerning their constitutive and regulated expression, modulation of the immune response and their role in the evasion of host immune surveillance. We also discuss recent therapeutic strategies that aim to improve immune-cell recognition of tumors and induce tolerance to autoreactive immune responses in normal tissues by manipulating B7 functions.

Introduction

It is generally accepted that the activation of naïve T cells is initiated by association of the T-cell receptor (TCR) with the major histocompatibility complex (MHC)–peptide complex (see Glossary). Optimal T-cell activation, however, requires a second signal, known as costimulation, which leads to clonal T-cell expansion, cytokine secretion and execution of effector functions. Lack of costimulation, however, results in the induction of T-cell tolerance, named anergy. After initial activation, coinhibitory molecules are engaged and counteract T-cell activation. Consequently, a balanced interplay between costimulatory and coinhibitory signals is required to control priming, functional maturation and limitation of the T-cell response at distinct times and locations. The identification of the B7–CD28– cytotoxic T lymphocyte-associated-4 (CTLA-4) pathway provided the basis for our understanding of costimulatory and coinhibitory signals, which are generated by binding of B7-1 (CD80) and B7-2 (CD86) expressed on professional antigen-presenting cells (APCs) to both the costimulatory CD28 molecule and its coinhibitory counterpart, the CTLA-4 protein, on T cells. During the past decade, five new members of the B7 family have been identified – B7-H1, B7-DC, B7-H2, B7-H3 and B7-H4 – each with different, although overlapping, functions controlling the priming, proliferation and maturation of T cells [1,2]. The efficacy of immune responses directed against target sites, including tumors, is obviously defined by an orchestrated balance between these factors [3,4]. Increasing knowledge about costimulatory

and coinhibitory B7 family members has recently led to the manipulation of these pathways for the treatment of malignant diseases.

Characteristics of the B7 family members

The B7 family members are classified into three groups according to their functions (Table 1). Group I B7 molecules include B7-1, B7-2 and B7-H2. B7-1 and B7-2 exhibit a restricted expression pattern and are inducibly expressed on APCs, such as macrophages, dendritic cells (DCs) and hematopoietic cells, and are upregulated on activated T cells. B7-1 and B7-2 molecules exhibit a degree of promiscuity in binding to their cognate stimulatory receptor CD28 and inhibitory receptor CTLA-4 because both ligands bind to either receptor. Engagement of B7-1 or B7-2 on APCs with CD28 on T cells provides costimulatory signals that are required for the activation of naïve T cells, and T-cell responses are severely inhibited in *Cd28*-knockout (KO) mice [5,6]. The interaction of CTLA-4 with the same ligands acts as a negative regulator of CD28-dependent T-cell activation [7,8], and loss of CTLA-4 leads to severe lymphoproliferation and fatal multiorgan destruction via autoimmunity [9]. In this way, the B7-1/B7-2–CD28/CTLA-4 pathway represents either a costimulatory or coinhibitory pathway in modulating the T-cell response. B7-H2 expressed on B cells, macrophages and non-lymphoid tissues mediates costimulatory signals upon binding to another member of the CD28 family, the inducible costimulator (ICOS), on T and B cells [10–12]. Analysis of mice deficient in B7-H2 or ICOS demonstrated that this pathway is required for CD4⁺ T-cell activation, effector-cell differentiation and the execution of effector functions [13,14].

The group II B7 molecules comprise B7-H1 (PD-L1) and B7-DC (PD-L2), the ligands of the programmed cell death-1 (PD-1) receptor [15–17]. Evidence is emerging that, in addition to PD-1, other receptors might exist that can mediate B7-H1 function [18]. B7-H1 is abundantly and constitutively expressed by many cells and in various tissues, such as spleen, APCs, T cells, natural killer (NK) cells, endothelial cells, mesenchymal stem cells, mast cells, immune privileged cells and non-hematopoietic cells, including vascular endothelial cells and pancreatic islet cells [19–22]. In contrast to B7-H1, the expression of B7-DC is more restricted, and the protein is constitutively found on resting peritoneal B1 cells but can be induced on DCs, macrophages and bone-marrow-derived mast cells. The receptor of the ligands B7-H1 and B7-DC, PD-1, is expressed on activated T cells, B cells, DCs, NKT cells and on activated monocytes [23–25]. The interaction of B7-H1 and B7-DC with PD-1 modulates TCR and B-cell receptor (BCR) signaling, thereby controlling the induction and maintenance of peripheral immune tolerance [26]. The same interaction is responsible for the functional impairment of antigen-specific CD8⁺ T-cell responses during chronic viral infections and malignant transformation [15–17,27]. The key role of group II B7 members in mediating immune tolerance is underlined by the observation that mice lacking the B7-H1 receptor only slowly develop autoimmune diseases and that B7-H1- or B7-DC-KO mice do not exhibit spontaneous autoimmune diseases [27]. PD-1, by contrast, mediates reverse signaling by delivering signals into B7-H1- and B7-H2-expressing DCs. Bi-directional signaling of B7-H1 and PD-1 could be the reason for the contradictory results reported so far, and further analyses will have to assign the functional consequences to the individual pathways.

Group III B7 members include B7-H3 and B7-H4, which are type I-transmembrane proteins with an as-yet-unidentified receptor. B7-H3 mRNA and protein expression has been found in many lymphoid and non-lymphoid cells and peripheral organs [28]. Its expression on DCs is upregulated by lipopolysaccharides [29]. Although B7-H4 mRNA transcription occurs widely in peripheral tissues and in most stromal and hematopoietic cells, protein expression is absent in most somatic tissues and only detected in epithelial cells of kidney, lung and pancreas. Because the receptor for B7-H3 has not yet been identified, functional analyses are currently difficult to perform, and the role of B7-H3 and B7-H4 in T-cell regulation has still to be defined.

The experimental evidence, however, implies that B7-H3 is involved in the regulation of cell growth and differentiation of non-hematopoietic tissues. Although B7-H3 stimulates T-cell proliferation, cytokine secretion and cytolytic activity [30], other studies have demonstrated inhibitory functions of B7-H3, including the impairment of T helper 1 (Th1) responses and NK-cell-mediated lysis [30]. B7-H4, by contrast, exhibits negative costimulatory functions, including blocking of antigen-driven CD8⁺ and CD4⁺ T-cell proliferation and cytokine production and rendering tumor cells refractory to apoptosis [31,32].

Roles of B7 molecules in tumor immunology

The physiological functions of costimulatory B7 family members are to enhance T-cell proliferation, to increase cytokine secretion and to prevent apoptosis, thereby stimulating T-cell responses. By contrast, the coinhibitory molecules provide key negative signals by limiting, terminating and attenuating T-cell responses, thereby preventing T-cell hyperactivation and avoiding tissue and organ damage during immune responses [33]. Although not elucidated in detail, there is accumulating evidence that B7 family members orchestrate the immune response by providing costimulatory 'GO' and coinhibitory 'STOP' signals (Figure 1). Their expression on professional APCs, as well as on immune cells of the tumor microenvironment, on stromal cells and on tumor cells, provides the basis for dynamic interactions between tumors and the host immune system, as discussed in the following sections.

Expression of B7 family members and their receptors in tumors

B7 family members are expressed on a variety of hematopoietic malignancies, solid tumors and on infiltrating immune cells (Table 2). B7-H1 is expressed at high levels in hematopoietic malignancies, such as leukemia, thymic neoplasms and multiple myeloma, and in most human solid cancers, including breast, colon, esophageal, gastric, head and neck squamous cell, kidney, liver, lung, ovarian, pancreatic, salivary and urothelial carcinomas, as well as in glioblastoma, Wilms' tumor and melanoma [34–43]. In glioblastoma, B7-H1 protein expression correlates with loss of the phosphatase and tensin homolog (PTEN) phosphatase, thus linking tumor-suppressor gene loss with immune inhibitory capacities [44]. In this context, it is noteworthy that, in murine and human tumor cell lines, B7-H1 is only expressed rarely and at low levels, which might be due to the lack of tumor microenvironment during *in vitro* culture of established cell lines. In contrast to B7-H1, high levels of B7-DC expression have so far only been described on mantle cell lymphoma and non-small-cell lung cancer (NSCLC) cells [45,46].

As well as B7-H1, B7-H4 is expressed on the surface of a variety of cancer types, including breast cancer, NSCLC, ovarian cancer, prostate cancer, renal cell cancer (RCC) and uterine endometrial carcinoma. Recently, B7-H4 has been found to be expressed also by nondividing brain tumor cells, in particular in a subset of brain tumor stem cells [47]. Despite their overlapping expression patterns in many tumor types, some cancers express only B7-H1 or B7-H4. For example, melanoma cells express B7-H1 with high frequencies but do not express B7-H4.

Only limited and conflicting information is available for B7-H2 and B7-H3 expression in tumors. B7-H2 is invariably coexpressed with other B7 molecules on solid tumors, promoting immune inhibition [48]. In hematologic diseases, B7-H2 expression promotes tumor cell expansion and is associated with poor prognoses [34]. High levels of B7-H3 have been detected on tumors (and tumor vasculature) of distinct origin, such as RCC, NSCLC and prostate cancer [49–52]. Although these data suggest a role for B7-H3 in immune escape, the function of B7-H3 on tumors needs to be defined.

Expression of B7 family members on host immune cells

Despite their expression by tumor cells, B7 family members are also expressed on cells of the tumor microenvironment. B7-H1 is expressed on tumor-infiltrating immune cells, such as tumor-infiltrating lymphocytes (TILs), macrophages, fibroblasts and tumor-associated myeloid DCs [53]. The expression of B7-DC is mainly limited to DCs and macrophages, and the receptor PD-1 is expressed on activated T cells, including TILs [54]. By contrast, high levels of B7-H3, as well as of B7-H4, have been detected on vascular endothelial cells of the tumor microenvironment. Furthermore, B7-H4 is expressed on tumor-associated macrophages (TAMs) of ovarian carcinoma, but it is not detectable in healthy tissues [31,55].

Regulation of the expression of B7 family members in tumor tissues

The understanding of the molecular mechanisms regulating expression of B7 molecules is still in its infancy. For instance, B7-H1 and B7-H4 mRNA is nearly ubiquitously expressed in a variety of tissues, which is in clear contrast to their pattern of protein expression, implying a form of regulation at the post-transcriptional level, the mechanisms of which are still unresolved. Inflammatory mediators in the tumor microenvironment, such as interferon- γ (IFN- γ), interleukin-4 (IL-4), tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor (VEGF), upregulate the expression of B7-H1 on many cell types, including tumor cells, owing to the presence of different cytokine-responsive elements in the promoter region, like that for the interferon regulatory factor (IRF)-1 [56]. Upon IFN- γ stimulation, B7-H1 is expressed on T cells, NK cells, macrophages, myeloid DCs, B cells, epithelial cells and vascular endothelial cells [3], providing a coinhibitory B7 ligand to PD-1 on activated T cells. Upregulation of B7-H1 by sustained low IFN- γ levels, which can occur under chronic inflammatory conditions, recruits host cells for the repression of tumor immunity, thereby promoting tumor development. In addition to IFN- γ , type I IFNs also stimulate the expression of B7-H1 on hepatocytes, monocytes, DCs and tumor cells. In contrast to B7-H1, B7-H2 is upregulated by granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4 [24]. B7-H4 expression on TAMs, monocytes and myeloid DCs is strongly induced by IL-6 and IL-10, both of which are cytokines present in the tumor microenvironment [57]. By contrast, IFN- γ only marginally induces B7-H4 expression. GM-CSF and IL-4 decrease the B7-H4 expression of tumor-infiltrating immune cells. These data suggest that expression of the coinhibitory ligands B7-H1 and B7-H4 is controlled by distinct mechanisms, all of which are likely to contribute to the repression of a successful anti-tumor immune response.

Clinical relevance of B7 family members in tumors

Given the evidence that B7 family members have immune-suppressive capacities, aberrant expression of these coinhibitory molecules might negatively interfere with the host immune response, leading to disease progression. Indeed, B7-H1, B7-H3 and B7-H4 expression on tumors and/or TILs is often associated with poor prognosis and aggressive behavior of tumors (Table 2). In breast cancer, B7-H1 expression is associated with progression, histological grading, loss of progesterone and estrogen receptors and expression of the proliferation marker Ki-67 [37]. The survival rate of RCC is significantly lower in patients with B7-H1-expressing tumors than in patients with tumors lacking B7-H1 (the survival rates are 42% and 83%, respectively [58]) implying that B7-H1 can serve as an independent predictor of RCC prognosis. Similar results have been obtained in bladder, oesophageal, gastric and pancreatic carcinomas, as well as in Wilms' tumor, in which B7-H1 expression correlates with an advanced tumor stage and an increased risk of relapse and/or post-operative mortality of patients with organ-limited diseases [42,43,59–61]. Accordingly, B7-H1⁺ pancreatic tumor cells exhibit an increased prevalence of tumor-infiltrating regulatory T (T_{reg}) cells [42] repressing the anti-tumor T-cell response. The role of B7-H1 as an indicator of poor prognosis holds also for its expression on TILs. The use of B7-H1 as a prognostic marker might allow the identification

of high-risk patients who could benefit from a more aggressive initial treatment. Similarly, PD-1 expression is correlated with disease progression in urothelial cancers [43]. Although its function is still controversial, B7-H3 expression is associated with disease progression. In urothelial carcinoma and clear cell RCC, B7-H3 expression predicts disease progression and the patients' outcome [43,49]. Recent studies indicate also an association between B7-H4 expression and the progression of some tumor entities, including RCC and prostate cancer [52,62]. Combined expression of both B7-H1 and B7-H4 in RCC, however, was associated with reduced survival when compared with those patients whose tumors were either negative for both B7-H molecules or expressed either B7-H1 or B7-H4 [62]. The clinical significance of the coexpression of other B7-H molecules, however, has so far not been defined in detail.

The 'GO' signals

Although an interaction between B7 and CD28 was the basis of the initial definition of costimulation, a large number of other receptors and ligands have been found to modulate positively the activation of T cells (Table 1). T-cell activation is initiated and promoted by TCR engagement with the cognate antigenic peptide–MHC complex on APCs in concert with B7-1- and B7-2-mediated CD28 costimulation. B7-1 and B7-2 are structurally related, but distinct, glycoproteins expressed on professional APCs, such as DCs, B cells and macrophages [1,3]. Each B7 molecule binds to either the costimulatory CD28 or the coinhibitory CTLA-1 receptor. The costimulatory CD28 signal is required to drive clonal expansion and differentiation of naïve T cells into effector cells, which is initiated by a cascade of signals, resulting in cytokine production, entry into the cell cycle and blockade of apoptosis. In the absence of CD28 costimulation, antigen-experienced T cells become anergic and nonresponsive.

Recent data suggest that B7-H1 is an important regulator of anti-tumor immunity [36]. B7-H1 on NK cells increases cytotoxic T lymphocyte (CTL) responses in the absence of PD-1 expression [20], delivering signals into immune as well as into tumor cells [63–65], whereas blockade of B7-H1 inhibits the T-cell response in a mouse colitis model [66]. By contrast, B7-H1 expression by tumor cells increases apoptosis of antigen-specific CTLs [15]. The seemingly contradictory properties of B7-H1 might point to an alternative stimulatory receptor independent of inhibitory PD-1 [67,68]. The induction of Th1 cytokines and of CTL anti-tumor responses implies that B7-DC functions as a costimulatory molecule, whereas the strong coinhibitory capacities of B7-H1 on the same tumor cells, however, makes the elucidation of the intrinsic properties of B7-DC in this context difficult. The costimulatory activity of B7-DC is supported by more progressive growth of transplanted tumors in B7-DC^{-/-} mice than in wild-type mice. B7-DC exhibits costimulatory functions in a PD-1-independent manner [69, 70], whereas B7-DC represses a T-cell response in a PD-1-dependent fashion [70]. Moreover, different splice variants might contribute to the reported discrepancies with respect to the immune-modulatory effects of B7-DC. Because B7-DC interacts with both inhibitory and stimulatory receptors, B7-DC on tumor cells has a dual effect depending on the activation of infiltrating T cells – that is, repression when interacting with activated PD-1⁺ effector T cells versus costimulation when interacting with PD-1⁻ naïve or early-stage effector T cells.

B7-H2, the other costimulatory member of the B7-H family, induces strong CTL-mediated tumor regression but also NK-cell-mediated killing in an ICOS-dependent manner when expressed on solid tumors. Optimal induction of the tumor antigen-specific immunity through B7-H2, however, requires coexpression of B7-1 or B7-2 on APCs in the tumor microenvironment, suggesting that B7-H2 acts in the post-priming phase of CTL activation [11]. Selective downregulation of B7-H2 on solid tumors is frequently found to be associated with the escape from immune surveillance. Like B7-DC, B7-H2 seems to exhibit dual functions

because it not only costimulates CTLs to enhance immunity against many solid cancers but also sustains progression of B cell malignancies by inducing Th2 cytokines [34].

The role of B7-H3 has been subject to controversy in this context. B7-H3 inhibits the NK-mediated immune response [71] or downregulates the Th1 response [30], whereas B7-H3 enhances tumor immunity by expansion of antigen-specific CD8⁺ CTLs [72]. The costimulatory activity is supported by the observation that B7-H3 expression in gastric cancer correlates with survival [60].

The 'STOP' signals

A variety of coinhibitory signals provided by the B7 family promote immune suppression by different mechanisms. Although tumor-associated antigens are recognized by specific T lymphocytes, their immunological clearance is rare. This is often associated with an immune-suppressive milieu provided by the tumor. Both B7-H1 and B7-H4, expressed alone or in concert with other signals, represent components of the suppressive tumor microenvironment (Table 2).

The coinhibitory activities of B7-H1 on the anti-tumor immune response—First identified in ovarian carcinoma, B7-H1 on tumor cells promotes immune suppression by binding to PD-1 on activated T cells, thereby sustaining tumor growth [73]. The B7-H1-mediated immune repression is even more complex because B7-H1 is not only expressed on tumor cells but also by APCs and cells of the tumor-associated microenvironment, particularly stroma, implying a more general role for tumor-associated B7-H1 in the repression of tumor immunity. In a variety of cells, B7-H1 expression is upregulated by IFN- γ , leading to the speculation that IFN- γ secretion occurring during chronic inflammatory processes might promote tumor development by upregulating B7-H1 and other inhibitory molecules. B7-H1 induced by IFN- γ causes CTL apoptosis and induces nitric oxide and prostaglandin E2 production in macrophages, suggesting that B7-H1–PD-1 engagement results in a negative-feedback loop that leads to the inhibition of the anti-tumor immune response. The model is supported by the observation that the *Pd-1*-KO mouse and blocking of B7-H1 by antibodies leads to increased cytokine production and anti-tumor activity [54]. Accordingly, B7-H1⁺ tumors are resistant to CTL attack. Moreover, B7-H1 expression on host cells contributes to suppression of tumor immunity by T cells: T-cell communication through PD-1–B7-H1 and B7-DC interactions results in repression of proliferation and secretion of IFN- γ and IL-10 [74]. The recent elucidation of the crystal structure of the PD-1–B7-H1 complexes provides information about the localization and signaling within the immunological synapse and will help in the design of new ways to manipulate this pathway [75].

Immune repression is reinforced, moreover, by IFN- γ , which increases B7-H1 expression, leading to the speculation that chronic inflammation might promote tumor development by repressing the T-cell anti-tumor immune response [53]. Furthermore, B7-H1 on NK cells and macrophages sustains inhibition of cytotoxic T cells in their anti-tumor activity [58]. This conclusion is in line with the observation that expression of a *B7-H1* transgene in parenchymal cells protects from autoimmune reactions, and B7-H1 expression on both RCC cells and TILs is associated with poor prognoses. In addition, B7-1 can bind to B7-H1, thereby leading to an inhibition of T-cell proliferation and cytokine production [18] and underscoring the complexity of T-cell costimulation.

Distinct mechanisms of B7-H4 action—In contrast to B7-H1, the functions of the negative-regulatory molecule B7-H4 have not yet been resolved in detail. B7-H4 overexpression on pre-malignant cells promotes their malignant transformation by protecting them from apoptosis. Accordingly, B7-H4 inhibition by siRNA leads to increased apoptosis

of B7-H4⁺ tumor cells. B7-H4 strongly inhibits immune responses by the downregulation of T-cell-mediated immunity, thereby contributing to accelerated tumor growth. Recently, it has been shown that engagement of B7-H4 enhances Fas-mediated, caspase-dependent apoptosis [76]. B7-H4 expressed on TAMs strongly inhibits the tumor antigen-specific T-cell response [57], which is in accordance with the observations that TAMs lacking B7-H4 showed reduced inhibitory activity and that suppression of B7-H4 on TAMs by antisense oligonucleotides reversed B7-H4-mediated repression [77]. Interestingly, the expression of B7-H4 on TAMs inversely correlates with survival of cancer patients [57]. Furthermore, professional APCs, when induced by T_{reg} cells to produce IL-10, upregulate B7-H4, thereby converting immunostimulatory APCs to repressor cells.

Targeting B7 molecules or their receptors for cancer therapy

Currently, approaches towards upregulation of costimulatory molecules and blockade of coinhibitory signals on tumor cells, T cells and other immune cells are being evaluated in preclinical models, as well as in clinical trials.

Induction of B7-1 and B7-2 costimulatory molecules

Owing to the frequent lack of B7-1 and B7-2, tumor cells are not able to deliver the costimulatory signals necessary for efficient effector T-cell activation. In preclinical tumor models, induction of T-cell activation, tumor rejection and lysis of tumor cells have been shown upon transgenic expression of B7-1 and/or B7-2 in tumor cells [78,79]. In a clinical trial, transgenic expression of B7-1 in autologous melanoma cells upon application of recombinant vaccinia virus vectors encoding B7-1 has been shown to repress tumor growth [80], demonstrating the therapeutic benefit of manipulating B7 costimulation. However, expression of B7-1 and B7-2 alone failed to induce immunity against poorly immunogenic tumors [81]. These studies led to the development of phase I/II trials using B7-1-manipulated allogeneic or autologous tumor cells in combination with cytokines and/or hematopoietic stem cell transplantation (Table 3). Trials conducted in patients with RCC [82,83], NSCLC [84] and acute myeloid leukemia [85] were promising, producing partial responses (i.e. reduction in size of multiple metastases) to stable disease. In a phase II trial, post-vaccination increases in IFN- γ responses to autologous tumor cells and lymphocyte infiltrates in tumor biopsies were reported [83].

Inhibition of CTLA-4 repressive capacities

Blocking of the immune-suppressive function of CTLA-4 has been shown to be a powerful tool for activating T-cell responses *in vivo* and enhancing anti-tumor immunity in different preclinical models [86,87]. Monotherapy with an antibody against CTLA-4 promoted rejection of transplantable tumors of various origins, although it failed to reject poorly immunogenic tumors [87]. Based on promising preclinical studies, the clinical potential of antibodies against CTLA-4 in different human malignancies, including melanoma, ovarian carcinoma, prostate carcinoma, non-Hodgkin's lymphoma and RCC, was evaluated using the two human antibodies against CTLA-4 – MDX-101 (ipilimumab) and CP-675.206 [88-92] (Table 3). Taken together, objective response rates in melanoma were obtained in ~20% of patients, with response durations ranging between 18 and 35 months. These data suggest that blocking CTLA-4 enhances or generates effective anti-tumor immune responses to some extent. The optimal protocol concerning dosage and duration of treatment, however, has still to be determined, and the effect of repetitive applications of blocking CTLA-4 antibodies has recently been explored [90]. It is noteworthy that some common adverse events involving the skin and the gastrointestinal tract occurred after this treatment, and these events led to the cessation of the antibody monotherapy against CTLA-4 and necessitated use of combinations with additional treatments.

Blockade of coinhibitory B7-H molecules

Although, as described above, several antagonistic antibodies targeting the CTLA-4 coinhibitory molecule have been developed, only a small number of antagonistic antibodies targeting B7-H family members are so far available. For instance, a crosslinking antibody against B7-DC potentiates the immune response in both prophylactic and preclinical models [70] by activating DCs through reverse signaling [93]. Administration of crosslinking B7-H2 fusion proteins might also prove useful for costimulating activated T cells. The coexpression of the repressors B7-H1 and B7-H4 in cancers with poor prognosis provides a rationale for blocking these repressors. Blockade of B7-H1 in tumors with antagonistic antibodies abrogated tumor resistance to a CTL attack. Accordingly, PD-1 blockade by antibodies not only resulted in tumor immunity but also in autoimmune reactivity. Induction of B7-H1 on tumor-infiltrating T_{reg} cells, however, partially reverts the B7-H1 blockade on tumor cells. The therapeutic potential of blockade of both B7-H1 and B7-H4, however, becomes obvious by their extraordinary high frequency of coexpression in a variety of tumor entities (Table 2). This is reflected by the observation that patients with B7-H1⁺ B7-H4⁺ RCC have a significantly lower survival rate than patients with double-negative and single-positive tumors.

Moreover, soluble B7-H receptors could block the inhibitory activity while preventing binding to the inhibitory ligand. For instance, B7-H1-mediated inhibition could be blocked by soluble PD-1 (sPD-1), thereby reducing tumor progression in preclinical models [94]. Alternatively, an increase in the costimulatory activity of B7-DC, B7-H2 or B7-H3 might be of benefit for the treatment of cancer patients. Indeed, intratumoral, transgenic B7-H3 expression induced tumor-antigen-specific immunity that could be enhanced by combining with anti-angiogenic therapy in a preclinical model [95]. Noteworthy in this context, several additional inhibitory mechanisms repress the immune response in the tumor microenvironment. These include T_{reg} cells, myeloid suppressor cells, inhibitory cytokines and inhibitory cell-surface molecules such as HLA-G. Along this line, it becomes evident that understanding the complex network of coinhibitory and costimulatory mechanisms of action will be essential for the implementation of successful tumor immunotherapy, which also includes the modulation of these immune stimulators and repressors. Based on this hypothesis, a combined blockade of the various coinhibitory molecules and/or suppressive immune cells might be beneficial for tumor patients.

Concluding remarks

There is increasing evidence that the tumor microenvironment has significant impact on the outcome of T-cell-based immunotherapy in the treatment of cancer. Combinations of costimulatory and coinhibitory molecules regulate T-cell function in healthy tissues and in tumors to maintain self-tolerance and anti-tumor immunity [88]. Tumor immunity and protection from autoreactivity seems to be linked to B7-H inhibitory molecules. Their manipulation consequently has the potential to reconstitute a balanced immune reaction [96]. In addition to inhibiting tumor infiltration, negative-regulatory mechanisms repress the T-cell anti-tumor response even if recruitment is successful. B7-H inhibitory molecules have crucial roles in preventing tissue attack by infiltrating T cells and, moreover, in preventing tumor cells from undergoing immune eradication, the manipulation of which has the potential to reconstitute an effective anti-tumor immune reaction [97,98]. Forced tumor immunity, however, might also be linked with severe autoreactivity. Currently, the costimulatory signals are well understood, whereas less is known about the inhibitory processes. The studies discussed here, along with others, suggest that multiple checkpoints exist that are mediated by the negative costimulatory molecules B7-H1 and B7-H4, the expression of which is moreover regulated by pro- and anti-inflammatory cytokines. The balance of coinhibition and costimulation in regulating T-cell anti-tumor activity is further modulated by the tumor

microenvironment itself. To improve current immunotherapeutic strategies, the following questions in particular need to be resolved:

- A variety of coinhibitory molecules exist, but what are the different consequences of their inhibition?
- Is induced costimulation required or is inhibition of inhibitory signals sufficient for the induction of tumor- (and auto-) reactivity of infiltrating T cells?
- Which inhibitory signals repress auto-immunity, and are these the same as those that prevent tumor immunity? Can tumor immunity be repressed without inducing auto-immunity?
- Which combination of blockade of the inhibitors is the most efficient for de-repressing the immune response?

It is obvious, however, that the negative regulatory B7-H network is one of a panel of repressive regulatory mechanisms that protects tumor cells from immune destruction. Although it is likely that additional members of the B7-H costimulatory and coinhibitory family remain to be elucidated, therapeutic strategies for blocking the inhibitors and/or enhancing the stimulators will probably prove to be powerful tools that can be implemented in the design of immunotherapy approaches for malignant diseases.

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Figure 1.

GO and STOP signals provided by B7 family members in the network of interactions between the tumor-stroma tissue and cytotoxic T lymphocytes (CTLs). **(a)** Go signals. (i) B7-H1 on activated natural killer (NK) cells binds to an as-yet-unidentified B7-H1 receptor ('X') mediating PD-1-independent CTL activation. (ii) Activated antigen-presenting cells (APCs) provide stimulatory signals by binding of B7-1 and B7-2 to CD28 expressed on CTLs, thereby preventing CTL apoptosis and sustaining their activation, proliferation and cytokine secretion. (iii) Furthermore, CTLs are activated by interaction of ICOS with B7-H2 on tumor tissues. **(b)** STOP signals. The tumor microenvironment provides an immunosuppressive shield by expression of B7-H1, B7-DC (the ligand for both of which is PD-1) and B7-H4, which binds to an as-yet-unidentified receptor ('Y') on CTLs. These interactions mediate CTL cell-cycle arrest, repressed cytokine production and entry into apoptosis, resulting in the repression of the anti-tumor attack. CTLs themselves can directly inhibit other CTLs by PD-1-B7-H1 interaction (i). APCs express B7-DC and B7-H1, which bind to PD-1 on CTLs (ii), and both mediate the repression of CTLs. In the presence of IL-10 and IL-6, B7-H4 is expressed on APCs (iii) and tumor-associated macrophages (TAMs) (iv), providing inhibitory signals to CTLs.

Table 1
B7 family ligands and their receptors of the CD28 family

Group		B7 ligands		CD28 family receptors		Comodulatory function
		Name	Expressing cells	Name	Expressing cells	
I	B7-1 (CD80)		Activated APCs	CD28	T cells	Stimulation
				CTLA-4	Activated T cells	Inhibition
	B7-2 (CD86)		Activated APCs	CD28	T cells	Stimulation
				CTLA-4	Activated T cells	Inhibition
II	B7-H2 (L-ICOS, B7h, B7RP-1)		B cells, macrophages, non-lymphoid tissues	ICOS	Activated T cells	Stimulation
				PD-1	Activated T cells, B cells, myeloid cells	Inhibition
	B7-DC (PD-L2)		NK cells	Not yet identified	Activated T cells	Stimulation
				PD-1	Activated T cells, B cells, myeloid cells	Inhibition, activation
III	B7-H3		APCs, lymphoid and non-lymphoid tissues, tumors	Not yet identified	Activated T cells	Inhibition, activation?
	B7-H4 (B7x, B7S1, VTCN1)		Epithelial tissues, tumors	Not yet identified	Activated T cells	Inhibition

Table 2

Expression of B7-H molecules in human tumors

Molecule	Cancer type	Specimen analyzed	Samples analyzed (n)	Frequency (%)	Clinical association	Refs
B7-H1	Acute myeloid leukemia (AML)	Blood	58	2.8	Poor survival	[34]
	Bladder urothelial carcinoma	Lesions	65	100	High frequency of relapse and poor survival	[61]
		Lesions	280	36.1	Grading and infiltration with mononuclear cells	[38]
	Breast cancer	Lesions	69	60	High risk grading, Ki-67 expression	[37]
		Lesions	44	50	Grading and hormone receptors	[99]
		TIL	44	34	Tumor size, grading, HER-2/neu (ERBB2) status	
		Lesions	52	100	Not associated with metastasis, focal expression, reciprocal correlation with TIL	[46]
	Pancreatic cancer	Lesions	40	38.2	Associated with poor tumor differentiation, advanced tumor stage	[41]
	RCC	Lesions	306	23.9	Disease progression, increased overall mortality	[100,101]
		Lesions	298	23.5	Increased risk of death	[102]
B7-H3	Gastric carcinoma	Lesions	196	37.2	Threefold increased risk of death	[103]
		TIL	267	56.6	Advanced disease, increased risk of death	[58]
		Vasculature	259	18.2	Threefold increased risk of death	[62]
		Lesions	102	85.8	Tissue infiltration depth and survival time	[60]
		Lesions	70	37	High levels of expression associated with metastasis, reduced numbers of TILs associated with lymph node metastasis	[51]
	Prostate cancer	Lesions	823	93	Metastatic spread, increased risk of recurrence and death, poor outcome	[52]
		Lesions	338	100	Disease progression and clinical outcome, variable staining pattern associated with Gleason score	[50]
	Breast	Primary	173	95.4	Increased staining intensity in invasive carcinomas associated with negative hormone receptor status, independent of tumor grade and stage	[104]
		Metastases	246	97.6		
B7-H4	NSCLC	Lesions	70	43	Reduced number of TILs associated with lymph node metastasis	[51]
		Lesions	90	100	Inverse correlation with the number of tumor-associated lymphocytes	[105]
	Ovarian cancer	Cells	12	<5	-	[77]
		Macrophages	12	70		
	Primary endometrioid	Serous	32	100	Poor prognostic factor for survival, potential diagnostic marker	[106]
		Endometrioid	12	100		
		Metastases	30	100		
	Pancreatic cancer	Lesions	51	27.5	Poor survival, inversely associated with TILs	[107]
		Lesions	36	92	None	
	Prostate cancer	Lesions	823	99	Metastatic spread, increased risk of recurrence and death, poor outcome	[52]
		Vasculature	259	59.1 ^d	Threefold increase in risk of death	[62]

Molecule	Cancer type	Specimen analyzed	Samples analyzed (<i>n</i>)	Frequency (%)	Clinical association	Refs
		Serum	101	81.5 ^{<i>b</i>} 6.5 ^{<i>c</i>} 53	Correlation with advanced tumor stage	[108]

^{*a*}RCC lesions.
^{*b*}Tumor endothelium.
^{*c*}Normal kidney.

Table 3
Targeting B7 molecules or their receptors for cancer therapy

Therapeutic strategy	Therapeutic agents	Malignancy	Clinical outcome	Refs
Cellular vaccines	Vaccinia virus encoding B7-1	Melanoma	50% SD	[80]
	B7-1/HLA-A-modified allogeneic cells	NSCLC	5% PR, 26% SD	[84]
Antibodies	Anti-CTLA-4 antibody (MDX-010, BMS-734016)	Prostate carcinoma	16% PR	[90]
	Anti-CTLA-4 antibody (CP-675 206)	Melanoma	6% CR + PR	[91]
	Anti-CTLA-4 antibody (MDX-010)	Melanoma	11% PR	[92]
	Anti-CTLA-4 antibody (CP-675 206)	Melanoma	12% PR	[91]
	Anti-CTLA-4 antibody (CP-675 206)	Melanoma	31% OR	[109]
	Anti-CTLA-4 antibody (MDX-010)	Renal cell carcinoma	13% PR	[110]
	Anti-CTLA-4 antibody (MDX-010) plus gp100 peptide vaccine	Melanoma	21% CR + PR	[88]
Combined therapy	Anti-CTLA-4 antibody (MDX-010) plus IL-2	Melanoma	22% CR + PR	[89]
	B7-1 modified autologous tumor cells plus IL-2	Renal cell carcinoma	8% CR + PR, 64% SD	[83]
	B7-1 modified autologous tumor cells plus IL-2	Renal cell carcinoma	25% SD + PR	[82]
	B7-1 modified AML cells plus IL-2	Acute myeloid leukemia	Ongoing	[85]
	Anti-CTLA-4 antibody (MDX-010) after vaccination with GM-CSF tumor cells	Melanoma	72% SD + PR	[92]
	Anti-CTLA-4 antibody (MDX-010) after vaccination with GM-CSF tumor cells	Ovarian carcinoma	44% SD + PR	[92]
	Anti-CTLA-4 antibody (MDX-010) plus peptide vaccine	Melanoma	14% OR	[111]
	Anti-CTLA-4 antibody (MDX-010) plus peptide vaccine	Melanoma	50% SD + PR	[112]
	Anti-CTLA-4 antibody (MDX-010) plus dacarbazine	Melanoma	17% CR + PR	[113]

Abbreviations: CR, clinical response (less pain, better performance, but not measurable); OR, objective response (measurable response); PR, partial response (decrease in size of tumor in response to treatment); SD, stable disease (no decrease, no increase).