The role of B7 family molecules in hematologic malignancy

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The B7 family consists of structurally related, cell-surface proteins that regulate immune responses by delivering costimulatory or coinhibitory signals through their ligands. Eight family members have been identified to date including CD80 (B7-1), CD86 (B7-2), CD274 (programmed cell death-1 ligand [PD-L1]), CD273 (programmed cell death-2 ligand [PD-L2]), CD275 (inducible costimulator ligand [ICOS-L]), CD276 (B7-H3), B7-H4, and B7-H6. B7 ligands are expressed on both lymphoid and nonlymphoid tissues. The importance of the B7 family in regulating immune responses is clear from their demonstrated role in the development of immunodeficiency and autoimmune diseases. Manipulation of the signals delivered by B7 ligands shows great potential in the treatment of cancers including leukemias and lymphomas and in regulating alloergic T-cell responses after stem cell transplantation.

Introduction

Cancer and the immune system are fundamentally interrelated. Cancer cells express tumor-specific aberrant antigens and must therefore evade immune detection to survive, either by inducing immunosuppression or deriving survival signals from tumor-infiltrating immune cells. T cell–mediated antitumor immunity requires recognition of cancer-associated antigen by the major histocompatibility complex (MHC), and appropriate costimulatory and repressive secondary signals arising from complex interactions with other immune, stromal, and tumor cells. Dysfunction of costimulation pathways may contribute to failed antitumor immunity.

The B7 system is one of the most important secondary signaling mechanisms and is essential in maintaining the delicate balance between immune potency and suppression of autoimmunity. Potential therapeutic applications include immune-boosting adjuvants to conventional anticancer therapy, hematopoietic stem cell transplantation (HSCT), antitumor vaccines, bioengineered T cells as well as attenuation of graft-versus-host disease (GVHD). The B7 family is only one aspect of a complex signaling network that comprises other immunoglobulin superfamilies (IGSF) members, the tumor necrosis factor superfamily (TNFRSF), chemokines, cytokines, and adhesion molecules. However, based on a substantial evidence base and a growing therapeutic armory, the B7 family requires particular attention, as summarized in Table 2.

The standardized approach to nomenclature is the cluster of differentiation (CD), which has not yet been applied to all members of the family. While many researchers are more familiar with their original names, which are still widely used in the literature, for the purposes of this review, the CD designation is used wherever possible.

B7 founder members

CD80, CD28, and CD152 (CTLA-4)

The first B7 family member was a molecule discovered on activated, proliferative splenic B lymphocytes, and termed B7 in an early nomenclature convention attributed to B cell–defining markers. Subsequent cloning and sequencing of the B7 gene revealed sequence homology with IGSF members and expression in a variety of lymphoid malignancies. B7 was found to bind CD28, an IGSF member on T cells, which augmented their activation. Further molecular profiling identified a CD28 homologue, CD152 (cytolytic T cell–associated sequence-4 [CTLA-4]), which mapped to the same chromosomal region as CD28 and bound B7 more potently. The B7 signal blockade using a CTLA-4.Ig construct led to suppression of humoral and cell-mediated immune responses, while transfection of immunogenic murine melanoma cell lines with B7 enhanced antitumor immunity, which could be abrogated by CTLA-4.Ig.

Initial experiments suggested that both anti-CD28 and anti–CTLA-4 monoclonal antibodies (mAbs) were T-cell activators, but contrary evidence showing a suppressive role for CTLA-4 soon emerged and was conclusively demonstrated in Ctl-1−/− mice, which rapidly develop fatal lymphoproliferation and multisystemic autoimmunity. Multiple mechanisms for CTLA-4–mediated immunosuppression are proposed, including competition for CD28 binding and induction of suppressive intracellular signaling pathways (reviewed in Rudd and Schneider).

Differential cellular binding of 2 B7-directed mAbs (anti–BB-1 and anti-B7) suggested distinct molecular targets (B7-1/CD80 and B7-2/CD86), a finding confirmed when the B7-2 gene was cloned from a CTLA-4.Ig-binding activated-B-cell cDNA library. Both molecules were demonstrated on antigen-presenting cells (APCs) including dendritic cells (DCs) and activated macrophages.

CD80/CD86 expression in malignancy

A role for B7 in antitumor immunity was confirmed by the demonstration that cytotoxic T cells better eradicated murine malignancies transfection to express B7-1 and B7-2. In contrast to solid tumors, both molecules are expressed innately in many hematologic malignancies. After in vitro activation, follicular lymphoma (FL) cells up-regulate CD80/CD86 and other costimulatory and adhesion molecules, thereby increasing APC activity and augmenting primed T-cell responses.

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Sternberg (HRS) cells of classic Hodgkin lymphoma (CHL) express high levels of CD80/CD86. Expression in multiple myeloma (MM) is variable, and may influence prognosis. Expression is low in chronic lymphocytic leukemia (CLL) unless the cells are stimulated, and rare in acute leukemia. However, expression of these costimulatory molecules alone is clearly inadequate for effective antitumor immunity because even malignancies showing high expression levels inevitably progress without therapy. Both molecules are widely expressed in the tumor immune microenvironment, and loss of expression may contribute to the failed antitumor responses.

Therapeutic modulation of CD28, CD152, and CD80/86

CD80 has been targeted using the mAb galiximab in phase 1 and 2 trials in non-Hodgkin lymphoma (NHL) and CHL, both alone and combined with rituximab. Off-target immune suppression through CD80 blockade may limit the efficacy of such an approach. Anti-CD28 mAb therapy showed preclinical promise as an agent that could specifically expand regulatory T-cell subsets as therapy for autoimmune disorders. However, the subsequent clinical trial led to disastrous toxicity resulting from the use of too high a dose of the antibody and an unanticipated massive cytokine storm, urging caution in future therapeutic approaches to B7 pathway modulation. The first B7 pathway-targeting agent, anti–CTLA-4 mAb (ipilimumab) has now been FDA approved for the treatment of metastatic melanoma. Evidence is accumulating for its efficacy in hematologic malignancies, including in vitro enhancement of antitumor T-cell responses.

Expansion of the B7 family and an immunosuppressive axis: CD274 (B7-H1/PD-L1), CD273 (B7-DC/PD-L2), and CD279 (PD-1)

A GenBank database B7 homology search identified a gene (B7-H1/CD274) encoding a protein distinct in amino acid sequence and tissue distribution from the founder B7 members, but with a similar tertiary protein structure. Expression in hematologic tissue was restricted to activated T cells and monocytes, although nonhematologic expression was widespread. Its natural ligand was neither CD26 nor CTLA-4 and proved elusive. In contrast to the effects of CD80/CD86, B7-H1 induced proliferation in...
activated T cells, but induced IL10 and IFN-γ production with negligible IL4 and IL2.

A subtractive cDNA library comparing DCs and macrophages revealed another homologous protein (B7-DC) whose structure and genomic sequence was similar to B7-H1, and which failed to bind CD80 or CTLA-4.Ig. B7-DC.Ig stimulated activated T cells more potently than CD80.Ig, inducing IFN-γ and IL2 production and, in contrast to B7-H1, very little IL10. Its ligand, programmed cell death 1 (PD-1), a molecule discovered a decade before, also bound to T-cell lines and identified a cell death–specific gene. CD279 (PD-1) and its ligand PD-1 conveyed potent suppressive signals. Evidenced for bidirectional signaling emerged from murine B cell– and –independent pathways. Growth of pancreatic cancer cells in immunocompetent B6 mice is impaired by administration of anti–PD-L1 antibody. Tumor-specific T cells fail to lyse melanoma cells in host 2C/Rag2−/− mice despite stimulatory DC80/86 mAb binding to their ligand PD-1 conveyed potent suppressive signals. Evidence for bidirectional signaling emerged from murine B cell– and –independent pathways. Knock-out of T cell–specific PD-1 or B cell–specific PD-L2 impaired long-lived plasma cell formation, implying a stimulatory role for reverse signaling. Receptor/ligand pairing is also more promiscuous than originally believed. A search for binding partners for CD80 using Cita-4−/−Cd28−/− activated CD4+ T cells suggested PD-1 as a candidate, with CD80 signaling through PD-1 independent of CTA-4 or CD28, to inhibit T-cell proliferation. The complexity of the B7 system is becoming clear: context and direction of signaling determines responder cell outcome.

### Table 2. Summary of evidence for role and therapeutic potential of the B7 family molecules in hematological malignancy, with citations discussed in this review

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mAb indicates monoclonal antibody; NK, natural killer; CTLA, cytolytic T cell–associated sequence-4; PD, programmed cell death; ICOS, inducible costimulator; CAR, chimeric antigen receptor; and X, there are currently no data or therapeutic agent available.

The PD-1/PD-L1/PD-L2 axis has been widely demonstrated to contribute to failed antitumor immunity. $\text{PD-1}^{−/−} \text{ mice and } \text{PD-L1}^{−/−}$ mice treated with anti–PD-L1 antibody demonstrate superior rejection of PD-L1–expressing tumors through PD-1–dependent and –independent pathways. Growth of pancreatic cancer cells in immunocompetent B6 mice is impaired by administration of anti–PD-1 or PD-L1 mAbs. Tumor-specific T cells fail to lyse melanoma cells in host $2C/Rag2^{−/−}$ mice despite stimulatory IFN-γ treatment because IFN-γ also induces up-regulation of immunosuppressive PD-L1. $\text{PD-L1}$ is expressed in many tumors and cell lines and appears to be associated with poorer outcome.
in solid malignancy, while PD-1 expression by tumor-infiltrating lymphocytes (TILs) may also confer poorer outcomes. After treatment using a tumor-vaccination strategy of murine acute leukemia, long-lived residual leukemia cells were found to up-regulate PD-L1, suggesting a mechanism of leukemia persistence and relapse. These cells resisted CTL-mediated cell lysis, which could be overcome by anti–PD-L1 mAbs. However, blockade using anti–PD-1 mAbs did not reverse CTL resistance, while blocking CD80 had a similar effect to a PD-L1 block. The B7 system complexity is further illustrated by these findings: CD274 appears to have further immunosuppressive binding partners, while CD80 may be capable of mediating suppressive as well as costimulatory signals.

There is sparse and conflicting evidence for CD274 (PD-L2) expression in acute leukemia, although the molecule is apparently up-regulated on malignant cell exposure to IFN-γ or Toll-like receptor (TLR) ligand, and at relapse. Evidence for reduced T cell–mediated specific lysis in vitro correlates with CD274 expression and is reversible by blocking CD274. Functional work in CLL has demonstrated a clear role for the PD-1/PD-L1 axis in mediating systemic, as well as antitumor, immune defects. Expression of CD279 by circulating T cells and CD274 by leukemia and FL cells is greater than in nonmalignant control cells, while CD274 expression is associated with poorer clinical outcome. T cells derived from patients with various malignancies have impaired in vitro p-GTPase signaling with reduced immune synapse formation. This defect is reversed by treatment with anti-CD1 and anti–PD-L1 mAbs, along with blockade of further inhibitory molecules CD200, CD270, and CD276 (B7-H3). Lenalidomide exposure also reverses this defect, coincident with down-regulation of CD274 and CD276 expression. These findings, along with evidence that CD80 expression is up-regulated on lenalidomide-exposed CLL cells, provide some insight into the drug/Es mechanism of action. In patients with adult T-cell leukemia/lymphoma (ATLL), CD279 expression on CD8+ T cells is up-regulated compared with ATLL-free HTLV1+ (human T-cell lymphotropic virus) controls.

CD279 is invariably expressed in angioimmunoblastic T-cell lymphoma, frequently in CLL, but absent in most other NHL. CD279 is expressed in the immune microenvironment of NHL, where it may have a prognostic significance, and there are conflicting reports of expression in the CHL microenvironment. Evidence for expression of CD274 in NHL is sparse and inconsistent. Immunohistochemistry and flow cytometric studies have shown expression by the malignant cell in the majority of primary mediastinal large B-cell lymphoma (PMBL) and anaplastic large cell lymphoma (ALCL), a minority of diffuse large B-cell lymphoma (DLBCL), and not at all in most other NHL. CD274 is overexpressed in the HRS of CHL associated with copy number aberrations and activating translocations involving the 9p24 chromosomal region containing the CD273 and CD274 genes, and upstream through the CD274 and CD273 expression-regulating JAK2 pathway. Limited in vitro evidence suggests that there is CD274-dependent suppression of T cells in CHL, evidenced by suppressed proliferation and IFN-γ production.

There is variable expression of CD274 in myeloma with evidence for an important role in myeloma-induced immunosuppression. CTL generated in vitro by exposure to IFN-γ-treated myeloma cell lines show impaired cytolyis of subsequently presented target myeloma cells. NK-cell trafficking and cytotoxic potency in vitro is augmented by pretreatment with anti–PD-1 mAb. An autologous transplantation and costimulator/vaccine-based treatment strategy for myeloma in a cell line–based mouse model, which had showed evidence for the emergence of dysfunctional circulating CD279-expressing CD8+ T cells, is augmented by the administration of PD-L1 antibodies.

While somewhat conflicting and lacking functional corroboration, these findings suggest that therapeutic modulation of this axis in hematologic malignancy may have a role.

Therapeutic modulation of CD274, CD279, and CD273

Fully humanized IgG4 mAbs targeting the PD-1/PD-L1 axis have reached clinical trials. Anti PD-L1 therapy (BMS936559) was administered to 160 heavily pretreated patients with a diverse group of solid tumors in a phase 1 trial. Objective and often durable responses were observed in up to 19% of patients, along with autoimmune and infusion reactions consistent with the murine PD-L1−/− models. A similar clinical trial of an anti–PD-1 monoclonal antibody therapy (BMS936558) demonstrated comparable response rates in 296 patients. There was an association between response and level of tumor PD-L1 expression. Further combination therapy approaches using anti–PD-1 mAbs are under way, including combination with the anti–CTLA-4 mAb ipilimumab in melanoma (NCT01024231), with conventional combination chemotherapy in non-small cell lung cancer (NCT01454102) and with tyrosine kinase inhibitors sunitinib and pazopanib in renal cell carcinoma (NCT01592370). Another PD-1 antibody (MK-3475) has also shown activity in patient with advanced solid tumors. A phase 1 safety and pharmacokinetic study of the anti–PD-1 mAb in advanced diverse hematologic malignancies showed a reasonable safety profile and a 33% overall response rate with further monotherapy and combination therapy trials proposed. The results are promising, but these drugs remain at an early stage of development and are likely to find their most active role as immune adjuvants to conventional therapy.

B7 pathway modulation to augment immunotherapy

Allogeneic HSCT

Failure of allogeneic HSCT may in part be mediated by suppressive B7 signaling. Anti–CTLA-4 mAb therapy has been applied successfully in phase 2 clinical trials to treat relapsed CHL, myeloma, and leukemias after allogeneic HSCT without the anticipated exacerbation of GVHD seen in murine transplantation experiments. Use of PD-L1/PD-1 axis blockade to enhance allogeneic T-cell responses has not yet reached clinical trial, although there is promising preclinical data in a murine AML model using TCR-engineered AML antigen-specific allogeneic T cells. Late transfer of these cells led to poorer antitumor responses accompanied by PD-1 overexpression. Responses were restored by treatment with anti–PD-L1 mAb. PD-1 blockade, however, accelerated GVHD lethality.

Tumor vaccination strategies

B7 molecules may play an essential role in cancer vaccination strategies. Mouse model immunization strategies using highly immunogenic cancer cell lines showed early success, particularly with chemokine costimulatory approaches. Tumor lysate-pulsed DCs have been incorporated into some vaccination strategies, showing modest success in patients with advanced lymphoma. Idiotype-specific tumor vaccines have been produced for myeloma and NHL, while immunogenic tumor-specific antigens have been identified in CLL and acute leukemia. However, vaccination has yet to make any real impact on cancer therapy: an informal meta-analysis incorporating almost
1000 patients with a variety of malignancies showed an overall response rate of < 4%. The combining vaccination strategies with immunosuppressive B7 pathway blockade may improve these disappointing outcomes. CTLA-4 mAb-enhanced melanoma rejection has been demonstrated in a clinical trial, but studies in NHL have been disappointing, with minimal responses achieved in vaccinated patients treated with ipilimumab. Encouraging in vitro evidence suggests that a similar strategy could be applied to PD-1/PD-L1 axis blockade, although this has yet to be incorporated into any clinical trial. One group showed PD-L1/PD-L2 siRNA silencing improved DC-induced leukemia-specific T-cell responses, while another demonstrated enhanced T-cell responses after PD-1 antibody treatment in a myeloma/DC fusion vaccination strategy.

Bioengineered T cells

In contrast to the active immunization strategies outlined in the previous paragraph, an alternative strategy is to expand and reinfuse autologous bioengineered T cells. Autologous TILs extracted from melanoma biopsies, expanded and reinfused into immunosuppressed recipients, have shown some therapeutic success but failed to translate to other malignancies. Attempts to augment autologous T-cell specificity for tumor antigens led to TCR bioengineering approaches, first against EBV-driven lymphoproliferative tumors and subsequently other tumor-specific antigens. While clinical responses are reported, reactive clones tend to rapidly lose efficacy or die off altogether. Subsequent developments used tumor antigen-inoculated transgenic mice whose T cells express human MHC, expanding tumor-specific T-cell clones and hence deriving already rearranged TCR genes for transfection into patient-derived T cells. Newer techniques incorporate novel receptor constructs into autologous T cells using chimeric antigen receptors (CAR). Here, a rearranged, antigen-specific immunoglobulin variable region gene is combined with intracellular domains of the TCR gene, thus bypassing MHC-restricted antigen recognition. CARs have the advantage of being amenable to further augmentation with costimulatory motifs, of which CD28 and CD80 have demonstrated efficacy. CARs incorporating costimulatory modifications have reached phase 1 clinical trials in NHL using an anti–CD19-CD28-TCR chain construct, and CLL, using an anti–CD19-CD137 construct (a TNFRSF member). Promising results have been observed, even in refractory patients. Further optimization is needed and B7 family members will play a central role in this.

GVHD attenuation

Allogeneic organ transplantation tolerance may be induced through CD80/CD86 blockade, and this finding has encouraged research into a role for the B7 molecules in GVHD. Fully HLA-mismatched mixed lymphocyte reactions are partially abrogated by CTLA-4.Ig or CD80/CD86 antibody treatment, and fully by combination treatment. This observation led to a promising clinical trial using CTLA-4.Ig to anergize donor lymphocytes and attenuate GVHD after mismatched allogeneic HSCT for hematologic malignancy. Early immune reconstitution with relatively low rates of acute and chronic GVHD and viral infection was observed, without the high relapse rates seen after T-cell depletion. Another potential GVHD-attenuating strategy, through PD-1/PD-L1/2 axis manipulation, is a promising hypothetical concept.

Regulatory T cells

The role of regulatory T cells (Tregs) in suppression of successful antitumor immunity is becoming more evident. There is an...
association between adverse outcome in solid tumor and increased FOXP3-expressing cells in the microenvironment\textsuperscript{130} suggesting that their presence suppresses antitumor immunity.\textsuperscript{131,132} However, in B-cell malignancies the converse has been found, with increased infiltrate associated with improved outcome.\textsuperscript{133-135} CTLA-4\textsuperscript{136,137} and PD1/PD-L1\textsuperscript{138-140} are key pathways used by Tregs to mediate their suppressive activity, hence therapeutic interference with these pathways may contribute by depleting this profoundly suppressive population. These possibilities are under active investigation.

Another costimulatory B7 pathway: ICOS and B7-H2

A further avenue of B7 family costimulatory molecules opened with the discovery of another CD28-like molecule (CD28-related peptide-1 [CRP-1]).\textsuperscript{141} The human equivalent was confirmed to be a stimulatory molecule expressed only on activated T cells, hence termed inducible costimulator\textsuperscript{142} (ICOS/CD278). A second clone from the same library, termed B7-related peptide-1 (B7RP-1), had structural similarities to CD80/CD86. ICOS failed to bind CD80/CD86, B7RP-1 failed to bind to CD28 or CTLA-4, but ICOS and B7RP-1 specifically bound each other. B7RP-1 had already been discovered and functionally characterized by an independent group and named \( \delta \)7h\textsuperscript{143}; its human homolog was identified and named B7-H2.\textsuperscript{144} This was the same molecule, ICOS-ligand (B7RP-1/\( \delta \)7h/ICOS-L/CD275), and a later study showed that ICOS-L could bind and costimulate via CD28.\textsuperscript{145}

ICOS may play a role in maintaining durable immune reactions\textsuperscript{146} and is expressed at particularly high levels in germinal center T cells or TFH (follicular helper) cells.\textsuperscript{147,148} ICOS-L is constitutively expressed by APCs as well as diverse nonhemato-logic tissues\textsuperscript{143} and down-regulated with ongoing inflammation,\textsuperscript{149,150} in contrast to the activation-induced CD28 ligands CD80/CD86.\textsuperscript{141,151} In CD28\textsuperscript{−/−} mice, germinal center formation is rescued by ICOS overexpression, while inappropriate overexpression in CD28\textsuperscript{wt/wt} mice induces systemic autoimmunity. T cells derived from ICOS\textsuperscript{−/−} mice show normal thymic T-cell development but dysfunctional proliferation, cytokine and immunoglobulin production, and germinal center formation while paradoxically conferring greater susceptibility to Th1/IFN-\( \gamma \)-mediated disease including experimental autoimmune encephalitis (EAE).\textsuperscript{152} The contradictory functions of ICOS suggest a more complex role in T-cell regulation, perhaps favoring IL4/IL10/IL13 and humoral responses at the expense of IFN-\( \gamma \)-mediated cytotoxic/Th1 responses.\textsuperscript{153} ICOS also plays a role in maintaining immunosuppressive CD4\textsuperscript{+} T-cell subsets, with ICOS\textsuperscript{+} enriched CD4\textsuperscript{+} cell cultures secreting more IL10 and less TNF\( \alpha \) than ICOS-depleted systems\textsuperscript{154} and ICOS, with FOXP3, essential for proper Treg development in the context of autoimmune diabetes and airway immune tolerance in ICOS\textsuperscript{−/−} mice.\textsuperscript{155}

Mutations in the human ICOS gene lead to an attenuated adult-onset common variable immunodeficiency (CVID),\textsuperscript{156} a predominantly B-cell defect likely arising from a loss of TFH cell function. The disease manifests with a variety of autoimmune phenomena as well as cancer and infection susceptibility.\textsuperscript{157} The complexity of ICOS makes any therapeutic applications difficult to predict, but its importance is clear.

ICOS and B7-H2 in cancer

Angioimmunoblastic T-cell lymphoma cells express ICOS, PD-1, and CXCR5, consistent with being the malignant counterpart of TFH lymphocytes.\textsuperscript{158} ICOS expression has also been described as a potential survival mechanism in NPM-ALK fusion anaplastic large cell lymphomas.\textsuperscript{159}
cell lymphoma cells.  

Although induced ICOS-L expression enhances cytotoxic T cell–mediated tumor rejection, there is sparse and conflicting evidence for its expression in solid tumors, while expression in myeloma and acute leukemia is widely heterogeneous. Despite these sometimes contradictory findings, the ICOS/ICOS-L axis may yet have an indirect role in enhancement of tumor immunity. ICOS is inducible on NK cells, which demonstrate cytotoxicity against ICOS-L–transfected murine leukemia cell lines. Cytokine-induced killer cells (CIK) demonstrate MHC-independent cytotoxicity and antitumor activity, enhanced after ICOS transfection. Optimal immunostimulatory therapy using CTLA-4 mAbs may be dependent on an intact ICOS/ICOS-L pathway, with CTLA-4 blockade increasing tumor-specific IFN-γ–secreting T cells in cancer patients.

**Therapeutic modulation of ICOS/ICOS-L**

ICOS is overexpressed during acute allogeneic solid organ rejection and tolerance is improved with pathway blockade, which suggests that this pathway may have importance in GVHD. Murine acute GVHD is reduced if donor Icos−/− mice or ICOS-blocking antibodies are used, with less gut and liver GVHD and improved survival despite intact Icos−/− donor cell cytotoxic function, cytokine-secretion and tissue trafficking, while Icos−/− recipient mice better tolerate allogeneic HSCT with more rapid and complete donor chimerism. However, a further study demonstrated more severe GVHD arising from allogeneic CD8+ Icos−/− cell transplantation compared with wild type, a contradiction perhaps explained by the differing mouse genetic backgrounds and variable use of Treg-depleting anti-CD25 mAbs. ICOS appears central to GVHD modulation, although because of these controversies, a therapeutic application has yet to emerge.

**Controversial immunosuppressive pathways: B7-H3 and B7-H4**

CD276 (B7-H3) was identified soon after ICOS, not detectable in resting blood cells but present in various normal tissues, in several tumor cell lines and induced on activated DCs and monocytes. B7-H3.Ig stimulates and increases IFN-γ secretion by activated T cells. Its ligand was eventually isolated from a CD28 family database homology search of a murine splenocyte cDNA library. Only TLT-2 showed specific binding, a member of the family database homology search of a murine splenocyte cDNA library. Although TLT-2 showed specific binding, it was the same molecule and proposed a ligand, a molecule that had been identified during previous gene expression profiling of Th1 polarized cells: BTLA (B and T lymphocyte attenuator). BTLA-binding properties suggested immunosuppressive functions analogous to those mediated by CTLA-4 and PD-1, which was supported by the Btla−/− mouse, which demonstrates enhanced Th1 activity. However, subsequent investigations failed to confirm that BTLA is the ligand of B7-H4. A TNFRSF molecule, HVEM, seems to be the major BTLA-interacting immunosuppressive molecule.

Although B7-H3 and B7-H4 are structurally related B7 family members, there is little consensus on function or binding partners. Crosstalk between other pathways, stimulatory as well as repressive functions, inducible as well as constitutive, tissue-dependent expression and 2–way signaling without clear receptor/ligand polarity are likely complexities existing across all molecules of this class and not only these more recently described members.

**B7-H6: a tumor-specific family member**

Investigation into natural killer cell (NK) effector mechanisms revealed the most recently identified member of the B7 family, B7-H6, a PD-L1/B7-H3 homologue specifically binding the CTLA-4–homologous NK-effector molecule NKP30. Membrane-bound B7-H6 activates NK cells, leading to degranulation and IFN-γ secretion. Unlike other B7 family members, B7-H6 is not expressed in any normal tissue even after activation, but is expressed in a variety of primary tumors and cell lines. The potential for therapeutic manipulation is clear, already inspiring incorporation of NKP30 into CAR constructs.

**An extended B7 definition? Butyrophilin and Skint**

Other B7 homologues have been described with potential importance in cancer immune surveillance and immune modulation, including the diverse Butyrophilin family and the epidermal T cell–associated Skint (Selection and upKeep of INTrappepithelial T cells) family. Butyrophilin subfamily 3 (BTN3) has a structural similarity to B7-H4 and is expressed on immune cells as well as human ovarian cancer, while BTN3 agonists may induce immunosuppression. However, functional data are conflicting; no ligand has been determined and no in vivo data generated. These molecules are intriguing but their role in cancer immunology remains speculative.

**Conclusion**

Our understanding of the B7 family has expanded enormously because the costimulatory pathways were first described. Contemporary models of immune regulatory networks reflect this complexity. The promise of immune-mediated enhancement of autologous and allogeneic responses through pharmacologic manipulation of this pathway shows substantial promise. Ongoing studies investigating the role of the most recently described members of the family: B7-H3, B7-H4, B7-H6, Butyrophilin, and Skint will likely reveal further potentially modifiable pathways as therapeutic targets for intervention. Whereas manipulation of the B7 family members and their ligands in suppressing GVHD and augmenting GVl after allogeneic HSCT has shown benefit in murine studies, and promising results in limited phase 2 clinical studies, this remains an area for clinical trial development. The contribution of ICOS/
ICOS-L to GVHD appears to be fundamental; however, on our current understanding of this pathway, the complexities of the interactions make predictions of clinical responses difficult to predict. After decades of study, strategies incorporating B7 family members, such as CTLA-4 and PD-1 pathway-inhibiting molecules and CAR-T cells, have finally demonstrated clear clinical benefit, fulfilling the early promise of the importance of this family in hematologic malignancies.

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The role of B7 family molecules in hematologic malignancy

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