

The contrasting role of B7-H3

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T lymphocytes of adaptive immunity provide vertebrates with the ability to survey for and respond specifically to an incredible diversity of antigens, whether foreign or native. Appropriate T cell response is required to eradicate pathogens, whereas abnormal T cell function could lead to autoimmune diseases, cancer, and transplantation rejection. The outcome of T cell engagement of antigen is determined by positive costimulation and negative coinhibition; both are primarily generated by the interaction between the B7 family and their receptor CD28 family (1). Recent years have seen the identification of several new members of B7/CD28 families. The growing B7 family now comprises seven members: B7-1 (CD80), B7-2 (CD86), B7h (CD275), PD-L1 (CD274), PD-L2 (CD273), B7-H3 (CD276), and B7x (B7-H4 or B7S1). Almost 5 years ago, we divided the B7 family into three groups by phylogenetic analysis: group I includes B7-1, B7-2, and B7h; group II consists of PD-L1 and PD-L2; and group III contains B7x and B7-H3 (2, 3). Receptors for group I are CD28, CTLA-4, and ICOS, and the receptor for group II is PD-1 (Fig. 1). B7-1 also binds PD-L1. One prediction of the phylogenetic comparison was that receptor(s) for B7-H3 would not be real homologue(s) of receptors for group I and II (3). Five years later, this prediction has been proven true (3). In this issue of PNAS, Hasiguchi *et al.* (3, 4) have identified one such receptor, triggering receptor expressed on myeloid cell (TREM)-like transcript 2 (TLT-2, or TREML2), which binds B7-H3 and costimulates activation of CD8 T cells in particular. TLT-2 is a member of the TREM receptor family (5, 6), which includes TREM-1, -2, and -3, as well as TLT-1 and -2. B7-H3 is the first ligand to be identified for the TREM receptor family. Because the immunological function of B7-H3 has been controversial, the discovery of the B7-H3/TLT-2 pathway is a significant step toward resolving the polarizing data involving the roles of B7-H3 in immune responses as well as the functions of the TREM receptor family.

B7-H3 has two forms. Mouse B7-H3 has extracellular IgV-IgC domains, whereas human B7-H3 contains tandemly duplicated IgV-IgC-IgV-IgC domains because of exon duplication (7). No func-

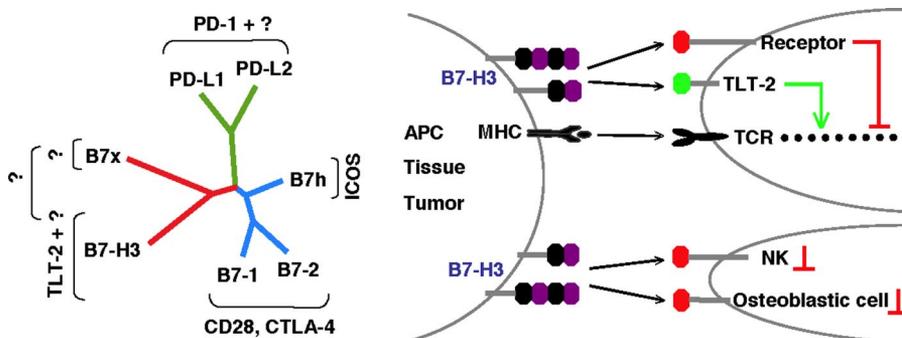


Fig. 1. B7-H3 acts through T cell costimulation and coinhibition and beyond. Phylogenetic tree of the B7 family generated by PAUP (2, 3). This analysis divides the B7 family into three groups. B7-H3 is broadly expressed. It binds to TLT-2 and costimulates T cell activation, whereas binding to unidentified receptor(s) leads to coinhibition of T cells. In addition, B7-H3 is an inhibitor for NK cells and osteoblastic cells by ligating unknown receptor(s).

tional difference has been observed between these two forms. B7-H3 is broadly expressed, in contrast to B7-1 and B7-2 whose expression is largely limited to professional antigen-presenting cells (APCs) such as dendritic cells (DCs), macrophages, and B cells. At the transcriptional level, B7-H3 is found in most organs (8, 9). At the protein level, B7-H3 is found in human liver, lung, bladder, testis, prostate, breast, placenta, and lymphoid organs. The exact cell type of B7-H3-positive cells in the organs has yet to be determined. The different expression pattern between mRNA and protein suggests that this molecule has posttranscriptional regulation. However, the molecular mechanisms regulating B7-H3 expression are still unclear. The expression of B7-H3 is induced on T cells, natural killer (NK) cells, and APC (8, 10, 11). B7-H3 is up-regulated during the maturation from monocytes to DC or during the interaction between DC and regulatory T cells. In addition, B7-H3 is found on fibroblasts, fibroblast-like synoviocytes, and epithelial cells. Finally, some human cancers overexpress B7-H3 (1). It has been observed to be overexpressed in prostate cancer, non-small-cell lung cancer, gastric carcinoma, and ovarian cancer. Structurally, B7-H3 is a type I transmembrane protein. However, the majority of this protein is found in the cytoplasm of tumor cells (12, 13). Factors that regulate B7-H3 mRNA translation and protein access to the cell surface can spatially and temporally determine the extent to which tumor-associated B7-H3 regulates T cell function.

B7-H3 acts as a T cell costimulator. The work that initially identified human

B7-H3 showed that it has a costimulatory effect on T cells (8). In the presence of anti-CD3 antibody, B7-H3 was able to increase proliferation of both the CD4 and CD8 T cell populations and selectively stimulated IFN- γ production. In addition, B7-H3 transiently transfected melanoma cells can enhance induction of human primary CD8 cytotoxic T cells (CTLs). Subsequently, in several mouse cancer models it has been shown that ectopic expression of B7-H3 leads to activation of tumor-specific CTLs that are able to slow tumor growth or even completely eradicate tumors (14–16). Mice with a B7-H3-transfected colon cell line had significantly prolonged survival times (16). In a P815 mastocytoma model, an immunogenic and B7-H3 negative tumor line, expression of B7-H3 on P815 led to tumor regression in half of the mice (14). P815-associated B7-H3 appears to induce rapid expansion of tumor antigen-specific CTL *in vivo*. CD4 T cells are not required for the induction of CD8 CTL for tumor immunity because depletion of CD4 T cells did not diminish the resistance of mice to the B7-H3-transfected P815 tumor. In a hepatocellular carcinoma model, intratumoral administration of a B7-H3-expressing

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plasmid and arsenic trioxide synergized to completely eradicate an established tumor (15), whereas neither arsenic trioxide nor B7-H3 monotherapy was effective. In these mouse cancer models, it seems that tumor-associated B7-H3 preferentially regulates CD4-independent induction of CD8 CTL responses. B7-H3 action through T cell costimulation is also implied by the fact that rapamycin treatment induced permanent cardiac and islet allograft survival in B7-H3 knockout mice (17), indicating that B7-H3 functions to promote T cell responses that mediate acute and chronic allograft rejection. These lines of evidence have now been augmented by the work showing that B7-H3 functions through the TLT-2 receptor on CD8 T cells as a costimulator (4). TLT-2 is constitutively expressed on CD8 T cells, B cells, NK cells, macrophages, DC, and neutrophils, and is induced on activated CD4 T cells. Unlike other members of the TREM family, TLT-2 is not associated with DAP12 for signaling, but contains a potential SH3-binding motif and an endocytosis motif in the cytoplasmic tail (5, 6). The downstream mechanism mediated by the B7-H3/TLT-2 pathway remains elusive.

B7-H3 acts as a T cell coinhibitor. Most data published so far support the notion that B7-H3 inhibits T cell activation. Both mouse and human B7-H3 inhibit CD4 T cell activation and the production of effector cytokines such as IFN- γ and IL-4 (7, 11, 18). The inhibition may govern through NFTA, NF- κ B, and AP-1 factors, three major signaling pathways through which T cell receptor (TCR) regulates gene transcription. Anti-B7-H3 antibodies augmented the severity of experimental autoimmune

encephalomyelitis (EAE) and allergic conjunctivitis (18, 19), suggesting B7-H3 inhibits Th1, Th2, or Th17 *in vivo*. Compared with wild-type mice, B7-H3 knockout mice developed EAE earlier as well as more severe airway inflammation under conditions in which T helper cells differentiated toward Th1 rather than Th2 (11). These results are in direct contrast to the previously discussed observations in another B7-H3 knockout

Clinical observations suggest B7-H3 is exploited by tumors as an immune evasion pathway.

model. The molecular mechanisms underlying the opposing phenotypes between these two types of knockout mice are currently unknown. In contrast to mouse tumor models, several independent studies have shown that human malignant tumor cells had a marked increase in expression of B7-H3 protein that was associated with increased disease severity (1). Prostate cancer patients with strong expression of B7-H3 were more likely to have disease spread at the time of surgery, and were at increased risk of clinical cancer recurrence and cancer-specific death (13). Intriguingly, B7-H3 was found in ovarian tumor vessels, which was associated with poor clinical outcome. These clinical observations suggest B7-H3 is exploited by tumors as an immune evasion pathway,

because its expression leads to down-regulation of T cell-mediated antitumor immunity. Consequently, it was suggested that tumor-associated B7-H3 offers a new therapeutic opportunity for enhancement of antitumor immunity or as a drug target (1). An explanation for B7-H3-mediated T cell coinhibition is that there are unexplored receptor(s) that may be preferentially expressed on CD4 T cells (Fig. 1).

The function of B7-H3 reaches beyond T cells. B7-H3 can inhibit NK cell function through unidentified receptor(s). It was reported that neuroblastoma can inhibit NK cell cytotoxicity through overexpressed B7-H3 (20). In addition, B7-H3 is a molecule that has a dual role in the bone-immune interface. It is highly expressed in developing bones during embryogenesis. B7-H3-deficient calvarial cells exhibit impaired osteogenic differentiation, whereas knockout mice are susceptible to bone fracture (21). It was reasoned that B7-H3 functions by binding to a putative counterreceptor on the osteoblastic cell surface.

The intense effort toward understanding T cell costimulatory and coinhibitory molecules over the past decade has shaped much of our understanding regarding the immune system. Elucidation of the action of B7-H3 will suggest additional potential targets for various immunotherapies for cancer, autoimmune disorders, infectious diseases, and transplantation rejection. The contrasting roles of B7-H3 can likely be attributed to multiple receptors on different cells. Although a piece of the B7-H3 puzzle has been solved by the identification of TLT-2 as a costimulatory receptor for B7-H3, coinhibitory receptor(s) for B7-H3 still need to be found!

- Zang X, Allison JP (2007) The B7 family and cancer therapy: Costimulation and coinhibition. *Clin Cancer Res* 13:5271–5279.
- Swofford DL (1998) PAUP*, Phylogenetic Analysis Using Parsimony (and Other Methods) (Sinauer, Sunderland, MA).
- Zang X, et al. (2003) B7x: A widely expressed B7 family member that inhibits T cell activation. *Proc Natl Acad Sci USA* 100:10388–10392.
- Hashiguchi M, et al. (2008) Triggering receptor expressed on myeloid cell-like transcript 2 (TLT-2) is a counter-receptor for B7-H3 and enhances T cell responses. *Proc Natl Acad Sci USA* 105:10495–10500.
- King RG, Herrin BR, Justement LB (2006) Trem-like transcript 2 is expressed on cells of the myeloid/granuloid and B lymphoid lineage and is up-regulated in response to inflammation. *J Immunol* 176:6012–6021.
- Klesney-Tait J, Turnbull IR, Colonna M (2006) The TREM receptor family and signal integration. *Nat Immunol* 7:1266–1273.
- Ling V, et al. (2003) Duplication of primate and rodent B7-H3 immunoglobulin V- and C-like domains: Divergent history of functional redundancy and exon loss. *Genomics* 82:365–377.
- Chapoval AI, et al. (2001) B7-H3: A costimulatory molecule for T cell activation and IFN- γ production. *Nat Immunol* 2:269–274.
- Sun M, et al. (2002) Characterization of mouse and human B7-H3 genes. *J Immunol* 168:6294–6297.
- Steinberger P, et al. (2004) Molecular characterization of human 4lg-B7-H3, a member of the B7 family with four Ig-like domains. *J Immunol* 172:2352–2359.
- Suh WK, et al. (2003) The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol* 4:899–906.
- Roth TJ, et al. (2007) B7-H3 ligand expression by prostate cancer: A novel marker of prognosis and potential target for therapy. *Cancer Res* 67:7893–7900.
- Zang X, et al. (2007) B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome. *Proc Natl Acad Sci USA* 104:19458–19463.
- Luo L, et al. (2004) B7-H3 enhances tumor immunity *in vivo* by costimulating rapid clonal expansion of antigen-specific CD8⁺ cytolytic T cells. *J Immunol* 173:5445–5450.
- Luo L, et al. (2006) Arsenic trioxide synergizes with B7H3-mediated immunotherapy to eradicate hepatocellular carcinomas. *Int J Cancer* 118:1823–1830.
- Lupu CM, et al. (2006) An orthotopic colon cancer model for studying the B7-H3 antitumor effect *in vivo*. *J Gastrointest Surg* 10:635–645.
- Wang L, et al. (2005) B7-H3 promotes acute and chronic allograft rejection. *Eur J Immunol* 35:428–438.
- Prasad DV, et al. (2004) Murine B7-H3 is a negative regulator of T cells. *J Immunol* 173:2500–2506.
- Fukushima A, et al. (2007) B7-H3 regulates the development of experimental allergic conjunctivitis in mice. *Immunol Lett* 113:52–57.
- Castriconi R, et al. (2004) Identification of 4lg-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis. *Proc Natl Acad Sci USA* 101:12640–12645.
- Suh WK, et al. (2004) The immune regulatory protein B7-H3 promotes osteoblast differentiation and bone mineralization. *Proc Natl Acad Sci USA* 101:12969–12973.