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Soluble B7-H1: Differences in production between dendritic cells and T cells

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Abstract

Tumor cells aberrantly express several T cell inhibitory molecules including members of the B7-H co-regulatory family. Presumably tumor-expressed B7-H1 and B7-H3 confer resistance to elimination by the immune system. In addition, elevated levels of soluble B7-H1 (sB7-H1) has been identified in the sera of cancer patients, including renal carcinoma patients and is associated with increased cancer related death. Here we report that sB7-H1 is produced and released by activated mature dendritic cells (mDC). Immature DC, macrophages, monocytes, or T cells are refractory to releasing sB7-H1. Exposure of CD4+ and CD8+ T cells to mDC-derived sB7-H1 molecules induced apoptosis. These data suggest that the immunobiology of B7-H1 is perhaps more complex than previously thought. sB7-H1 molecules may represent an unanticipated

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Conflict of interest statement

EDK, HD, XF, BAI and SMH, have filed for patents of B7-H ligands as prognostic markers and therapeutic targets for cancer.

Author contributions

Xavier Frigola, Brant Inman, Haidong Dong, Al Dietz, and Eugene Kwon: conception and design. Xavier Frigola and Haidong Dong: data analysis; Xin Liu, Susan Harrington and Peggy Bulur: technical support. Xavier Frigola, Brant Inman, Haidong Dong, Al Dietz, Christopher Krco, Eugene Kwon: interpretation of data; Xavier Frigola and Christopher Krco: manuscript writing. Eugene Kwon: final approval of manuscript.

contributing factor to immune homeostasis. That both immune and tumor cells can be sources of sB7-H1 suggests that optimization of co-regulatory blockade immunotherapy for solid malignancies of necessity will require impact of targeting tumor and immune-derived B7-H1 molecules.

Keywords

B7-H1; Soluble; T cell; Dendritic cell; Coregulatory; Tumor cell

1. Introduction

B7-homologues (B7-H) – B7-H1, B7-H2, B7-H3 and B7-H4 and B7-DC – predominantly function as negative costimulatory molecules [1,2]. B7-H molecules were initially discovered as membrane-bound ligands in myeloid-lineage cells and activated T and B cells [3–6]. It was later observed that tumors aberrantly express several types of B7-H molecules, which was associated with increased resistance to immune or pharmacological attack [7]. In particular tumor expressions of B7-H1, B7-H3 or B7-H4 were associated with an increased risk of cancer-related death [8–10].

B7-H ligands have been detected in serum of patients with varying disorders [11,12], including sera from patients with solid malignancies [13–15]. We have detected soluble B7-H1 and B7-H4 molecules in the sera of ccRCC[1] patients [13,16]. Tumor-derived sB7-H1[2] is biologically active and is capable of delivering cell death inducing signals [13].

In addition to tumor cells, immune cells may also release soluble B7-H ligands in the course of immune reactions. For example, sB7-H3 has been detected in cultures of monocytes, DC[3] and activated T cells [17]. sB7-H3 has also been detected in serum from healthy individuals [17]. Given the poor prognosis associated with ccRCC expression of B7-H1 and the observation that activated DC and T cells express membrane-bound B7-H1 we investigated whether immune cells have the capacity to release sB7-H1. Understanding the immunobiology of sB7-H1 generation may have applications in clinical settings, notably optimizing B7-H1 blockade immunotherapy for solid malignancies. sB7-H molecules, tumor or immune-derived, may deliver systemic inhibitory messages that could globally adversely impact anti-tumoral immune responses.

2. Materials and methods

2.1. Human cells

Human PBMC[4] were isolated from leukoreduction filters (Pall) as previously described [18]. Purified CD3+, CD4+, CD8+ and CD14+ cells were obtained by negative-selection (Miltenyi Biotech). To induce differentiation of DC, CD14+ cells (purity > 90%) were placed in 24-well plates at 2×10^6 cells/well and cultured in complete media supplemented with IL-4 (1000 IU/mL, R&D Systems) and GM-CSF (2800 IU/mL, Bayer). After 3 days of culture, iDC[5] were harvested, washed and dispensed at 1×10^6 cells/well in 24-well plates and cultured for 2 days in fresh media containing IL-4, GM-CSF and either TNFα (1100 IU/mL, R&D Systems) and PGE-2 (1 μg/mL, Sigma), poly I:C (20 μg/mL, Sigma), or LPS (100 ng/mL, Sigma) to drive the generation of mDC[6]. Expression of CD80, CD83, MHC-I, HLA-DR and membrane B7-H1 on DC preparations was determined on days 0, 3 and 5 of culture. Purified CD3+ cells were placed in 24-well plates at 1×10^6 cells/well and cultured in media containing anti-CD3/anti-CD28 microbeads (25 μL/well, Invitrogen), PHA (20 μg/mL, Sigma), CD28 (2 μg/mL BD Biosciences), IFNγ (100 IU/mL, eBioscience), TNFα (100

ng/mL, R&D Systems) or IL-10 (500 ng/mL, BD Biosciences), with or without anti-CD3 (2 μg/mL, BD Biosciences).

2.2. DC: T cell co-cultures

Purified CD4+ or CD8+ T cells were plated at 1×10^6 cells/well at the bottom of 24 well transwell plates pre-coated with azide-free anti-human CD3 (2 μg/mL, BD Biosciences). iDC cells or PBS was added to the upper chamber of the wells along with LPS (100 ng/ml, Sigma) to produce stimulated mDC. Extent of target CD4 and CD8 apoptosis was determined by Annexin-V (BD Biosciences) and propidium iodide (Sigma) staining at day 5 of culture.

2.3. sB7-H1 supernates

Corresponding supernates from intact PBMC (day 3), T cells (day 3), iDC and mDC cultures (days 0, 3 and 5) and transwell cultures (day 5) were clarified twice by centrifugation and stored at −20 °C for later testing.

2.4. Sandwich ELISA for sB7-H1

Soluble B7-H1 ELISA was performed as previously described [13]. High-binding polystyrene plates (Corning Life Sciences) were coated for 2 h at RT with 0.2 μg/well of capture antibody 2.2B. After each step, plates were washed three times (PBS +0.05% Tween-20). Free binding sites were blocked with 200 μL/well of Superblock (Pierce) 1 h at RT. After washing, 50 μL of standard, control or test culture supernate were added in duplicates to 50 μL of assay buffer (PBS +1% BSA) and incubated overnight at 4 °C. Biotinylated 5H1-A3, detection antibody was added (100 μL/well at 1 μg/mL diluted in PBS +0.1% BSA) to the wells and incubated 1 h at RT. Next, 100 μL/well of horseradish peroxidase-conjugated streptavidin (BD Biosciences), diluted in PBS +0.1% BSA, was added and incubated 1 h at RT. Plates were developed with TMB (Pierce), the reaction was stopped using 0.5 N H_2SO_4 and read at 450 nm using a Benchmark Plus plate reader and associated software (Bio-Rad). For calibration, each plate was loaded with duplicate parallel dilutions of recombinant B7-H1 fusion protein (R&D Systems) ranging in concentration from 0.01 to 20 ng/mL. To normalize the reporting of production of sB7-H1, all results are expressed in picograms of sB7-H1 per day, per mL and million cells (pg/day × mL × 10^6 cells).

2.5. Statistical analyses

Calibration of the B7-H1 ELISA against standard B7-H1 dilution curves was done as previously described [13]. To compare apoptosis in T cells we used paired *t*-tests. All *p*-values were two-sided and considered statistically significant if <0.05.

3. Results and discussion

Unfractionated, normal donor PBMC, isolated using Ficoll density gradients and challenged with the mitogen PHA, released sB7-H1 (mean sB7-H1: 33.4 pg/day × mL × 10^6 cells; 95% CI: 10.2–56.6; Fig. 1A). The release of sB7-H1 was maximal between days 2 and 3, a time point corresponding to both maximal proliferation and induced membrane expression B7-H1 (data not shown). Unstimulated control PBMC cultures did not release detectable sB7-H1 (Fig. 1A), suggesting immune cell activation may be necessary for sB7-H1 production.

As a first approximation in identifying the immune cell source of sB7-H1, PBMC were fractionated into plastic adherent and non-adherent populations and challenged with PHA. Non-adherent PBMCs did not produce detectable sB7H1 whereas PBMC which adhered to plastic produced comparable levels of sB7-H1 (mean sB7-H1: 36.1 pg/day × mL × 10^6

cells) as unfractionated PBMCs treated similarly (mean sB7-H1: 22.4 pg/day × mL × 10^6 cells, $p = 0.426$, Fig. 1B). These observations suggest that cells of myeloid lineage (monocytes, macrophages and DC; plastic adherent) may be a source of sB7-H1.

T cell populations were then isolated, challenged and culture fluid levels tested for the presence of sB7-H1. While activation of CD3+ T cells induced expression of membrane-B7-H1 (Fig. 2A) and a panel of commonly accepted membrane markers of activation (Fig. 2B), undetectable or only trace amounts of sB7-H1 (Fig. 2B and C) were released following optimal antigen independent stimulation (<14 pg/day × mL × 10^6 cells) suggesting that CD3+ T cells are refractory to releasing sB7-H1.

To investigate the capacity of myeloid lineage cells to release sB7-H1, CD14+ cells were isolated by immunomagnetic selection and induced to differentiate into either iDC or mDC. Both iDC and mDC expressed membrane-B7-H1 (Fig. 3A). Although iDC did not release sB7-H1 (Fig. 3B), mDC released sB7-H1 following maturation induced with TNFα/PGE-2, poly(I:C) or LPS (mean sB7-H1: 94.8, 184.3 and 293.4 pg/day × mL × 10^6 cells respectively, Fig. 3B).

Tumor cell-derived sB7-H1 is biologically active and can induce apoptosis in T cells [13]. To determine whether immune cell-derived sB7-H1 can induce T cell apoptosis, mDC were cocultured with CD4+ or CD8+ T cells. Coincubation of CD4+ or CD8+ T cells with sB7-H1-producing cells mDC induced an increase of T cells undergoing apoptosis (mean: 20.8% for CD4; 26.6% for CD8; Fig. 4). In contrast, co-culture of T cell populations with iDC did not induce detectable apoptosis.

This explorative investigation revealed, rather surprisingly, that myeloid derived cells have the capacity to release sB7-H1 molecules and may represent a natural source of sB7-H1. In contrast T cells, even when optimally stimulated only contributed trace amounts of sB7-H1. The immunological significance of these findings is not fully understood. That both myeloid and T cell exhibit increased expression of membrane B7-H1 but release of sB7-H1 is a feature of myeloid populations suggests that distinct regulatory mechanisms may be controlling sB7-H1 production. Whether the disparity between mDC and T cells with respect to sB7-H1 release is a reflection of distinct membrane dynamics or intracellular processing is an unresolved question. It has been reported that matrix metalloproteinases can affect release of sB7-H3 from cells [17]. Whether differential membrane enzymatic activity or more sophisticated intracellular regulatory mechanisms account for sB7-H1 release between DC and T cells will require ongoing detailed analyses.

B7-H1 blockade is of intense interest and is undergoing phase I clinical trials. That sB7-H1 can be generated from tumors and subpopulations of immune cells suggest that not all administered anti-B7-H1 immunotherapeutic antibody may reach the surface of tumor cells, with a potentially appreciable proportion being sequestered by sB7-H1 within the circulation. Compensating for potential tie-up of antibody needs to be considered in optimizing B7-H1 blockade therapies.

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Abbreviations

ccRCC	clear cell renal carcinoma
sB7-H1	soluble B7-H1
DC	dendritic cells
PBMC	peripheral blood mononuclear cells
iDC	immature dendritic cells
mDC	mature dendritic cells

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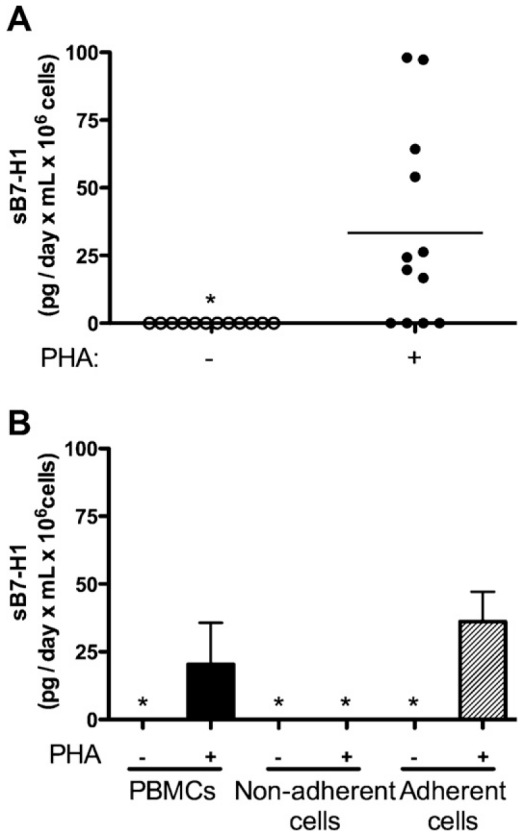

Fig. 1.

Immune cells naturally release sB7-H1 upon activation. (A) Human PBMCs release sB7-H1 upon PHA stimulation. Horizontal line represents mean sB7-H1. (B) PHA-stimulated plastic-adherent myeloid PBMCs release sB7-H1. Whole PBMCs were incubated −/+ PHA for 2 days. The non-adherent cells were removed, washed and placed in new wells along with fresh media. Fresh media was also added to the remaining adherent cells. Cells were incubated for 2 more days, and sB7-H1 was measured in the supernatant. Vertical bars represent standard deviation. Star (*) represents undetectable sB7-H1 levels. Data are representative of 6 to 12 individual samples.

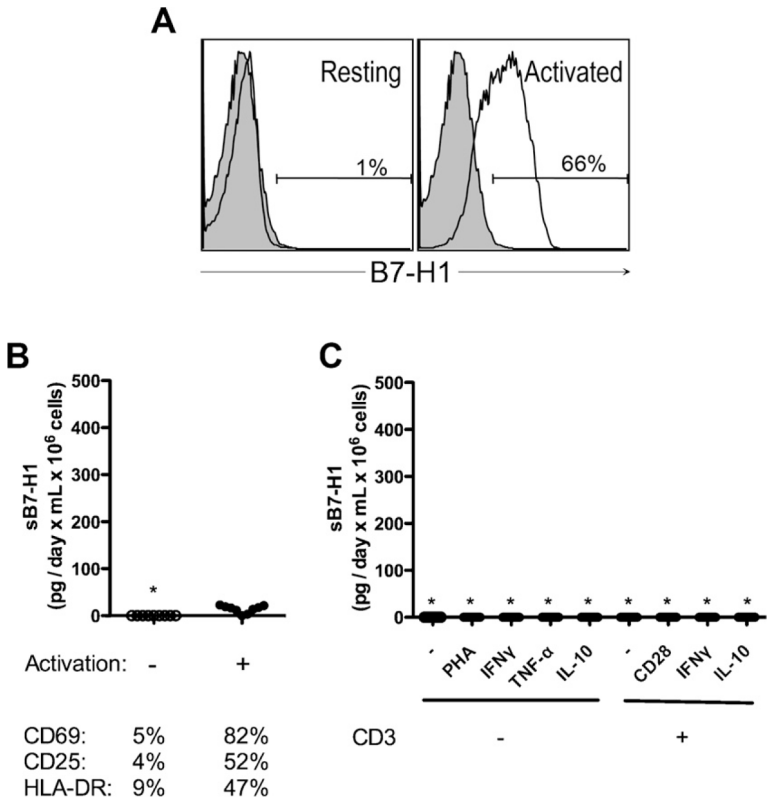

Fig. 2.

T cells do not release measurable amounts of sB7-H1. (A) Expression of membrane-bound B7-H1 (open histogram) in resting (left panel) and activated (right panel) T cells. Shaded histograms represent isotype-matched controls. (B) Measure of sB7-H1 in purified T cells after 3 days of CD3/CD28 bead activation (top panel) and assessment of corresponding activation markers (lower panel). (C) Activation of purified T cells and measure of sB7-H1 after 3 days. Star (*) represents undetectable sB7-H1. Data are representative of 7 to 10 samples analyzed per condition.

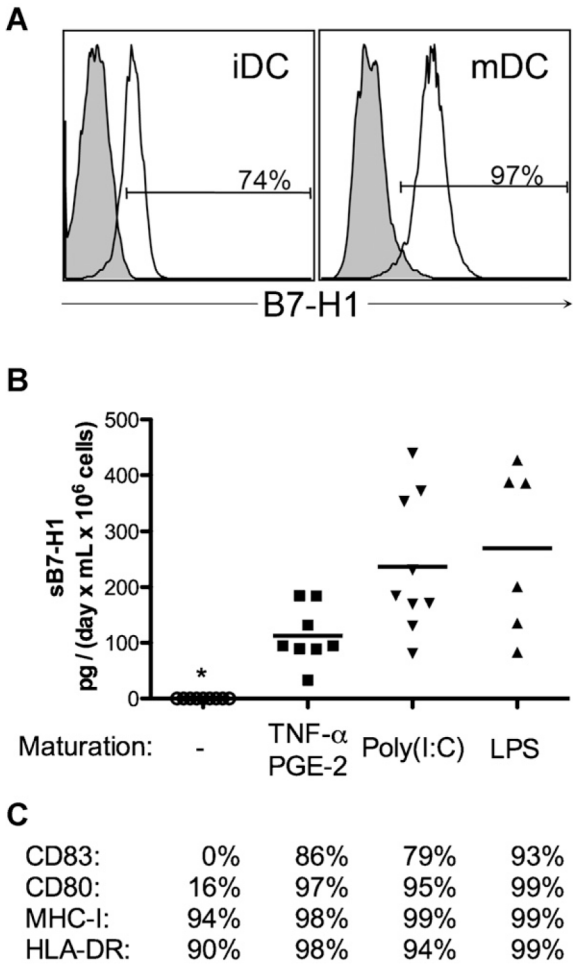

Fig. 3.

DCs release measurable amounts of sB7-H1 after maturation. (A) Expression of membrane-bound B7-H1 (open histograms) in immature (left panel) and mature (right panel) DCs. Shaded histograms represent the isotype-matched controls. (B) sB7-H1 production is independent on which TLR is stimulated. Horizontal lines represent mean sB7-H1. Star (*) represents undetectable levels of sB7-H1. (C) Median % of cells expressing different costimulatory markers representative of DC maturation. Data were obtained using 7 to 10 different samples per experimental condition.

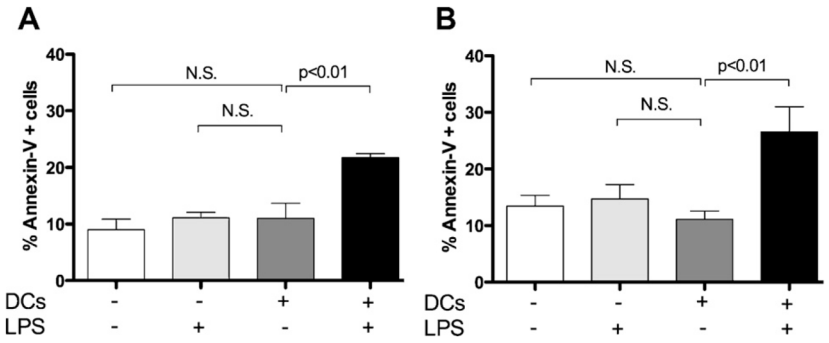

Fig. 4.

DC-released sB7-H1 produces T cell apoptosis in vitro. Apoptosis of purified (A) CD4+ and (B) CD8+ cells in transwell co-culture experiments without (−) or with (+) LPS and DCs. Bars represent mean sB7-H1 and vertical lines standard deviation. Data are representative of 5 samples analyzed per condition. N.S., statistically not significant.