Biofunctions of Three New B7 Family Members

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Abstract

Three new B7 family members, B7-H5 (VISTA, Gi24, or Dies1), B7-H6, and B7-H7 (HHLA2) were recently identified, but their functions remain uncertain. We expressed the human B7-H5, B7-H6, and B7-H7 extracellular domain human IgG_1 Fc Chimeras in a mammalian cell line and investigated the functions of the purified recombinant B7-H5/Fc, B7-H6/Fc, and B7-H7/Fc proteins *in vitro*. To determine the functions of B7-H5 and B7-H7, human T cells were treated with plate-bound anti-CD3 and either B7-H5/Fc, B7-H7/Fc or control IgG_1 Fc. The levels of multiple cytokines were measured in cell culture supernates using R&D Systems Proteome Profiler™ Human Cytokine Array, followed by measuring individual cytokines using a series of cytokine-specific Quantikine® ELISA kits. B7-H5 significantly reduced eight T-cell derived cytokines including IFN-γ, IL-2, IL-17, IL-4, IL-8, IL-13, IL-16, and TNF-α. Similarly B7-H7 markedly decreased five cytokines including IFN-γ, IL-2, IL-17, IL-8, and TNF-α. To investigate the bioactivity of B7-H6, NK-92 cells were treated with plate-bound B7-H6/Fc or control IgG_1 Fc. B7-H6/Fc induced IFN-γ production in NK cells in a dose-dependent manner. Furthermore, B7-H6/Fc showed binding to recombinant human NKp30/Fc. Taken together, our data suggest that B7-H5 and B7-H7 are co-inhibitory molecules for T cell activation, and that B7-H6, a major NKp30 ligand, triggers NKp30-dependent NK cell activation.

Introduction

The B7 family is a group of cell surface glycoproteins that share structural features with immunoglobulin (Ig). This family is comprised of ten members including B7-1, B7–2, B7-DC (PD-L2), B7-H1 (PD-L1), B7-H2 (ICOS-L), B7-H3, B7-H4, B7-H5, B7-H6 and B7-H7.[1] The B7 family proteins deliver co-stimulatory or co-inhibitory signals by binding to receptors in the presence of peptide/MHC complex-mediated TCR signaling. Integration of the signals generated by the B7 family contributes to the outcome of T cell responses.[2] In addition, B7-H6 is involved in NK cell activation through NKp30.[3] In this *in vitro* study, we examined the functional roles of the ectodomains of recombinant human B7-H5/Fc (rhB7-H5, a.a. 33-194/Fc), B7-H6/Fc (rhB7-H6, a.a. 25-262/Fc), and B7-H7/Fc (rhB7-H7, a.a. 23-344/Fc).

B7-H5, also known as VISTA, Gi24, or Dies1, is a 55-65 kDa transmembrane glycoprotein with homology to B7-H1. Its extracellular domain contains a single extracellular Ig-V domain of 136 amino acids. B7-H5 is expressed on macrophages, neutrophils, dendritic cells (DCs), naïve $CD4^+$ T cells, and activated regulatory T cells. Its expression is also inducible during an inflammatory response. B7-H5 functions as a co-inhibitory ligand through an unknown receptor by inhibiting T cell proliferation and cytokine production and by arresting the cell cycle.[4] Consistent with previous studies, our results show that the recombinant human B7-H5 protein significantly inhibits IFN-γ, IL-2, IL-17, IL-4, IL-8, IL-13, IL-16, and TNF-α in anti-CD3 activated human T cells.

B7-H6, also known as NCR3LG1, consists of an IgV-IgC extracellular domain, a transmembrane region, and a long cytoplasmic tail. B7-H6 acts as a co-stimulatory ligand that delivers a stimulatory signal to natural killer (NK) cells through the receptor NKp30.[3] B7-H6 is expressed in a wide range of hematopoietic, carcinoma, and melanoma tumor cells, which is consistent with the detection of NKp30 binding sites on many tumors. Our data support previous studies showing that immobilized recombinant human B7-H6 protein stimulates the secretion of IFN-γ from NK cells.

B7-H7, previously known as human endogenous retrovirus-H long terminal repeat associating 2 (HHLA2), has three signature Ig domains (IgV-IgC-IgV) in its extracellular region. B7-H7 is expressed on T, B, NK cells, and monocytes freshly isolated from human PBMCs. It is also induced by poly (I:C), suggesting that B7-H7 is largely an inducible molecule on antigen-presenting cells in response to proinflammatory stimuli. In spite of the accumulating data on B7-H7, the exact functions of this ligand remain uncertain. Previous studies have shown that B7-H7-Ig significantly decreased T cell activation induced by anti-CD3.[5] However, a current study indicates that B7-H7 is a specific ligand for human CD28H. The B7-H7-CD28H pathway strongly promotes $CD4^+$ T cell proliferation and cytokine production via an Akt-dependent signaling cascade in the presence of TCR signaling, suggesting that B7-H7 induces a new co-stimulatory pathway.[6] In our studies, treatment of human T cells with recombinant human B7-H7 protein significantly inhibited anti-CD3 induced IFN-γ, IL-2, IL-17, IL-8, and TNF-α, suggesting that B7-H7 acts as a co-inhibitory molecule and is able to down-regulate T cell activation.

Results

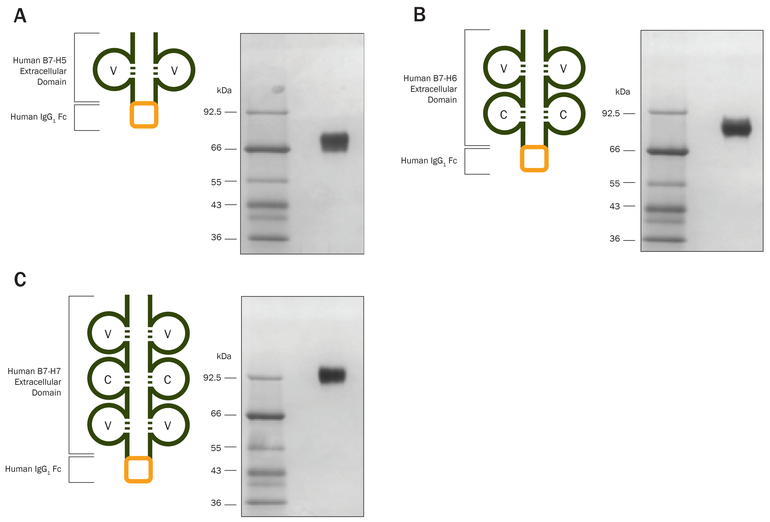

Figure 1. SDS-PAGE Analysis of rhB7-H5/Fc (A), rhB7-H6/Fc (B) and rhB7-H7/Fc (C) under reducing conditions. Recombinant Human B7-H5/Fc (Catalog # 7126-B7), Recombinant Human B7-H6/Fc (Catalog # 7144-B7) and Recombinant Human B7-H7/Fc (Catalog # 8084-B7) were visualized on silver-stained, SDS polyacrylamide gels under reducing conditions.

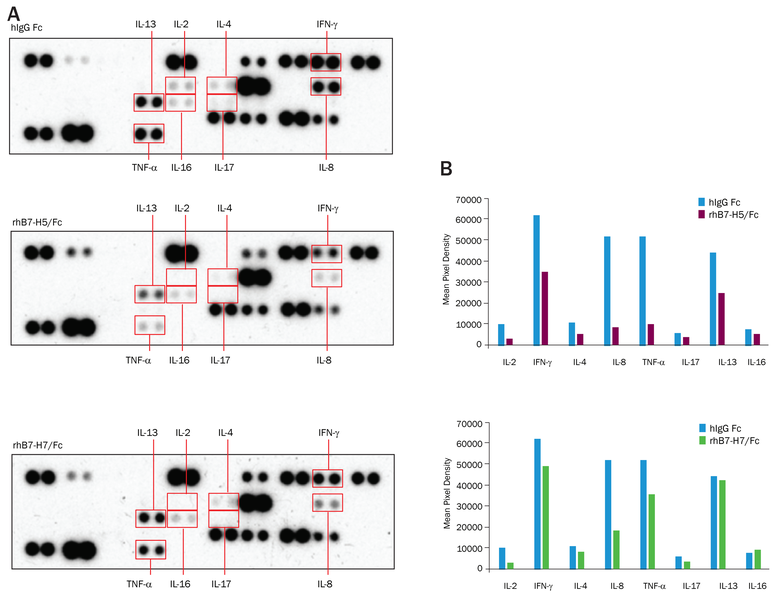

Figure 2. B7-H5 and B7-H7 Inhibit Anti-CD3-induced Cytokine Production in Human T Cells. Human PBMCs were stimulated with PHA (2 µg/mL) and IL-2 (50 U/mL, Catalog # 202-IL) for 10 days to generate human T cells. Human T cells were incubated with immobilized Mouse Anti-human CD3ε Monoclonal Antibody (3 µg/ml, Catalog # MAB100) and rhB7-H5/Fc (10 µg/mL, Catalog # 7126-B7), rhB7-H7/Fc (10 µg/mL, Catalog # 8084-B7), or rhIgG/Fc (10 µg/mL, Catalog # 110-HG) for 24 hours. **A.** Cytokine secretion in the cell culture supernatants was measured using the Proteome Profiler Human Cytokine Array Panel A (Catalog # ARY005). **B.** Pixel densities were collected and analyzed using a transmission-mode scanner and image analysis software.

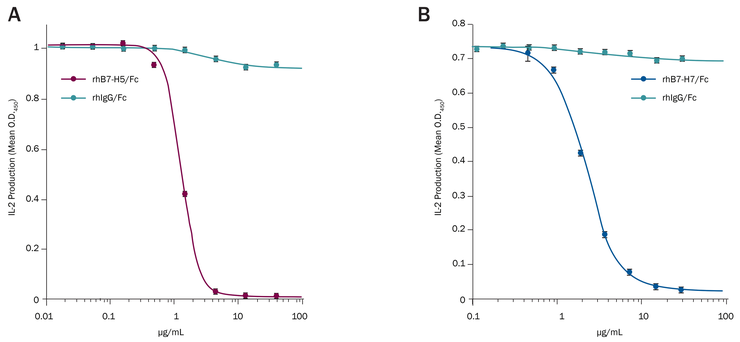

Figure 3. B7-H5 and B7-H7 Inhibit Anti-CD3-induced IL-2 Secretion by Human T Cells in a Dose-dependent Manner. Human T cells were incubated with immobilized Mouse Anti-human CD3ε Monoclonal Antibody (3 µg/ml, Catalog # MAB100) and the indicated concentrations of rhB7-H5/Fc (A, Catalog # 7126-B7), rhB7-H7/Fc (B, Catalog # 8084-B7), or rhIgG/Fc (Catalog # 110-HG) for 24 hours. IL-2 secretion was measured using the Human IL-2 Quantikine® ELISA kit (Catalog # D2050).

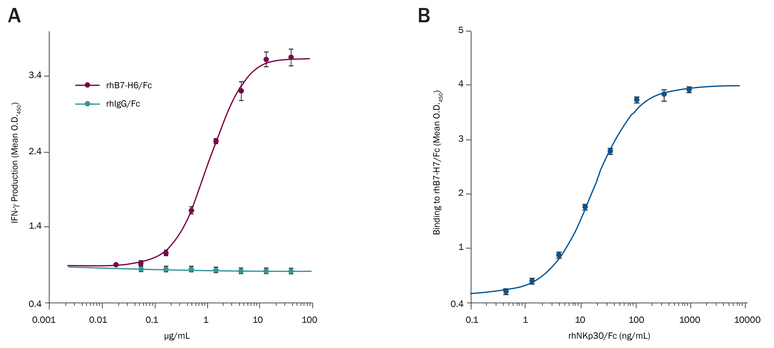

Figure 4 (A). B7-H6 Induces IFN-γ Secretion from NK-92 cells. The NK-92 human natural killer lymphoma cell line was incubated with the indicated concentrations of immobilized rhB7-H6/Fc (Catalog# 7144-B7) or rhIgG/Fc (Catalog # 110-HG) for 24 hours. IFN-γ secretion was measured using the Human IFN-γ Quantikine ELISA Kit (Catalog # DIF50). **(B). Recombinant Human B7-H6/Fc binds to NKp30/Fc in a Functional ELISA.** Immobilized rhB7-H7/Fc (0.5 µg/mL; 100 µL/well) bound to rhNKp30/Fc (Catalog # 1849-NK) in a dose-dependent manner.

Summary

- Treatment of human T cells with Recombinant Human B7-H5 or B7-H7 inhibits anti-CD3 induced cytokine production.
- Treatment of NK cells with Recombinant Human B7-H6 induces IFN-γ production in a dose-dependent manner.
- Recombinant Human B7-H6 binds to the receptor, Recombinant Human NKp30, in a functional ELISA.

Conclusions

- B7-H5 and B7-H7 are co-inhibitory ligands for T cell activation.
- B7-H6 acts as NKp30 ligand to induce NK cell activation.

References

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