VSIG8 is a Co-inhibitory Ligand and an Immune Checkpoint Molecule for Human T Cells

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Abstract

Cell surface-localized and secreted immunoglobulin superfamily (IgSF) proteins play central roles in regulating adaptive and innate immune responses, and are the primary targets for the development of new immunotherapeutics. In this work, we provide biologic and functional insight on VSIG8, one member of this important class of proteins. VSIG8 inhibits the production of cytokines (IL-2, IFN-γ, IL-17, IL-6, and IL-19), chemokines (MCP-1, MCP-3, and IP-10), and other proteins (IGFBP3 and RBP4) on anti-CD3 activated human CD3$^+$ T cells. Furthermore, VSIG8 significantly reduces the production of IFN-γ and IL-2 from both CD4$^+$ and CD8$^+$ T cells in the presence of T cell receptor signaling. In addition, VSIG8 markedly suppresses anti-CD3-induced human T cell proliferation and profoundly decreases the differentiation of naïve CD4$^+$ T cells into Th1 cells. Thus, we have identified VSIG8 as a new immune checkpoint molecule that is able to inhibit human T cell activation. This novel human T cell co-inhibitory ligand may be a unique target for developing new immunotherapy strategies for the treatment of human cancers, autoimmune disorders, and infectious diseases.

Introduction

VSIG8 (V-set and immunoglobulin domain containing 8), also known as C1orf204, is an approximately 45 kDa type I transmembrane protein belonging to the immunoglobulin superfamily (IgSF). Mature human VSIG8 consists of a 242 amino acid (aa) extracellular domain (ECD) containing two V-type Ig-like domains, a 21 aa transmembrane domain, and a 130 aa cytoplasmic domain. Within the ECD, human VSIG8 shares 88% and 89% aa identity with mouse and rat VSIG8, respectively. Alternative splicing generates a long isoform of human VSIG8 with a substitution in the cytoplasmic juxtamembrane region and a 124 aa extension at the C-terminus. VSIG8 was identified by proteomic analysis of human hair shafts,[1,2] and was found to be expressed in the hair follicle and shaft, and superficial layers of the nail matrix and oral epithelium.[3] A published US patent (WO2016090347 A1) has reported that VSIG8 is a receptor for VISTA/B7-H5 and can mediate the suppressive effects of VISTA/B7-H5 on T cell immunity. Furthermore, VSIG8 has been reported to be the counterpart of CXAR, serving the analogous function of maintaining tight junctions in stratified epithelia through homophilic trans-dimerization.[4] However, the role of VSIG8 as a co-inhibitory ligand capable of modulating T cell immunity, has not been previously described.

In this study, VSIG8-Fc fusion proteins were produced by cloning the extracellular domain of human VSIG8 (aa 1—263) fused to the Fc region of human IgG_1 in mammalian expression vectors. This VSIG8-Fc fusion protein showed negative regulation of human T cells in various *in vitro* experimental systems, suggesting that VSIG8 is a co-inhibitory ligand involved in regulating T cell-mediated immunity. As an IgSF protein with robust T cell inhibitory activity, VSIG8 represents a novel B7-like ligand that exerts negative immune modulation via interaction with a receptor expressed on activated T cells, thereby defining it as a novel immune checkpoint molecule.

Results

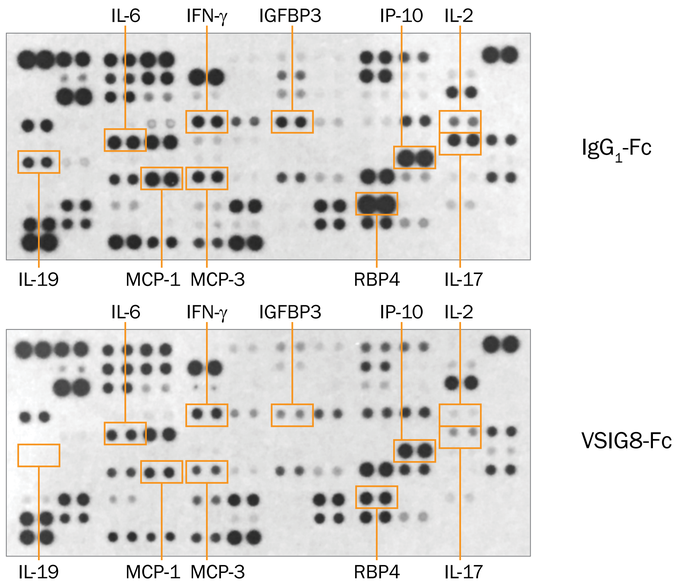

Figure 1. VSIG8 inhibits the production of cytokines (IL-2, IL-17, IL-6, IL-19, and IFN-γ), chemokines (MCP-1, MCP-3, and IP-10), and other proteins (IGFBP-3 and RBP4) by anti-CD3-activated human CD3$^+$ T cells. Human CD3$^+$ T cells were isolated from PBMCs using the MagCellect™ Human CD3$^+$ T Cell Isolation Kit (Catalog # MAGH101). The isolated cells were then treated with a combination of plate-bound Mouse Anti-Human CD3 Monoclonal Antibody (1 µg/mL; Catalog # MAB100) and either plate-bound Recombinant Human VSIG8-Fc (10 µg/mL; Catalog # 9200-VS) or Recombinant Human IgG_1-Fc (10 µg/mL; Catalog # 110-HG) for 24 hours. Cytokine levels in the supernatants were measured using the Proteome Profiler™ Human XL Cytokine Array Kit (Catalog # ARY022B).

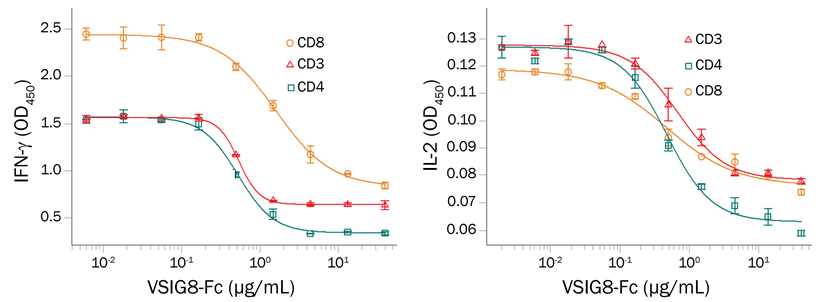

Figure 2. VSIG8 inhibits anti-CD3-induced IL-2 and IFN-γ production by human CD3$^+$, CD4$^+$, or CD8$^+$ T cells in a dose-dependent manner. Human CD3$^+$, CD4$^+$, or CD8$^+$ T cells were isolated from PBMCs using the MagCellect™ Human CD3$^+$, CD4$^+$, or CD8$^+$ T Cell Isolation Kits (Catalog # MAGH101, # MAGH102, or # MAGH112, respectively). Human CD3$^+$, CD4$^+$, or CD8$^+$ T cells were then incubated with an immobilized Mouse Anti-Human CD3 Monoclonal Antibody (1 µg/mL) and the indicated concentrations of Recombinant Human VSIG8-Fc or Recombinant Human IgG_1-Fc for 24 hours. The levels of IL-2 and IFN-γ in the cell culture supernatants were measured using the Human IL-2 or IFN-γ Quantikine® ELISA Kits (Catalog # D2050 or # DIF50, respectively). IgG_1-Fc controls did not alter anti-CD3 induced IL-2 or IFN-γ secretion by CD3$^+$, CD4$^+$, or CD8$^+$ T cells (data not shown).

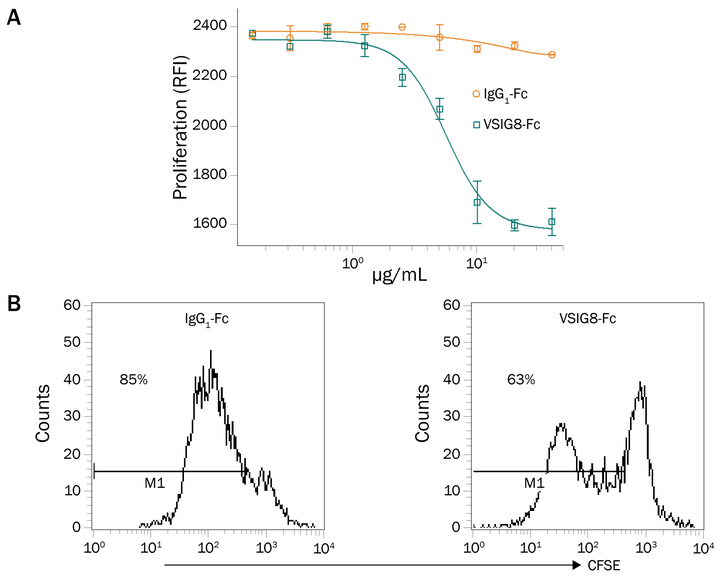

Figure 3. VSIG8 inhibits anti-CD3 induced human CD3$^+$ T cell proliferation in a dose-dependent manner. (**A**) Human T cells were incubated with an immobilized Mouse Anti-Human CD3 Monoclonal Antibody (1 µg/mL) and the indicated concentrations of Recombinant Human VSIG8-Fc or Recombinant Human IgG_1-Fc for 3 days. Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye, Alamar Blue (Resazurin). IgG_1-Fc controls did not alter anti-CD3-induced cell proliferation of CD3$^+$ T cells. (**B**) CFSE-labeled T cells were treated with a combination of plate-bound Mouse Anti-Human CD3 Monoclonal Antibody (1 µg/mL) and either plate-bound Recombinant Human VSIG8-Fc (10 µg/mL) or Recombinant Human IgG_1-Fc (10 µg/mL) for 5 days. T cells were analyzed by flow cytometry.

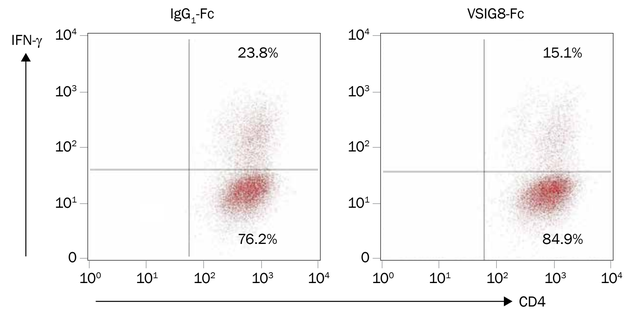

Figure 4. VSIG8 inhibits the differentiation of naïve CD4$^+$ cells to Th1 cells. Human naïve CD4$^+$ T cells were isolated from PBMCs using the MagCellect™ Human Naive CD4$^+$ T Cell Isolation Kit (Catalog # MAGH115). To induce Th1 cell differentiation, naïve CD4$^+$ cells were treated for 5 days with immobilized Mouse Anti-human CD3 Monoclonal Antibody (1 µg/mL) in 5% FBS-RPMI1640 medium supplemented with Recombinant Human IL-2 (10 ng/mL; Catalog # 202-IL) and Recombinant Human IL-12 (10 ng/mL; Catalog # 219-IL). To determine the effect of VSIG8 on Th1 cell differentiation, naïve CD4$^+$ cells were treated with immobilized Recombinant Human VSIG8-Fc (10 µg/mL), or Recombinant Human IgG_1-Fc (10 µg/mL). On day 5, the cells were fixed, permeabilized, and stained using an APC-conjugated Mouse Anti-Human IFN-γ Monoclonal Antibody (Catalog # IC285A) and a Fluorescein-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791F). Quadrants were set based on staining with the appropriate isotype controls.

Summary

- VSIG8 inhibits cytokine and chemokine production by human T cells.
- VSIG8 suppresses human T cell proliferation.
- VSIG8 decreases the differentiation of naïve CD4$^+$ T cells to Th1 cells.

In conclusion, VSIG8 is a novel negative regulator of T cell responses, with potential roles in the modulation of immune responses.

References

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4. Eng-Hui Y. *et al.* (2014) J. Mol Biol. **426**:945.

Acknowledgments

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