VSIG-3/IGSF11 is a Ligand of VISTA/PD-1H and Inhibits Human T Cell Function

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Abstract

B7 family members and their receptors play a central role in the regulation of T cell responses through T cell co-stimulatory and co-inhibitory pathways that constitute very attractive targets for the development of immunotherapeutic drugs. In this study, we report that VSIG-3/IGSF11 is a ligand of the B7 family member VISTA/PD-1H that inhibits human T cell functions through a novel VSIG-3/VISTA pathway. An extensive functional ELISA binding screening assay reveals that VSIG-3 binds the new B7 family member VISTA, but does not interact with other known members of the B7 family. Furthermore, VSIG-3 inhibits human T cell proliferation in the presence of T cell receptor signaling. In addition, VSIG-3 significantly reduces cytokine and chemokine production by human T cells including IFN-γ, IL-2, IL-17, CCL5/RANTES, CCL3/MIP-1α, and CXCL11/I-TAC. Anti-VISTA neutralization antibodies attenuate the binding of VSIG-3 to VISTA, as well as VSIG-3-induced T cell inhibition. Thus, we have identified a novel B7 pathway that is able to inhibit human T cell proliferation and cytokine production. This unique VSIG-3/VISTA co-inhibitory pathway may provide new strategies for the treatment of human cancers, autoimmune disorders, infection, and transplant rejection, and may help to design better vaccines.

Introduction

Critical modulators of the immune system, also referred to as immune checkpoint regulators, generate co-stimulation or co-inhibition of T cell responses.[1] Checkpoint blockage can generate potent anti-tumor responses by enhancing the immune system's ability to seek and destroy cancer cells. Members of the B7 family have emerged as important checkpoint regulators and recently the United States Food and Drug Administration has approved the use of anti-PD-1 and anti-CTLA-4 antibodies for cancer immunotherapy.[2,3] There is an urgent need for more agents to enter clinical use.

V domain-containing Ig suppressor of T cell activation (VISTA), also termed Differentiation of Embryonic Stem Cells 1 (Dies1), Gi24, and PD-1 homolog (PD-1H), is a 55- to 65-kDa type I Ig membrane protein with an extracellular domain homologous to PD-L1. VISTA is highly expressed on mature CD11b^{high} myeloid-derived APCs and to a lesser extent on CD4$^+$ T cells, CD8$^+$ T cells, and regulatory T cells, and is also found on tumor infiltrating lymphocytes.[4] It has been demonstrated that VISTA is a co-inhibitory receptor on CD4$^+$ T cells or a co-inhibitory ligand for T cells. *In vitro*, a VISTA-Ig fusion protein inhibits CD3-stimulated T cell activation, proliferation, and production of cytokines, such as IL-2 and IFN-γ.[5] Another group reported that VISTA$^{-/-}$ CD4$^+$ T cells showed stronger Ag-specific proliferation and cytokine production than wild-type CD4$^+$ T cells, which supports the thesis that VISTA functions as an inhibitory receptor on CD4$^+$ T cells. The analysis using both VISTA-Ig and genetic ablation has suggested that VISTA is a negative checkpoint regulator of T cell activation. The binding partners of VISTA that mediate these effects may be potential targets for therapeutic intervention, but have remained unknown to date.

V-Set and Immunoglobulin domain containing 3 (VSIG-3), also known as BT-IgSF and IGSF11 was originally identified as a member of the immunoglobulin superfamily that mediates homophilic adhesion in a calcium-independent manner.[7] VSIG-3 expression is elevated in colorectal cancers and hepatocellular carcinomas, as well as intestinal-type gastric cancers. Suppression of VSIG-3 by siRNA retarded the growth of gastric cancer cells, suggesting that VSIG-3 is a good candidate for gastric cancer immunotherapy.[8] A recent study showed that VSIG-3 regulates synaptic transmission and plasticity through interactions with the postsynaptic scaffolding protein PSD-95 and AMPA glutamate receptors (AMPARs).[9] However, the biological functions and binding partners of VSIG-3 outside of the brain have not yet been identified.

Here we report that VSIG-3 is a novel ligand for VISTA, and the engagement of VSIG-3 with VISTA on activated T cells inhibits T cell proliferation as well as cytokine and chemokine production. The co-inhibitory functions of VSIG-3 on activated T cells, combined with the highly elevated expression of VSIG-3 in colorectal cancers, hepatocellular carcinomas, and intestinal-type gastric cancers, suggest that blockade of the VSIG-3/VISTA pathway represents a new cancer immunotherapeutic strategy.

Results

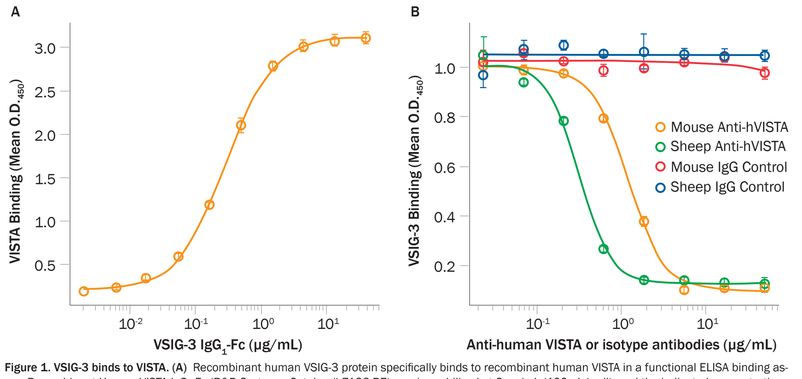


Figure 1. VSIG-3 binds to VISTA. (**A**) Recombinant human VSIG-3 protein specifically binds to recombinant human VISTA in a functional ELISA binding assay. Recombinant Human VISTA IgG_1-Fc (R&D Systems, Catalog # 7126-B7) was immobilized at 2 µg/mL (100 µL/well), and the indicated concentrations of Recombinant Human VSIG-3 IgG_1-Fc (R&D Systems, Catalog # 9229-VS) were added. The concentration of VSIG-3 IgG_1-Fc that produced 50% of the optimal binding response was approximately 0.25 µg/mL (3.4 nM). (**B**) Anti-human VISTA antibodies neutralize the binding of VSIG-3 and VISTA. Biotinylated Recombinant Human VISTA IgG_1-Fc (1 µg/mL) was pretreated with the indicated concentrations of Anti-Human VISTA Antibodies (R&D Systems, Catalog # AF7126 or # MAB71261) or Isotype Controls (R&D Systems, Catalog # 5-001-A or # MAB0041) and then added to ELISA plates containing immobilized Recombinant Human VSIG-3 IgG_1-Fc (2 µg/mL). After washing away any unbound proteins, streptavidin-HRP (R&D Systems, Catalog # DY998) and substrate solution (R&D Systems Cat# DY999) were added to the wells. The color development was stopped and the intensity of the color was measured using an ELISA plate reader.

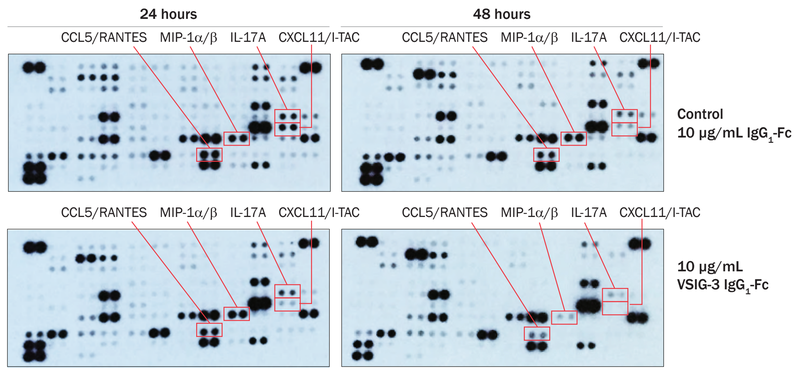


Figure 2. VSIG-3 inhibits cytokine and chemokine production by PBMCs. Human PBMCs were stimulated with a combination of plate-bound Mouse Anti-Human CD3 antibody (1 µg/mL; R&D Systems, Catalog # MAB100) and either plate-bound Recombinant Human VSIG-3 IgG_1-Fc (10 µg/mL) or Control IgG_1-Fc (10 µg/mL; R&D Systems, Catalog # 110-HG) for 24 or 48 hours. Cytokine levels in the cell culture supernatants were measured using the Proteome Profiler™ Human XL Cytokine Array Kit (R&D Systems, Catalog # ARY022B).

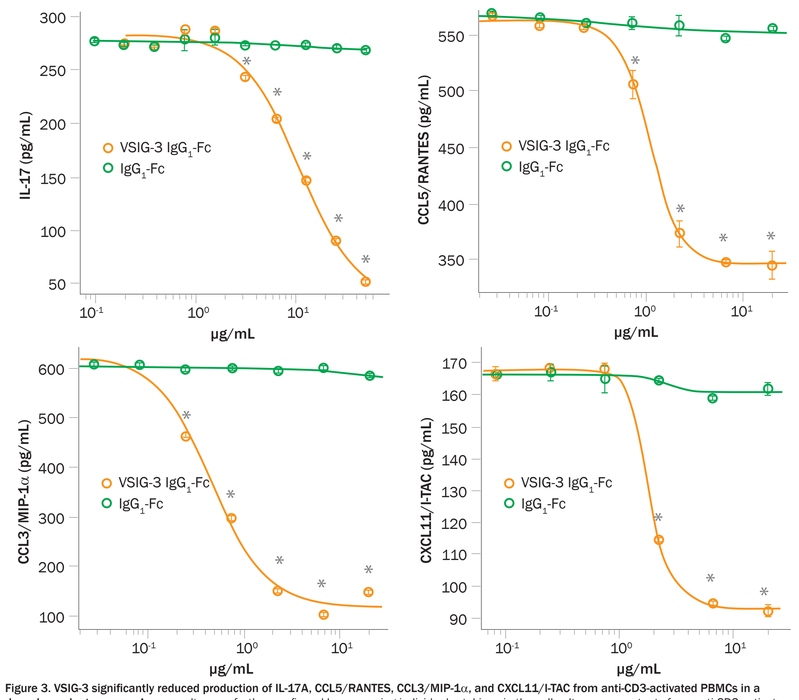
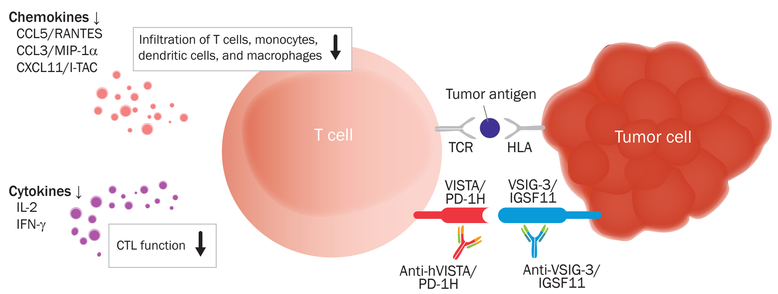


Figure 3. VSIG-3 significantly reduced production of IL-17A, CCL5/RANTES, CCL3/MIP-1α, and CXCL11/I-TAC from anti-CD3-activated PBMCs in a dose-dependent manner. Array results were further confirmed by measuring individual cytokines in the cell culture supernatants from anti-CD3-activated human PBMCs using the Human IL-17, CCL5/RANTES, CXCL11/I-TAC, and CCL3/MIP-1α Quantikine® ELISA Kits (R&D Systems, Catalog # D1700, DRN00B, DCX110, and DMA00). (*) indicates $p<0.01$, compared with the IgG_1-Fc controls. Results are representative of three independent experiments.

Summary



1. VSIG-3 is a novel ligand of VISTA.
2. VSIG-3 interacts with VISTA to inhibit human T cell activation.
3. Similar to the cell surface molecular interaction between B7-H1 and PD-1, the interaction between VSIG-3 and VISTA represents a new, independent T cell co-inhibitory pathway.

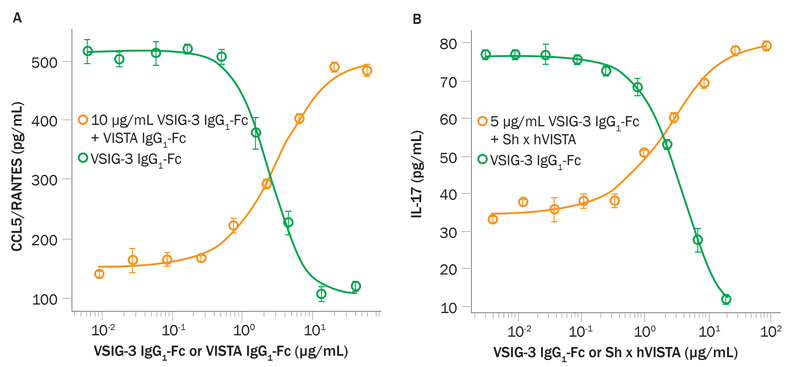
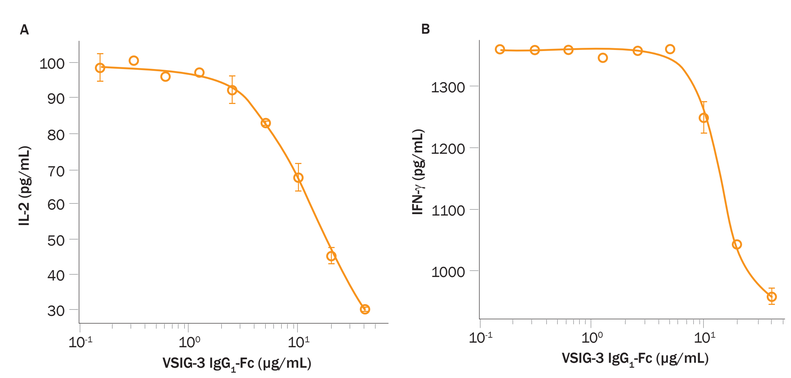


Figure 4. VSIG-3 is a specific ligand of VISTA. (**A**) The extracellular domain of VISTA attenuates VSIG-3 functions. Pretreatment of immobilized Recombinant Human VSIG-3 IgG_1-Fc (10 µg/mL) with the indicated concentrations of Recombinant Human VISTA IgG_1-Fc for 1 hour significantly attenuated the ability of VSIG-3 to inhibit CCL5/RANTES secretion from anti-CD3 activated PBMCs. (**B**) Anti-Human VISTA Antibody attenuates VSIG-3 inhibitory functions. Human PBMCs were treated with immobilized Anti-Human CD3 (1 µg/ml), and various concentrations of Recombinant Human VSIG-3 IgG_1-Fc, or 5 µg/mL of Recombinant Human VSIG-3 IgG_1-Fc plus the indicated concentrations of a Sheep Anti-Human VISTA Antibody (R&D Systems, Catalog #AF7126), or Sheep IgG (R&D Systems, Catalog # 5-001-A) as a control. VSIG-3 significantly inhibited anti-CD3 induced IL-17 production in a dose-dependent manner. Anti-human VISTA Antibody attenuated the inhibitory effect of VSIG-3 on IL-17 secretion from anti-CD3-activated PBMCs. The Sheep IgG control did not alter the inhibitory effect of VSIG-3 on IL-17 secretion (data not shown). Results are representative of three independent experiments.



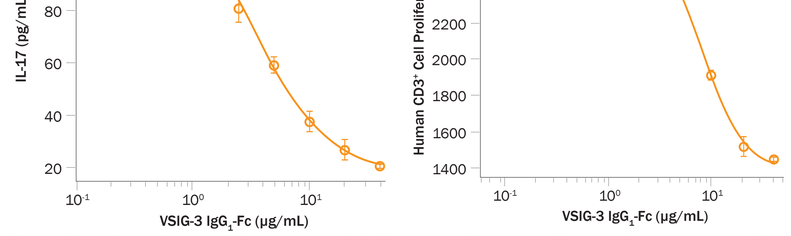


Figure. 5. VSIG-3 negatively regulates human T cell activation. (**A-C**) VSIG-3 inhibits anti-CD3 induced IL-2, IFN-γ, and IL-17 production by human CD3$^+$ T cells in a dose-dependent manner. Human CD3$^+$ T cells were isolated from PBMCs using a MagCellect™ Human CD3$^+$ T Cell Isolation Kit (R&D Systems, Catalog # MAGH101). Human T cells were then incubated with an immobilized Mouse Anti-Human CD3 Monoclonal Antibody (1 µg/mL) and the indicated concentrations of Recombinant Human VSIG-3 IgG_1-Fc or IgG_1-Fc for 24 hours. The levels of IL-2, IFN-γ, and IL-17 in the cell culture supernatants were measured using the Human IL-2, IFN-γ, and IL-17 Quantikine® ELISA Kits (R&D Systems, Catalog # D2050, # DIF50, and # D1700). IgG_1-Fc controls did not alter anti-CD3-induced IL-2, IFN-γ, or IL-17 secretion (data not shown). Results are representative of three independent experiments. (**D**) VSIG-3 inhibits anti-CD3-induced human CD3$^+$ T cell proliferation in a dose-dependent manner. Human T cells were incubated with an immobilized Mouse Anti-Human CD3 Monoclonal Antibody (1 µg/mL) and the indicated concentrations of Recombinant Human VSIG-3 IgG_1-Fc or IgG_1-Fc for 72 hours. Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye Resazurin (R&D Systems, Catalog # AR002). IgG_1-Fc controls did not alter anti-CD3-induced CD3$^+$ cell proliferation (data not shown). Results are representative of three independent experiments.

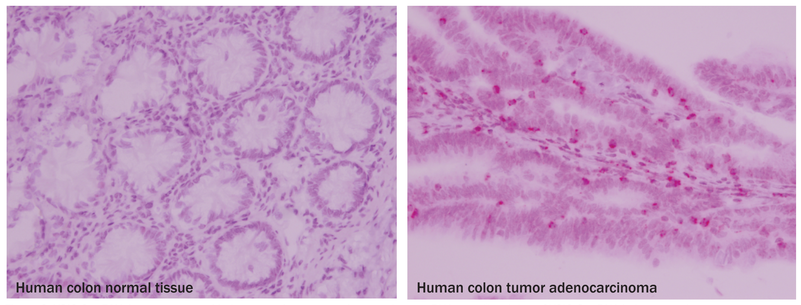


Figure 6. VSIG-3 mRNA is overexpressed in human colon tumor adenocarcinoma. RNAscope® staining of VSIG-3 mRNA in human colon tumor adenocarcinoma and colon normal tissue. VSIG-3 transcript levels were detected using RNAscope® 2.0 HD Red Detection Kit (Advanced Cell Diagnostics, a Bio-Techne brand). A custom-designed human VSIG-3 RNAscope® probe was used to stain following the instructions contained in the RNAscope® 2.0 HD Red Detection Kit.

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Acknowledgments

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