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P-selectin Glycoprotein Ligand-1 Modulates the Functions of Human T Cells and Macrophages In Vitro

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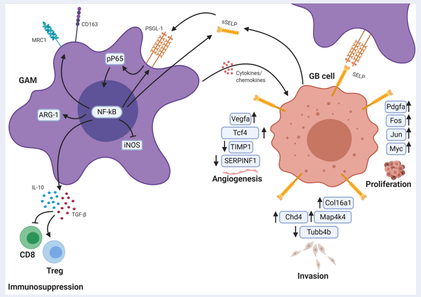
Abstract

P-selectin glycoprotein ligand-1 (PSGL-1) is a type I transmembrane protein expressed on the surface of most hematopoietic cells. PSGL-1 can engage multiple ligands (eg, selectins, VISTA, Siglec-5, versican, CCL19, and CCL21). Apart from being a key adhesion molecule involved in immune cell trafficking, PSGL-1 has been shown to function as a negative immune checkpoint receptor in both T cells and macrophages. PSGL-1 is highly expressed in tumor-infiltrating T cells (TILs) and in tumor-associated macrophages (TAMs) in the tumor microenvironment (TME). PSGL-1 signaling in TILs induces development of T-cell exhaustion, and, in TAMs, it promotes immunosuppressive activity of macrophages. In the TME, PSGL-1 is also highly expressed on the surface of myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and some types of cancer cells. We hypothesized that PSGL-1 may also act as a ligand to modulate the effector functions of human T cells and macrophages in the TME. In the present study, flow cytometry analysis showed that monocytes and granulocytes had a higher PSGL-1 expression than CD4$^+$ and CD8$^+$ T cells isolated from normal human blood cells. To mimic the cell-expressing PSGL-1, a recombinant human PSGL-1-Fc protein (rhPSGL-1-Fc, R&D Systems) was coated onto 96-well plates overnight. Human peripheral blood mononuclear cells (PBMCs), purified human CD4$^+$ T cells, or CD8$^+$ T cells were cultured in the PSGL-1–coated plates for 3 days in the presence of anti-CD3 antibody. In some experiments, a commercial tool PSGL-1 monoclonal antibody (mAb) with the ability to block PSGL-1/P-selectin interaction was added into the culture system. Cytokine secretion in the culture supernatants was measured using Meso Scale Discovery assays. Similar experiments were also conducted with commercially supplied human M1 and M2 macrophages. The results demonstrated that plate-bound rhPSGL-1-Fc dose-dependently inhibited IFN-γ, IL-2, and TNF-α cytokine production in human PBMCs, CD4$^+$ T cells, and CD8$^+$ T cells following T-cell receptor stimulation. The tool PSGL-1 mAb was able to partially rescue the suppression of cytokine production. Plate-bound rhPSGL-1-Fc also inhibited TNF-α secretion from human M1 macrophage, and the PSGL-1 mAb significantly enhanced TNF-α production in both human M1 and M2 macrophages treated with plate-bound PSGL-1-Fc. Together, these data indicate that PSGL-1 expressed on immune cells in TME may act as both an inhibitory ligand and a receptor to impact on T-cell and macrophage function.

Introduction

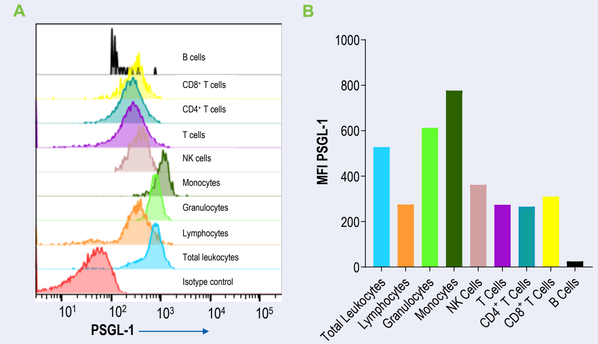
- PSGL-1 acts as an inhibitory checkpoint receptor promoting T-cell exhaustion[1,2]
- PSGL-1 has been reported as a novel macrophage checkpoint receptor with a role in maintaining a suppressive functional macrophage state[3,4]



Reproduced from Yeini E, et al. P-selectin axis plays a key role in microglia immunophenotype and glioblastoma progression. *Nat Commun*. Springer Nature (2021).
ARG-1, arginase1; GAM, glioma-associated microglia/macrophages; GB, glioblastoma; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; MRC1, mannose receptor C type 1; NF-kB, nuclear factor kappa B; PSGL-1, P-selectin glycoprotein ligand-1; SELP, P-selectin; sSELP, soluble SELP; TGF-β, transforming growth factor-β; Treg, regulatory T cells.

- PSGL-1 is highly expressed on the surface of Tregs,[2] TAMs,[3] MDSCs,[5] and some cancer cells[6] in the TME, interacting with multiple binding partners, such as VISTA, P-selectin, E-selectin, L-selectin, versican, Siglec-5, CCL19, and CCL21
- In the present study, we have demonstrated that in addition to functioning as a checkpoint receptor, PSGL-1 can act as an inhibitory ligand to modulate T-cell and macrophage functions

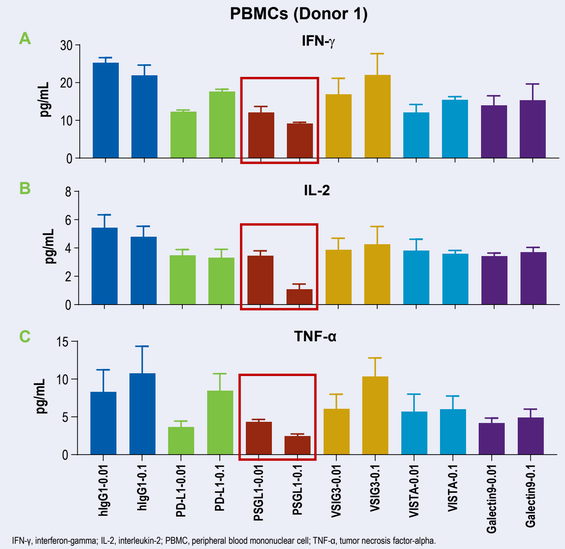
PSGL-1 Expression on the Surface of Normal Whole Blood Cells



MFI, median fluorescence intensity; NK, natural killer; PSGL-1, P-selectin glycoprotein ligand-1.

- Flow cytometry analysis of PSGL-1 expression on the surface of human normal blood cells showed that monocytes and granulocytes had a higher PSGL-1 expression than CD4$^+$ and CD8$^+$ T cells, natural killer cells, and B cells (A and B)

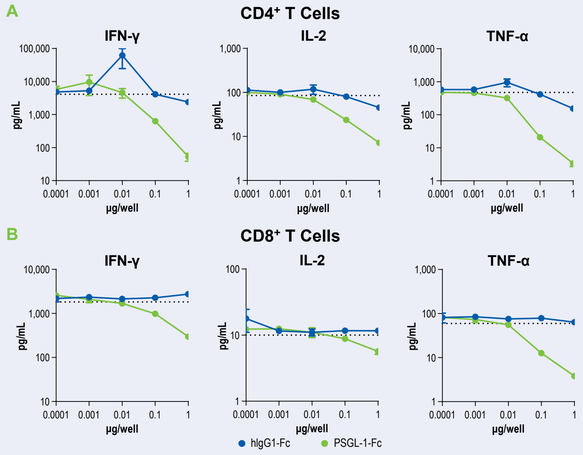
Plate-Bound rhPSGL-1 Inhibited Cytokine Production in Human PBMCs Following TCR Stimulation



IFN-γ, interferon-gamma; IL-2, interleukin-2; PBMC, peripheral blood mononuclear cell; TNF-α, tumor necrosis factor-alpha.

- Plate-bound rhPSGL-1 dose-dependently inhibited IFN-γ, IL-2, and TNF-α production in human PBMCs following T-cell receptor (TCR) stimulation, compared with hIgG1 control (A, B, and C). Human PBMCs isolated from a healthy donor were cultured in a 96-well plate coated with rhPSGL-1, hIgG1, or other immune checkpoint ligands at 0.1 or 0.01 μg/well (R&D Systems, Minneapolis, MN) for 3 days in the presence of antihuman CD3 antibody

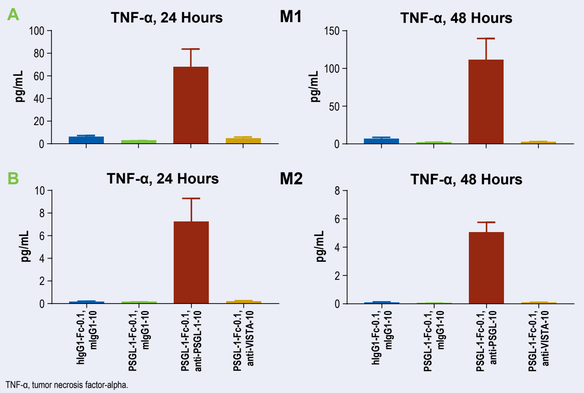
Plate-Bound rhPSGL-1 Inhibited Cytokine Production in Human T Cells Following TCR Stimulation



IFN-γ, interferon-gamma; IL-2, interleukin-2; PSGL-1, P-selectin glycoprotein ligand-1; TNF-α, tumor necrosis factor-alpha.

- Plate-bound rhPSGL-1 dose-dependently inhibited INF-γ, IL-2, and TNF-α production in CD4$^+$ (A) and CD8$^+$ T cells (B) following TCR stimulation, compared with hIgG1 control. CD4$^+$ and CD8$^+$ T cells purified from normal human PBMCs using EasySep Isolation Kits (StemCell Technologies Inc., Vancouver, BC, Canada) were cultured in 96-well plates coated with rhPSGL-1 or hIgG1 at the indicated concentrations for 3 days in the presence of antihuman CD3 antibody

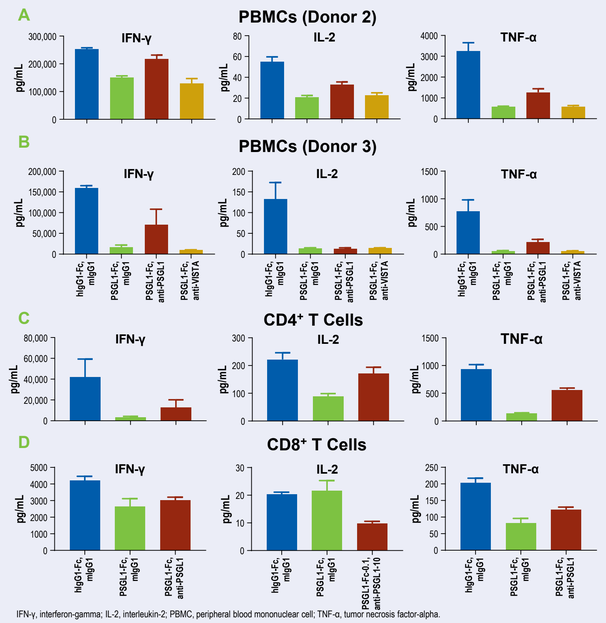
PSGL-1 mAb Enhanced TNF-α Production in Human M1 and M2 Macrophages



TNF-α, tumor necrosis factor-alpha.

- A mouse antihuman PSGL-1 mAb (10 μg/mL, R&D Systems) blocking PSGL-1/P-selectin interaction reversed the suppression of TNF-α production in M1 macrophages (A) induced by plate-bound rhPSGL-1-Fc at 0.1 μg/well at 24 or 48 hours
- The antihuman PSGL-1 mAb also significantly enhanced TNF-α production in both M1 and M2 macrophages (HemaCare, Los Angeles, CA), compared with mouse IgG1 control or a mouse antihuman VISTA mAb (A and B)

PSGL-1 mAb Partially Rescued rhPSGL-1 Induced Suppression on Cytokine Production in PBMCs/T Cells



IFN-γ, interferon-gamma; IL-2, interleukin-2; PBMC, peripheral blood mononuclear cell; TNF-α, tumor necrosis factor-alpha.

- A mouse antihuman PSGL-1 mAb (10 μg/mL, R&D Systems) blocking PSGL-1/P-selectin interaction partially reversed the suppression of INF-γ, IL-2, and TNF-α production in human PBMCs (A, B), and in CD4$^+$ and CD8$^+$ T cells (C, D) induced by plate-bound rhPSGL-1-Fc at 0.1 μg/well for 3 days in the presence of antihuman CD3 antibody, compared with mouse IgG1 control or a mouse anti-VISTA mAb

Conclusions

- **PSGL-1 is highly expressed on monocytes and granulocytes in human peripheral blood, which is consistent with the report of high PSGL-1 expression observed on the surface of monocytic (M-MDSC) and granulocytic myeloid-derived suppressor cells (PMN-MDSC) in the TME[5]**
- **Plate-bound rhPSGL-1 protein dose-dependently inhibited INF-γ, IL-2, and TNF-α production in PBMCs and CD4$^+$ and CD8$^+$ T cells following TCR stimulation, whereas an antihuman PSGL-1 mAb blocking its binding to P-selectin was able to partially reverse the suppression in vitro**
- **The antihuman PSGL-1 mAb blocking PSGL-1/P-selectin interaction also significantly increased TNF-α production in both M1 and M2 macrophages in the presence of plate-bound rhPSGL-1-Fc**
- **Taken together, these data suggest that PSGL-1 may modulate T-cell and macrophage function by acting as a ligand and a receptor**

Disclosures

Guan, Dees, Chadderton, Amador Arjona: Employment and stock ownership – *Incyte Corporation*.

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