A15153G mAb, a novel tool for human TIGIT study

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Abstract

T cell immunoglobulin and ITIM domain receptor (TIGIT) is a recently identified member of the PVR (poliovirus receptor) family of immunoglobulin-like domain containing proteins that is expressed on subsets of T cells and NK cells. With mouse model studies, TIGIT has been established as a new co-inhibitory, or "checkpoint" molecule in immune regulation. It is thus considered a potential therapeutic target for cancer and autoimmune/inflammatory disorders. However, due to limited availability of antibody reagents to human TIGIT, the biological function of this molecule in human cells, as well as its detailed mechanisms, have not been fully elucidated. We have developed a novel monoclonal antibody, clone A15153G, specific to human TIGIT. As expected, this antibody reacts with a subset of human peripheral blood T cells and most CD56$^+$ NK cells. When A15153G antibody was applied in culture, it strongly suppressed anti-CD3 induced human T cell proliferation, suggesting that A15153G is an agonistic antibody. By multi-color flow cytometric analysis using this antibody, we found that the expression patterns of human TIGIT on the surface of CD4$^+$ and CD8$^+$ T cells are different. In the CD4$^+$ T cells, TIGIT is mainly expressed in memory-like population; in contrast, a significant percentage of "naïve" CD8$^+$ T cells also expressed high levels of TIGIT in addition to memory populations. Studies of cytokine production profiles of TIGIT-expressing cells are ongoing.

Methods

Anti-TIGIT mAb development: BALB/c mice were immunized with recombinant human TIGIT in the presence of adjuvants. Hybridomas were developed by fusion of immune cells with mouse myeloma cell Sp2/0. Stringent screening by ELISA and flow cytomety resulted in several specific clones for human TIGIT. Clone A15153G (mouse IgG_{2a} κ) was selected for this study.

Cell staining and flow cytometry: Human peripheral blood cells were stained with fluorophore conjugated antibodies for cell surface markers as indicated. After RBC lysis, cells were washed and fixed. Flow cytometry data acquisitions were performed on BD™ LSR II, BD LSRFortessa™, or BD FACSCanto™ II instruments.

FoxP3 staining: After cell surface staining as described above, cells were fixed and permeablized using BioLegend True-Nuclear™ Transcription Factor Buffer Set, and then stained with anti-human FoxP3 mAb.

Intracellular cytokine staining: Human PBMCs were stimulated with PMA and ionomycin in the presence of Brefeldin A for 5 hours. Cells were stained with antibodies for surface makers. After fixation and permeablization, cells were intracellularly stained with indicated anti-cytokine antibodies.

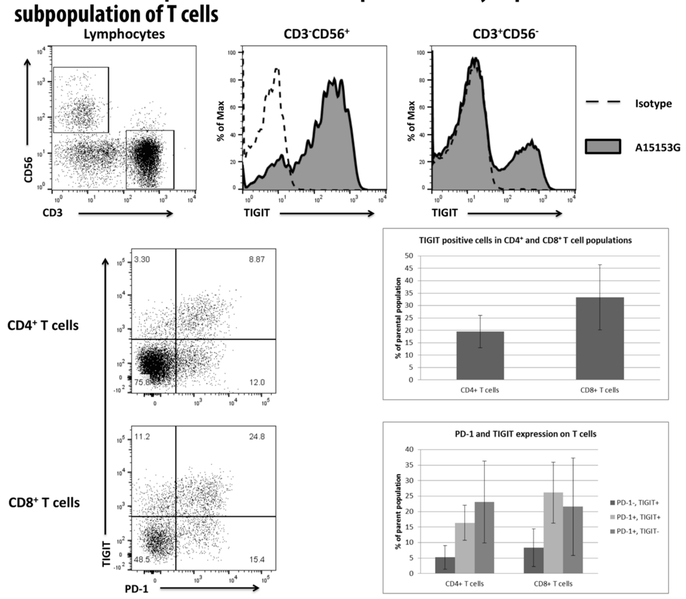
TIGIT-negative T cell isolation: Human peripheral blood T cells were isolated by using the BioLegend MojoSort™ Human CD3 T Cell Isolation Kit. TIGIT-expressing T cells were then depleted by using biotinylated A15153G and Mojosort™ Nanobeads Streptavidin magnetic separation.

T cell activation assay: CFSE-labeled human PBMCs or total T cells were cultured in 12-well plates that were coated with anti-CD3 with or without anti-CD28 or A15153G mAbs and then analyzed by flow cytomerty.

Conclusions

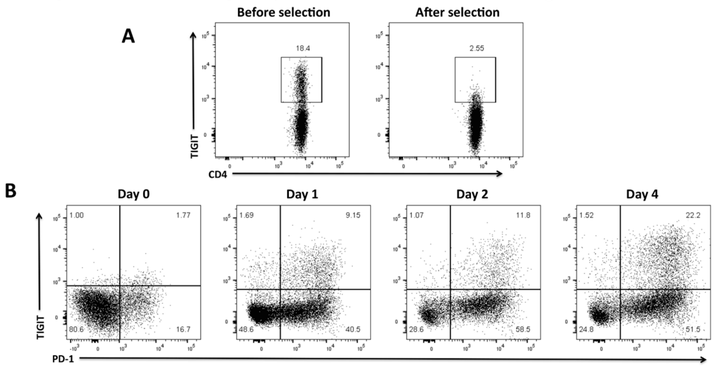
A15153G is a specific mAb for human TIGIT. It can be used for cell surface staining of TIGIT for flow cytometric analysis and for isolation or depletion of TIGIT-expressing cells. It can also be used as agonistic antibody for TIGIT signaling in functional studies. A15153G proves useful as a tool in TIGIT biology research.

Figure 1: mAb A15153G is specific for TIGIT that is predominantly expressed on NK cells and a subpopulation of T cells



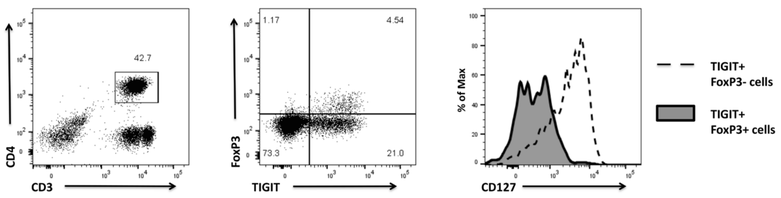
A15153G stains subpopulations of CD4$^+$ T cells, CD8$^+$ T cells, and most CD56$^+$ NK cells in human peripheral blood. Higher percentages of CD8$^+$ T cells than CD4$^+$ T cells express TIGIT, but B cells and myeloid cells do not express TIGIT. The expressions of PD-1 and TIGIT only partially overlap in *ex vivo* T cells. Data shown are representative of more than 20 healthy donors, which are summarized in bar graph.

Figure 3: Expression of TIGIT is quickly induced on activated TIGIT-negative T cells



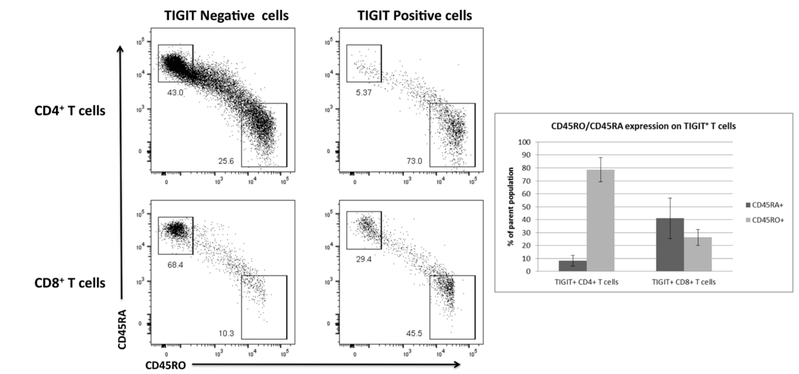
A: Total T cells were isolated from human PBMCs. Biotinylated A15153G and Mojosort™ Streptavidin Nanobeads were used to deplete TIGIT$^+$ cells.
B: TIGIT-negative isolated T cells were stimulated with anti-CD3/anti-CD28 coated plate for different periods of time. Cells were stained for TIGIT and PD-1. TIGIT surface expression is upregulated quickly and it is mainly expressed on PD-1 high cells. Data shown are CD3$^+$CD4$^+$ gated cells.

Figure 5: FoxP3$^+$ Treg cells reside in CD4$^+$ TIGIT$^+$ T cell population, which express low levels of CD127 (IL-7Ra)



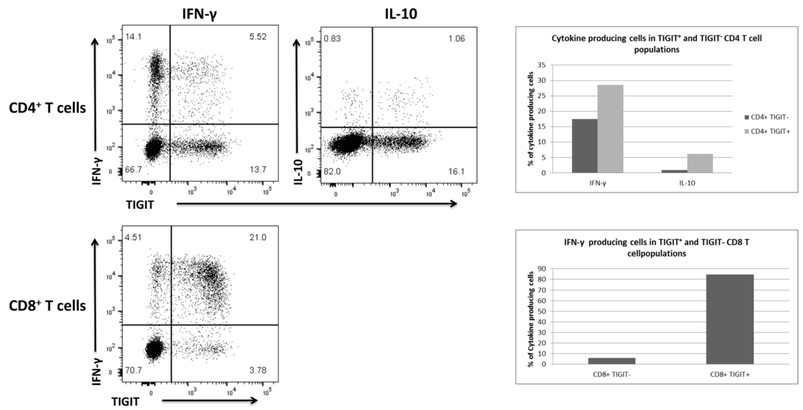
Human PBMCs were surface stained with CD3, CD4, CD127, and TIGIT (A15153G), and intracellularly with anti-FoxP3. Data shown are representative of 10 random healthy donors.

Figure 2: Different TIGIT-expression profiles of naïve vs memory cell populations in CD4$^+$ and CD8$^+$ T cells



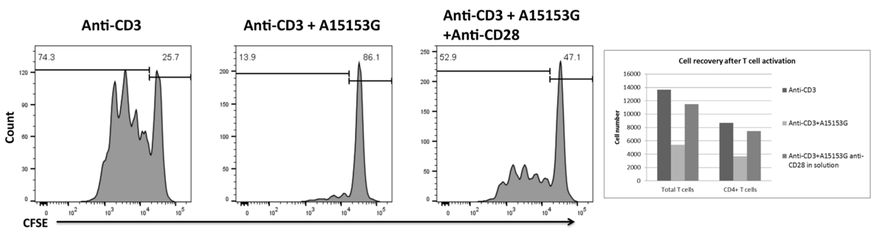
TIGIT is mainly expressed on CD45RO$^+$ cells in the CD4$^+$ T cell population, but CD8$^+$ T cells do not have a similar TIGIT expression pattern. Data shown are representative of 20 random healthy donors, which are summarized in the bar graph.

Figure 4: Higher percentage of TIGIT$^+$ T cells express IL-10 and IFN-γ compared to TIGIT$^-$ T cells



Intracellular cytokine staining were performed as describe in Methods. TIGIT$^+$CD4$^+$ T cells have a higher percentage of IL-10 expressing cells compared to TIGIT$^-$ cells. IL-10 expression was not detected in CD8$^+$ T cells (data not shown). A majority of CD8$^+$TIGIT$^+$ T cells produce IFN-γ.

Figure 6: mAb A15153G suppresses anti-CD3 induced T cell proliferation



PBMCs were cultured in anti-CD3 or anti-CD3/anti-TIGIT (A15153G) coated plates. Soluble anti-CD28 was added to culture medium as indicated. After 4 days of culture, CFSE dilution was analyzed by flow cytometry for T cell proliferation. CD4$^+$ T cell recovery was summarized in bar graph. mAb A15153G can suppress anti-CD3 induced T cell activation/proliferation, which was partially rescued by anti-CD28 costimulation.

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