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 “naive” 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 cytometry resulted in several specific clones for human TIGIT. Clone A15153G (mouse IgG1, κ) was selected for this study.

Cell staining and flow cytometry: Human peripheral blood cells were stained with fluorescein-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 permeabilized using BioLegend TrueNuclear® 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 4 hours. Cells were stained with antibodies for surface makers. After fixation and permeabilization, 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 MoJoSet® Human CD3 T Cell Isolation Kit. TIGIT-expressing T cells were then depleted by using biotinylated A15153G and MoJoSet® Streptavidin Nano beads.

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 cytometry.

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 an agonistic antibody for TIGIT signaling in functional studies. A15153G proves useful as a tool in TIGIT biology research.