Abstract

HIGHER ORDER CLUSTERING

PRIMING variants of this antibody with diminished binding to the inhibitory factor receptor superfamily (TNFRSF). Like other T cell co-stimulatory TNFR family members, GITR utilizes multiple other immunomodulatory or immune education strategies. The formation of receptor superclusters, comprised of two or more trimeric molecules, has been defined for multiple TNFRs as a means of regulating downstream signal amplification. For co-stimulatory TNFRs, like GITR, utilizing multiple signaling mechanisms from therapeutic utility in enhancing the activity of tumor-reactive T cells, either as single agents or in combination with other immunomodulatory or immune education strategies.

Here we describe a structure-activity analysis of a bispecific anti-human GITR antibody that ablates unique immunosuppressive features of the inhibitor, INCAGN1876. A human GITR receptor and GITR antibody was engineered to form a conformational epitope located within a Pru of the receptor domain of GITR. This antibody binding also modified the ligand of GITR monomer, dimer and trimers to promote receptor oligomerization, resulting in downstream NF-kB signaling. Notably, this mode of antibody engagement seems to be a unique mechanism by which these antibodies are capable of potentiating TCR stimulation by recruiting cytokine production. Therefore, antibodies that specifically target GITR antigen-driven activation and the ability of antibodies to engage conformationally modified GITR antibody required for receptor oligomerization with increased activity in TCR signaling. This antibody binding also modified the ligand of GITR monomer, dimer and trimers to promote receptor oligomerization, resulting in downstream NF-kB signaling. Notably, this mode of antibody engagement seems to be a unique mechanism by which these antibodies are capable of potentiating TCR stimulation by recruiting cytokine production. Therefore, antibodies that specifically target GITR antigen-driven activation and the ability of antibodies to engage conformationally modified GITR antibody required for receptor oligomerization.

The GITR Pathway Promotes T Cell Co-stimulation

INCGA1876 Promotes T cell co-stimulation in the context of TCR activation through selective T cell activation, cytokine production, and the activation of professional APCs.

INCGA1876 Promotes GITR Signaling in Recently Activated T Cells, Including in the Absence of Concomitant TCR Stimulation

INCGA1876 Favors Binding to Higher Order GITR Clusters

INCGA1876 Mediates GITR Clustering on the Surface of Cells That Correlates With T Cell Activation

INCGA1876 Promotes T Cell Co-stimulation

INCGA1876 Demonstrates Increased Activity as an IgG1 Fc and Cooperates With Other Immunomodulatory Antibodies

INCGA1876 promotes total cytokine production in vitro in freshly isolated PBMCs cultured with anti-OX40 alone or in combination with anti-PD-1 and anti-CTLA4. INCAGN1876 efficiently promotes the formation of GITR clusters on the surface of cells that correlates with GITR forward signaling.

Summary

INCGA1876 potently binds to viable, human, and higher order T cell complexes, as compared with other GITR-targeting antibodies. INCAGN1876 promotes GITR forward signaling in freshly isolated T cells in primary and secondary cultures of T cell complexes.

INCGA1876 efficiently promotes the formation of GITR clusters on the surface of cells that correlates with GITR forward signaling.

INCGA1876 promotes total cytokine production in vitro in freshly isolated PBMCs cultured with anti-OX40 alone or in combination with anti-PD-1 and anti-CTLA4. INCAGN1876 has shown potential mechanisms of action.

The GITR pathway provides an attractive therapeutic strategy for enhancing the function of tumor-specific T cells in the absence of APCs.

Acknowledgments

This research was supported by grants from the National Institutes of Health and the Department of Defense. The authors thank the members of the Heller lab for their support and assistance.

References