Functional optimization of agonistic antibodies to OX40 receptor with novel Fc mutations to promote antibody multimerization

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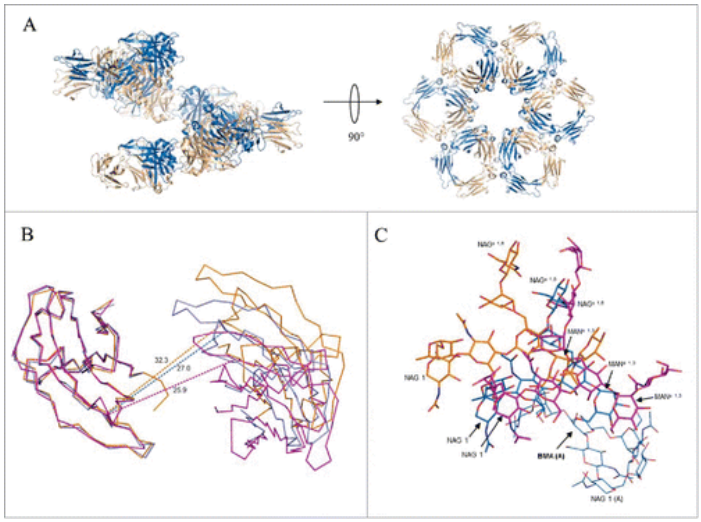
Immunostimulatory receptors belonging to the tumor necrosis factor receptor (TNFR) superfamily are emerging as promising targets for cancer immunotherapies. To optimize the agonism of therapeutic antibodies to these receptors, Fc engineering of antibodies was applied to facilitate the clustering of cell surface TNFRs to activate downstream signaling pathways. One engineering strategy is to identify Fc mutations that facilitate antibody multimerization on the cell surface directly. From the analyses of the crystal packing of IgG1 structures, we identified a novel set of Fc mutations, T437R and K248E, that facilitated antibody multimerization upon binding to antigens on cell surface. In a NF-κB reporter assay, the engineered T437R/K248E mutations could facilitate enhanced agonism of an anti-OX40 antibody without the dependence on FcγRIIB crosslinking. Nonetheless, the presence of cells expressing FcγRIIB could facilitate a boost of the agonism of the engineered antibody with mutations on IgG1 Fc, but not on the silent IgG2σ Fc framework. The Fc engineered antibody also showed enhanced effector functions, including antibody-dependent cell-meditated cytotoxicity, antibody-dependent cellular phagocytosis, and complement-dependent cytotoxicity, depending on the IgG subtypes. Also, the engineered antibodies showed normal FcRn binding and pharmacokinetic profiles in mice. In summary, this study elucidated a novel Fc engineering approach to promote antibody multimerization on a cell surface, which could enhance agonism and improve effector function for anti-TNFR antibodies as well as other therapeutic antibodies.



Local environment about K248E and T437R mutations in crystal structure of SF2IgG1_RE Fc.

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