THE CD16 L48H/R POLYMORPHISM ENABLES MORE
COMPACT RECEPTOR CLUSTERING AND ENHANCES
NATURAL KILLER CELL-MEDIATED ANTIBODY-
DEPENDENT CELLULAR CYTOTOXICITY

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Background Antibody-dependent cellular cytotoxicity (ADCC) is a critical component of many monoclonal antibody cancer immunotherapies. The binding affinity between IgG Fc and FcγRIIIa (CD16) is a major determinant of ADCC, and increasing this affinity through the high affinity CD16 polymorphism F158V can significantly potentiate the response. However, the effects of other CD16 polymorphic variants are not well understood. Here we investigated the effects of the L48H/R CD16 polymorphism on ADCC.

Methods NK cells derived from healthy donors and NK-92 cells retrovirally transduced with different CD16 variants were tested for cytotoxicity against SKOV-3 target cells treated with or without trastuzumab using both xCELLigence RTCA and real-time microscopy. The expression of CD16 on the surface of NK-92 cells was tested before and after cytotoxicity assays using flow cytometry. Synapse formation and cytolytic polarization in NK-92 cells were also tested using confocal microscopy. The clustering of different CD16 variants on supported lipid bilayers was measured using confocal microscopy, and calcium influx from CD16 engagement was assessed via flow cytometry.

Results We found that 48H or 48R CD16 significantly enhanced ADCC in vitro when expressed in NK-92 and primary NK cells. This effect was accompanied by increased rates of cytolytic vesicle polarization and loss of CD16 surface expression after tumor cytolysis. We also found that 48H CD16 formed more compact immunological synapses, suggesting that these phenotypes were being driven by more efficient receptor clustering. Upon directly engaging CD16 with antibody, we measured significantly stronger calcium influx through CD16 48H, as compared to 48L. This effect was also observed by directly conjugating NK cells to target cells, though this was attenuated by the presence of CD2 ligands CD48/58 on the surface of the target cell.

Conclusions These results are intriguing due to the established physical interaction between CD16 and CD2, which the 48H polymorphism has been shown to disrupt. Our data support a model that L48H/R promotes tighter CD16 clustering, potentially through the loss of CD2 binding, which leads to a stronger ADCC response. These findings suggest that patients with L48H/R CD16 polymorphisms may respond better to treatment with ADCC-inducing immunotherapies, particularly with solid tumors lacking CD2-stimulating ligands.

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