Modeling CD16 signal kinetics reveals the role of adaptor CD3ζ in NK cell signaling in humans and mice

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***Abstract*—Natural killer (NK) cells provide important protection against viral infections and tumors. Stimulation of CD16, an activating NK receptor that recognizes antibodies, leads to lysis of antibody-coated target cells by NK cells. However, engagement of CD16 in mouse NK cell produces weaker cytotoxic responses than in human NK cell [1]. We developed a CD16 signaling model to investigate the above differences in NK cell responses between humans and mice. Our study suggests higher number of ITAMs in CD3ζ, along with increased association between CD16 and CD3ζ are crucial for greater recruitment of downstream signaling molecules, resulting in higher cytotoxic responses.**

***Keywords* — NK cell signaling, CD16 receptor, CD3ζ, FcεR1γ, in silico modeling**

I. Background

Human and mouse NK cells express activating CD16 receptors. CD16 in human NK cells can associate with both CD3ζ and FcεR1γ adaptor molecules. When CD16 binds to cognate ligands, phosphorylation of tyrosine residues on immunoreceptor tyrosine-based activation motifs (ITAMs) associated with these adaptors recruit kinases such as ZAP70 and Syk leading to propagation of signals further downstream and NK cell activation. FcεR1γ and CD3ζ carry one and three pairs of ITAMs per chain respectively, in their transmembrane region. They associate with CD16, and a single mutation (46L→ 46I) in the CD3ζ chain found in mouse leads to poor association of CD3ζ with CD16 in mouse NK cells [1]. This results in lower NK cell signaling (e.g., Ca^{2+} flux) and activation (e.g., mobilization of lytic granules) in mouse NK cells compared to human NK cells. In order to glean mechanisms that underlie the above difference in NK cell stimulation in humans and mice, we combined experiments with a T lymphoma cell line (BWZ) and mechanistic *in silico* modeling. BWZ cells were transduced with mouse and human CD16 along with different combinations of mouse/human FcεR1γ and CD3ζ adaptors which included a variety of amino acid residue mutations in their transmembrane regions.

II. Sample collection

BWZ cells were transduced with MSCV retroviruses expressing human or mice CD16. Then they are transduced with MSCV retroviruses expressing human CD3ζ, mouse CD3ζ−WT, mouse FcεR1γ−WT and mutants of mouse CD3ζ. Cells were then stained with biotinylated anti-CD16 antibody and Ca^{2+} influx is measured in flow cytometer upon addition of streptavidin.

III. Model

We consider a well-mixed system consisting of CD16 receptor, IgG-antibody, CD3ζ (or, FCεR1γ) adaptor, Src-family kinase Lck, Syk-family kinase ZAP70 and phosphatase SHP- 1 in a simulation box of size $5\times5\times1$ μm^3 representing a small region proximal to the cell membrane. The signaling process is modeled using rule-based stochastic modeling in Vcell (vcell.org). *Signaling reactions:* CD16 binds with the ligand IgG to form the IgG-CD16 complex. CD3ζ (or, FcεR1γ) homo-dimer associates with the IgG-CD16 complex. The tyrosine residues on CD3ζ (or, FcεR1γ) are phosphorylated by Lck. ZAP70 and SHP-1 bind to the phosphorylated residues on the adaptor. ZAP70 is further phosphorylated by Lck, whereas SHP-1 bound to adaptors dephosphorylate the phosphorylated ZAP70. We obtained the resulting Ca^{2+} flux using a coarse-grained ODE based model.

IV. Results

We modeled the CD16 signaling pathways for different mutants of CD3ζ and FcεR1γ and computed the kinetics of the abundance of phosphorylated ZAP70 (pZAP70) and Ca^{2+} flux. Comparison of the Ca^{2+} flux kinetics between our model and experiments indicates that the increase in the number of ITAMs plays a crucial role in increasing Ca^{2+} flux. CD3ζ, which has 6 ITAMs are more efficient in producing phosphorylated ZAP70 compared to FcεR1γ, which has 2 ITAMs. We further explain Ca^{2+} signals from experiments using our model for different mutants of CD3ζ. Our model suggests, mutants that are better at producing Ca^{2+} signal have higher affinities to associate with the CD16 receptor. Our model can be extended to describe signaling kinetics for various receptors that can associate with multiple types of adaptors.

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References

[1] O. A. Aguilar, L. K. Fong, K. Ishiyama, W. F. DeGrado, and L. L. Lanier, "The CD3ζ adaptor structure determines functional differences between human and mouse CD16 Fc receptor signaling," *Journal of Experimental Medicine*, vol. 219, no. 5, p. e20220022, 2022.