

## DISTINCTION OF Fc $\gamma$ RECEPTOR ISOFORMS BY FLOW CYTOMETRY

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The human leukocytes bind IgG isotype antibodies through their Fc $\gamma$  receptors, therefore these receptors have an important role to link the adaptive and the innate immune systems. Human Fc $\gamma$  receptors consist of three main groups: Fc $\gamma$ RI, Fc $\gamma$ RII and Fc $\gamma$ RIII. Fc $\gamma$ RII have three isoforms: Fc $\gamma$ RIIA, Fc $\gamma$ RIIB and Fc $\gamma$ RIIC. Fc $\gamma$ RIII have two isoforms: Fc $\gamma$ RIIIA and Fc $\gamma$ RIIIB. Because of the high degree of homology these isoforms are often difficult to distinguish. We set out to characterize expression of Fc $\gamma$ R isoforms using affinity reagents and a biochemical method.

We examined the Fc $\gamma$ RII and the Fc $\gamma$ RIII isoform expression of cells in whole blood by flow cytometry, along with the U937 monocytoid cell line. We determined the Fc $\gamma$ RII isoform expression with receptor specific antibodies and Fc $\gamma$ RIII expression by digesting the GPI-anchored Fc $\gamma$ RIIIB with phosphatidylinositol-specific phospholipase C (PI-PLC). We used U937 cells as reference, as these cells express the Fc $\gamma$ RIIA and the Fc $\gamma$ RIIB receptors as well.

We confirmed that the Fc $\gamma$ RIIA isoform specific antibody (clone IV.3) bound only to the Fc $\gamma$ RIIA isoform, no binding to B cells carrying only Fc $\gamma$ RIIB was observed. On the other hand, we found the Fc $\gamma$ RIIB specific antibody (clone 2e1) to bind Fc $\gamma$ RIIA, as well. Using U937 cells we quantitated differences in the ratio of Fc $\gamma$ RII isoforms on neutrophil granulocytes of various donors. We successfully distinguished the Fc $\gamma$ RIIIA and the Fc $\gamma$ RIIIB expressing cells with the PI-PLC enzyme. Following the removal of the GPI-anchored Fc $\gamma$ RIIIB, the Fc $\gamma$ RIII specific antibody (clone 3g8) showed lower binding compared to the undigested control in populations where Fc $\gamma$ RIIIB isoform is expressed.

These studies lay the grounds for a multiplex, protein microarray-based method for the characterization of cell adherence to arrayed human IgG subclasses. The U937 cells are suitable as a reference cells for examining Fc $\gamma$ RII isoforms' ratio of neutrophil granulocytes in human donors.

These studies are supported by OTKA 109683 grant of the National Science Fund.

Poster

Experimental research