

DISTINCTION OF Fc γ RECEPTOR ISOFORMS BY FLOW CYTOMETRY

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

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

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