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The Ability of NK Cell and Granulocyte Forms of CD16 to Trigger Cytolytic Function

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The FcγIII receptor (CD16) is expressed on NK cells, granulocytes, cultured monocytes and liver macrophages [1]. Two allelic forms of CD16 exist in granulocytes. They have been designated NA1 and NA2 and migrate in SDS-PAGE with different relative molecular masses (M_r) [1]. We recently showed that granulocyte CD16 is anchored to the cell membrane by a phosphatidylinositol-glycan (PIG) moiety [2]. CD16 is released from granulocytes by treatment with phosphatidylinositol-specific phospholipase C (PIPLC) and its expression is deficient in granulocytes of patients with paroxysmal nocturnal hemoglobinuria (PNH), an abnormality of hematopoietic cells affecting the biosynthesis or attachment of PIG tailed molecules [3]. Subsequent experiments have indicated that an alternative, PIPLC-resistant form of CD16 is expressed on NK cells (fig. 1) [4]. Other proteins on NK cells which are attached via PIG anchor, such as decay accelerating factor (DAF, CD55) or LFA-3 (CD58) are PIPLC sensitive (fig. 1), indicating that the difference between NK cell and granulocyte CD16 is not due to a cell-type-specific resistance to PIPLC. In addition, immunoprecipitation of surface-labelled cells with CD16 mAb after PIPLC treatment results in a decrease of CD16 from the granulocyte surface and an appearance of CD16 in the supernatant, whereas the amount of CD16 from the NK cell surface is unaffected [4].

After removal of N-linked carbohydrates the M_r of the granulocyte CD16 protein backbone is either 26 or 28 kd depending the NA1/NA2 allelic form of the donor. In contrast, the M_r of the NK cell CD16 protein

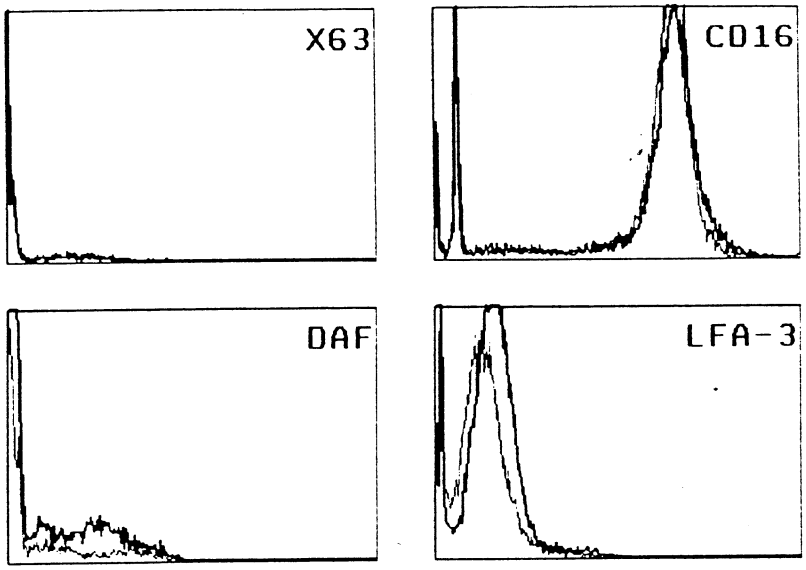

Fig. 1. Cell surface analysis of NK cells. Highly purified (81% $CD16^+$) human peripheral blood NK cells were treated with or without PIPLC and stained with control mAb (X63), CD16 mAb (CLB/FcGran1), DAF mAb (1A10) or LFA-3 mAb (TS2/9) followed by FITC-goat-anti-mouse $F(ab')_2$ and analyzed by FACS. Bold lines represent staining without and thin lines after PIPLC treatment.

backbone is 33 kd. The observed 5–7 kd larger size [4–6] and PIPLC resistance indicate that NK cell CD16, in contrast to granulocytes, is a transmembrane protein.

Functional Difference between NK Cell and Granulocyte CD16

CD16 is the only FcR on NK cells, whereas granulocytes also express FcγRI (CD64) and FcγRII (CDw32) [1]. Both NK cells and IFNγ-treated granulocytes mediate antibody-dependent cellular cytotoxicity (ADCC) [7, 8] but in granulocytes this function has been mainly attributed to FcγRI and FcγRII. To specifically compare the ability of the different membrane-anchored forms of CD16 to trigger ADCC, CD16 mAb-bearing hybridomas (CLB/FcGran1, 3G8) were used as targets in redirected killing [8].

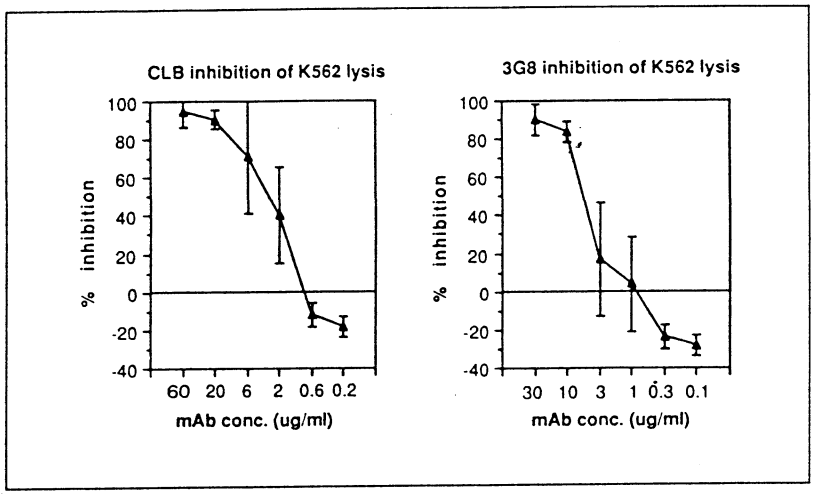

Fig. 2. The effect of CD16 mAb on NK cell-mediated lysis. Freshly isolated human NK cells were incubated for 30 min at +4 °C with the indicated concentrations of CD16 mAb CLB/FcGran1 or 3G8. ^{51}Cr-labelled K562 target cells were added in E:T ratio 10:1 and a standard 4-hour cytotoxicity assay was performed. Mean ± SD of 4 experiments.

These experiments demonstrated that NK cells but not IFNγ-activated granulocytes mediated killing [4]. The lysis by NK cells was specifically triggered by CD16, since hybridomas bearing mAb against other NK cell antigens were not lysed and the killing of anti-CD16 hybridomas could be blocked by soluble CD16 mAb. The failure of granulocytes to kill CD16 mAb-bearing hybridomas was not due to deficient cytolytic machinery because the cells could mediate cytotoxicity through other FcR if the target cells were opsonized with mAb reacting with hybridoma cells. Thus, the type of CD16 membrane anchor profoundly affects its ability to trigger cytolytic function.

The Role of CD16 in NK Cell-Mediated Lysis

The signal-transducing capacity of NK cell CD16 is well established. Activation through CD16 triggers ADCC and induces second messenger formation, IL-2R expression, cytokine secretion and cytotoxicity of NK

cell clones towards resistant target cells [7, 9–11]. Whether CD16 plays any role in NK cell-mediated lysis is, however, controversial. We analyzed the effect of the CD16 mAb from the IV Leukocyte Typing Workshop on killing by fresh NK cells [12]. Six of 11 mAb (ascites, 1:200 dilution) significantly inhibited NK cell lysis of K562 targets (mean inhibition 49%, range 41–69) and 9 of 11 mAb blocked lysis of Molt-4 targets (mean 80%, range 56–96). In further studies purified CD16 mAb CLB/FcGran1 and 3G8 had a concentration-dependent effect on NK-mediated lysis when included in the ^{51}Cr-release assay (fig. 2). High concentrations (2–30 μg/ml) efficiently inhibited killing of K562 targets, whereas low mAb concentrations enhanced the lytic activity. Neither mAb affected effector:target cell conjugate formation. The mechanism of the dual concentration-dependent effect of CD16 mAb is not known. CD16 mAb may act in triggering the release of cytolytic molecules from NK cells in an undirected way and thus lead to impaired lytic activity. Alternatively, CD16 mAb could block interaction of CD16 with an uncharacterized molecule on the target cell surface required in the cytolytic process. Although our recent studies using purified CD2 molecules to block NK activity indicate that CD2/LFA-3 interaction is the major activation pathway in NK-mediated killing [Carpén, unpubl.], a possible role for CD16 as a synergistic or alternative signal-transducing molecule during NK cell lysis cannot be ruled out.

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