The use of CD107a (LAMP-1) as a biomarker of Natural Killer Cell functionality in nonclinical toxicology studies and clinical trials

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ABSTRACT

Background: Natural killer (NK) cells are a subset of lymphocytes that play a central role in the innate immune response to tumors and infections, particularly those caused by viruses. These cells kill by a mechanism involving the release of small cytoplasmic granules containing granzyme B and Perforin-1 that induce cell death in the target cell. Although a number of chemicals have been documented to impair natural killer cell activity, direct evidence for clinically significant pathologic consequences is lacking. In addition, few immunosuppressive drugs have been shown to reproducibly suppress NK cell activity. Although NK cell numbers can be enumerated by immunophenotyping on the flow cytometer as CD3-CD16+CD56+ cells (human) and CD3-CD159a+ cells (nonhuman primate or NHP), an important limitation in the study of NK cells is the deficiency of high throughput assays available for the detection of the functional activity of NK cells. As degranulation occurs, secretory lysosomes are released, and the lysosome-associated membrane protein-1 (LAMP-1, CD107a) is transported to the surface of the NK cells. Once on the surface, it becomes accessible for antibody binding and allows identification of NK cells which have potentially been activated, making this an attractive biomarker for identifying the cytotoxic activity of NK cells. **Methods:** Here we describe the use of CD107a as a biomarker of NK cell functional activity by multi-parameter flow cytometry. As a comparison, effector cells from PBMCs of cynomolgus monkeys and healthy human donors were isolated and the K562 cell line was used as a target of cytotoxicity, with cell damage and death determined by intercalation of the DNA dye propidium iodide (PI). It is well known that IL-2 activates NK cells, so to explore the effect of IL-2 on CD107a expression; isolated PBMCs were co-cultured with K562 cells in the presence of IL-2 and PMA/Ionomycin. Expression of CD107a on NK cells was also evaluated by flow cytometry. **Results:** In this study we demonstrate that CD107a is upregulated on both NK cells from human donors and cynomolgus monkey in response to stimulation. The relationship between the expression of CD107a and cytotoxic activity in NK cell has been determined and increased expression of CD107a after IL-2 stimulation of NK cells correlated well to the increase of cytotoxicity. **Conclusions:** Our results suggest that CD107a expression is a sensitive biomarker for the cytotoxic activity determination that could be employed in both nonclinical toxicology studies and clinical trials.

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INTRODUCTION

NK cells contribute to a variety of innate immune responses to viruses, tumors and allogenic cells. An important aspect of many toxiclogy and drug safety studies is the ability to not only evaluate the impact of a potential immunotherapeutic on NK cell numbers but NK cell activity as well. At the present time, most NK functional assays are time consuming and labor intensive, making these determinations difficult. CD107a is a protein that is transported to the surface of the NK cells during activation, making this an attractive surface marker for measuring potential of NK cell activity.

Our goal was to compare the antibody staining of CD107a as a biomarker of NK cell functional activity with a classic cytotoxicity assay in which cell damage and death are determined by intercalation of the DNA dye propidium iodide (PI) in target K562 cells. To evaluate the universality of this assay, effector cells from whole blood and PBMCs of cynomolgus monkeys and human donors were also evaluated.

MATERIALS AND METHODS

Cells – Human and cynomolgus monkey whole blood specimens were obtained in sodium heparin vaccutainers. PBMCs were isolated by centrifugation on Lymphoprep™ density gradients (Greiner Bio-One). NK cells were also enriched by incubation RosetteSep® Human NK Cell Enrichment Cocktail (StemCell Technologies) at 50 µL/mL of whole blood. The target cell line K562 is a human myelogenous leukemia cell line that is devoid of MHC Class I surface antigens (ATCC) .

Flow cytometry - Phenotype analyses were performed by Flow cytometry. 1x10^6 cells were stained at ambient temperature for 20 - 25 min with a cocktail of monoclonal Abs (BD Pharmingen). The human specific cocktail included CD3, CD56 and CD107a, while the NHP cocktail included CD3, CD159a and CD107a. Cells were gated on forward versus side scatter. After staining the cells were washed once in PBS and directly subjected to cytofluorometric analysis performed by a BD FACSCanto™ equipped with BD FACSDiva™ 6.0 software.

Cytotoxicity assay - NK cell cytotoxicity was tested within a 4-hour incubation of PKH67 (Sigma) labeled target K562 cells and PBMC effector cells. Cell death was determined by intercalation of the DNA dye propidium iodide (PI, Sigma) in labeled target K562 cells.

RESULTS

CD107a Antibody Surface Staining

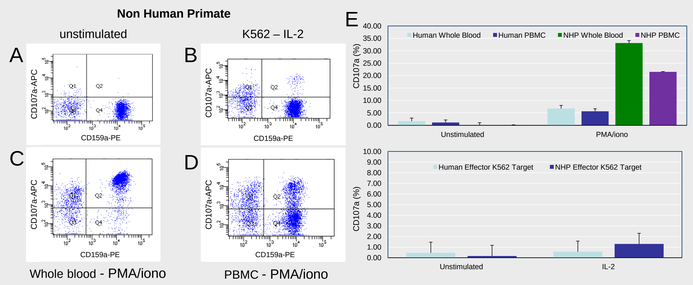

Figure 1. CD107a is expressed at detectable levels on the surface of NK cells following stimulation.
Flow cytometry figures represent the percent positive CD3-CD159a+ cells that express CD107a following no stimulation **(A),** stimulation with K562 cells in the presence of 200U/mL IL-2 (Peprotech) **(B)** or with phorbol 12-myristate 12-acetate (PMA, 50 ng/mL, Fisher) and Ionomycin (iono, 1 mg/mL, Sigma) with whole blood **(C)** or with PMA/iono with PBMC **(D)** for a single representative cynomolgus monkey. The bar graphs **(E)** represent the percentage of cells expressing CD107a under different stimuli. Results are shown as mean ± SD of the study population (n = 3) for CD107a expression

Capability of Effector Cells to Kill Target Cells

Figure 2. Cytotoxic activity of PBMC effector cells prepared 24 hrs after blood draw to PKH67 labeled K562 target cells depends on the ratio and is IL-2 inducible. Effector to target ratios tested were 100:1, 50:1, 25:1 with and without 200 U/mL IL-2.

NK Cell Enrichment

Figure 3. The percent positive of CD107a is increased on enriched NK cells following stimulation.
Flow chart of enrichment of NK cells from human whole blood based on RosetteSep® technology (StemCell Technologies) **(A),** stimulation of the expression of surface CD107a with PMA/iono of enriched NK cells **(B)** in three representative donors.

RESULTS (cont.)

CD107a Staining on Enriched NK Populations

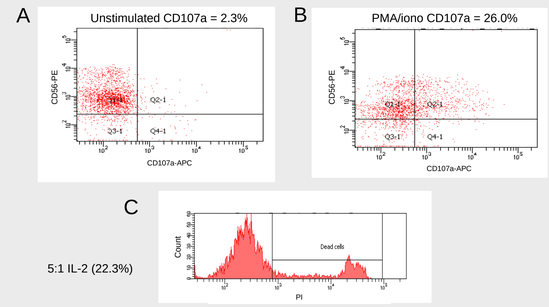

Figure 4. CD107a staining on NK enriched populations from human whole blood correlates well with cytotoxic activity.
Flow cytometry figures represent the percent positive CD3-CD56+ cells that express CD107a for a single representative human donor following no stimulation **(A)** or stimulation with PMA/iono **(B)**. Percent cytotoxicity is also shown **(C)**.

CONCLUSIONS

- CD107a is expressed at detectable levels on the surface of NK cells following stimulation.
- Cytotoxic activity of PBMC effector cells prepared 24 hrs after blood draw to K562 target cells depends on the ratio and is IL-2 inducible.
- CD107a staining is increased on RosetteSep® NK enriched populations from human whole blood and correlates well with cytotoxic activity.
- In the future, we may be able to improve the signal of CD107a staining with K562 stimulation by extending the time of incubation.

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