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Natural killer cell subsets and receptor expression in peripheral blood mononuclear cells of a healthy Korean population: Reference range, influence of age and sex, and correlation between NK cell receptors and cytotoxicity

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ABSTRACT

Background: The purpose of this study was to identify CD56^{bright} and CD56^{dim} natural killer (NK) cell subsets and analyze their receptors expression in a healthy Korean population, and to determine whether receptor expression correlates with age, sex, and cytotoxicity.

Materials and methods: We performed multicolor flow cytometry assays to analyze the expression of various NK cell receptors (CD16, NKG2A, NKG2C, NKG2D, CD57, DNAM-1, CD8a, CD62L, NKp30, and NKp46) on both CD3$^-$/CD56^{dim} and CD3$^-$/CD56^{bright} NK cells in whole-blood samples from 122 healthy donors. The expression of these receptors was compared according to age (<30 years, n = 22, 30 –60 years, n = 73 and >60 years, n = 27) and gender (male, n = 61, female, n = 61). NK cell cytotoxicity assays were performed with peripheral blood mononuclear cells (PBMCs) from 18 individuals. The results were compared to the expression levels of NKp30 and NKp46 receptors.

Results: A normal reference range for NK cell receptor expression in two NK cell subsets was established. NKp46 and NKG2D expression gradually decreased with age ($p < 0.01$ and $p < 0.05$, respectively) whereas NK cell proportion and numbers, frequencies of CD56^{dim} cells, and CD57 expression increased with age ($p < 0.01$ in all cases). Men showed greater NK cell proportion and numbers, frequencies of CD56^{dim} cells, and CD57 expression compared to those of women ($p < 0.05$ and $p < 0.001$; $p < 0.01$ and $p < 0.01$, respectively). Notably, the expression of NKp46 was negatively correlated with NK cell frequency ($r = -0.42$, $p < 0.001$). Furthermore, NK cell cytotoxicity was found to positively correlate with NCR expression ($p = 0.02$), but not NK cell proportion ($p = 0.80$).

Conclusion: We have established a profile of NK cell surface receptors for a Korean population, and revealed that age and gender have an effect on the expression of NK cell receptors in the population. Our data might explain why neither NK cell numbers nor proportions correlate with NK cell cytotoxicity.

Abbreviations: CBC, complete blood count; NK, natural killer; PBMC, peripheral blood mononuclear cell; pbNK, peripheral blood natural killer; WBC, white blood count.

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1. Introduction

Natural killer (NK) cells are lymphocytes that are critical for cellular and innate immunity. They comprise 10–20% of lymphocytes in the human peripheral blood [1–4]. Immunophenotypically, NK cells are known to express various levels of CD56 and CD16, and lack expression of the pan T cell marker CD3 and the B cell marker CD19. The expression intensity of CD56 further divides NK cells into two subsets: specifically, $CD56^{dim}$ cells comprise the majority of NK cells, and exhibit cytotoxic properties, while $CD56^{bright}$ cells exhibit less cytotoxicity, but secrete various cytokines for the regulation of immune functions. NK cells have the capacity to induce apoptosis or cell lysis in tumor cells and virus-infected cells; the underlying mechanism for this function is known to be regulated by a balance between various activating and inhibitory receptors [5,6]. Previous studies have demonstrated that NK cell cytotoxicity correlates with certain NK cell surface receptors, specifically natural cytotoxicity receptors (NCRs) and NKG2D [5,7–11].

Changes in peripheral blood NK (pbNK) cell count, percentage of NK cell subtypes, NK cell receptor expression, and NK cell activity have been reported in various disease conditions and in healthy populations [4,12–14]. To date, studies on the subset distribution of NK cells have been performed in healthy populations and in patients with cancer, infections, and autoimmune diseases [12,15]. Baseline NK cell activity in the general population was found to have an impact on cancer related risk [14], and it is speculated that NK cell functions determine the outcome in certain cancer types [16]. From a prognostic perspective, the evaluation of NK cell surface receptor expression and the correlation of this expression with NK cell activity can be beneficial in order to predict the anti-tumor immune response.

It has been reported that the proportion of $CD56^{bright}$ and $CD56^{dim}$ NK subsets might vary based on an individual's age, gender, and ethnic background. Aging itself is reported to influence the redistribution of NK cell subsets with notable changes including a decrease in $CD56^{bright}$ cells and an increase in $CD56^{dim}$ cells [4,13]. Gender can also affect the distribution of lymphocytes, and a few studies have shown that men have a higher percentage of NK cells [17,18]; however, NK cell cytotoxicity was shown to be higher in women [19–21]. In terms of ethnic factors, numbers and subset distribution of NK cells among different races (Chinese Han, Caucasian, and African) have been studied [22], with results showing that the absolute NK cell count in Chinese Han subjects was significantly higher than that in Caucasians. Therefore, NK cell proportions and functions are diverse depending on age, sex, and ethnicity, suggesting the importance of establishing local reference ranges in healthy volunteers. Herein, we analyzed pbNK cell subsets and receptor expression in a healthy Korean population and established reference ranges relative to age and sex. Furthermore, we analyzed the association between NK cell surface receptors and NK cell cytotoxicity against K562 cancer cells.

2. Materials and methods

2.1. Study population

Healthy individuals who visited the Samsung Medical Center for regular medical checkups between July 2015 and August 2016 were randomly recruited. Our Institutional Review Board (No. SMC 2015-01-131) approved this study and waived the requirement for a written informed consent, as we did not use any data for personal identification. All samples were acquired from leftover blood from routine health checkups. All participants were examined and a questionnaire was administered to confirm general health and past medical history. Subjects who had no apparent underlying medical issues were selected. All selected subjects were of Korean ethnicity. We obtained blood samples in ethylenediaminetetraacetic acid anticoagulant tubes (BD Vacutainer, Franklin Lakes NJ, USA) after routine ABO/RhD blood typing. NK cell cytotoxicity assay required another blood draw from selected subjects; we received informed written consent and obtained 10 ml of whole blood in heparinized vacutainer tubes (BD Vacutainer) for this matter.

2.2. Flow cytometry analysis

Complete blood count (CBC) was performed using the XE-2100 analyzer (Sysmex, Kobe, Japan), and NK cell receptor expression was analyzed by flow cytometry. The parameters for analysis of CBC were the numbers of white blood cells (WBC) and the percentages and numbers of lymphocytes. Whole blood (100 μl) was incubated with an appropriate combination of fluorochrome-conjugated monoclonal antibodies for the analysis of surface markers in NK cells using flow cytometry. The NK cell surface antigens analyzed in this study are listed in Supplementary Table 1. Samples were incubated for 15 min at room temperature in the dark and red blood cells were lysed by the addition of 2 ml of FACS lysing solution (BD Biosciences, San Jose, CA, USA) for 10 min. After centrifugation and washing with phosphate buffered saline (PBS), 200 μl of FACS buffer was added to each tube and assessed using a FACS Canto II (BD Biosciences) flow cytometer. For eight–color analysis, 20,000–50,000 events in the lymphocyte collection gate were collected and the data were analyzed by Kaluza software version 1.3 (Beckman Colter, Brea, CA, USA). Our gating strategy for analyzing NK cells is described in Fig. 1; we selected bright CD45 events with low side scatter patterns and then gated for $CD3^-$/$CD56^+$ events. This population was then evaluated for its expression of surface receptors including CD16, NKG2A, NKG2C, NKG2D, NKp30, NKp46, CD57, DNAM-1, CD8a, and CD62L.

2.3. NK cell cytotoxicity

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood using Ficoll-Paque 1.077 g/mL density gradient centrifugation (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). NK cell cytotoxicity against K562 cells was assessed as shown previously using PBMCs stained with Calcein AM (CAM) followed by flow cytometry analysis [23]. PBMCs were stained with 0.05 mmol/L CAM in RPMI1640 medium for 2 min at room temperature in a volume of 10 ml, followed by washing twice with media. Target cells (1×10^4) were mixed with various numbers of CAM-stained viable effector cells at 10:1, 20:1, and 40:1 effector-to-target ratios and then incubated for 3 h at 37 °C in 5% CO_2. The mixed cells were transferred to FACS tubes after 3 h. Before acquisition (5–10 min), 10 μl of 1 μg/ml propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) was added to each tube. The cells were acquired on a FACS Canto II and analyzed using Kaluza software (Beckman Coulter).

2.4. Statistical analysis

Statistical evaluation was performed with Prism 5 software (GraphPad Software, San Diego, CA, USA). Intergroup comparisons were assessed with the Kruskal-Wallis test, followed by the Dunn's post-analysis test to define significance between pairs of groups. The Mann-Whitney test (non-parametric) was also applied to analyze the specific sample pairs for significant differences. Pearson's correlation analysis was performed on age or NK cell proportions with NK cell surface receptor expression. All tests were two-tailed, and differences were considered statistically significant when the p-value was less than 0.05.