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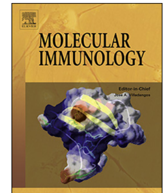
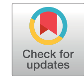


Impaired cytolytic activity of asthma-associated natural killer cells is linked to dysregulated transcriptional program in energy metabolism



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ABSTRACT

Natural killer (NK) cells are a cytotoxic subset of the innate lymphoid cells, playing essential roles in host defense against tumors and infections, which, however, are usually functionally compromised in chronic diseases. Atopic diseases, such as allergic asthma, characterized by type 2 immune responses, are usually associated with chronic inflammations. Whether asthma -associated immune environment affects the cytolytic function of NK cells has not been elucidated. Here, YTS, a human NK cell line, was exposed to serum from healthy donors or asthma patients for analysis of its cytolytic function. We found that, serum from asthma patients reduced the cytolytic activity of YTS cells against Raji human B lymphoblasts, in comparison with normal serum. The impairment of cytolytic activity of these YTS cells was accompanied with decreased degranulation potentials, weakened conjugation formation with Raji cells, and premature termination of ERK phosphorylation upon stimulation. Meanwhile, apoptosis or cell death of YTS cells was not increased after exposure to serum from asthma patients. Importantly, such impairment of cytolytic activity of asthma -associated YTS NK cells was accompanied with aberrantly enriched genes involved in oxidative phosphorylation. Taken together, these results demonstrate that the serum of asthma patients directly suppresses the cytolytic function of NK cells, possibly through dysregulation of energy metabolism in NK cells.

1. Introduction

Natural killer (NK) cells are cytotoxic effector cells of the innate immune system, playing critical roles in host anti-viral/anti-tumor immune defense (Vivier et al., 2008). NK cells directly eliminate transformed cells or infected cells through exocytosis of lytic perforin/granzyme granules, or through triggering death receptors on target cells upon target cells recognition.

Allergic disorders, such as allergic asthma, are characterized by type 2 immune responses, and are also usually associated with chronic inflammations (Galli et al., 2008). Although increased activity of pulmonary NK cells was reported in the early phase of allergic airway inflammations (Bi et al., 2017), orthodox functions of NK cells, such as their cytokine production profiles, were shown to be detrimentally altered in later phases. Compared with healthy individuals, asthma patients had fewer NK cells in the bronchoalveolar lavage (BAL), and displayed decreased cytolytic activity towards tumor cells (Duvall et al., 2017). Importantly, NK cells in the PBMCs of asthma patients with chronic rhinosinusitis exhibited a severe impairment in IFN-γ production (Kim et al., 2013). In addition, NK cells in the peripheral blood of asthma patients were reported to express type II cytokines, and to show constitutive activation of STAT6 (Wei et al., 2005). These studies suggest that allergic asthma might affect not only local, but also systemic immunologic state, as well as the immune cells in the peripheral. Despite the direct analyses of effector functions NK cells in asthma patients, and in mouse models, however, the effects of the asthma -associated immune environment on the cytolytic function of NK cells have not been investigated.

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Metabolism is essential for normal lymphocyte functions. Generally, quiescent lymphocytes engage oxidative phosphorylation (OXPHOS) for bioenergy, which is efficient, yet at a lower rate. On the other hand, aerobic glycolysis is usually required to fuel robust proliferation and biosynthesis of effector molecules upon lymphocyte activation (Pearce et al., 2013). NK cells were reported to require OXPHOS for normal effector functions both in humans (Keating et al., 2016) and in mice (Keppel et al., 2015). Despite these knowledge on NK cell metabolic requirement at normal state, however, the metabolic levels or metabolism-related transcriptional program linked to the aberrant functions of NK cells under disease settings have not been investigated.

In this study, we showed that NK cells exposed to serum from asthma patients displayed impaired cytolytic activity, possibly due to decreased expression of cytotoxic molecules, weakened conjugation formation, as well as prematurely terminated MAPK signaling. Gene sets enrichment analysis further showed that such dysfunction of asthma -associated NK cells was linked to the aberrantly enriched genes involved in the OXPHOS metabolic pathway, which is usually engaged by quiescent, rather than activated, lymphocytes.

2. Materials and methods

2.1. Cell lines and serum exposure

YTS cells (a subclone of the YT NK leukemia cell line) and Raji human B lymphoblast (ATCC® CCL-86™) were maintained in RPMI-1640 medium (Corning) supplemented with 10% (vol/vol) FBS (Hyclone), 2 mM L-Glutamine, 1 mM HEPES, pH 7.4, 100 U/ml of penicillin and 100ug/ml of streptomycin. Data reported in this study were obtained from 8 healthy donors and 20 asthma patients. The recruited male or female asthmatic patients fulfilled the criteria for asthma, and their allergic status was further confirmed by elevated serum IgE (Fig. 1A). Healthy individuals displayed no evidence of atopy, and showed low levels of serum IgE (Fig. 1A). Information for patients and healthy individuals was listed in Table 1. Serum isolated from healthy individuals and asthma patients was preserved at −80 °C before use. Exposure to serum was performed by incubation of YTS cells with the above -mentioned medium mixed with 30% (vol/vol) serum from healthy individuals or asthma patients for 12 h before washed and experiments. For RNA sequencing and transcriptome analysis, pooled serum from healthy individuals or asthma patients was used for serum exposure. Informed consent was obtained from the patients and healthy donors, and the study was approved by the ethical committee of Shenzhen Institutes of Advanced Technology.

2.2. ELISA

Amounts of human IgE in the serum were determined by an ELISA kit from DAKEWE (Shenzhen, China) according to the manufacturer's instructions. Briefly, serum from patients or healthy controls was added to ELISA plate pre-coated with anti-human IgE antibody, and the plate was incubated for 2 h at room temperature. After washing, biotinylated anti-human IgE antibody from the kit was applied for 1 h at room temperature, followed by streptavidin-HRP conjugate from the kit for additional 30 min at room temperature after washing. Finally, TMB substrate from the kit was applied after extensive washing, and the 450 nm absorbance was obtained with a Multiskan GO microplate reader (Thermo Scientific, MA, USA).

2.3. Antibodies and reagents

We purchased APC-anti-CD56, purified mouse anti-CD28, APC-anti-mouse IgG, FITC-anti-Granzyme B, PE-anti-Perforin, Foxp3 Fix/Perm Buffer Set from Biolegend (San Diego, CA, USA); rabbit anti-ERK, and mouse anti-p-ERK from Cell Signal Technology (Danvers, MA, USA); goat anti-rabbit IgG-HRP and goat anti-mouse IgG-HRP from Bosterbio (Wuhan, China). Flow cytometric anaysis of CD28 expression was performed using purified mouse anti-CD28 staining followed by washing and APC-anti-mouse IgG staining. Both Phorbol 12-myristate 13-acetate (PMA) and 5(6)-Carboxyfluorescein diacetate N-succinimidyl ester (CFSE) were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

2.4. Cytolytic assay

Cytolytic activity of YTS cells was assessed by a cytolytic assay using CFSE-7AAD staining. Briefly, YTS cells were incubated with CFSE -labeled Raji cells at the ratio of 10:1 for 4 h. 7AAD was then added into the cell suspensions, and incubated on ice for 10 min, before flow cytometric analysis of the percentages of $7AAD^+$ cells among $CFSE^+$ cells.

2.5. Apoptosis detection

Apoptosis of YTS cells was determined by Annexin V - PI staining using an apoptosis detection kit (Transgen, Beijing, China) according to the manufacturer's instructions.

2.6. Conjugation assay

Conjugation formation assay was performed by incubation of CD56 -stained YTS cells with CFSE -labeled Raji cells at 37 °C for 12 min or for the indicated time, followed by fixation using Foxp3 Fix/Perm Buffer Set (Biolegend, San Diego, CA, USA) and flow cytometric analysis on a cytoflex (Beckman Coulter, Brea, CA, USA). Data were analyzed with FlowJo software (Tree Star). Conjugates formed would display double positive signals for both CD56 and CFSE.

2.7. RNA sequencing and gene set enrichment analysis (GSEA)

Total RNA of YTS NK cells after 12 h of 30% pooled serum -containing complete medium exposure was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA). Next generation sequencing library preparations were contructed using Ultra RNA Library Prep Kit for Illumina according to the manufacturer's protocol (New England Biolabs, Ipswich, MA, USA). The libraries were multiplexed and loaded on an Illumina HiSeq instrument according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Sequencing was carried out using a 2 × 150bp paired-end configuration. The sequences were processed and analyzed by GENEWIZ (Suzhou, China).

GSEA using Broad Institute software (http://www.broadinstitute.org/gsea/) was performed on RNA sequencing data from each samples. GSEA used gene sets from the Molecular Signature Database v.6.1 (Subramanian et al., 2005; Liberzon et al., 2015, 2011) including KEGG pathway gene lists obtained from http://www.genome.jp/kegg/pathway.html, as well as BIOCARTA gene sets obtained from http://software.broadinstitute.org/gsea/msigdb/genesets.jsp?collection=CP:BIOCARTA. Enrichment scores (ES) obtained by GSEA were used to compare different pathways between exposure with control serum and asthma patients -derived serum.

2.8. Statistics

Statistically significant differences were determined by Student's t-tests. Values of $*P < 0.05$, or $**P < 0.005$ were considered significant.

3. Results

3.1. Exposure to serum from asthma patients impaired the cytolytic activity of NK cells

To evaluate the effects of asthma -associated immune environment

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