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## SHORT COMMUNICATION

# Investigation of Clinical Value of NKT-like Cells in Tumor Diseases

Yingying Chen [1], Xinhong Yang [1], Cuihong Gu [1], Zhihua Zhang [1], Xiaofeng Yang [2]

[1] *Department of Hematology, The Affiliated Hospital of Chengde Medical College, Chengde, Hebei Province, China*
[2] *Department of Pediatric Surgery, The Affiliated Hospital of Chengde Medical College, Chengde, Hebei Province, China*

## SUMMARY

***Background:*** **The goal is to study the changes of natural killer T-like (NKT-like) cells with age and explore the value of NKT-like cell changes in the evaluation of immune function and prognosis of tumor patients.**
***Methods:*** **From January 2021 to December 2021, 19 patients with lung cancer, 37 patients with lymphoma, 16 patients with multiple myeloma (MM), 13 patients with acute lymphoblastic leukemia (ALL), 70 patients with acute myeloid leukemia (AML) treated in the Affiliated Hospital of Chengde Medical College and 141 healthy volunteers included in the healthy control group were recruited to study the change trend of NKT-like cells with age and changes in different tumor patients.**
***Results:*** **With the increase of age, NKT-like cells in peripheral blood increased gradually in healthy people, mainly composed of CD8+NKT-like cells and CD8-CD4-NKT-like cells. The proportion of NKT-like cells in the lymphoma group and AML group was significantly higher than that in the control group ($p < 0.05$). The proportion of CD8+NKT-like cells decreased in the Lymphoma, AML, ALL, and lung cancer groups ($p < 0.05$), there was no statistical significance between MM group and control group ($p > 0.05$).**
***Conclusions:*** **With the increase of age, NKT-like cells in peripheral blood gradually increased in healthy people and were mainly composed of CD8+NKT-like cells and CD8-CD4-NKT-like cells. The increase of CD8+NKT-like cells in peripheral blood has important reference value for the evaluation of immune function and prognosis of patients with lymphoma, AML, ALL, and lung cancer and provides direction for immunotherapy.**
**(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240509)**

**Correspondence:**
Xinhong Yang
Department of Hematology
The Affiliated Hospital of
Chengde Medical College
Chengde, Hebei Province
China
Email: caccine.tumor@aliyun.com

Short Communication accepted May 21, 2024

**KEYWORDS**

natural killer T-cells, NKT-like cells, cytokines, immunotherapy

## INTRODUCTION

NKT-like cells are natural T cells with T cell and NK cell properties that express both the NK cell receptor NK1.1 (CD161) and the constant T cell antigen receptor (TCR)αβ. According to the recognition of surface receptors and antigens, NKT-like cells are divided into three different populations: classical type I NKT-like cells (iNKT, invariant NKT-like cells), type II NKT-like cells (vNKT, variant NKT), and NKT-like cells. The iNKT-like cells express constant TCR-αβ, which is composed of the germ cell Vα gene (human Vα24, mouse Vα14) and Jα18 fragment and a more diverse set of non-germ cell Vβ genes (mouse Vβ8.2, Vβ7 or Vβ2

and human Vβ11) [1]. The expression of vNKT-like cells are more abundant than iNKT-like cells, the expression of TCR-α and TCR-β chains are relatively complex. A major cellular subset of vNKT-like cells express an oligoclonal TCR sequence, which mainly use the gene fragments of Vα3/Vα1-Jα7/Jα9 and Vβ8.1/Vβ3.1-Jβ2.7 [2]. Antigen recognition of the iNKT and vNKT-like cells dependent on CD1d, whereas the NKT-like cells are non-CD1d-dependent. In the current study, NKT-like cells were defined as CD3+CD56+ cells, which may include cells that are unrelated to the NKT cell lineage, such as TCRγδ+T cells. Based on the expression of CD4 and CD8 on the cell surface, NKT-like cells can be divided into three groups, which are CD4+ CD8- (12 - 36%), CD4-CD8+ (1 - 5%) and CD4-CD8- (60 - 85%). According to Romero-Olmedo et al. [3], human peripheral blood CD3+CD56+ cells constitute a phenotypic continuum of different natural T cell subsets, such as CD4-CD8+ or CD4-CD8-, γδ+, and MAIT cells. CD3+CD56+T cells mainly composed of CD8+ and TCRγδ+ cells (median 36.1% and 27.1%, respectively), while the less abundant CD4+, CD4-CD8-, and MAIT cells accounted for 6.4%, 9.0%, and 4.9%, respectively. CD4-NKT-like cells mainly produce Th1-type cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α), while CD4+NKT-like cells mainly produce Th2-type cytokines (IFN-γ, TNF, IL-4, IL-5, IL-10, and IL-13) and Th1-type cytokines. Effector NKT-like cells produce high levels of cytokines immediately after stimulation [4,5], which facilitates the transcriptional activation of other immune cells. NKT-like cells have NK cell-like cytotoxic activity after activation.

NKT-like cells participate in regulation [6,7] of immune responses to cancer, autoimmunity, infection, allergy, and transplantation. NKT-like cells have become the main focus in the development of effective cancer immunotherapy [8,9]. The purpose of this paper is to investigate the changing trend of NKT-like cells and their subsets in healthy people with age and to investigate the changes of NKT-like cells and their subsets in the peripheral blood of tumor patients.

## MATERIALS AND METHODS

### Experimental materials and reagents

General data were collected from January 2022 to December 2023 from 141 patients with lung cancer, 37 patients with lymphoma, 16 patients with multiple myeloma (MM), 13 patients with acute lymphoblastic leukemia (ALL), and 70 patients with acute myeloid leukemia (AML).

The following antibodies of CD45/CD4/CD8/CD3/CD56/CD19 (BioLegend, San Diego, CA, USA; BD) were used for the test of CD8+NKT-like cells, CD4+ NKT-like cells, and CD4-CD8-NKT-like cells in peripheral blood. Data were acquired on a Flow Cytometer (BECKMAN COULTER NAVIOS) and analyzed using Kaluza software.

### Experiments and methods

For flow cytometry and analysis, the lymphocyte population was delineated with CD45/SSC. The percentage of CD3+CD56+ region is the rate of NKT-like cells. Then the ratio of each subsets of NKT-like cells was analyzed, such as CD3+CD4+NKT-like cells, CD3+CD8+ NKT-like cells and CD3+CD8-CD4-NKT-like cells.

### Statistical analysis

Data were analyzed using SPSS Statistics 25.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Groups were compared using independent sample *t*-test. Data were reported as means ± SE. A p-value $< 0.05$ denoted a statistically significant difference.

## RESULTS

The trend of NKT-like cells in peripheral blood with age in healthy individuals NKT-like cells gradually increased with age in healthy individuals (Table 1).

The proportion of NKT-like cells in peripheral blood of patients with different tumors and the composition ratio of each subgroup.

NKT-like cells were higher in the lymphoma and AML groups compared with the control group ($p < 0.05$). CD8+NKT-like cells were lower in the lymphoma, lung cancer, AML, and ALL groups compared with the control group ($p < 0.05$) and with no significant change in CD4+NKT-like cells ($p > 0.05$). CD4-CD8-NKT-like cells were higher in the lymphoma and AML groups compared with the control group ($p < 0.05$). There was no statistical significance between the MM group and control group ($p > 0.05$). When CD8+NKT-like cells, CD4+NKT-like cells, and CD4-CD8-NKT-like cells in MM group were compared with the control group, there was no statistical significance ($p > 0.05$) (Table 2).

## DISCUSSION

NKT-like cells have strong antitumor responses and thus have become a major focus in the development of effective cancer immunotherapy. Currently, the types of killer cells used in cellular immunotherapy are as follows: CD8+T cells include tumor-infiltrating lymphocytes (TIL) and chimeric antigen receptor T (CAR-T) cells, NK cells, and natural killer T (NKT) cells. The data showed that these CD8+NKT subsets may have innate and adaptive immunity, and showed more cytotoxic functions compared with conventional NK and CTL cells. More granzyme granules were found in the cytoplasm of CD8+NKT-like cells. Different subsets of NKT-like cells have distinct functions *in vivo*. CD8+ NKT-like cells may be very effective in promoting Th1 cell-mediated immunity, while other NKT cell subsets

**Table 1. The trend of NKT-like cells in peripheral blood with age in healthy people (%, X ± s).**

| Group | n | Age (year) | Gender (male/female) | NKT-like cells |
|---|---|---|---|---|
| 1 | 59 | 1.65 (1 - 2) | 37/21 | 0.32 ± 0.33 ▲ |
| 2 | 36 | 3.91 (3 - 6) | 22/14 | 0.63 ± 2.37 |
| 3 | 21 | 11.10 (7 - 15) | 11/10 | 1.27 ± 1.38 △ |
| 4 | 25 | 40.48 (24 - 73) | 14/9 | 5.48 ± 4.67 * |
| F value | | | | 5.089 |
| p-value | | | | 0.010 |

▲ group 1 compared with group 2, group 3, and group 4, $p < 0.05$. △ group 3 compared with group 2, $p < 0.05$. * group 4 compared with group 3, $p < 0.05$.

**Table 2. The proportion of NKT-like cells in peripheral blood of patients with different tumors and the composition ratio of each subgroup (%, X ± s).**

| Group | n | Age (year) | NKT-like cells | CD8+NKT-like cells | CD4+NKT-like cells | CD8-CD4-NKT-like cells |
|---|---|---|---|---|---|---|
| Control group | 25 | 40.48 (24 - 73) | 5.48 ± 4.67 | 75.31 ± 22.22 | 9.25 ± 11.05 | 15.70 ± 15.29 |
| MM group | 16 | 61.78 (57 - 71) | 3.95 ± 3.42 △ | 70.82 ± 21.31 △ | 9.47 ± 12.94 △ | 18.75 ± 23.10 △ |
| Lymphoma | 37 | 60.83 (46 - 72) | 11.64 ± 9.57 * | 60.55 ± 21.54 * | 7.92 ± 9.23 △ | 29.82 ± 23.08 * |
| Lung cancer | 19 | 59.68 (49 - 72) | 8.71 ± 5.24 △ | 63.42 ± 26.91 * | 12.87 ± 21.43 △ | 22.20 ± 22.31 △ |
| AML group | 70 | 55.25 (26 - 73) | 11.68 ± 9.06 * | 62.24 ± 21.91 * | 11.43 ± 17.39 △ | 25.52 ± 21.28 * |
| ALL group | 13 | 38.15 (27 - 44) | 9.77 ± 5.50 △ | 57.2 ± 17.95 * | 13.4 ± 16.11 △ | 27.28 ± 18.71 |
| F value | | | 5.108 | 17.721 | 49.091 | 2.988 |
| p-value | | | < 0.01 | < 0.01 | < 0.01 | < 0.01 |

Compared with the control group * $p < 0.05$, △ $p > 0.05$.

may be more effective in immunosuppression.

In our study, the percentage of NKT-like cells in each age group were as follows: 1 - 2 years 0.32 ± 0.33%, 3 - 6 years 0.63 ± 2.37%, 7 - 13 years 1.27 ± 1.38%, 24 - 73 years 5.48 ± 4.67%, indicating that NKT-like cells gradually increased with age, and NKT-like cells were almost undetectable in neonates, which indirectly proved that the generation of NKT-like cells gradually increased with the maturation of the thymus.

The proportion of CD8+CD4-NKT-like cells, CD4+ CD8-NKT-like cells, and CD8-CD4-NKT-like cells in healthy controls were 75.31 ± 22.22%, 9.25 ± 11.05%, and 15.70 ± 15.29%, respectively. CD8+CD4-NKT-like cells and CD8-CD4-NKT-like cells mainly produce Th1-type cytokines, which can improve the immune response effectively.

This study showed a higher proportion of NKT-like cells in the lymphoma, AML, and lung cancer groups compared to control group. The subset proportions of CD4+NKT-like cells and CD4-CD8-NKT-like cells were increased. The proportion of CD8+NKT-like cells significantly decreased. Therefore, for tumor patients, the low proportion of CD8+NKT-like cells has important reference value for immune function evaluation and prognosis judgment, indicating low immune response and the progression of disease. Therefore, in the future, it is possible to induce different subsets of NKT lymphocytes into CD8+NKT-like cells, or inject more CD8+NKT-like cells to achieve an anti-tumor goal. Although the proportion of NKT-like cells in the MM group were compared to the control group, there were no statistically significant differences. This could be related to the small size of specimen.

## CONCLUSION

In conclusion, with the maturity of the thymus, the generation of NKT-like cells are gradually increased, and CD8+NKT-like cells are predominant. CD8+NKT-like cells have important reference value for assessment of immune function and prognosis of tumor patients, and provide a new direction for NKT cell immunotherapy.

**Source of Support:**
Foundation of Health Commission Scientific Research of Hebei (No. 20220423).

**Declaration of Interest:**
None of the authors have a conflict of interest to disclose.

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