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Progress on the Distribution of NK Cells in Healthy Human Peripheral Blood

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Abstract: NK cells are an important subset of lymphocytes in the human immune system and play a key role in antiviral and anti-tumor immunity. In this paper, the distribution characteristics and dynamic changes of peripheral blood NK cells in healthy people were systematically reviewed, focusing on the influence of age, gender, ethnicity, and environmental factors. Studies have shown that NK cells show a relatively stable distribution ratio in healthy adults, while in the development stage of children, they show dynamic characteristics that increase with age. The receptor expression and function of NK cells in the elderly population showed an age-related downward trend, indicating that the quality of NK cells, rather than the quantity, was a key indicator of age-related immune changes. Different ethnic groups and high-altitude environments also had a significant impact on NK cell distribution. This paper not only summarizes the quantitative characteristics of NK cell distribution but also analyzes the mechanism of its functional phenotypic changes, provides a scientific basis for clinical immune assessment, and points out the direction for future basic research and clinical application of NK cells.

Keywords: NK cells; Peripheral blood; Lymphocyte subsets; Age correlation; Immune function

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1. Introduction

Quantitative analysis of lymphocyte subsets is an important means to assess the immune response in health and disease states, and is of great value for the diagnosis and monitoring of diseases such as immunodeficiency, autoimmune diseases, infections, and cancer. Natural killer cells (NK, CD3-CD56+CD16+), as key effector cells of the human innate immune system, play an irreplaceable role in defending against viral infections, tumorigenesis, and other pathogen invasion [1,2]. NK cells are mainly distributed in the peripheral blood of the human body, usually accounting for 5% to 20% of the total number of lymphocytes in peripheral blood [3–6]. In addition, NK cells are also found in lymphoid tissues such as lymph nodes, bone marrow, spleen, and other peripheral tissues, but their distribution density in these sites is usually lower than that in the periphery [7].

The distribution characteristics of NK cells are influenced by various factors, including age, gender, genetic background, health status, lifestyle, and environmental factors [1,7]. Understanding the normal distribution range and dynamic changes of NK cells in healthy people is of great significance for establishing clinical diagnostic reference standards, assessing immune function status, guiding immunotherapy, and developing NK cell-related immunotherapy. This article aims to systematically review the research progress of NK cell distribution in the peripheral blood of healthy people, with special attention to the characteristics of NK cell distribution in different age groups, genders, and ethnic groups, and to deeply analyze the relationship between NK cell quantity stability and functional changes, to provide scientific reference for clinical immunology research and practice.

2. Relative stability of the proportion of NK cells in peripheral blood in adult healthy populations

A number of studies have focused on the distribution characteristics of NK cells in healthy adults, revealing the relative stability of this immune cell population in different age and gender groups, providing valuable biological references for clinical medicine and basic scientific research. This stability reflects the functional consistency of NK cells as a core component of the immune surveillance system, but behind it lies a complex immune regulatory mechanism.

In a major study conducted in China [8], researchers conducted a detailed analysis of 193 healthy individuals, divided into three age groups: youth (≤39 years), middle-aged (40–59 years), and elderly (≥60 years), taking into account gender differences, including 92 men and 101 women. Using standardized flow cytometry techniques, the study accurately measured the percentage of NK cells (CD3-CD56+) within the peripheral blood lymphocyte population. The results showed that the proportion of NK cells in all age groups and genders remained relatively stable, with the youth group (18.07 ± 4.74%), the middle-aged group (18.49 ± 7.05%), and the elderly group (25.74 ± 10.88%) ($P > 0.05$), and there was no significant difference in the proportion of NK cells between males and females. These data are highly consistent with the results of previous studies in China, indicating that the distribution ratio of NK cells in healthy adults has relative stability across age and gender under normal physiological conditions.

Similarly, a large sample study in Cuba confirmed this phenomenon [9]. The study included 129 healthy adults aged 18 to 80 years, including 61 males and 68 females, and were further divided into groups less than 50 years and greater than or equal to 50 years by age. All study subjects had an exclusion of a history of chronic disease and did not use substances such as tobacco, alcohol, or certain medications that may affect the number and function of immune cells. Using dual-platform flow cytometry, the researchers determined the absolute values and percentages of T lymphocytes (CD3+), helper T cells (CD3+/CD4+), cytotoxic T cells (CD3+/CD8+), B cells (CD19+), and NK cells (CD3-/CD56+) in peripheral blood. Data analysis showed that, despite individual differences, the proportion of NK cells remained relatively stable among different age groups (<50-year-old group: 10.28 ± 4.13%; ≥50-year-old group: 9.87 ± 3.95%), a finding that further supports the stability theory of NK cell distribution.

It should be pointed out that this "relative stability" of the proportion of NK cells is not absolutely constant, but exists in a specific range of fluctuations. This stability may reflect the dynamic balance mechanism of the immune system in adulthood: despite individual exposure to different environmental factors and pathogens, the immune system maintains basic stability in NK cell numbers through a complex regulatory network to ensure continuous and effective immune surveillance function. However, this quantitative stability does not equate to functional constancy, and the functional phenotype and receptor expression of NK cells may vary with age, as discussed in depth below, which constitutes the key to understanding the differences in immune function of NK cells at different ages.

Taken together, the distribution of NK cells in the peripheral blood of healthy adult individuals, both in Asia and the Americas, shows a relative stability across age and gender, emphasizing the functional consistency of NK cells as a key component of immune surveillance. These findings not only deepen our understanding of the dynamic distribution of NK cells in normal immune states but also lay the foundation for the use of NK cells as disease diagnostic markers or therapeutic targets. However, clinicians should also fully consider the physiological range of NK cells when assessing the immune status of patients and avoid over-interpreting the results of a single measurement.

3. Characteristics of dynamic changes in the proportion of NK cells during child development

Unlike the relative stability of adulthood, the distribution of NK cells during childhood development shows significant dynamic changes, reflecting the development and maturation of the immune system in the early stages of life. This dynamic change not only involves the increase in the number of NK cells, but also contains the biological significance of the functional maturity and diversification of NK cells, which is of great value for understanding the development of the children's immune system.

In recent years, a series of studies [10] on the distribution of NK cells in the peripheral blood of Chinese children have provided valuable longitudinal data. These studies show that the percentage and absolute number of NK cells show a clear upward trend with the age of children. Specifically, the proportion of NK cells is relatively low from neonatal to infancy (first month of life), about 2.51 ± 1.12%, and the absolute count is approximately 82 to 1431 cells per microliter of blood (median: 391/μL). This low proportion reflects that the newborn's immune system is not yet fully mature, and the function of NK cells is relatively limited, mainly relying on maternal transmission of immune protection.

Subsequently, the proportion of NK cells increased significantly to 4.12 ± 1.53% from infancy to early childhood (2 months to 1 year of age), and the absolute count increased to 210 to 660 cells/μL (median: 483 cells/μL). From early childhood to preschool age (1 to 3 years), the proportion of NK cells further increased to 5.11 ± 1.78%, with counts ranging from 210 to 2,001 cells/μL (median: 657 cells/μL). The proportion of NK cells in preschool to school-aged children (4 to 6 years) jumped to 7.92 ± 2.35%, with absolute counts ranging from 233 to 1,946 cells/μL. By school age until adolescence (7 to 18 years of age), the percentage of NK cells peaks at about 9.96 ± 2.84%, and its absolute number is widely distributed between 144 and 2,551 cells/μL.

These data clearly show the stepwise increase in the proportion of NK cells with age, reflecting the gradual improvement of the NK cell bank during the development of the child's immune system. This dynamic change has important biological significance: as children's exposure to the external environment and pathogens increases, the simultaneous increase in the number and function of NK cells provides the body with stronger innate immune defenses. In particular, the rapid growth stage from preschool to adolescence coincides with the critical period of the transformation of children's immune system from "naïve" to "mature," indicating that the development and maturity of NK cells is an important part of children's overall immune capacity building.

It is important to note that environmental and genetic factors can also significantly affect the development trajectory of NK cells in children. A study of Tibetan children in China [11] found that there were statistically significant differences in the percentage of NK cells in Tibetan children between different age groups (preschool group and adolescent group), and the proportion of NK cells in Tibetan children was generally higher than that of Han children of the same age. Researchers believe that this difference may be related to physiologically adaptive immunomodulatory mechanisms in high-altitude environments. Prolonged exposure to hypoxic environments may induce adaptive changes in the immune system, including upregulation of the proportion of NK cells in response to the specific environmental challenges at high altitudes. This finding highlights the synergistic role of ethnic genetic background and environmental factors in NK cell development.

In summary, the proportion of NK cells in children's peripheral blood shows a dynamic upward trend with age, which not only provides an important reference value for assessing children's immune development status but also provides key clues for understanding the maturation process of the innate immune system. Clinicians must consider age when interpreting NK cell test results in children and use age-specific reference ranges. In addition, the differences in the distribution of NK cells in different ethnic and geographical environments also suggest that it is of great significance to establish a localized and ethnic-specific reference value system for accurate assessment of children's immune status.

4. Age-related functional phenotype of NK cells and receptor expression in old age

As we age, the immune system undergoes a series of complex changes, a process known as immunosenescence. While several studies discussed earlier have shown that the absolute number and relative proportion of NK cells in healthy adults remain relatively stable, in-depth analysis shows that the quality and function of NK cells change significantly in old age. This change is mainly reflected in changes in NK cell subset distribution, functional phenotype, and receptor expression, rather than a simple decrease in number. Understanding this phenomenon of "quantitative stability and qualitative change" is of key significance for understanding the changes in immune function in the elderly.

A thorough study conducted by Korean scholars [12] provides important insights into understanding the functional changes of aged NK cells. The study used high-parameter flow cytometry to comprehensively analyze peripheral blood samples from 122 healthy volunteers (including two age groups under 60 years old and over 60 years old, with a balanced proportion of men and women in each group), focusing on NK cell subsets and their receptor expression. The study not only determined the overall proportion of NK cells, but also deeply analyzed the distribution of the two main NK cell subsets of CD56bright and CD56dim, as well as the expression levels of various activated receptors such as CD16, NKG2A, NKG2C, NKG2D, CD57, DNAM-1, CD8a, CD62L, NKp46, and NKG2D.

The results showed that although there was no significant difference in the overall proportion of NK cells in peripheral blood between the elderly group (≥60 years old) and the young group (<60 years old), the expression level of NK cell function-related receptors showed significant age-dependent changes. In particular, the expression of NKp46 and NKG2D, two key activated receptors, gradually decreased with age ($P < 0.01$ and $P < 0.05$, respectively). These receptors play a central role in NK cell recognition and killing of target cells, and their decreased expression is directly related to the decline of NK cell function. This finding reveals an important concept: the immune function of NK cells depends primarily on their quality (functional phenotype and receptor expression) rather than on quantity. In old age, although the number of NK cells remains relatively stable, their functional receptor expression decreases, leading to a decrease in overall cytotoxicity.

This phenomenon of "stable quantity but decreased quality" may be one of the important immunological mechanisms of increased susceptibility to infection and tumors in the elderly population.

In addition, the study also found that the distribution of NK cell subsets changed with age. The proportion of CD56bright (regulatory/cytokine-producing type) NK cells was relatively increased in the elderly group, while the proportion of CD56dim (cytotoxic/killer) NK cells was relatively decreased. This subset switch further explains the mechanism of functional changes in aged NK cells: the elderly immune system may shift from an emphasis on direct cytotoxicity to a more cytokine-mediated immune modulation, a shift that may reflect the body's adaptive adjustment to chronic low-grade inflammatory states.

In summary, the study of NK cells in the elderly stage should go beyond simple quantitative assays and analyze their functional phenotypes, receptor expression, and subpopulation distribution in the elderly. The Korean study not only constructed a characteristic map of NK cell surface receptors for the Korean population, but more importantly, revealed the profound impact of age on the functional quality of NK cells. This framework of "quantitative stability and qualitative change" helps to unify the seemingly contradictory research results and provides a new perspective for understanding the changes in immune function in the elderly. Future i mmune intervention strategies for the elderly should focus on improving the functional quality of NK cells rathe r than simply increasing their number, such as modulating the functional status of NK cells through cytokine the rapy or receptor agonists.

5. NK cell subset heterogeneity: The regulatory role of age and sex

NK cells are not a homogeneous population, but are composed of multiple functional and phenotypic subpopulations that play different roles in the immune response. An in-depth understanding of the heterogeneity of NK cell subsets and their regulatory mechanisms by host factors such as age and sex is crucial to fully grasp the biological characteristics of NK cells. In recent years, with the development of high-parameter flow cytometry, researchers have been able to analyze the distribution characteristics of NK cell subsets in more detail, revealing the complex regulatory network of age and gender on the composition of NK cell subpopulations.

A landmark study in Switzerland [13] provides a valuable reference for understanding the distribution of NK cell subsets in healthy adults. The study systematically determined the absolute count and percentage of lymphocyte subsets, including T cells, B cells, and NK cells, through multiparametric flow cytometry analysis of peripheral blood from 150 healthy volunteers (ages 18–60 years), with special attention to marker expression related to cell activation status and maturity. The study confirmed that the average proportion of NK cells in the peripheral blood of healthy adults (CD3-CD56+CD16+) was 11.70 (5.35–30.93)%, and the absolute count was 184 (77–427)/μL, which were highly consistent with other European studies. The relative stability of NK cell distribution in adulthood was verified.

However, when analyzing the distribution of NK cell subsets in depth, the gender differences became significant. The study found that the percentage of CD56+CD5+NK cells, a subset of mature NK cells with highly cytotoxic activity, was significantly higher in male participants than in females [male: 13.70 (5.22–32.86)% vs female: 9.80 (5.33–20.25)%, $P < 0.05$], suggesting that male NK cells may have a higher baseline activation status. This gender difference may be related to the regulatory effect of sex hormones on the immune system. E strogen has been shown to inhibit the cytotoxicity of NK cells, and testosterone may enhance NK cell function, providing a potential mechanism for understanding gender differences in immune responses [14].

In terms of the effect of age on NK cell subsets, no significant age-related changes in the overall proportion of NK cells were found, which is consistent with the results of the Chinese and Cuban studies mentioned above. It is important to note that there are differences in the definition and typing strategies of NK cell subsets in different studies, which may lead to inconsistencies in research results. For example, some studies have classified NK cells into two main subpopulations, CD56brightCD16- and CD56dimCD16+, while others have further subdivided them based on receptor expression, such as KIRs (killer cell immunoglobulin-like receptors) and NKG2A. This technical difference underscores the need to establish a standardized NK cell subset typing protocol [15].

Taken together, the distribution of NK cell subsets is finely regulated by various factors such as age, gender, genetics, and environment. In adult healthy populations, although the overall proportion of NK cells remained relatively stable, their subset composition, receptor expression, and functional status showed significant individual differences. This subpopulation heterogeneity explains why it is difficult to comprehensively assess immune function based solely on the total number of NK cells, emphasizing the need for comprehensive evaluation in clinical practice in conjunction with NK cell functional phenotypes. Future research should further explore the molecular mechanisms that regulate the distribution of NK cell subsets to provide a theoretical basis for personalized immune intervention.

6. Research outlook

Although current research has broadly understood the distribution characteristics of NK cells in healthy populations, there are still many urgent problems and promising research directions in this field. Based on the existing literature and clinical needs, we believe that the following directions are worth exploring in depth.

First, there is an urgent need to conduct large-scale, multi-center longitudinal cohort studies to track the dynamic changes of NK cells throughout an individual's life. Most current studies are cross-sectional designs, which are difficult to accurately depict the real change trajectory of NK cells during ontogeny [15–17]. Ideal longitudinal studies should cover all stages from birth to old age, with regular sample collection, combined with detailed health records, and systematic analysis of NK cell numbers, subpopulation distribution, temporal changes in receptor expression and functional activity, as well as associations with environmental exposures and disease events. This kind of research will provide a solid foundation for the establishment of a true "NK cell life trajectory map," far exceeding the current level of understanding based on cross-sectional data of different populations.

Secondly, the application of single-cell multi-omics technology will revolutionize the study of NK cell heterogeneity. Traditional flow cytometry can usually only analyze 10–15 markers simultaneously, making it difficult to capture the full heterogeneity of NK cells. Technologies such as single-cell RNA sequencing (scRNA-seq), single-cell ATAC-seq, and high-dimensional mass spectrometry flow cytometry (CyTOF) can analyze dozens or even hundreds of molecular signatures simultaneously, promising to reveal new NK cell subpopulations, differentiation trajectories, and functional states [18]. Particularly valuable is the application of these techniques to samples of different ages, genders, and disease states, potentially uncovering NK cell profiles associated with aging, disease susceptibility, or treatment response, providing new tools for precision immunoassessment.

Third, the standardization and clinical translation of NK cell function assessment are important directions. Current NK cell function assay methods are diverse and lack standardization, which limits clinical application [19]. Future research should focus on: (1) developing a simple and stable NK cell function detection platform; (2)

establishing a reference range for NK cell function in different age, gender, and ethnic groups; (3) exploring the correlation between NK cell function indicators and clinical outcomes. These efforts will move NK cell evaluation from laboratory research to clinical routine applications, providing practical tools for immune status monitoring, disease risk prediction, and treatment efficacy evaluation.

Fourth, multi-omics integration analysis will deepen the understanding of NK cell regulatory networks. Combining genomics (e.g., KIR gene polymorphisms), epigenomics (DNA methylation, histone modification), transcriptomics, and proteomics data, it can systematically analyze how genetic, epigenetic, and environmental factors synergistically regulate the development, differentiation, and function of NK cells. Of particular interest is how these multi-level regulations change with age and how they respond to immune challenges such as vaccination, infection, or tumors [20]. This systematic understanding will provide precise strategies for immune interventions targeting NK cells.

Finally, it is crucial to establish a global reference database of NK cells. Given the significant influence of race, geography, and environmental factors on NK cell distribution, it is difficult to universalize the reference value of a single population. The international academic community should collaborate to establish a shared NK cell reference database covering the multidimensional characteristics of NK cells across different ethnic, geographical regions, ages, and gender groups [21]. This resource will provide clinical laboratories around the world with a localized basis for calibration, facilitating the standardization and realization of clinical value for NK cell assays on a global scale.

7. Conclusion

As the core effector cells of the innate immune system, NK cells in the peripheral blood of healthy people reflect the dynamic balance and adaptability of the immune system. Based on the existing research, we can draw the following important conclusions.

In healthy adults, the proportion of NK cells in peripheral blood lymphocytes remains relatively stable, usually 5% to 10%, with no significant differences between age groups and genders. This stability reflects the immune system's conservative strategy to maintain basic immune surveillance capabilities, ensuring continuous monitoring of pathogens and tumor cells. However, this quantitative stability of the surface masks the deep functional changes, especially in the elderly population, where NK cell receptor expression and cytotoxic activity show age-related declines, indicating that the functional quality of NK cells, rather than the quantity alone, is a key indicator for assessing immune status.

The distribution of NK cells showed significant dynamic changes during child development, gradually increasing from a low proportion in the neonatal period (about 2.5%) to a near-adult level in adolescence (about 10%). This growth trajectory coincides with the overall development and maturation of the immune system, reflecting the body's gradual establishment of a complete innate immune defense system. It is worth noting that this developmental trajectory is affected by genetic background, environmental exposure, and other factors, and there may be specific NK cell distribution patterns in children of different ethnic groups and regions, emphasizing the importance of establishing localized reference values.

The heterogeneity of NK cell subsets provides a new perspective for understanding the regulation of NK cell function by age and gender. Studies have shown that men generally have a higher proportion of activated NK cells, while older adults present with decreased NK cell receptor expression and functional activity. In addition, studies in Tibetan populations at high altitudes have revealed the impact of environmentally adaptive immune remodeling on NK cell distribution, highlighting the importance of genetic-environmental interactions in shaping immune phenotypes.

NK cell research is moving from simple quantitative assays to a new era of multidimensional functional assessment. Future research should focus on longitudinal cohort design, single-cell multi-omics technology application, functional assessment standardization, and global reference database construction to comprehensively analyze the role of NK cells in health and disease. In clinical practice, it is necessary to go beyond the limitation of the total number of NK cells and integrate multi-dimensional indicators such as subset distribution, receptor expression, and functional activity to achieve an accurate assessment of individual immune status.

In conclusion, the complex picture of NK cell distribution reflects the adaptive regulation of the immune system across different life stages, genders, and environmental contexts. In immune assessment and intervention, these individual differences must be fully considered, and individualized strategies must be adopted to truly realize the potential of NK cells in disease prevention, diagnosis, and treatment. By deepening our understanding of NK cell biology, we are expected to develop more effective immune intervention strategies to improve human health.

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Disclosure statement

The authors declare no conflict of interest.

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