# Natural killer cell biology

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## ABSTRACT

Innate lymphocytes can acquire phenotypes and functions determined by an organ or tissue, or by a niche within these microenvironments. On the other hand, they can exhibit transient phenotypes and functions—with different amounts or densities of the same receptors. Natural killer cells, as innate lymphocytes, can also have different functions or phenotypes and are primarily tissue-resident lymphocytes.

**Keywords:** Killer Cells, Natural. Lymphocytes. Cell Biology.

## INTRODUCTION

Natural killer (NK) cell biology is still a matter of debate. Because of its complexity and biological behavior, it took almost 30 years to define which hematopoietic lineage it belonged to. The main point of discussion has been the unquestionable fact that it behaves like an innate cell, although it is a lymphocyte. Up to then, the understanding was that lymphocytes were synonymous with the adaptive immune system response. It took another 30 years to learn that there are several innate lymphocytes, all of them tissue-resident cells, and some with ambiguous phenotypical and functional characteristics. Accordingly, before getting into NK cells biology, it is essential to discuss the characteristics of the innate immune system and contextualize the role of NK cells in it.

## METHODS

At the seminars in science held by our group of scientists and science students at the good manufacturing practices facility of Hospital de Clínicas de Porto Alegre, RS, Brazil, we reviewed innate lymphocyte biology and, for this manual, the relevant functions of NK cells. Based on the reported transient persistence of adoptively transferred NK cells and on our own experience[1], these studies, as well as studies on other innate immune cell responses and adaptive lymphocyte biology, are designed to support the characterization of immune reconstitution in our upcoming phase II trial: a randomized, double-blind clinical trial on the use of NK cells for consolidation in acute myeloid leukemia complete remission.

## RESULTS

Innate lymphoid cells (ILCs) are long-lived, tissue-resident cells analogous to T lymphocytes subsets except for the lack of antigen-specific receptors[2–4]. Their main function is to rapidly react to altered cells through the

production of immune mediators and cytotoxic activity, delivering to the microenvironment molecules that trigger adaptive immune response development. Immature NK cells produce several cytokines, chemokines, and growth factors, and mature NK cells are cytotoxic; the latter can also develop antibody-dependent cellular cytotoxicity (ADCC)[5,6].

ILCs are divided into five reasonably defined subtypes: ILC1, ILC2, ILC3, NK cells, and ILC precursors. In animal models, several interesting characteristics of these innate lymphocytes are better understood, although defining a particular subset is difficult since they share the same receptors at many intensities. Mature ILC2, for example, can also express receptors that are present in immature NK cells. On the other hand, some ILCs can transdifferentiate between subtypes, showing a remarkable plasticity[3].

The microenvironment exerts an effect on the ILCs precursors' development and fate, as it has been reviewed by Vivier et al.[7]: adoptively transferred, genetically modified ILC precursors for fate tracking can give rise to different ILCs according to the tissue/organ they infiltrate, each one producing various amounts of, sometimes, the same cytokines. In addition, as already pointed out, according to the surrounding condition, one ILC can transdifferentiate from one type to another. ILCs can also behave differently (phenotypically and functionally) according to distinct niches within the same tissue/organ[2]. Among their subtypes, only ILC1 and NK cells can develop cytotoxicity[8].

In summary, innate lymphocytes are tissue-resident cells. As part of the tissue/organ immunosurveillance system, the characterization of the transition of one type of ILC to another is poorly defined because of the different amounts of the same receptors they express and the plasticity ILCs are capable of.

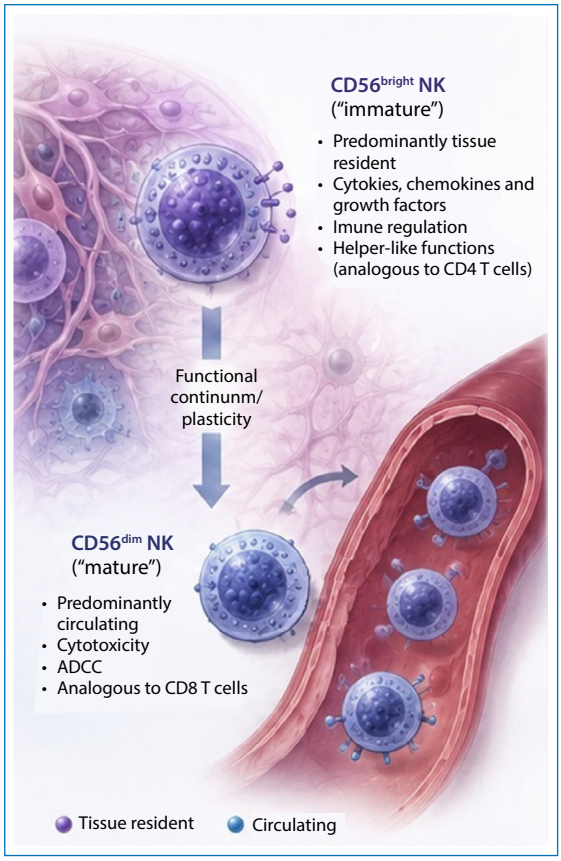
Among them all, NK cells are the best characterized and defined ILC. Their function is analogous to CD8 T cells and CD4 T cells in terms of cytotoxicity (CD56 dim) and helper activity (CD56 bright), respectively[7].

## Natural killer cells

NK cells are divided into two major subsets: CD56bright or "immature" NK cells, which are predominantly tissue-resident cells, and CD56dim or "mature" NK cells, which compose most peripheral blood circulation NK cells[9]. It is important to point out that *immature* and *mature* refer to ontogeny, since the *immature* subtype is, as seen in the previous section, analogous to T helper cells—that is, they modulate both the innate and adaptive immune system through secretion of immune mediator molecules and growth factors. As also pointed out, cytotoxic NK cells (CD56dim) are analogous to CD8 T cells, since both are cytotoxic and can develop memory, except that NK cells are active producers of cytokines and, as ILCs, lack antigen-specific receptors.

Like the other innate lymphocyte subtypes, the transition between bright and dim subsets is also difficult to define because of the promiscuity between receptors—as in the case of ILC2, that shares receptors with immature or "helper" NK cells (Fig. 1). In animal models, it was suggested that the different expression or density of the same set of receptors provides a broader combination that ensures the recognition of several ligands and effective immunosurveillance[9].

The role of NK cells in virus-infected cells has been defined and is better known in cytomegalovirus-infected animal models. In these models, it was recently recognized that NK cells can undergo antigen-specific proliferation, as well as memory formation, just like T CD8 cytotoxic lymphocytes. It was also shown that a single NK cell can give rise to a diverse population of cells. However, a single NK cell clone undergoes a relatively smaller expansion (1,000 times) as compared to the expansion (40,000 times) of a single $CD8^+$ T cell clone[10]. It is suggested that epigenetic differences between NK and T cytotoxic cells can determine their different ability to expand. Finally, as T cells in the blood circulation increases in number, followed by a slow decay phase of contraction, NK cells behave similarly, but on a much lower scale then T lymphocytes[11].




NK: natural killer; ADCC: antibody-dependent cellular cytotoxicity. Source: Elaborated by the authors.

**Figure 1.** Phenotypic and functional continuum of NK cell subsets between tissue residency and peripheral circulation.

If NK cells can behave like that against viruses, there is no reason to believe they cannot behave against malignancy. The difficulty in observing this behavior by measuring peripheral blood expansion and persistence of NK cells in clinical trials can be attributed to their innate lymphocyte characteristic behavior—they are primarily tissue-resident cells and apparently have smaller ability to expand as compared to T lymphocytes.

## Natural killer cells receptors

NK cells, as ILCs, are constitutively in a pre-activation state, regardless of the stage of development they are in, and as such, they can react against a target cell in a matter of hours. This natural and quick activation is modulated by the presence of inhibitory and stimulatory receptors such as the killer immunoglobulin-like receptors (KIR), particularly observed in cytotoxic NK cells. Inhibitory KIRs for self-human leukocyte antigen (HLA) class I ligands protect normal cells from being destroyed—the so-called missing-self theory[12]. However, when parasitized, malignant, transformed, or overly stressed, a self-cell can express an excess of ligands for stimulatory receptors that overcome the inhibitory effect of KIR-HLA class I. In addition to stimulatory KIR receptors also expressed in NK cells, there are several others, known as natural receptors, that can activate or inhibit NK cell reactivity as well[13].

Based on the set of KIRs expressed by NK cells of an individual, two haplotypes are recognized: haplotype A, composed of fewer activating KIRs, and haplotype B, with a higher number. In animal models, the main inhibitory or stimulatory set of receptors has been studied according to, respectively, the absence or presence of its related ligand on the target cell—the so-called KIR mismatch—and its analysis can reasonably predict NK cells alloreactivity. It must be kept in mind, as shown above, that the microenvironment can affect innate lymphocytes. In tumor microenvironments, there are nearby inhibitory cells such as Tregs[14] or myeloid suppressor cells[15] that can negatively alter the activation of NK cells, irrespective of their receptor.

### Natural killer cells antibody-dependent cellular cytotoxicity

NK cell ADCC activity refers to the ability of NK cells to kill a target cell opsonized by antibodies. NK cells recognize antibodies' Fc portion through its FcyRIIIa (CD16) receptor. CD16-Fc portion engagement triggers the release of perforin, granzymes, and the secretion of IFN-, inducing target cell death. NK cell ADCC is active in anti-tumor and anti-viral immune responses and mediates the effectiveness of several therapeutic monoclonal antibodies such as rituximab, trastuzumab, and cetuximab[5,6].

### Natural killer cells anti-tumor activity

Under certain *in-vitro* conditions, NK cells can kill every tumor cell line tested[16]. There are several well-designed clinical trials that show non-genetically modified NK cells have anti-myeloid leukemia activity despite their lack of persistence in peripheral blood[17]. In our experience, the adoptive transfer of non-genetically modified NK cells, *in-vitro* expanded in the presence of genetically modified K562 cells to express interleukin (IL)-21 and 41BB (mbIL21K562 cells), resulted in a 78.6% overall response rate in patients with relapsed/refractory acute myeloid leukemia[1]. NK cells obtained in the same production platform were also tested in hematopoietic stem cell transplantation with encouraging results. Incidentally, the role of NK cells in hematopoietic stem cell transplantation is unquestionable. NK cells (mostly with the immature CD56bright phenotype) are the first lymphoid cells to recover, and their number and rate of recovery are related to better stem cell transplantation outcomes[18].

The adoptive transfer of non-genetically modified NK cells, using different products, has been studied in solid tumors without meaningful anti-tumor activity. At the time of most of those studies, knowledge of the immunosuppressive tumor microenvironment (TME) was just emerging. Bone marrow and lymphatic tumors—the so-called liquid tumors—with an extensive capillary net designed for leukocytes transit or circulation through an intricate net of lymphatic channels that allow only mononuclear cells, either innate or adaptive immune cells, respectively, are sensitive to adoptive immunotherapy strategy despite the TME.

Finally, many years ago, we were able to show that NK cells' cytotoxic activity can be significantly enhanced by a bi-specific antibody that engages it to the tumor cell[19], paving the way for the development of numerous engagers, either bi-specific or chimeric modified cytotoxic immune cells, to express the desired engager on their membrane—chimeric antigen receptor (CAR) cells.

The emergence and rapidly spreading CAR cell technology has introduced CAR-NK cells immunotherapy for hematological cancers. CAR-NK cells, discussed in a separate chapter of this manual, have remarkable potential because, in the few published clinical trials, in addition to their anti-tumor activity, and as opposed to CAR-T cells, only a few and clinically manageable off-target side effects were observed.

## CONCLUSION

ILCs share several functional characteristics with adaptive lymphocytes, although they lack antigen-specific receptors and are predominantly tissue-resident cells. Among ILCs, NK cells have a unique capacity to combine rapid cytotoxic activity with cytokine production and the ability to develop memory. During an immune response, NK cells expand to a lesser extent than T cells and can contribute to the activation of adaptive immunity. Most NK cells detected in peripheral blood display a mature cytotoxic phenotype, but peripheral blood may not fully reflect NK cell distribution and activity within tissues.

This distinction is particularly relevant in the context of adoptive NK-cell immunotherapy. Although the persistence and expansion of transferred NK cells in peripheral blood may be limited, this characteristic does not preclude their clinical activity. The anti-tumor activity observed with adoptively transferred non-genetically modified NK cells in myeloid malignancies supports their continued investigation as a cancer immunotherapy strategy.

Important translational questions remain, particularly regarding NK-cell trafficking and tissue distribution, the relationship between peripheral blood persistence and clinical activity, and the influence of the tumor microenvironment on NK-cell function. A better understanding of these aspects may help explain why anti-tumor effects can occur without a direct correlation with NK cell persistence or expansion in peripheral blood. Further studies addressing these knowledge gaps will be important to define how the biological characteristics of non-genetically modified NK cells can be better exploited therapeutically and to clarify their role in modern cancer immunotherapy.

## CONFLICT OF INTEREST

Nothing to declare.

## DATA AVAILABILITY STATEMENT

All data are presented in this paper.

## AUTHORS' CONTRIBUTION

**Substantive scientific and intellectual contributions to the study:** Pezzi A, Valim V, Amorin B, Zambonato B, Paz A, Furlan J, Teixeira M, Martins D, Domingues MF, Rodrigues RM, Spagnol F, Trzesniak JN, Sekine L and Silla L; **Conception and design:** Silla L; **Acquisition of data:** Pezzi A, Valim V, Amorin B, Zambonato B, Paz A, Furlan J, Teixeira M, Martins D, Domingues MF, Rodrigues RM, Spagnol F, Trzesniak JN, Sekine L and Silla L; **Analysis and interpretation of data:** Silla L; **Manuscript preparation:** Pezzi A, Amorin B, Rodrigues RM and Silla L; **Manuscript writing:** Silla L; **Critical revision:** Sekine L; **Final approval:** Rodrigues RM.

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## DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

Generative artificial intelligence (AI) was used to preparation of a figure. The authors reviewed and approved all AI-assisted content and take full responsibility for the final manuscript.

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