Immunology Letters 190 (2017) 58–63

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Historical overview on the morphological characterization of large granular lymphocytes/natural killer cells

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ARTICLE INFO

Keywords:
Immunology
LGL cells
Morphology
NK cells
Ultrastructure

ABSTRACT

Natural Killer (NK) cells were discovered when research on T cells produced an unexplained background, or "natural" cytotoxicity in non-immunized mice. The previously unknown cells were thus named "natural" killer cells, or NK cells. They are roughly defined by a lack of the T cell marker CD3 and a presence of CD56 (or NCAM, neural cell adhesion molecule) and correspond to the large granular lymphocytes (LGLs). This article is focused to the description the morphological features of these cells and of the LGLs and to the fundamental contribution of the Italian scientist Carlo E. Grossi to the ultrastructural characterization of these cells.

1. Introduction

Natural killer (NK) cells were discovered more than 30 years ago the term NK was used for the first time by Kiessling and co-workers [1], as large granular lymphocytes (LGLs) that belong to the innate immune system because unlike T or B lymphocytes of the adaptive or antigen-specific immune system, NK cells do not rearrange genes encoding T cell or B cell antigen receptors.

A decade later the work of Kiesseling [1], Kärre and colleagues formulated the "missing self hypothesis" based on the finding that NK cells target cells with low or absent expression of Major Histocompatibility Complex (MHC) class I molecules [2]. This was based on the observation that NK cells could kill efficiently murine lymphoma cell lines that had lost MHC-class I molecules, while the parental MHC-class I-positive lymphoma cells were resistant to lysis. In the absence of MHC class I molecules, the target cell is lysed. Consequently, NK cells are involved in the immune response to tumors, particularly when tumors down-regulate MHC class I expression.

A possible interpretation of the "missing self hypothesis" was the expression, on NK cells, of receptors capable of sensing MHC-class I molecules and modulating NK cell function. This concept was reinforced by the discovery, by two Italian scientists, Lorenzo and Alessandro Moretta (Fig. 1) and coworkers working at the University of Genoa School of Medicine, of new surface molecules that could inhibit NK cell function when cross-linked by specific monoclonal antibodies [3,4].

NK cells were defined as "null" cells, which meant they were non-B, non-T, non-phagocytic and non adherent cells that are largely Fc receptor positive, mostly complement receptor negative, and have low affinity to form rosettes with sheep erythrocytes [5–7].

1.1. NK cells distribution in healthy tissues

NK cells originate from hematopoietic stem cells (HSC) and undergo maturation primarily in the bone marrow. During HSC differentiation into NK cells, the expression of CD34 is progressively lost while other surface antigens specific for the NK lineage appear. In addition, NK cells progressively acquire the expression of inhibitory and activating receptors as well as their functions of cytokine secretion and cytotoxicity. Bone marrow stromal cells release factors involved in the proliferation and maturation of NK cell progenitors. Evidence has been accumulated that NK precursors at different stages of differentiation are present in tonsils, lymph nodes, decidua, and gut-associated lymphoid tissues.

NK cells account for 5–15% of the peripheral blood lymphocytes. More than 90% of NK cells are found in the blood circulation, where they express high amounts of CD16 and low amounts of CD56 (or NCAM, neural cell adhesion molecule), whereas less than 10% are found in healthy tissues, such as skin, gut, spleen, liver, lungs, and uterus during pregnancy.

In mice, the percentages of NK cells are higher in non-lymphoid than in lymphoid organs and the order for NK cell frequency is lung > liver > peripheral blood > spleen > bone marrow > lymph node > thymus. However, differences in NK cell proportions exist and, conversely to mice, human NK cell numbers are ten-fold higher in lymph nodes than in peripheral blood. Whether NK broad distribution is due to continuous cell recirculation, to preferential homing of

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http://dx.doi.org/10.1016/j.imlet.2017.07.014
Received 27 June 2017; Received in revised form 15 July 2017; Accepted 26 July 2017
Available online 27 July 2017

Fig. 1. A port trait of Alessandro (on the right) and Lorenzo Moretta (on the left).

Fig. 2. A port trait of Carlo E. Grossi (courtesy of Prof. C. Tacchetti).

particular NK subsets, or local differentiation/maturation in these sites is still an open question.

1.2. NK cells in pathological conditions

Based on the knowledge regarding immunity and cancer immunology at 1975, T and B lymphocytes were responsible for the rejection of tumors and virally infected cells. Studies of virally induced tumors [8,9] implicated a thymus-derived lymphocyte in the elimination of the tumors [9,10]. In vivo and in vitro studies provided further support that the tumor regression or killing was indeed T cell mediated [11,12]. According to a T-cell mediated model of tumor destruction, athymic and/or nude mice, which lack T cells, should have unrestrained tumor growth. However, unimpeded growth of tumors in the absence of T cells was never observed in these models [13,14] suggesting that another cell type is mediating tumor cytolysis. There was now a large amount of data demonstrating "spontaneous" T cell-independent cell-mediated cytotoxicity that could not be ignored. Taken together, these findings eventually led to the coining of the terms "natural killing" and "natural cytotoxicity" [15]

Under pathological conditions and during inflammation, NK cells extravasate into the lymph nodes and accumulate at sites of tumor growth. Mice with compromised NK cell function are more susceptible to carcinogen-induced cancers, and individuals lacking NK cells suffer from persistent viral infections and as a consequence die prematurely.

NK function was first identified on a functional basis according to the ability of human peripheral blood lymphocytes to lyse the K562 cell line in the absence of prior stimulation [16].

NK cells exert cytotoxic functions mainly directed against virus-infected cells and tumor cells [17]. They also display immunomodulatory functions through secretion of immunoregulatory cytokines, like Interferon-gamma (IFN-γ,) tumor Necrosis Factor-alpha (TNF-α), interleukin-10 and −13 (IL-10, IL-13), and granulocyte colony stimulating factor (GM-CSF), but they have a low natural cytotoxicity [18]. In particular, IFN-γ enables the NK cells to call in other types of immune cells to co-operate in killing cancer cells, thereby amplifying the immune system's response.

The activation of NK cells depends on an intricate balance between activating and inhibitory signals that determines if a target will be susceptible to NK-mediated lysis. To "see" and discriminate between normal and transformed cells, NK cells express activating and inhibitory membrane receptors that recognize ligands at the surface of target cells.

While T cells must be educated by antigen-presenting cells before they recognize tumors, NK cells spontaneously lyse tumor targets in vivo and in vitro without requiring immunization or pre-activation. Cytokines including IFN-α/-β, IL-2, IL-12 and IL-15 enhance NK cell-mediated cytotoxicity, whereas IL-2, IL-12 induce NK proliferation, and IL-1, IL-2, IL-12, IL-15, IL-18 and TNF induce NK lymphokine-production.

1.3. Morphological characterization

LGLs constitute 2–6% of the peripheral white cells and approximately 10–15% of peripheral blood lymphocytes. LGLs are larger than the typical lymphocytes (10–12 μm) with a large amount of cytoplasm containing peroxidase-negative granules. Most NK cells have LGL morphological aspects [19]. However, not all NK cells have LGL morphology and not all LGL cells are NK cells. Human LGLs are medium-large size lymphocytes with round or indented nuclei, condensed chromatin and prominent nucleoli. In the cytoplasm, primary lysosomes are present ranging in diameter from 50 to 800 nm and containing an electron dense core surrounded by a layer of lesser opacity. In addition to lysosomal enzymes, the granules contain phospholipids, proteoglycans, and proteins important for cytotoxic lymphocyte functions, including serine esterase (granenzymes) and performing proteins (perforins) [20]. The granules consist of an electron dense center that contain the perforin and may be enclosed by a thin membrane. A layer of lesser opacity containing the granenzymes surround the core. Both perforin and granenzymes are important for their cytotoxic function [21]. The combined functions of these proteins lead to the generation of pores on the plasma membrane and activation of the caspase cascade. The majority of NK cells found in reactive lymph nodes and peripheral tissues are poor cytolitic (perforin low), while in the peripheral blood most NK cells are high cytolytic (perforin high) [22]. The core of lytic granules is surrounded by a lipoid bilayer that contains Fas ligand and lysosomal-associated membrane glycoproteins (LAMPs) [23].

The Italian scientist Carlo E. Grossi (Fig. 2) working at the University of Genoa School of Medicine, has produced fundamental contributions to the morphological characterization of LGLs. In 1982, Grossi et al. [5] isolated the granules of LGL from human peripheral blood and analyzed by enzyme cytochemistry and electron microscopy (Fig. 3). In the single cells, granules at different stages of maturation could be detected; in addition, packaging of the granules took place in the proximity of the Golgi apparatus. Acid phosphatase (AP) was observed within the granules and the vesicles located in the Golgi area; while the Golgi apparatus identified through its thiamine pyrophosphatase-positivity was consistently negative for AP. Alpha naphthyl-acetate esterase (ANAE) activity was localized in the granules as well as