Cytokine & Growth Factor Reviews 26 (2015) 113–120

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

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

NK cells and interferons

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ARTICLE INFO

Article history:
Available online 13 November 2014

Keywords:
NK cells
Type I interferon
Interferon-γ
Cytotoxicity
Antiviral response

ABSTRACT

The role of Natural Killer cells in host defense against infections as well as in tumour surveillance has been widely appreciated for a number of years. Upon recognition of "altered" cells, NK cells release the content of cytolytic granules, leading to the death of target cells. Moreover, NK cells are powerful producers of chemokines and cytokines, particularly Interferon-γ (IFN-γ), of which they are the earliest source upon a variety of infections. Despite being armed to fight against pathogens, NK cells become fully functional upon an initial phase of activation that requires the action of several cytokines, including type I IFNs. Type I IFNs are now recognized as key players in antiviral defense and immune regulation, and evidences from both mouse models of disease and *in vitro* studies support the existence of an alliance between type I IFNs and NK cells to ensure effective protection against viral infections.

This review will focus on the role of type I IFNs in regulating NK cell functions to elicit antiviral response and on NK cell-produced IFN-γ beneficial and pathological effects.

1. Introduction

Natural Killer (NK) cells are large granular lymphocytes that belong to the innate arm of the immune system and play an important role in the immune responses against certain microbial pathogens and tumour cells. They have been recently described as members of the group 1 of Innate Lymphoid Cells (ILCs), as they do not undergo RAG-dependent receptor rearrangement, and are strong producers of IFN-γ [1]. NK cells develop from a common lymphoid precursor resident in the bone marrow (BM) that is considered the main site of their maturation.

During viral infections, inflammation, tumour growth and invasion, NK cells are rapidly recruited from the blood and accumulate in the parenchyma of injured organs where activated NK cells can kill target cells and release inflammatory cytokines and chemokines, thus participating in the recruitment and activation of other leukocytes and in the modulation of accessory cell function [2,3].

Unlike B cells and T cells that express a single antigen specific receptor, NK cells are endowed with a multiple germ-line encoded cell surface receptor system recognizing ligands on virus-infected or tumour cells. All the receptors expressed by NK cells are not unique to this cell type, but are also present on cells of other lineages such as T cells or myeloid cells. Receptor expression on NK cells is highly regulated, with some receptors being oligoclonally distributed and/or expressed on subsets of NK cells.

This complex receptor system is acquired during NK cell development, and consists of both inhibitory and activating receptors belonging to highly polygenic and polymorphic families [4,5].

Most inhibitory receptors specifically interact with MHC class I antigens. In humans, they belong to two distinct groups: the KIR family that comprises molecules binding to groups of human leucocyte antigen (HLA)-A, -B, -C alleles, and the C-type lectin receptors (i.e. CD94/NKG2A) specific for the widely expressed non-classical HLA-class I molecule, HLA-E. In the mouse, the functional surrogates of the Killer Ig-like receptors (KIR) are the Ly49 receptors. NK cells also express activating counterparts of KIR and Ly49 receptors, which share the same ligand specificities of the inhibitory receptors, but are endowed with lower affinity.

Among the receptors capable of triggering natural killing, the C-type lectin family NKG2D receptor recognizes the MHC class

Abbreviations: cDC, conventional dendritic cells; HCMV, human cytomegalovirus; IFNAR, type I interferon receptor; IFN, interferon; LCMV, Lymphocytic Choriomeningitis Virus; MCMV, murine cytomegalovirus; NK cells, Natural Killer cells; pDC, plasmacytoid dendritic cells; KIR, Killer Ig-like receptors.

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http://dx.doi.org/10.1016/j.cytogfr.2014.11.003
1359-6101/

I-related A and B proteins (MICA and MICB) and the members of a family of proteins named UL16-binding proteins (ULBPs) in humans and of *retinoic acid early inducible*-1 gene (*RAE*-1) and UL-16 binding protein-like (MULT)-1 in mice [6]. These ligands are mainly expressed on the surface of tumour cells of different histotypes, infected, and stressed cells, and can be induced in response to DNA damage [7,8]. Other activating receptors, namely the Ig-like molecules NKp46, NKp44, and NKp30 belong to the Natural Cytotoxicity Receptor (NCR) family, but their cellular ligands are still elusive.

Moreover, NK cells express a number of receptors acting as activating or co-stimulatory molecules such as CD2, CD244 (2B4), NKp80, beta1 and beta2 integrins and DNAM-1 (CD226).

Based on the receptor complexity, activation of NK cell functions is, thus, the result of concomitant engagement of various activating and inhibitory receptors by the particular set of ligands on target cells. In most instances the inhibitory signals override the triggering ones [9], while during infection or transformation when MHC class I molecules can undergo down-regulation, activation of NK cells prevails.

Despite already being armed for attack, NK cells require activation by type I interferons (IFN-α or IFN-β) or pro-inflammatory cytokines, such as interleukin-15 (IL-15), IL-12 and IL-18, in order to become fully functional and provide optimal host defense against infections [2,3].

This review overviews the role of type I IFNs in regulating NK cell anti-viral functions with an emphasis also given to the protective and detrimental effects of NK cell produced IFN-γ.

2. Regulation of NK cell functions by type I IFNs

NK cells have been classically considered capable of exerting their effector functions upon their first encounter with a potential target cell, such as a viral infected cell. However, they display effector functions following an initial phase of activation provided by dendritic cells (DCs) via a direct contact and/or through the release of several cytokines including IL-12, IL-15, IL-18 and type I IFNs, being this latter family of cytokines critical for early NK cell responses to several viral infections [2,3].

Type I IFNs are a family of innate cytokines consisting of several IFN-α subtypes in mice and humans, and one IFN-β subtype in both species [10]. As result of binding to their heterodimeric type I IFN receptor (IFNAR) broadly expressed on most cells, type I IFNs trigger a series of signalling cascades leading to phosphorylation of STAT (signal transducer and activator of transcription) molecules. STAT phosphorylation allows the formation of a transcriptional complex and the subsequent induction of several IFN-stimulated gene products endowed with antiviral activity [11]. Thus, exposure of cells to type I IFNs before infection induces an antiviral state that prevents productive viral infection.

Type I IFNs are among the most potent regulators of NK cell activation [3]. The pivotal role of type I IFNs in the induction of NK cell cytotoxicity was firstly established in 1977 and 1978 from several independent groups. Trinchieri and Santoli [12] showed that type I IFNs activate human NK cell cytotoxicity *in vitro* against virus-infected cells. Welsh and Zinkernagel [13] demonstrated that NK cells are activated *in vivo* upon mouse infection with the Lymphocytic Choriomeningitis Virus (LCMV) and that this activation was promoted by virus-induced IFN production, whereas Gidlund and co-authors [14] showed enhanced NK cell cytotoxicity against sensitive target cells in mice treated with IFN inducers or with type I IFNs. In addition, Herberman's group provided evidence that the level of NK-mediated cytotoxicity can be rapidly boosted in rats and mice upon inoculation with viruses or IFN inducers [15,16].

Subsequent reports from other groups demonstrated that type I IFNs potentiate NK cell cytotoxic activity increasing perforin-dependent cytotoxicity [17,18] and inducing TRAIL expression [19]. Moreover, type I IFNs, with the coordinated action of IL-12, activate NK cells to produce large amounts of IFN-γ [17,20], contribute to NK cell homeostasis [21] and support NK cell proliferation driving the expression of IL-15 [18].

Although many cell types are capable of releasing type I IFNs in response to viral infection, the capacity to secrete high amounts of these cytokines appears to be restricted to specialized DCs. Several years ago, unusual cells capable of secreting high amount of type I IFNs were identified in human blood [22,23]. Subsequently, these cells were found to correspond to a population of cells with plasmacytoid morphology that are present in T cell-rich area of inflamed lymph nodes and are able to differentiate into mature DCs [24,25]. Notably, cells endowed with similar morphology and functions were also identified in mice and termed mouse IFN-α-producing cells (MIPCs) [26,27]. Thus, this subset of DCs, in both human and mice, represent the major producer of circulating type I IFNs in response to many viral infections.

A pioneering work of Fernandez and co-authors formally demonstrated that also conventional DCs (cDCs) are able to activate NK cells through the contribution of both cell contact-dependent and -independent mechanisms [28]. This observation was then confirmed in a variety of experimental settings [29,30], which clearly documented the existence of a complex bidirectional crosstalk between cDC and NK cells. cDC-mediated activation of NK cells results in increased NK cell cytotoxic activity and/or IFN-γ production and can be induced by both resting and activated cDCs, the latter being more potent primers. Activated cDCs up-regulate the expression of MHC, co-stimulatory and adhesion molecules and, can also express ligands for NK cell activating receptors depending on the stimuli received. In particular, the NKG2D ligands, namely MICA and MICB, are induced on human cDCs upon type I IFN stimulation and contribute to NK cell activation during DC/NK cell contact [31]. Moreover, cDCs produce most of the NK cell activating cytokines including type I IFNs [30].

Thus, type I IFNs play a multifaceted role in the survival, expansion and activation of NK cells particularly during primary infections. However, how type I IFN regulates NK cell activation is still a controversial field.

2.1. Type I IFN-mediated direct and indirect mechanisms of action

Many studies have shown that type I IFNs can directly activate NK cells [32–34]; however counterevidence argues that a direct action of type I IFNs is not necessarily required to efficiently activate NK cells [35–37].

In 1978, Trinchieri and Santoli showed that addition of type I IFNs on *in vitro* cultured NK cells enhanced their cytotoxic potential [12], thus supporting a direct action of type I IFNs. More recently, adoptive transfer experiments have shown that a direct action of type I IFN on NK cells is necessary for the innate immune defence against vaccine virus infection [32] and adenoviral vectors [33]. Indeed, wild type (WT) NK cells transferred into $IFNAR^{-/-}$ knockout recipient mice were efficiently primed upon viral infection. Furthermore, in LCMV infection, Mack and co-authors demonstrated that direct type I IFN-induced signalling on NK cells is required for secretion of IFN-γ *in vivo* [34].

In support to an indirect route of activation, a first study from Lucas and co-authors demonstrated that the action of type I IFNs on accessory DCs, but not on NK cells, was required for NK cell activation in response to TLR ligands [35]. In this context, type I IFNs would exert their stimulatory function by eliciting DC production of IL-15, which can then be trans-presented by DCs to NK cells [35].