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Journal of Nutritional Biochemistry 46 (2017) 1–12

Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Dietary flavonoids and modulation of natural killer cells: implications in malignant and viral diseases

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Received 19 December 2016; received in revised form 30 December 2016; accepted 14 January 2017

Abstract

Flavonoids are a large group of secondary plant metabolites present in the diet with numerous potentially health-beneficial biological activities. In addition to antioxidant, anti-inflammatory, cholesterol-lowering, and many other biological functions reported in the literature, flavonoids appear to inhibit cancer cell proliferation and stimulate immune function. Although the immunomodulatory potential of flavonoids has been intensively investigated, only little is known about their impact on natural killer (NK) cells. Enhancing NK cell activity, however, would have strong implications for a possible clinical use of flavonoids, especially in the treatment and prevention of diseases like cancer and viral infections. Therefore, the purpose of this review is to summarize the currently available information on NK cell modulation by flavonoids. Many of the structurally diverse flavonoids stimulate NK cell activity and have thus great potential as diet-derived immune-modulatory chemopreventive agents and may even serve as therapeutic compounds or lead structures for the development of novel drugs for the treatment of both malignant and viral diseases.

Keywords: Natural killer cell modulation; Innate immunity; Cancer; Viral infection; Flavonoid; Isoflavones

1. Introduction

1.1. Natural killer cells

Natural killer (NK) cells are a subset of cytotoxic lymphocytes that exhibit some features of cytotoxic T cells and are part of the innate immune system [1]. There is growing evidence that NK cells share some characteristics with cells of the adaptive immune system (as described for mice and rhesus macaques), such as antigen-specific immunological memory [2,3]. The latter might improve their response to secondary infections with the same antigen [3]. NK cells originate from the same types of lymphoid progenitor cells as B and T lymphocytes and are found, for example, in peripheral blood (5%–20% of lymphocytes), liver (10% of lymphocytes) and spleen (3% of murine splenocytes) [4]. They differentiate and develop in bone marrow, lymph nodes, spleen, tonsils, and thymus [5]. NK cells are

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; BCECF, 20–70-bis(carboxyethyl)-5(6)-carboxyfluorescein; BW, body weight; Ca^{2+}, calcium; CD, cluster of differentiation; CREB, cAMP response element-binding protein; DG, daidzein glucuronide; ERK, extracellular-signal-regulated kinase; E/T, effector to target; EGTE, epigallocatechin-3-gallate fraction of green tea extract; F1, filial 1; FAA, flavone acetic acid; Fc, fragment crystallizable; FDA, Food and Drug Administration; GG, genistein glucuronide; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFNγ, interferon γ; IgG, immunoglobulin G; IL, interleukin; ip, intraperitoneal; iv, intravenous; HDAC, histone deacetylase; HLA, human leukocyte antigen; KIR, killer-cell immunoglobulin-like receptors; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; MICA, major histocompatibility complex, class I-related chain A; MICB, major histocompatibility complex, class I-related chain B; NCAM, neural cell adhesion molecule; NCR, natural cytotoxicity receptor; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NK cell, natural killer cell; NKG2, natural-killer group 2; NKp, NK cell protein; NKT cell, natural killer T cell; P_3IK, phosphatidylinositol-4,5-bisphosphate3-kinase; PBMC, peripheral blood mononuclear cell; PD-1, programmed death; PD-L1, programmed death ligand; ppm, part per million; PE, phycoerythrin; PXD, phenoxodiol; SAMP10, senescence-accelerated mice-prone 10; SCID, severe combined immunodeficiency; TCR, T cell receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; ULBP1, UL16-binding protein 1; ULBP2, UL16-binding protein 2.

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http://dx.doi.org/10.1016/j.jnutbio.2017.01.006
0955-2863/

activated by specific cytokines and in response secrete interferon γ (IFNγ), tumor necrosis factor and granulocyte/macrophage colony-stimulating factor, which then induce inflammatory responses [6]. Importantly, NK cells differ from natural killer T (NKT) cells in number, origin and function. NKT cells express T-cell receptors, in contrast to NK cells, and may promote NK cell function by the release of IFNγ [7]. Interestingly, NK cells are able to act without prior antigen sensitization and their function strongly depends on both activating [e.g., aberrant expression of major histocompatibility complex (MHC) class I by target cells or distinct ligands that are expressed by "stressed" cells] and inhibitory signals (e.g., intact MHC class I expression) [8]. They do not require antibodies or the presence of MHC, also known as human leukocyte antigen (HLA), for their activation. This phenomenon, which is termed "non-MHC-restricted cytotoxicity" or "missing self," seems to be a unique feature of NK cells [8,9].

A number of proteins are secreted by NK cells and facilitate their cytolytic function. Perforin is a cytolytic protein found in the granules of NK cells that forms pores in the plasma membranes of target cells [10]. Granzymes (e.g., granzyme B) belong to the class of serine proteases and are found in the same granules as perforin. Granzymes are released by NK cells and cytotoxic T cells to induce apoptosis in target cells [11].

NK cells show a fast response (within 3–5 days in mice) [12] and are a first line of defense against malignantly transformed cells (especially cancer cells that acquired the capability to evade the immune system *via* down-regulation of MHC molecules) or virus-infected cells [1,13]. Whether or not NK cells are important in the defense against bacterial diseases is still discussed controversially [14–17].

A number of methods are frequently employed to determine NK cell activity *in vitro* and *ex vivo*. The chromium release assay quantifies the release of the mineral from cultured NK cell-target cells that were pretreated with radiolabeled ^{51}Cr. It is the most commonly used assay to determine NK cell activity (specific lysis, lytic units and cytolytic units), even though it has some caveats, such as the use of radioactive compounds and high spontaneous release independent of NK cell activity [18]. NK cell-mediated tumor cell lysis is species-dependently measured against established target cell lines like K562 (human lymphoblast) [19–21] and YAC-1 (murine lymphoblast) [22–24]. Human NK cell activity is commonly determined either in peripheral blood mononuclear cells (PBMCs) [20,21] or directly with NK cells isolated from PBMCs. Splenocytes isolated from excised spleen are mainly used to evaluate murine NK cell activity [13,23,25].

1.2. NK cell surface receptors

NK cells express a multitude of surface receptors (Table 1), including cluster of differentiation (CD) molecules, that determine NK cell behavior. Healthy cells are able to activate inhibitory receptors on the surface of NK cells and thus prevent their NK cell-mediated cell lysis. Stressed or malignantly transformed cells have reduced inhibitory signaling toward NK cells and/or an overexpression of activating signals, which ultimately result in the activation of NK cells, which induces lysis of such target cells.

NK cells are commonly characterized by the absence of the T-cell co-receptor CD3 and in turn expression of CD56. CD56, which is also expressed on other cell types, is also known as neural cell adhesion molecule (NCAM) and important for cell to cell adhesion [26]. The NK cell population can be divided into two major subsets depending on their level of CD56 expression [27]. $CD56^{dim}$ (diminished expression of CD56) NK cells are known for their cytolytic capabilities and account for about 90% of the human NK cell population, whereas $CD56^{bright}$ (strong expression of CD56) NK cells (about 10% of the human NK cell population) mainly perform immunoregulatory tasks *via* cytokine production [6]. CD16 [a low-affinity immunoglobulin G fragment crystallizable (Fc) receptor], is another surface marker, which is linked to the distinct tasks of each NK cell subtype. It is particularly highly expressed on $CD56^{dim}$ NK cells, whereas $CD56^{bright}$ NK cells have either less or absent CD16 expression, depending on their respective function. CD16 can also be found on macrophages, monocytes and neutrophil leukocytes [6,28,29]. Two human isoforms of CD16 are known [29,30]. CD16a (FcγRIIIa) is an activating Fcγ receptor, which enables antibody-dependent cell-mediated cytotoxicity (ADCC), while the corresponding inhibitory receptor (FcγRIIIb) is missing on the NK cell surface [31,32].

One of the most important activating receptors found on NK and T cells is natural-killer group 2 member D (NKG2D) [33,34]. The expression of NKG2D ligands on healthy cells is low, whereas cancer and infected cells often overexpress them. Binding of NKG2D ligands activates NK cells and triggers cytolysis of ligand-expressing cells [33]. NKG2D ligand expression is suggested to be induced, for example, by inhibition of nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) and/or phosphatidylinositol-4,5-bisphosphate 3-kinase (PI_3K) [35,36]. Other receptors activating NK cell-mediated cytolysis belong to the family of natural cytotoxicity receptors (NCRs), such as NKp30, NKp44, NKp46 and NKp80, and have a broad spectrum of (partially unknown) ligands derived from viruses, parasites, bacteria and other cells [27,37].

The killer-cell immunoglobulin-like receptors (KIRs) represent a surface receptor family, which has predominantly inhibitory function (even though a subset of KIR has activating properties). KIRs are highly variable with many different allelic variants resulting in a broad spectrum of KIR haplotypes [38]. They are critical for the detection and elimination of cells with reduced levels of MHC (MHC I specific inhibitory KIR form the molecular basis for the "non-MHC-restricted cytotoxicity") and are capable of recognizing HLA-A, HLA-B and HLA-C (HLA-E is recognized by CD94-NKG2A) [6,38].

Taken together, a large number of activating and inhibitory receptors are found on the surface of NK cells, and cellular activity is tightly regulated by the predominance of either stimulating or repressive signals. Potential target cells are screened by NK cells and only evade NK-mediated cell lysis if they are able to induce sufficient inhibitory NK cell signaling.

1.3. Compounds increasing NK cell activity

A number of endogenous compounds increase NK cell activity by targeting distinct activating receptors on the surface of NK cells and/or

Table 1
Examples for surface receptors that are differentially expressed in the two main NK cell subsets

Marker	$CD56^{bright}$	$CD56^{dim}$
CD56 (NCAM-1)	++	+
CD16 (FcγRIIIa)	+/−	++
Inhibitory receptors		
Inhibitory KIR (2DL, 3DL)	−	+/−
CD94/NKG2A	++	+/−
ILT2	−	+/−
Activating receptors		
NKp30	+	++
NKp46	++	+
DNAM-1	+	++
Cytokine receptors		
IL-2Rα	+	−
IL-2Rβ	++	+
IL-18R1	++	+
Chemokine receptors		
CCR7	++	−
CXCR3	++	+/−
Other molecules		
Perforin	+	++
Granzyme B	+	++

++, strong expression (bright); +, weak expression (dim); +/−, expression only in a subpopulation; −, no expression.
Table modified from Ref. [116].