Shaping of NK cell subsets by aging

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Current Opinion in Immunology

Shaping of NK cell subsets by aging

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NK cells are key players in the innate immune response against virus infection and tumors. Here we describe the current knowledge on age-associated changes in NK cells and the role of persistent CMV infection in configuring NK cell compartment in the elderly. Aging but not CMV causes a redistribution of NK cell subsets as shown by a decrease of CD56bright cells and an increase of CD56−CD16+ NK cells. On the contrary the changes in CD56dimCD16+ NK cells are compatible with the accumulation of CD57+ long-lived NK cells that can also be observed in young CMV-seropositive individuals. NK cell function and dynamics in the elderly will be related not only with age but also with exposure to pathogens, especially CMV.

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Introduction

Natural Killer (NK) cells are members of the recently described innate lymphoid cell (ILC) family. NK cells belong to the group 1 of ILCs and represent the main cytotoxic population [1,2]. NK cells are specialized in destroying virus-infected cells and tumor cells without previous sensitization. NK cell cytotoxicity depends on a fine balance between activating and inhibitory signals transmitted through surface receptors that govern NK cell responses.

NK cells contribute directly to immune defense through their effector functions, as cytotoxicity and cytokine secretion, and indirectly by regulating dendritic cell maturation and function as antigen presenting cells. They use an array of innate receptors to detect changes in the environment and respond to alterations caused by infection, cellular stress and tumor transformation. Several studies have shown that usually no single activating receptor dominates; instead, synergistic signals from different receptors are integrated to activate cytotoxicity and cytokine production by NK cells. NK cells also express inhibitory receptors for Major Histocompatibility Complex (MHC) class I that have a crucial role in regulating NK cell responses. Through a process called NK cell licensing, only NK cells expressing inhibitory receptors for self-MHC class I molecules become functionally competent to be triggered by activating receptor signaling [3,4].

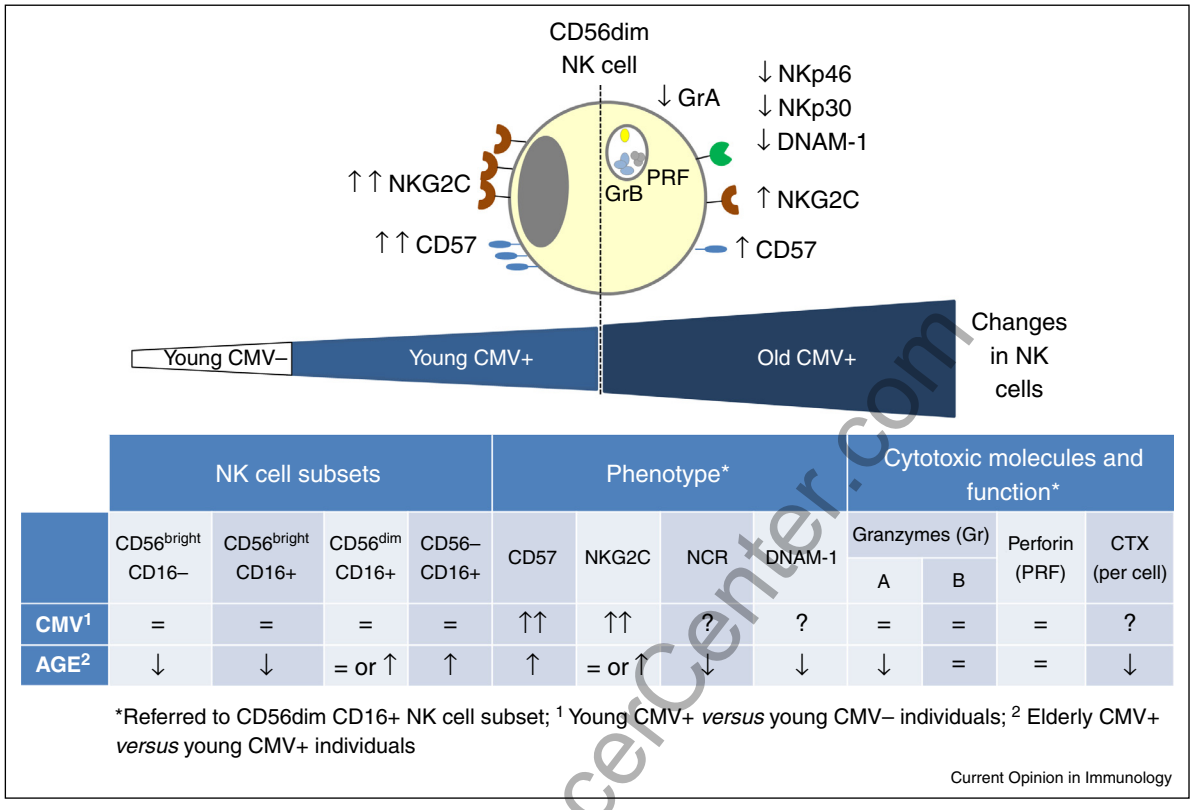
Human NK cells comprise several subpopulations with different phenotype and function, probably representing different stages of NK cell maturation or activation. NK cells can be classified in different subpopulations on the basis of the expression of CD56 and CD16 surface markers. CD56brightCD16− NK cells, that represent less than 10% of peripheral blood NK cells, have an immunemodulator role with high production of cytokines and chemokines in response to cytokines such as interleukin (IL-2), IL-12 or IL-18 (for review see [4]). These cells are particularly abundant in lymph nodes where they can differentiate to CD56dimCD16+ in the presence of inflammatory stimuli. CD56dimCD16+ cells represent the majority (approximately 90%) of peripheral blood NK cells, display high cytotoxic capacity and secrete cytokines as interferon (IFN)-gamma after direct contact with target cells. As CD56dimCD16+ NK cells represent the majority of peripheral blood NK cells most studies on the effect of aging on NK cells refer to these CD56dimCD16+ NK cells, unless specifically indicated. CD56dimCD16+ NK cells have a heterogeneous expression of activating and inhibitory receptors whose expression is affected by exposure to pathogens, especially, but not exclusively, to cytomegalovirus (CMV). Another minor subset of NK cells, characterized by the lack of CD56 and the expression of CD16, is also altered in old individuals [5•].

Aging is associated with changes in the frequency, phenotype and distribution of NK cells subsets and alterations of NK cell function. Given the relevant role of CMV in the generation of long-lived NK cells, in this review we summarize recent data on the effect of aging on the redistribution of NK cell subsets and the possible role of chronic CMV infection in NK cell homeostasis (Figure 1).

Effect of aging on CD56bright NK cells

CD56bright NK cells are immature peripheral blood NK cells with longer telomeres than the CD56dim NK cells [6•,7]. As shown in Figure 2 a linear differentiation pathway from hematopoietic stem cells and NK precursors in the bone marrow to immature CD56bright that leads to more mature CD56dim NK cells in the periphery has

Figure 1



	NK cell subsets				Phenotype*				Cytotoxic molecules and function*			
	$CD56^{bright}$ $CD16-$	$CD56^{bright}$ $CD16+$	$CD56^{dim}$ $CD16+$	$CD56-$ $CD16+$	CD57	NKG2C	NCR	DNAM-1	Granzymes (Gr) A	Granzymes (Gr) B	Perforin (PRF)	CTX (per cell)
CMV[1]	=	=	=	=	↑↑	↑↑	?	?	=	=	=	?
AGE[2]	↓	↓	= or ↑	↑	↑	= or ↑	↓	↓	↓	=	=	↓

*Referred to CD56dim CD16+ NK cell subset; [1] Young CMV+ *versus* young CMV– individuals; [2] Elderly CMV+ *versus* young CMV+ individuals

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Characteristics of NK cells in the elderly: possible role of cytomegalovirus (CMV). A remodeling process of NK cell subsets occurs with aging. A decreased percentage of CD56bright cells and an increase of CD56–CD16+ NK cells are observed in the elderly. By contrast, CMV serostatus does not associate with changes in NK cell subsets defined by CD56 and CD16 expression. Young CMV seropositive (CMV+) individuals show a higher expression of CD57 and NKG2C compared with young CMV seronegative (CMV–) individuals. Within CMV+ donors, a reduction of Natural Cytotoxicity Receptors (NCRs) activating receptors are observed associated to age that probably correlates with the reduced per cell cytotoxicity described in the elderly.

been proposed [8–10]. This model is supported by the recent demonstration that patients with deficiency in GATA2, required for the maintenance of the CD56bright pool, have a severe reduction in peripheral blood NK cells and marked functional impairment [11•]. CD56brightCD16+, representing 30–50% of CD56bright cells, closely resemble CD56brightCD16– NK cells and are considered an intermediate differentiation stage [12].

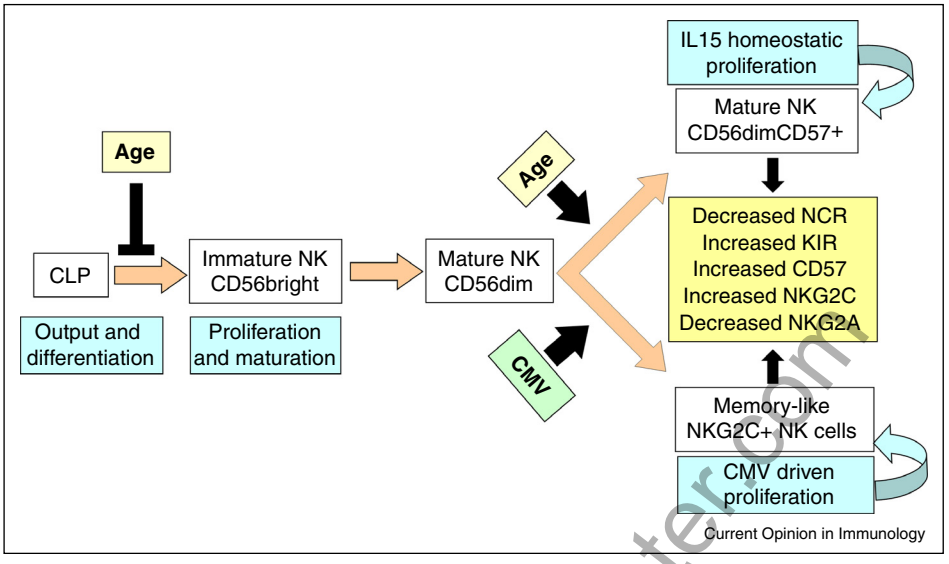
The proportion of peripheral blood CD56bright NK cells in elderly individuals is reduced [13,14] (Figure 1) including CD56brightCD16– and CD56brightCD16+ subsets [5•]. This decrease is likely due to a lower output of new NK cells from the bone marrow as a consequence of the age-associated changes in the number and function of hematopoietic stem cells [15] and the impairment of the production of new NK cell from the bone marrow observed in the elderly [16••].

Considering that these NK cells have a high capacity to produce different cytokines and chemokines in response to cytokines [4], their decreased proportion can be responsible of the defective production of cytokines and chemokines by NK cells stimulated with IL-2 or IL-12 observed in elderly individuals including nonagenarians [17]. On the contrary old individuals show an increased production of IFN-gamma by CD56bright cells, potentially representing compensatory mechanism to maintain the immunoregulatory role of these cells in older individuals [18].

CD56dimCD16+ NK cell homeostasis: effect of aging and CMV infection

In spite of the decrease in the CD56bright NK cell subset, the percentage and absolute numbers of NK cells, most of them CD56dimCD16+, have been reported to be maintained, increased or decreased in the elderly [13,19–23]. Considering the broad range of NK cell percentages in the elderly the reasons for these discrepancies are likely due to the selection criteria of the population analyzed. The number of NK cells in elderly donors depends on intrinsic and extrinsic factors. It has been shown that very old individuals carrying Killer Immunoglobulin Receptor (KIR) A haplotype have higher NK cell numbers and

Figure 2



Proposed model of the effect of aging and CMV infection on NK cell development and differentiation. NK cells develop from bone marrow common lymphoid progenitors (CLP) and differentiate in the periphery where they become functionally competent cytotoxic cells. Aging is associated with a decrease of the production of new immature CD56bright NK cells. In addition there is an increase of the percentage of long-lived CD56dim NK cells expressing CD57, likely as a consequence of cytokine induced homeostatic proliferation. CMV chronic infection is associated with an expansion of a memory-like NK cell subset characterized by the expression of the activating receptor NKG2C and low expression of NKG2A. CD57 can be considered a marker of NK cell maturation toward cytotoxic cells, with low proliferative response to cytokines, decreased levels of Natural Cytotoxicity Receptors (NCRs), high Killer Immunoglobulin-like Receptor (KIR) expression and low levels of NKG2A. Homeostasis of NK cells in the elderly is associated to the expansion of these subsets of NK cells.

percentages compared to KIR B carriers [24•]. However, extrinsic factors as nutrition or health status also influence the percentage of NK cells. For example, an inverse correlation exists between NK cells percentage and body mass index (BMI) [25] and there are also reports showing the association between decreased proportion of NK cells or decreased NK cytotoxicity with increased risk of severe infections or risk of death [26,27].

The effect of aging on the expression of NK cell receptors has been recently reviewed [21] (Figure 1). In brief neither CD16 expression nor CD16 function on NK cells are altered in the elderly [19], whereas the expression of the activating natural cytotoxicity receptors (NCRs) NKp30 and NKp46, and the co-stimulatory molecule DNAX accessory molecule (DNAM)-1 are decreased [28,29,30•]. The analysis of NK inhibitory receptors shows an age-related increase in KIR expression and a decrease in CD94/NKG2A expression [31] although discrepancies can be found in different studies probably due to the high variability of the results and the broad age ranges considered [28,29]. As a consequence, NK cytotoxicity against classic NK cell targets is impaired at the single-cell level but antibody dependent NK cell cytotoxicity is not affected by aging [19].

NK cells, like other cells of the innate immune system, have been classically considered short-lived effector cells. The half-life of human NK cells in healthy young individuals is about 12 days [16••], similar to that found in murine studies [32]. However, the analysis of NK cell homeostasis in the old donors shows decreased production of new NK cells and a maintained number of peripheral blood NK cells, indicating the existence of a high proportion of long-lived NK cells in the elderly [16••]. Although the bases of this observation were unclear, in the last few years studies in mice and humans have challenged the dogma that NK cells are short-lived effectors. Thus, it has been demonstrated that murine NK cells become long-lived cells after ligation of their activating receptor or stimulation by inflammatory cytokines. IL-15 plays a central role in NK cell homeostatic proliferation and subsequent cell longevity in these models [33••,34••,35••]. In addition, during murine CMV infection, naive NK cells generate 'memory-like' NK cells detectable months later (Figure 2). Interestingly, viral induced NK cell proliferation and survival are IL-15 independent but dependent on IL-12 [35••,36••].

Little is known on the factors involved in the generation of long-lived NK cells in humans. Recent evidence has demonstrated that CMV infection is associated with expansion of long-lived 'memory-like' NK cells characterized by the expression of CD94/NKG2C dimmers and CD57 [37••,38]. It has been proposed that during acute CMV infection, the NKG2C+ NK cells expand

[38,39•,40,41••] and acquire CD57, that could be considered a marker of 'memory-like' NK [37••,42••]. In CMV seropositive individuals these cells can be expanded in infections by Hantavirus [43], Chikungunya virus [44] or Hepatitis B and C virus [45] supporting that CMV plays a significant role in this effect.

Human CMV is a persistent beta-herpesvirus which infects all human populations with a variable prevalence. An effective defense against CMV in immune competent subjects requires the participation of NK cells and T lymphocytes. CMV seropositivity depends on geographic, ethnic and social factors and increases with age [46]. It has been shown that CMV chronic infection in old individuals is associated with accumulations of late-differentiated CD8+ T cells, characteristic of CD8T cell immunosenescence, and with the development of an 'Immune Risk Phenotype' (IRP), predictive of early mortality in the elderly [47,48] indicating that this virus is a major driving force of T cell immunosenescence.

The expansion of CD57+CD56dimCD16+ NK cells has been considered a hallmark of NK cell immunosenescence. These cells are defined as NK cells with a mature phenotype, high expression of KIR, low expression of NKG2A, high cytotoxic capacity, decreased sensitivity to cytokines and reduced replicative potential [49]. They constitute a stable NK cell subpopulation, that is absent at birth and increases with age [13,28]. However, this increased CD57 expression is associated to CMV infection rather than to aging as no significant differences are observed between young and old CMV seropositive donors [5•], suggesting that CD57 might be a marker of NK cells expanded in response to CMV infection [37••] and that accumulate with age, to maintain NK cell homeostasis. In addition the possibility that long-lived NK cells can be generated in human by the stimulation of NK cells via NK activating receptors or by cytokines as IL-15, similar to the observations in mice, has to be considered.

Finally aging per se may have a deleterious effect on the functional capacity of these long-lived expanded NK cells expressing CD57. It is well established that NK cells from old donors have shorter telomeres than NK cells from young donors [20,50] although whether this is due to telomere attrition of the stem cell precursor or the result of homeostatic or virus induced proliferation, or a combination of both factors is not known.

Effect of aging on CD56−CD16+ NK cells

An additional minor subpopulation of NK cells characterized by the lack of expression CD56 and the expression of CD16 and other NK receptors can be found in healthy subjects [51] (Figure 1). We have described that the proportion of these CD56−CD16+ NK cells is increased in elderly donors [5•]. An expansion of these NK cells was originally defined in untreated Human Immunodeficiency Virus (HIV)-1 infected patients [52,53] and hepatitis C virus (HCV) infection [54].

Although the function of these cells and their relationship with other NK subsets are still unclear they were defined as dysfunctional NK cells with low replicative and cytotoxic capacities, and reduced capacity to produce cytokines in HIV patients and HCV infection being functionally skewed toward MIP-1beta chemokine production [53,54]. A recent study of these cells in HIV patients confirms these results but shows that they degranulate after interaction with target cells [55]. Phenotypically CD56−CD16+ NK cells expressed high levels of inhibitory NK receptors and retained low level of NCRs or NKG2D compared with that of CD56+CD16+ NK cells [53,54]. The expansion of these cells in HCV infected patients was associated with an impaired ability to respond to antiviral treatment with IFN-alpha and ribavirin [54]. An expansion of this NK cell subset has been found in a fraction of adult patients with CMV replication episodes after umbilical cord blood transplantation [56••], but not in immunocompetent young CMV seropositive donors [5•], suggesting that the impaired ability of some patients to respond to viruses found in clinical situations associated with decreased adaptive immune response (HIV or HCV infection, cord blood transplantation or aging) is associated with the expansion of these dysfunctional NK cells.

Concluding remarks and future prospective

Aging is associated with a gradual loss of the CD56bright NK cell subset, probably due to limited production of its precursors, and with the expansion of highly differentiated mature CD57+CD56dimCD16+ and dysfunctional CD56−CD16+ NK cells. Persistent viral infections as CMV may play a role in these alterations observed on NK cells from elderly individuals. Since the majority of elderly individuals are CMV seropositive, CMV-driven changes on NK cells are difficult to differentiate from those ascribed to aging per se. By the comparative analysis of CMV seropositive and seronegative young individuals, we have shown that CMV seropositivity is associated to the increased expression of NKG2C and CD57 on the CD56dimCD16+NK cell subset. By contrast, changes in other NK cell subsets are not related with CMV serostatus. How this remodeling of NK cell subsets affects the capacity of elderly individuals to respond to different pathogens and cancer and the contribution to the age-associated decline in immune competence have received little attention so far. Further work is required to better understand the mechanisms involved in the decreased output of CD56bright NK cells and the survival of homeostasis expanded or virus induced long-lived NK cells to design protocols to improve NK cell function in the elderly.

In addition future studies aiming to define the molecular basis that permit the stabilization and extension of lifespan of human NK cells independently of age, are of potential interest as they would open new possibilities in NK cell based adoptive immunotherapy. Whereas up to now the use of NK cells in immunotherapy is considered a transient therapy, the possibility to exploit long-lived 'memory-like' NK cells might provide not only long-term protection against leukemia or other tumors but also protection against infectious agents (as CMV) in hematopoietic stem cell and solid organ transplantation.

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