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SYMPOSIUM-IN-WRITING PAPER

Age-related changes in natural killer cell repertoires: impact on NK cell function and immune surveillance

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Abstract A key feature of human natural killer (NK) cells, which enables efficient recognition of infected and malignant target cells, is the expression of HLA class I-specific receptors of the KIR and NKG2 gene families. Cell-to-cell variability in receptor expression leads to the formation of complex NK cell repertoires. As outlined here, NK cells go through major changes from newborns to adults characterized by downregulation of the inhibitory NKG2A receptor and concomitant upregulation of KIR family members. This process is completed in young adults, and in the majority of individuals, KIR/NKG2A repertoires remain remarkably stable until old age. Nonetheless, age-related factors have the potential to majorly influence the complexity of NK cell repertoires: Firstly infection with HCMV is associated with major clonal expansions of terminally differentiated NKG2C- and KIR-expressing NK cells in certain individuals. Secondly, ineffective hematopoiesis can lead to immature and less diversified NK cell repertoires as observed in myelodysplastic syndrome (MDS), a malignant disease of the elderly. Thus, whereas in the majority of elderly the NK cell compartment appears to be highly stable in terms of function and phenotype, in a minority of subjects a breakdown of NK cell repertoire diversity is observed that might influence immune surveillance and healthy aging.

Keywords KIR · NKG2 · Natural killer cell · Aging

Abbreviations

ADCC	Antibody-dependent cellular cytotoxicity
CD	Cluster of differentiation
HCMV	Human cytomegalovirus
HLA	Human leukocyte antigen
HSPC	Hematopoietic stem and progenitor cells
IFNγ	Interferon-γ
IgG	Immunoglobulin G
IL-2	Interleukin-2
IPSS	International prognostic scoring system
KIR	Killer cell immunoglobulin-like receptor
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MIC	MHC class I chain-related protein
MSC	Mesenchymal stem cells
NK	Natural killer
TNFα	Tumor necrose factor-α
ULBP	UL-16-binding proteins

Introduction

The occurrence of cancer is tightly associated with age and, with the exception of certain childhood-specific tumors such as acute lymphoblastic leukemia, neuroblastoma, and low-grade astrocytoma exhibits a significantly increased incidence in the elderly [1]. The contribution of the immune system to this phenomenon is a matter of debate for many years. In particular, the role of T cells in the aging immune system is seen as a critical component in the context of cancer immunosurveillance. It is well documented that the number and frequency of naïve T cells of CD4 and CD8 subsets declines with old age [2]. The age-associated loss

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of naïve T cells is partly due to the fact that progredient thymic involution leads to drastically reduced numbers of newly generated tolerant T cells, which is only partly compensated by homeostatic proliferation of existing naïve T cells [3]. Moreover, naïve T cells in adults are continuously exposed to antigenic challenge by newly encountered pathogens, further driving the depletion of the naïve T cell pool. In particular, the cytotoxic CD8 subset accumulates clonal expansions of the effector/memory type that are long-lived and exhibit markers of terminal differentiation such as the CD57 epitopes. Thus, starting from a maximally diversified T cell receptor repertoire at birth, antigen-specific memory T cells accumulate in an age-dependent way that is driven by an individual's immunological history and ultimately leads to a highly oligoclonal T cell repertoire in the majority of subjects beyond the age of 65 [4]. It appears tempting to speculate that this drastic reduction in complexity of the T cell repertoire also impairs T cell-mediated surveillance for malignant cells presenting tumor-specific antigens. However, longitudinal studies showing that the loss of T cell diversity is associated with increased incidence of cancer or mortality in old people are still lacking.

Together with T cells, NK cells are thought to be an important part of the tumor surveillance system in mammals, which makes them a highly relevant subject for aging research. In contrast to T cells, NK cells do not express any somatically recombined antigen receptors but use germline-encoded receptors to survey cells for proper expression levels of major histocompatibility complex (MHC) class I molecules. In humans this is achieved by clonally distributed expression of human leukocyte antigen (HLA) class I-specific inhibitory receptors encoded by the polymorphic killer cell immunoglobulin-like receptor (KIR) family in conjunction with the more conserved, HLA-E-specific NKG2A receptor [5]. Through recognition of MHC class I downregulation on malignant cells, NK cells close an important loophole in the system that emerges from the fact that adaptive T cell-mediated immunity is MHC-restricted and thus not able to cope with MHC class I-negative cells in an antigen-specific way. The concerted action of T and NK cells seems to provide a very effective way to eliminate aberrant cells including HLA class I-negative tumor escape variants, thereby conferring immune protection even through the extended life span of humans, being one of the most long-lived mammalian species. Nonetheless, the age-associated accumulation of genetic aberrations through intrinsic and extrinsic factors is an inevitable process and eventually leads to a significant increase in tumors in the elderly. We yet do not know whether this entropic process is aggravated by less efficient immune surveillance by an 'aged' NK cell compartment or whether on the opposite a trained and HLA class I-adapted NK cell repertoire is better suited to cope with the increasing incidence of dysregulated cells in an aging body. In this regard it is a fascinating question whether a young or rejuvenated NK cell compartment would lead to a decline of cancer incidence in elderly subjects back to levels observed in the young population? Future studies, for example, following up on adult leukemia patients transplanted with either neonatal or adult stem cells would represent suitable in vivo scenarios to address the question how these patients cope with secondary cancer events in the long run. In any case, it would be highly desirable to define what exactly characterizes a 'healthy aged' NK cell compartment.

Studies of age-related changes in the T cell compartment have defined a so-called immunosenescent phenotype that is characterized by late-stage memory markers, decreased proliferation, impaired effector functions and a profound loss of T cell repertoire diversity in the CD4 and CD8 compartments [6]. The transition is associated with an almost complete conversion of the naïve T cell pool into a memory pool. In a way, the 'hard disk' of the T cell compartment is completely filled with information from previous antigenic challenges with no space left to store information from newly encountered antigens. What do we know about comparable age-related changes/problems in the NK cell compartment and can we define an immunosenescent NK cell phenotype?

NK cell subsets and effector function

So far, only few things were consistently reported to change in an age-dependent context across the many studies analyzing human NK cell phenotype and function. First of all, in contrast to T cells, which are significantly reduced in frequency and number, NK cells exhibit slightly increased frequencies in the elderly in most studies [7–12]. The increase is due to an elevated frequency of the more mature $CD56^{dim}$ subset that is characterized by the expression of clonally distributed KIR and high levels of CD16, which is the low affinity IgG Fc receptor (Table 1). Expression of CD16 which is a key receptor for antibody-dependent cellular cytotoxicity (ADCC) together with higher perforin and granzyme content leads to superior cytotoxic function of this subset. In contrast, the number of immature $CD56^{bright}$ cells is significantly diminished in the elderly [13]. $CD56^{bright}$ NK cells are on the one hand the progenitors of mature $CD56^{dim}$ NK cells and on the other hand are also effector cells that are poorly cytotoxic but efficient in production of cytokines such as interferon-γ (IFNγ) and tumor necrose factor-α (TNFα). In spite of the diminished $CD56^{bright}$ and the increased $CD56^{dim}$ subset, NK cells from old subjects exhibit normal production of IFNγ but slightly reduced cytotoxicity against class I-negative target cell lines [7]. Of note, the perforin and granzyme B content

Table 1 Changes in NK cell subsets during differentiation and aging

	Differentiation				Aging	
	Immature $CD56^{bright}$ NK cells	Mature, naive $CD56^{dim}$ NK cells	Mature, terminally differentiated $CD56^{dim}$ NK cells	References	Age-related changes: young to elderly adults	References
Phenotype						
NKG2A	+++	++	(+)	[15, 16]	→	[7, 12]
KIR	(+)	++	+++	[15–18]	→	[7, 10]
CD57	–	+	+++	[15–18]	↗	[7, 9, 10, 12]
CD62L	+++	++	+	[15, 16, 18]	↘	[18]
NCR	++	++	+	[15, 17, 19, 20]	→	[7, 9]
CD16	–	+++[a]		[21]	→	[7, 9]
NKG2D	+++	+++	+++	[17, 20, 22]	→	[7]
Function						
CD107	+	++	++	[15, 16]	→	[9]
IFNγ	+++	++	+	[15, 16, 18, 23]	→	[7]
Cytotoxicity	–	+	++	[18, 21]	↘	[9]
ADCC	(+)	++	+++	[16]	→	[11]
Proliferation/apoptosis						
Telomere length	++	+[a]		[24]	↘	[25]
Proliferation (CFSE/Ki67)	+++	+	(+)	[10, 15, 17, 18]	→	[10]
Apoptosis (TUNEL)	(+)	+[a]		[10]	→	[10]

[a] No differentiation between naïve and terminally differentiated subset

of mature NK cells from young and old individuals is comparable as is CD107 mobilization [9, 12], the latter being a correlative marker for degranulation of secretory lysosomes [14]. A possible explanation for the less efficient cytotoxicity was suggested by a recent study demonstrating that the release of perforin into the immunological synapse was diminished in elderly subjects [9]. It is unclear in how far the NK cell compartment can compensate the observed reduction in killing efficiency on a per cell basis with the concomitant increase in frequency of $CD56^{dim}$ NK cells in the elderly.

Remarkably, the changes of NK cell subsets in mouse models of aging point into the opposite direction: Several studies have shown that the overall frequency of NK cells decreases in most organs and that mature NK cells, commonly identified as $CD11b^{low}CD27^{high}KLRG1^{+}$ subset, are diminished in aged mice leading to a bias toward more immature $CD11b^{-}CD27^{+}KLRG1^{-}$ NK cells [26, 27]. Beli et al. [26] suggested that the reduction in circulating mature NK cells is due to less efficient maturation and decreased egress of mature NK cells from the bone marrow, which in contrast to other organs did not show reduced NK cell frequencies. It can only be speculated why NK cell subsets in mice and humans are behaving differently during aging. One important aspect besides the obvious differences in life span and the many species-specific differences of the NK cell compartments is the fact that mice are usually kept under pathogen-free conditions and are thus not shaped by immunological history in the same way as humans. Since the accumulation of lymphocytes with memory phenotype is one of the most significant features of the aging human immune system, comparisons with mouse models have to be interpreted with caution. On the other hand, mouse studies enable to observe intrinsic age-related changes of the immune system without the 'noise' of infections, which is obviously not possible in humans. Moreover, the finding of organ-specific differences in the NK cell subsets in mice raises an important concern regarding the current picture of human NK cell aging, which is largely restricted to the analysis of peripheral blood NK cells. We thus do, for example, not know whether the decrease in immature $CD56^{bright}$ NK cells in blood may in fact be due to relocation to other tissues where they might be found at increased frequencies. Thus, systematic studies of NK cell subsets in other immunological organs such as secondary lymphoid organs, liver and bone marrow are needed to get a system-wide understanding of age-related changes in homeostasis of the NK cell compartment.

Repertoire of HLA class I-specific receptors

The specificity of NK cells is majorly guided by the clonally distributed expression of HLA class I-specific receptors with individual clones expressing specific combinations of receptors of the KIR and NKG2 family. Up to date, 15 *KIR* loci have been discovered in the *KIR* region on chromosome 19 resulting in 17 different *KIR* genes [28]. Eight genes display inhibitory (*KIR2DL1-3*, *KIR2DL5A-B*, *KIR3DL1-3*) and six genes stimulatory (*KIR2DS1-5*, *KIR3DS1*) structures, *KIR2DL4* can act both ways, whereas *KIR2DP1* and *KIR3DP1* are pseudogenes. The *KIR* gene family exhibits a remarkably high level of polymorphism; hence, the inter-individual diversity of the NK cell repertoire is majorly influenced by genetic and epigenetic determinants [29, 30]. Specifically, the genomic organization in haplotypes with different *KIR* gene contents (designated as *A* and *B* haplotypes), the copy number and the allelic variation of *KIR* lead to a multiplicity of different *KIR* genotypes. Between 16 (*A*/*A* haplotype) and more than 2000 different KIR combinations (certain *A*/*B* haplotype constellations) are found to be expressed on the NK cells of a given individual depending on the *KIR* genotype [5]. Moreover, clonal expression of *KIR* has to be considered in concert with clonal expression of *NKG2* genes that are part of the killer cell lectin-like receptor family and exhibit both inhibitory (NKG2A) and stimulatory (NKG2C, NKG2CD, NKG2CE, NKG2CF) functions. Except NKG2D, which forms a homodimer, the other NKG2-molecules form a heterodimer with CD94. The non-classical HLA-E serves as a ligand for NKG2A and NKG2C, whereas NKG2D binds to the stress-induced MHC class I chain-related proteins (MIC)-A and (MIC)-B as well as UL-16-binding proteins (ULBP) [31–35].

In terms of target cell specificity, the HLA class I-specific inhibitory members of the KIR and NKG2 families play a dominant functional role. The significance of these receptors is illustrated by the fact that NK cells that lack expression of suitable inhibitory receptors for self-HLA class I, often encompassing >20 % of the NK cell repertoire (Fig. 1b), are in a functionally anergic state irrespective of the expression of other stimulatory receptors. The process, referred to as education or licensing, ensures that only NK cells that are inhibited by autologous HLA class I are able to efficiently eliminate aberrant cells that have downregulated HLA class I expression [36–38]. In contrast to T cells that are efficiently eliminated in the thymus if they do not recognize self-MHC, unlicensed NK cells are thus kept in the repertoire but remain tolerant despite their potential to recognize ‘missing self’ on healthy cells.

Age-related transition from an NKG2A- to a KIR-driven NK cell repertoire

Information on age-related changes in KIR and NKG2 repertoires of NK cells are scarce, especially in children. A decrease in NKG2A expression was reported to occur from young to elderly adults [10] as well as in a second study comparing cord blood to adults [7]. In contrast, an increased frequency of KIR expression was observed from cord blood to adults without further increase in old and very old (60–100 years) individuals [7]. So far, to our knowledge, no studies are available tracking NK cell repertoire changes through all age segments, and consequently, it is presently not known when exactly the observed changes take place. We thus performed a comparative reanalysis of our own published data comprising samples of healthy donors of mostly Caucasoid origin from cord blood ($n = 86$), children ($n = 75$), as well as young and old adults of good health status ($n = 121$) [22, 39–41]. As shown in Fig. 1, in neonatal blood 70 % of NK cells expressed NKG2A with or without co-expression of KIR, whereas only 14 % expressed any inhibitory KIR without NKG2A, demonstrating that neonatal NK cell repertoires are dominated by NKG2A expression [40]. The expression frequency of NKG2A substantially decreased during childhood without significant further downregulation beyond the second decade of life. Reciprocally, KIR expression showed a significant increase during the first decade with a strong increase already in the first 2 years of life. Importantly, no further significant increase in KIR expression was observed in adults, suggesting that the major transitions in NK cell repertoires take place during childhood and puberty. Thus, when considering how many NK cells are either governed by expression of NKG2A (disregarding KIR co-expression) or KIR (NKG2A negative) in adults, both subsets fluctuated in the same frequency corridor of 30–50 % and were not associated with aging. Of note, albeit not significant the highest mean KIR frequency was found in the oldest age segment (71–90 years), which might indicate an increase in very old subjects (Fig. 1a right panel). Clearly, additional studies including detailed general health, vaccination and virus status information are necessary to further substantiate these observations.

Do immunosenescent NK cells accumulate with age?

It is yet unclear whether the observed changes in overall KIR and NKG2A expression in the first two decades of life are an intrinsic, genetically determined process or driven

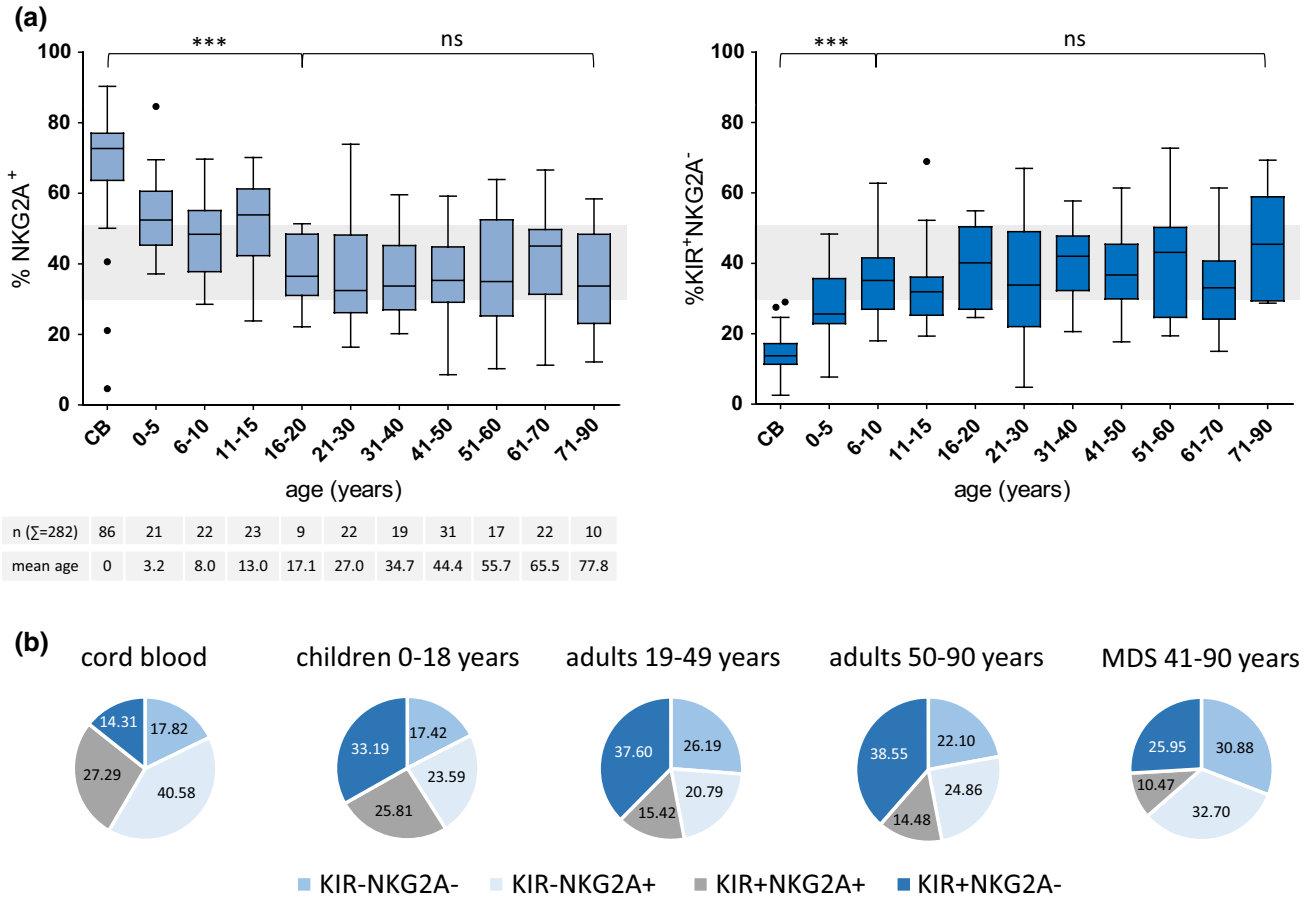

Fig. 1 Age-related expression of KIR and NKG2A on NK cells. **a** Frequency of NKG2A$^+$ (*left panel*) and KIR$^+$NKG2A$^-$ (*right panel*) subsets in different age segments presented as Tukey *box plots* with variability (*whiskers*), outliers (*single dots*) and median (*horizontal line inside the boxes*). A frequency corridor of 30–50 %, encompassing the median of all adult age segments, is indicated in *light gray*. The data represent a comparative reanalysis of previously published sample sets from cord blood [40], pediatric [39] and adult [22, 41] healthy donors. **b** Distribution of the four NK cell subpopulations KIR$^-$NKG2A$^-$ (*mid-blue*), KIR$^-$NKG2A$^+$ (*light-blue*), KIR$^+$NKG2A$^+$ (*gray*) and KIR$^+$NKG2A$^-$ (*dark-blue*) in the indicated age groups compared to MDS patients (data from [22]). Statistical significance was determined using nonparametric one-way ANOVA, ***$p < 0.001$

by immunological history such as pathogen encounters and vaccination. In this regard, it is important to note that NKG2A is preferentially found on naïve CD56^{dim} NK cells, whereas KIR expression without NKG2A is associated with experienced NK cells [15–18]. Compared to KIR$^-$NKG2A$^+$ NK cells, the KIR$^+$NKG2A$^-$ NK cell subset is characterized by a specific expression pattern of markers associated with maturity such as a decrease in CD62L and CD27 as well as an increase in CD57 expression (Table 1) [16, 18]. Similar to T cells, CD57 was already previously defined as marker of replicative senescence and lack of proliferation in NK cells [42]. As shown by Björkström et al., downregulation of NKG2A, acquisition and number of KIR and expression of CD57 correlated independently with terminal differentiation as shown by reduction in proliferation capacity, homing molecules, response to cytokines and expression of activation markers [15]. In terms of effector functions, CD57$^+$ NK cells exhibited a larger cytotoxic capacity and a higher sensitivity to stimulation via CD16 [17]. Thus, NK cell function changes from immature CD56^{bright} and mature but naïve CD56^{dim}CD57$^-$ NK cells produce high amounts of cytokines to experienced and terminally differentiated CD56^{dim}CD57$^+$ NK cells exhibiting strong target cell cytotoxicity and ADCC.

The changes associated with terminal differentiation are compatible with the observed transition in early life from a mostly NKG2A-governed NK cell repertoire toward a more KIR-driven NK cell compartment (Fig. 1). However, this view is not easy to reconcile with the fact that there is no further accumulation of NKG2A$^-$KIR$^+$ NK cells in the elderly: Rather it should be expected that due to the continuous antigenic challenge throughout life, these NK cells would further accumulate in the elderly similar to what is observed in

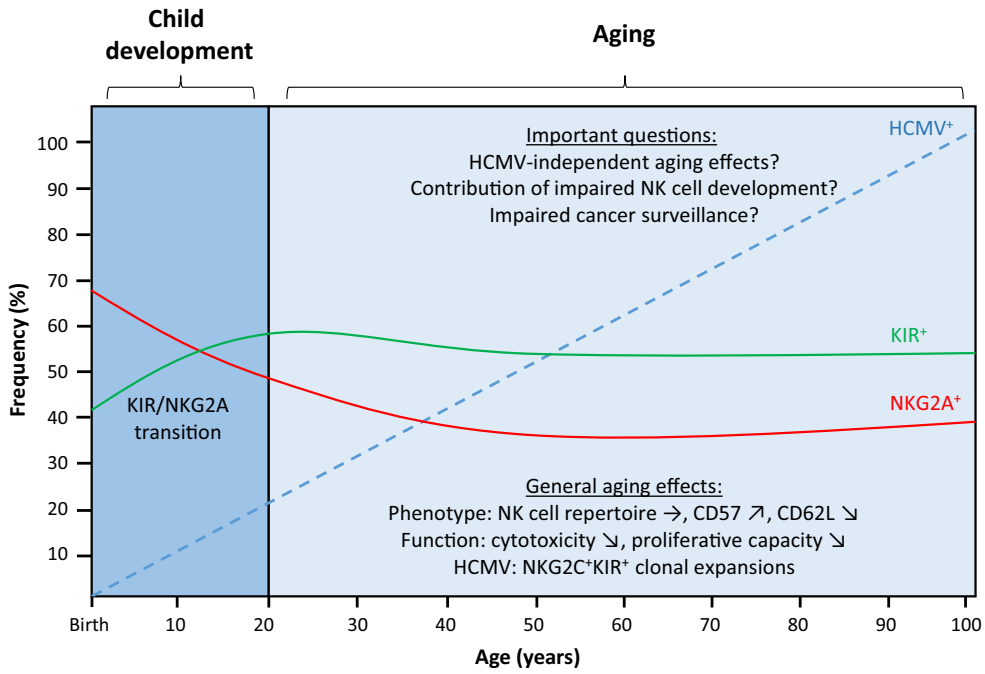

Fig. 2 Impact of aging on NK cell repertoire and function. The diagram summarizes age-dependent changes in the NK cell compartment and highlights important unanswered questions regarding the effect of aging on NK cell function. Changes in the frequency of KIR (*green solid line*) and NKG2A-expressing NK cells (*red solid line*) are primarily occurring during childhood and early adulthood and from there on remain stable in elderly and very old individuals. Nonetheless, in a minority of subjects, the KIR/NKG2A equilibrium is disturbed by HCMV-dependent clonal expansions or immaturity of NK cell repertoires due to impaired NK cell development, as exemplified in MDS patients. The data represent reanalysis of previously described samples [22, 39–41]. The age-related putative increase in HCMV infection is depicted by a *blue broken line*

the T cell compartment, which exhibits a highly oligoclonal repertoire of cytotoxic T cells in the elderly [43]. Furthermore, although it is convincingly shown that the frequency of $CD57^+$ NK cells is increased in the elderly, recent data suggest that the $CD57^+$ increase is associated with human cytomegalovirus (HCMV) infection: As shown by Campos et al., HCMV-seropositive young adults had a similar frequency of $CD57^+$ NK cells compared to $HCMV^+$ elderly adults and a much higher frequency than $HCMV^-$ young adults [12]. Due to the very high rate of HCMV infection in the elderly, there are so far no comparisons with uninfected elderly available. Taken together, the available data are similarly compatible with the view that the NKG2A-to-KIR transition in NK cell repertoires is a genetically determined process that is largely completed in the first two decades of life and does not include any further aging component (Fig. 2). Thus, although the accumulation of senescent NK cells in the elderly would be an intuitive finding, current studies do not conclusively support this expectation. Among the many potentially confounding factors, future studies of age-dependent NK cell senescence should aim at separating the contributions of KIR and HLA class I-based genetic variation as well as the individual immunological history including infection from true aging effects.

What is the role of HCMV in NK cell aging?

As indicated above, the HCMV status has become an important factor of immune aging research. Although HCMV infection is usually associated with mild subclinical symptoms and remains latent in healthy donors, reactivation is observed in the context of other virus infections and immune-compromised situations such as stem cell transplantation leading to severe clinical complications. As reviewed by Pawelec, the aging immune system devotes a remarkably large part of its resources to the control of HCMV latency, in particular with regard to HCMV-specific T cells [44]. In case of NK cells, a similarly fascinating but still enigmatic role emerges for HCMV from a series of recent reports. As initially identified by the group of Lopez-Botet, expression of the stimulatory, HLA-E-specific NKG2C receptor is tightly associated with HCMV infection [45]. In contrast to Ly49H that in mice is a dedicated receptor for the murine CMV-encoded m157 protein [46], NKG2C seems not to directly interact with any HCMV-encoded protein.

HCMV infection is associated with unusual clonal expansions of $NKG2C^+$ NK cells co-expressing KIR, which can make up more than 50 % of the whole NK cell

repertoire [47]. It is currently unknown, why only a minority of donors exhibit such a vigorous response, whereas the majority of infected subjects show either no or only minor expansions of NKG2C^{+} NK cells. Importantly, the signaling machinery of KIR^{+}NKG2C^{bright} NK cells (NKG2C^{dim} NK cells are also found in uninfected subjects) is apparently altered to respond in a primed- or memory-like fashion to further viral challenges by HCMV reactivation or newly encountered viral infections. In this regard, several studies have shown expansion of NKG2C^{bright} NK cells in the course of hantavirus, human immunodeficiency virus, or hepatitis B and C virus infection [48–50]. Of note, no association with aging was found for NKG2C^{+} NK cell frequency in two different studies with [12] or without [7] stratification for HCMV status, albeit the study groups were very small and samples of uninfected elderly were unavailable.

The unusual dominance of KIR^{+}NKG2C^{+} NK cell expansions in some donors is likely to lead to a substantial reduction in KIR repertoire complexity. This in turn could be detrimental for recognition of non-HCMV-related targets and tumor surveillance. On the other hand, reactivation of HCMV following stem cell transplantation for acute myeloid leukemia, a disease of the elderly, was shown to be associated with decreased risk of relapse suggesting a protective effect of HCMV infection in this immunologically compromised setting [51]. However, since the study was performed in patients transplanted with T cell-repleted grafts, it remains unclear whether the virus anti-leukemia effect was due to immune surveillance mechanisms of T cells, NK cells or both. Thus, it remains an open question whether HCMV infection in the long run supports or weakens anti-tumor responses. In any case, HCMV constitutes an important factor that has to be taken into account when analyzing NK cell-based immune aging and cancer surveillance in the elderly.

Failure of NK cell maturation in MDS, a hematopoietic malignancy of the elderly

The replenishment of the NK cell compartment with naïve NK cells and thus the maintenance of a diverse NK cell repertoire depend on a properly functioning NK cell differentiation pathway. Whereas the T cell compartment is majorly affected by the age-associated decline of thymus function leading to an eventual halt in central T cell production in elderly subjects [3], information on age-specific changes in human NK cell development is scarce. In contrast, a decline in mature naïve NK cells is well documented in mouse models of aging, which exhibit an accumulation of immature NK cells [26, 27]. Thus, although differently to mice mature NK cells are generally increased in healthy human aging, the question remains whether NK cell development and the associated stem cell niche are fully functional in the elderly or whether they can potentially fail with age.

Myelodysplastic syndrome (MDS) is the most common hematopoietic malignancy of the elderly (subjects aged >70 years). They constitute a heterogeneous group of clonal bone marrow disorders with defective hematopoiesis and an increased risk of transformation to acute myeloid leukemia [52]. Although originally defined as a disease of the myeloid lineage, our analyses revealed several serious defects within the NK cell compartment [22]. Firstly, the overall NK cell frequency as well as the absolute cell count was significantly reduced in the high-risk subgroup of MDS patients with poor prognosis [as classified by the international prognostic scoring system (IPSS)] compared to age-matched healthy volunteers. Secondly, a significant loss of cytotoxicity in the majority of patients was caused by a reduction in properly 'armed' NK cells and an impaired NK cell compartment since the frequency and absolute cell count of CD56^{dim} NK cells were decreased. And thirdly, the NK cell repertoire was biased toward the poorly cytotoxic KIR^{-}NKG2A^{-}CD56^{dim} NK cell subset (Fig. 1b). The expression of multiple KIR as a hallmark of terminal differentiation was significantly reduced in MDS patients, and KIR expression was mostly restricted to the 'early' receptor KIR2DL2/3. Furthermore, MDS NK cells expressed less CD57 and more of the naïve NK cell marker CD62L. Thus, NK cells of MDS patients clearly exhibit a more immature phenotype than the NK cells of age-matched controls [22]. In essence, the NK cell compartment of MDS patients phenotypically resembled that of cord blood [40]. Similar to the latter, decreased cytotoxicity and loading of cytotoxic granules in the elderly could be restored using in vitro IL-2 stimulation. Importantly, mesenchymal stem cells (MSC) derived from MDS patients exhibited impaired stromal support for myelopoiesis using hematopoietic stem and progenitor cells (HSPC) in vitro [53]. Together, these observations suggest that the hematopoietic stem cell niche is compromised in these patients. Notably, in aged mice NK cell maturation defects are not overcome by transfer of bone marrow from young animals pointing toward a dominant role of the stem cell niche for proper NK cell differentiation in this animal model [54]. Comparison of MSC from young and elderly subjects will be necessary to evaluate whether and how the stromal support for NK cell differentiation changes with age in humans and whether the integrity of the hematopoietic stem cell niche affects efficient tumor surveillance as suggested by the observations in MDS.

Conclusion

The incidence for cancer development is reciprocally associated with the level of NK cell-mediated cytotoxicity [55]. It is thus a highly relevant question how NK cell aging is connected to tumor surveillance in the elderly. Compared to the T cell compartment, the evidence for NK cell aging in a sense of a deteriorating and functionally compromised compartment is scarce. In this context, it is important to remember that NK cells are part of the natural immune system and that in steady-state conditions at least one-half of the complete circulating NK cell pool is exchanged every 2 weeks [10, 56]. The homeostasis of NK cells is thus in between the faster turnover of granulocytes and the slower kinetics of T and B lymphocytes. Analysis by others and us suggests that the NK cell compartment exhibits major changes from neonates to adults characterized by a transition from an NKG2A- to a KIR-dominated NK cell repertoire and a shift in effector functions from more cytokine-driven to more cytotoxic responses. However, compared to the major age-related changes in cytotoxic T cells, NK cell phenotype and function remain remarkably stable in the elderly (Fig. 2). Nonetheless, we suggest that in a minority of elderly subjects a pronounced loss of NK cell repertoire complexity might affect tumor surveillance function. Failure to replenish the naïve NK cell pool due to either inefficient NK cell differentiation or a highly skewed NK cell repertoire due to expansion of virus-specific NK cell clones both might impair NK cell function in elderly. In future studies, it will be important to find out how the differences in NK cell repertoire diversity in the elderly population affect healthy aging and tumor immune surveillance.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. WHO Globocan (2008) Cancer incidence and mortality worldwide. http://globocan.iarc.fr. Accessed 22 April 2015
2. Fulop T, Larbi A, Pawelec G (2013) Human T cell aging and the impact of persistent viral infections. Front Immunol 4:271. doi:10.3389/fimmu.2013.00271
3. Linton PJ, Dorshkind K (2004) Age-related changes in lymphocyte development and function. Nat Immunol 5(2):133–139. doi:10.1038/ni1033
4. Naylor K, Li G, Vallejo AN, Lee WW, Koetz K, Bryl E, Witkowski J, Fulbright J, Weyand CM, Goronzy JJ (2005) The influence of age on T cell generation and TCR diversity. J Immunol 174(11):7446–7452. doi:10.4049/jimmunol.174.11.7446
5. Manser AR, Weinhold S, Uhrberg M (2015) Human KIR repertoires: shaped by genetic diversity and evolution. Immunol Rev 267. doi:10.1111/imr.12316
6. Weng NP (2006) Aging of the immune system: how much can the adaptive immune system adapt? Immunity 24(5):495–499. doi:10.1016/j.immuni.2006.05.001
7. Le Garff-Tavernier M, Beziat V, Decocq J, Siguret V, Gandjbakhch F, Pautas E, Debre P, Merle-Beral H, Vieillard V (2010) Human NK cells display major phenotypic and functional changes over the life span. Aging Cell 9(4):527–535. doi:10.1111/j.1474-9726.2010.00584.x
8. Almeida-Oliveira A, Smith-Carvalho M, Porto LC, Cardoso-Oliveira J, Ribeiro Ados S, Falcao RR, Abdelhay E, Bouzas LF, Thuler LC, Ornellas MH, Diamond HR (2011) Age-related changes in natural killer cell receptors from childhood through old age. Hum Immunol 72(4):319–329. doi:10.1016/j.humimm.2011.01.009
9. Hazeldine J, Hampson P, Lord JM (2012) Reduced release and binding of perforin at the immunological synapse underlies the age-related decline in natural killer cell cytotoxicity. Aging Cell 11(5):751–759. doi:10.1111/j.1474-9726.2012.00839.x
10. Lutz CT, Karapetyan A, Al-Attar A, Shelton BJ, Holt KJ, Tucker JH, Presnell SR (2011) Human NK cells proliferate and die in vivo more rapidly than T cells in healthy young and elderly adults. J Immunol 186(8):4590–4598. doi:10.4049/jimmunol.1002732
11. Lutz CT, Moore MB, Bradley S, Shelton BJ, Lutgendorf SK (2005) Reciprocal age related change in natural killer cell receptors for MHC class I. Mech Ageing Dev 126(6–7):722–731. doi:10.1016/j.mad.2005.01.004
12. Campos C, Pera A, Sanchez-Correa B, Alonso C, Lopez-Fernandez I, Morgado S, Tarazona R, Solana R (2014) Effect of age and CMV on NK cell subpopulations. Exp Gerontol 54:130–137. doi:10.1016/j.exger.2014.01.008
13. Chidrawar SM, Khan N, Chan YL, Nayak L, Moss PA (2006) Ageing is associated with a decline in peripheral blood CD56^{bright} NK cells. Immun Ageing 3:10. doi:10.1186/1742-4933-3-10
14. Uhrberg M (2005) The CD107 mobilization assay: viable isolation and immunotherapeutic potential of tumor-cytolytic NK cells. Leukemia 19(5):707–709. doi:10.1038/sj.leu.2403705
15. Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA, Bjorklund AT, Flodstrom-Tullberg M, Michaelsson J, Rottenberg ME, Guzman CA, Ljunggren HG, Malmberg KJ (2010) Expression patterns of NKG2A, KIR, and CD57 define a process of CD56^{dim} NK-cell differentiation uncoupled from NK-cell education. Blood 116(19):3853–3864. doi:10.1182/blood-2010-04-281675
16. Beziat V, Descours B, Parizot C, Debre P, Vieillard V (2010) NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs. PLoS One 5(8):e11966. doi:10.1371/journal.pone.0011966
17. Lopez-Verges S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, Norris PJ, Nixon DF, Lanier LL (2010) CD57 defines a functionally distinct population of mature NK cells in the human CD56^{dim}CD16 + NK-cell subset. Blood 116(19):3865–3874. doi:10.1182/blood-2010-04-282301
18. Juelke K, Killig M, Thiel A, Dong J, Romagnani C (2009) Education of hyporesponsive NK cells by cytokines. Eur J Immunol 39(9):2548–2555. doi:10.1002/eji.200939307
19. Campos C, Lopez N, Pera A, Gordillo JJ, Hassouneh F, Tarazona R, Solana R (2015) Expression of NKp30, NKp46 and DNAM-1 activating receptors on resting and IL-2 activated NK cells from healthy donors according to CMV-serostatus and age. Biogerontology. doi:10.1007/s10522-015-9581-0
20. Ferlazzo G, Thomas D, Lin SL, Goodman K, Morandi B, Muller WA, Moretta A, Munz C (2004) The abundant NK cells in

human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. J Immunol 172(3):1455–1462. doi:10.4049/jimmunol.172.3.1455
21. Nagler A, Lanier LL, Cwirla S, Phillips JH (1989) Comparative studies of human FcRIII-positive and negative natural killer cells. J Immunol 143(10):3183–3191
22. Hejazi M, Manser AR, Frobel J, Kundgen A, Zhao X, Schonberg K, Germing U, Haas R, Gattermann N, Uhrberg M (2015) Impaired cytotoxicity associated with defective natural killer cell differentiation in myelodysplastic syndromes. Haematologica 100(5):643–652. doi:10.3324/haematol.2014.118679
23. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood 97(10):3146–3151
24. Romagnani C, Juelke K, Falco M, Morandi B, D'Agostino A, Costa R, Ratto G, Forte G, Carrega P, Lui G, Conte R, Strowig T, Moretta A, Munz C, Thiel A, Moretta L, Ferlazzo G (2007) CD56^{bright}CD16- killer Ig-like receptor- NK cells display longer telomeres and acquire features of CD56^{dim} NK cells upon activation. J Immunol 178(8):4947–4955. doi:10.4049/jimmunol.178.8.4947
25. Mariani E, Meneghetti A, Formentini I, Neri S, Cattini L, Ravaglia G, Forti P, Facchini A (2003) Different rates of telomere shortening and telomerase activity reduction in CD8 T and CD16 NK lymphocytes with ageing. Exp Gerontol 38(6):653–659. doi:10.1016/S0531-5565(03)00058-5
26. Beli E, Duriancik DM, Clinthorne JF, Lee T, Kim S, Gardner EM (2014) Natural killer cell development and maturation in aged mice. Mech Ageing Dev 135:33–40. doi:10.1016/j.mad.2013.11.007
27. Shehata HM, Hoebe K, Chougnet CA (2015) The aged nonhematopoietic environment impairs natural killer cell maturation and function. Aging Cell 14(2):191–199. doi:10.1111/acel.12303
28. Parham P, Norman PJ, Abi-Rached L, Guethlein LA (2012) Human-specific evolution of killer cell immunoglobulin-like receptor recognition of major histocompatibility complex class I molecules. Philos Trans R Soc Lond B Biol Sci 367(1590):800–811. doi:10.1098/rstb.2011.0266
29. Uhrberg M (2005) The KIR gene family: life in the fast lane of evolution. Eur J Immunol 35(1):10–15. doi:10.1002/eji.200425743
30. Uhrberg M (2005) Shaping the human NK cell repertoire: an epigenetic glance at KIR gene regulation. Mol Immunol 42(4):471–475. doi:10.1016/j.molimm.2004.07.029
31. Borrego F, Ulbrecht M, Weiss EH, Coligan JE, Brooks AG (1998) Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. J Exp Med 187(5):813–818. doi:10.1084/jem.187.5.813
32. Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, Lanier LL, McMichael AJ (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A. B and C. Nature 391(6669):795–799. doi:10.1038/35869
33. Lee N, Llano M, Carretero M, Ishitani A, Navarro F, Lopez-Botet M, Geraghty DE (1998) HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci USA 95(9):5199–5204. doi:10.1073/pnas.95.9.5199
34. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T (1999) Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 285(5428):727–729. doi:10.1126/science.285.5428.727
35. Cosman D, Mullberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny NJ (2001) ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity 14(2):123–133. doi:10.1016/S1074-7613(01)00095-4
36. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, French AR, Sunwoo JB, Lemieux S, Hansen TH, Yokoyama WM (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 436(7051):709–713. doi:10.1038/nature03847
37. Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH (2005) A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. Blood 105(11):4416–4423. doi:10.1182/blood-2004-08-3156
38. Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, Breso V, Frassati C, Reviron D, Middleton D, Romagne F, Ugolini S, Vivier E (2006) Human NK cell education by inhibitory receptors for MHC class I. Immunity 25(2):331–342. doi:10.1016/j.immuni.2006.06.013
39. Bienemann K, Iouannidou K, Schoenberg K, Krux F, Reuther S, Feyen O, Bienemann K, Schuster F, Uhrberg M, Laws HJ, Borkhardt A (2011) iNKT cell frequency in peripheral blood of Caucasian children and adolescent: the absolute iNKT cell count is stable from birth to adulthood. Scand J Immunol 74(4):406–411. doi:10.1111/j.1365-3083.2011.02591.x
40. Schonberg K, Fischer JC, Kogler G, Uhrberg M (2011) Neonatal NK-cell repertoires are functionally, but not structurally, biased toward recognition of self HLA class I. Blood 117(19):5152–5156. doi:10.1182/blood-2011-02-334441
41. Schonberg K, Sribar M, Enczmann J, Fischer JC, Uhrberg M (2011) Analyses of HLA-C-specific KIR repertoires in donors with group A and B haplotypes suggest a ligand-instructed model of NK cell receptor acquisition. Blood 117(1):98–107. doi:10.1182/blood-2010-03-273656
42. Phillips JH, Lanier LL (1985) A model for the differentiation of human natural killer cells. Studies on the in vitro activation of Leu-11 + granular lymphocytes with a natural killer-sensitive tumor cell, K562. J Exp Med 161(6):1464–1482
43. Nikolich-Zugich J (2008) Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. Nat Rev Immunol 8(7):512–522. doi:10.1038/nri2318
44. Pawelec G, McElhaney JE, Aiello AE, Derhovanessian E (2012) The impact of CMV infection on survival in older humans. Curr Opin Immunol 24(4):507–511. doi:10.1016/j.coi.2012.04.002
45. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M (2004) Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood 104(12):3664–3671. doi:10.1182/blood-2004-05-2058
46. Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL (2002) Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science 296(5571):1323–1326. doi:10.1126/science.1070884
47. Beziat V, Liu LL, Malmberg JA, Ivarsson MA, Sohlberg E, Bjorklund AT, Retiere C, Sverremark-Ekstrom E, Traherne J, Ljungman P, Schaffer M, Price DA, Trowsdale J, Michaelsson J, Ljunggren HG, Malmberg KJ (2013) NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. Blood 121(14):2678–2688. doi:10.1182/blood-2012-10-459545
48. Bjorkstrom NK, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M, Michaelsson J, Malmberg KJ, Klingstrom J, Ahlm C, Ljunggren HG (2010) Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J Exp Med 208(1):13–21. doi:10.1084/jem.20100762
49. Beziat V, Dalgard O, Asselah T, Halfon P, Bedossa P, Boudifa A, Hervier B, Theodorou I, Martinot M, Debre P, Bjorkstrom

NK, Malmberg KJ, Marcellin P, Vieillard V (2012) CMV drives clonal expansion of NKG2C + NK cells expressing self-specific KIRs in chronic hepatitis patients. Eur J Immunol 42(2):447–457. doi:10.1002/eji.201141826

50. Guma M, Cabrera C, Erkizia I, Bofill M, Clotet B, Ruiz L, Lopez-Botet M (2006) Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. J Infect Dis 194(1):38–41. doi:10.1086/504719
51. Elmaagacli AH, Steckel NK, Koldehoff M, Hegerfeldt Y, Trenschel R, Ditschkowski M, Christoph S, Gromke T, Kordelas L, Ottinger HD, Ross RS, Horn PA, Schnittger S, Beelen DW (2011) Early human cytomegalovirus replication after transplantation is associated with a decreased relapse risk: evidence for a putative virus-versus-leukemia effect in acute myeloid leukemia patients. Blood 118(5):1402–1412. doi:10.1182/blood-2010-08-304121
52. Germing U, Aul C, Niemeyer CM, Haas R, Bennett JM (2008) Epidemiology, classification and prognosis of adults and children with myelodysplastic syndromes. Ann Hematol 87(9):691–699. doi:10.1007/s00277-008-0499-3
53. Geyh S, Oz S, Cadeddu RP, Frobel J, Bruckner B, Kundgen A, Fenk R, Bruns I, Zilkens C, Hermsen D, Gattermann N, Kobbe G, Germing U, Lyko F, Haas R, Schroeder T (2013) Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia 27(9):1841–1851. doi:10.1038/leu.2013.193
54. Chiu BC, Martin BE, Stolberg VR, Chensue SW (2013) The host environment is responsible for aging-related functional NK cell deficiency. J Immunol 191(9):4688–4698. doi:10.4049/jimmunol.1301625
55. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K (2000) Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356(9244):1795–1799. doi:10.1016/S0140-6736(00)03231-1
56. Zhang Y, Wallace DL, de Lara CM, Ghattas H, Asquith B, Worth A, Griffin GE, Taylor GP, Tough DF, Beverley PC, Macallan DC (2007) In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection. Immunology 121(2):258–265. doi:10.1111/j.1365-2567.2007.02573.x