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REVIEW

# NK and NKT cell functions in immunosenescence

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## Summary

**Immunosenescence is defined as the state of dysregulated immune function that contributes to the increased susceptibility to infection, cancer and autoimmune diseases observed in old organisms, including humans. However, dysregulations in the immune functions are normally counterbalanced by continuous adaptation of the body to the deteriorations that occur over time. These adaptive changes are likely to occur in healthy human centenarians. Both innate (natural) and adaptive (acquired) immune responses decline with advancing age. Natural killer (NK) and natural killer T (NKT) cells represent the best model to describe innate and adaptive immune response in aging. NK and NKT cell cytotoxicity decreases in aging as well as interferon-γ (IFN-γ) production by both activated cell types. Their innate and acquired immune responses are preserved in very old age. However, NKT cells bearing T-cell receptor (TCR) γδ also display an increased cytotoxicity and IFN-γ production in very old age. This fact suggests that NKT cells bearing TCRγδ are more involved in maintaining innate and adaptive immune response in aging leading to successful aging. The role played by the neuroendocrine–immune network and by nutritional factors, such as zinc, in maintaining NK and NKT cell functions in aging is discussed.**

**Key words: adaptive immunity; aging; cytokine production; cytotoxicity; innate immunity; NK cell; NKT cells, successful aging.**

## Introduction

Immunosenescence is defined as the state of dysregulated immune function that contributes to the increased susceptibility to infection, cancer and autoimmune diseases observed in old organisms, including humans (Pawelec, 1999). Dysregulation in immune function is normally counterbalanced by a continuous adaptation of the body to the deterioration that occurs over time, leading to both loss and gain in the organism's resources. These adaptive changes occur in healthy human centenarians, who represent the best living example of successful aging (Paolisso *et al*., 2000).

Both innate (natural) and adaptive (acquired) components of the immune system undergo significant age-related changes (Pawelec, 1999). In this context, recent advances have been focused on natural killer (NK) and natural killer T (NKT) cells. The former, together with polymorphonuclear leucocytes (PMNs), dendritic cells (DCs), macrophages and the complement system, support innate immunity, which represents the first line of defence against pathogens. By contrast, NKT cells have been suggested as a bridge between the innate resource and the adaptive system (Taniguchi *et al*., 2003b).

The phylogenetically ancient defence mechanism, namely the innate immune response, has been considered for a long time as a separate entity from the adaptive immune response and has been regarded as of less importance in the hierarchy of immune functions. For the past few years, however, interest in innate immunity has grown enormously, based on the knowledge of its integration with specific immune effectors and its key role in stimulating the subsequent response of adaptive immunity. The cells with cytotoxic activity (NK and NKT cells), as well as their direct killing of target cells, also play a pivotal role in providing signals that are required to drive the adaptive immune response. Age-related alterations of the cellular components of innate immunity might therefore be involved in the impairment of the adaptive immunity observed in the elderly. In this short review, we report on the role played by NK and NKT cells during aging and the changes that lead to healthy aging and longevity.

## NK cell biology

Human NK cells are a population of heterogeneous lymphocytes that provide a critical complement of the innate immune response against a broad variety of infections and tumours. NK cells were originally identified as a population of large granular lymphocytes (LGLs) able to lyse target cells spontaneously, such as virus-infected cells or certain susceptible tumour cell lines, without presensitization or MHC restriction. NK cells derive from pluripotent haematopoietic stem cells. T and NK-cell lineages share a common precursor expressing FcγRIII. Whereas T progenitors are transferred into the thymus for differentiation and maturation, NK cells can develop independently of the thymus, as shown in nude (athymic) mice (Budzynski & Radzikowski, 1994). Moreover, NK precursor cells can differentiate and proliferate in the

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bone marrow under the stimulation of growth factors by mechanisms that are still not completely understood. It has been suggested that co-operative stimulation by interleukin-2 (IL-2), IL-15 and, more recently, also by IL-21, most likely produced by the bone marrow stromal cells, could play a key role in NK cell differentiation and maturation (Toomey *et al*., 2003).

NK cells do not rearrange immunoglobulin (Ig) or T-cell receptor (TCR) genes and therefore neither Ig nor the TCR/CD3 complex is expressed at the cell surface, except for the zeta (ζ) chain (Lanier, 1998).

Mature NK cells are characterized by the expression of peculiar markers such as CD56, an isoform of the neural cell adhesion molecule (N-CAM), CD16, the low-affinity IgG Fc receptor (FcRγIIIA), CD57, an oligosaccharide antigenic determinant, and CD2, an adhesion molecule that appears to be correlated with the acquisition of fas ligand-mediated cytotoxicity (Nakazawa *et al*., 1997).

The lysis of target cells by NK cells involves a secretory and a non-secretory mechanism. The secretory mechanism occurs by regulated secretion of specialized lysosomes, which induces the rapid apoptosis of target cells (Griffiths, 2003). These lysosomes contain the pore-forming protein perforins, which damage cellular membrane and are required to deliver to the target cells the serine proteases granzyme B and H, which in turn initiate the apoptotic cascade by cleaving substrates in the cytosol of the target cells. The non-secretory mechanisms involve the binding of tumour necrosis factor (TNF) receptor-like molecules, such as Fas/CD95, with their respective ligands (FasL). This results in the activation of pro-apoptotic molecules (e.g. caspase-3) and the subsequent induction of programmed cell-death pathways (Trapani, 1998).

NK cytotoxic function is regulated by activating and inhibitory cell surface receptors that permit NK cells to recognize specifically tumours and virus-infected cells and to sense the levels of MHC class I on prospective target cells in order to prevent unwanted destruction of the target cells. Both activating and inhibitory receptors can transduce, respectively, positive or negative signals to regulate NK cell cytotoxicity and cytokine release. All the inhibitory receptors [of the killer-cell immunoglobulin like receptors (KIR) family, such as p58, p70, p140, CD94/NKG2A, Ly-49] recognize MHC class I molecules and contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domains that recruit SH2 domain-containing protein-tyrosine phosphatases, resulting in inhibition of cytotoxicity and cytokine secretion by NK cells (Lanier, 1998). By contrast, activating NK receptors, such as CD16, CD2 and p46, which transduce the signal via the ζ chain, and CD94/NKG2C, NKp44 and the HLA-specific activation receptor p50, which transduce the signal via DAP12, trigger the NK cell cytotoxicity of target cells (Sun, 2003). Other NK receptors that trigger NK cytolysis are CD69 and NKRP1 (Lanier, 1998).

NK cells express several cytokines and chemokine receptors that modulate their cytotoxic ability and, at the same time, secrete immunoregulatory cytokines and chemokines, thus playing an intriguing role in the initiation of the adaptive immune response.

It has been shown that cytokines, such as IL-2, IL-12, IL-15, IL-18 and IFN-α/β, promote proliferation and activation, increase the migratory response, enhance cytotoxicity of NK cells and induce them to produce IFN-γ, TNF-α and GM-CSF (for review see Solana & Mariani, 2000; Farag *et al*., 2003).

NK cell activation and migration are also observed with chemokines, such as MIP-1α, MIP-1β, MCP-1,2,3, RANTES. Some chemokine receptors (CCR2, CCR5) expressed in NK cells favour NK cell migration as well as NK-target cell binding and NK recruitment (Kim *et al*., 1999). These immunoregulatory cytokines and chemokines will subsequently modulate the adaptive response mediated by Th lymphocytes, suggesting that the broad range of NK cell activators and responses provide an efficient early innate immune response against infections and tumours promoting the downstream adaptive response. Alterations of innate immunity mediated by NK cells may promote impairments in adaptive immunity occurring in aging with the appearance of age-related diseases, such as infections and tumours.

## NK cell function and aging

Several alterations have been described with advancing aging both in animals and humans at the level of NK cell number and function. In rodents, a peak value of basal NK cell activity is observed at the age of 5–8 weeks, whereas nearly undetectable levels are present in 25-month-old animals (Albright & Albright, 1983). In old humans, contradictory data exist due mainly to the different selection criteria of the elderly populations studied. NK cell cytotoxicity is not significantly affected by aging using strict selection criteria, such as the very healthy elderly (SENIEUR protocol; Kutza & Murasko, 1994). In other studies, using the same strict selection criteria, circulating NK cells from elderly subjects display decreased cytotoxic function both after sorting freshly isolated cells and after limiting dilution and cloning of NK cell precursors (Mariani *et al*., 1990). The decrement is more evident in old subjects aged between 75 and 85 years. Indeed, old subjects at this age range who met the criteria of the SENIEUR protocol showed that the only immune parameter to correlate with a history of severe infections or subsequent death due to infection was absolute NK cell cytotoxicity (Ogata *et al*., 1997). This finding was recently confirmed in a group of elderly subjects 75–85 years of age presenting with a high risk of mortality by infections in the subsequent 2 years of observation, despite the original strict selection criteria (Mocchegiani *et al*., 2003). Therefore, the decrement in NK cell cytotoxicity is dependent on the age of the old subject rather than on the strict selection criteria. Moreover, the impaired NK cell cytotoxicity represents a high risk factor for the appearance of infections in advanced age. Studies in nonagenarian/centenarians showed an NK cell cytotoxicity that was similar in old subjects (aged 60–65 years) but higher than that in slightly older subjects (75–85 years) (Sansoni *et al*., 1992; Mariani *et al*., 1999; Mocchegiani *et al*., 2003). This suggests that preservation of NK cell cytotoxicity in middle age may be a crucial immune function in order to escape some age-related diseases and to reach healthy

aging. Such preservation in the healthy elderly may be due to increased numbers of NK cells in order to compensate for a possible decrement of NK cell cytotoxicity. This increased number has been related to the higher number of $CD56^{dim}$ rather than $CD56^{bright}$ subsets (Borrego *et al*., 1999). As the $CD56^{dim}$ represents the mature NK cell subset, a phenotypic and functional shift in the maturity status of NK cells occurs in aging (Krishnaraj, 1997). However, such a shift might be irrelevant because it is important to maintain the $CD56^{bright}/CD56^{dim}$ ratio. It is not simply a coincidence that this ratio is maintained in human centenarians (E. Mocchegiani, unpubl. observ.). Moreover, the preserved innate immune response in older people may be due to the negligible telomere shortening and the maintained telomerase expression in NK cells, which could allow the NK cells of nonagenarians to delay replicative senescence (Mariani *et al*., 2003). However, the problem is not that simple and becomes problematic in aging because NK cell activity is controlled by cytokines and chemokines, which are altered in aging (Rink *et al*., 1998; Mariani *et al*., 2002). In addition, activated NK cells produce cytokines and chemokines for the adaptive immune response, which is in turn decreased in old age (Solana & Mariani, 2000). It has been reported that NK cell cytotoxicity is preserved in old mice after IL-2 stimulation, whereas the responsiveness to IFN is reduced (Provinciali *et al*., 1989). The situation is different in old humans. The *in vitro* activation of NK cells from old donors with IL-2 enhances killing of NK-sensitive targets (e.g. K562 cells) and induces killing of NK-resistant target cells (e.g. the Daudi cell line). The cytotoxic activity of NK cells by activation with IL-12 or IFN-α and IFN-γ is also well preserved in the healthy elderly (Krishnaraj, 1997). However, the capacity of these last cytokine-activated killer cells to lyse the NK-resistant Daudi cell line is decreased in the elderly as compared with young people (Kutza & Murasko, 1994). These last results indicate that, in general, NK cells from elderly donors are defective in their response to cytokines with a subsequent limited capacity to develop lymphokine activated killer (LAK) cells able to kill NK-resistant cell lines (Solana & Mariani, 2000). The cause of this defective response in old age may be related to a lack of cytokine receptors on NK cells (Krishnaraj, 1997) or to decreased production within the NK cells of perforin granules or granzymes (B and H), which are deputed to DNA degradation of NK target cells (Trapani & Smyth, 2002).

Although no significant decrement of perforin molecules in NK cells from young and old donors has been reported (Mariani *et al*., 1996), an age-related decline of perforin expression has been well documented in the elderly; however, this is significantly greater in elderly men than in elderly women (Rukavina *et al*., 1998). Moreover, the ability of NK cells to transduce signals that are normally mediated by negative modulators (i.e. ATP-induced suppression of NK cytolytic activity) is reduced to a greater degree in old females with a higher level of basal NK cell cytotoxicity (Krishnaraj, 1992). These two factors also raise the question of possible biological reasons for the existence of a greater number of women centenarians than men, despite other gender-intrinsic and environmental genetic differences obviously involved in healthy aging (IMUSCE study; Franceschi *et al*., 2000). However, immunological changes associated with senescence are particularly complicated when the profile of cytokine production by NK cells is considered. One of the main functions of NK cells in defence against viral and bacterial infections is the capacity to synthesize and release some cytokines, especially IFN-γ and TNF-α, that participate in the initiation of the Th1-dependent adaptive immune response. During aging, cytokine (IL-2, IL-12, IFN-α and IFN-γ) production, involved in activating NK cells, is reduced (Rink *et al*., 1998). Consequently, their production by activated NK cells also decreases, leading to an impaired adaptive immune response and to the possible appearance of age-related diseases. The production of IFN-γ by NK cells in the elderly is also reduced after stimulation with rIL-2, as compared with values observed in young individuals either in short- (18 h) or in long-term (7 days) cultures (Krishnaraj & Bhooma, 1996). Treatments with IL-12, however, seem more effective in inducing NK cell IFN-γ production in the elderly (Argentati *et al*., 2000) as well as the release of some chemokines associated with inflammatory processes (MP1-α, RANTES and IL-8) (Mariani *et al*., 2002). However, the total amount of both cytokines and chemokines is about the half that obtained from young NK cells (Mariani *et al*., 2002). Therefore, the production of cytokines and chemokines by NK cells is always low, or at least not sufficient for a normal adaptive immune response in the elderly, despite the activation by IL-2 or IL-12. Chronic treatment with IL-2 only develops LAK cell cytotoxicity and increases IFN-γ production, as observed in cancer (Provinciali *et al*., 1998). Toxicity of chronic IL-2 treatment is well known for its unexpected harmful side-effects, such as haemorrhagic shock (Schwartz *et al*., 2002). Therefore, the problem is very complicated in the elderly regarding possible corrections of NK cell cytotoxicity via cytokine stimulation or treatment. However, the problem may be circumvented with hormonal treatments or dietary interventions.

Without excluding nutritional factors reported in restoring innate immunity in the elderly (Chandra, 2002), the trace element zinc plays a pivotal role in the efficiency of the neuroendocrine–immune network in aging (Mocchegiani *et al*., 2004b). It has been reported that the neuroendocrine–immune pathway is relevant in immunosenescence (Straub *et al*., 2001). Some hormones of the hypothalamic–pituitary–gonadal axis (FSH, GnRH, ACTH, TSH, GH, PRL, testosterone and progesterone) as well as thyroid hormones (T3 and T4), DHEA, IGF-1, melatonin and insulin affect the innate immune response as a result of the presence of respective hormone receptors on immune cells, including NK cells. NK cells activated by hormones secrete cytokines, thereby affecting the adaptive immune response. This pathway with autocrine and paracrine mechanisms is necessary in order to counteract stressful conditions and antigenic insults (see review by Mocchegiani *et al*., 2004b). A deficient production of these hormones leads to impaired innate and adaptive immune response in aging. Hormonal treatments (T3, T4, melatonin, GH, IGF-1) in old mice restore NK cell cytotoxicity, and IL-2 and IFN-γ production (Mocchegiani *et al*., 2004a,b). However, it is noteworthy that the beneficial effects of T3, T4, GH, IGF-1 and melatonin are also mediated by a restoration of

the zinc pool because of the presence of specific hormone receptors on the gut allowing better intestinal absorption of zinc (Mocchegiani *et al*., 2004a,b). Therefore, the nutritional factor zinc is pivotal in maintaining the neuroendocrine–immune pathway, as well as directly affecting multiple aspects of the immune system (including cytokines of the Th1 response). In this context, several studies in old animals and humans describe decreased NK cell activity related to a zinc deficiency status (Mocchegiani *et al*., 1998). *In vitro* (1 μM) and *in vivo* zinc treatments (12 mg $Zn^{2+}$/day) for short periods induce complete recovery of NK cell cytotoxicity both in old mice and in humans (Mocchegiani *et al*., 1995, 2003). In addition, zinc treatment at physiological doses for 1 month in old infected patients, as well as increasing NK cell cytotoxicity, rescues IFN-γ production, leading to a reduction of 50% in infection relapses (Mocchegiani *et al*., 2003). This clearly indicates restoration of the adaptive immune response. Findings in centenarian subjects and in very old mice confirm the relevance of zinc in restoring NK cell function in the elderly. Indeed, they have a well-preserved NK cell cytotoxicity, good zinc ion bioavailability and satisfactory IFN-γ production (Miyaji *et al*., 2000; Mocchegiani *et al*., 2003) as well as preserved thyroid hormone turnover (Mariani *et al*., 1999). This last finding also supports, as described above, a sufficient maintenance of the neuroendocrine–immune network, via the zinc pool, in older individuals, contributing to avoidance of some age-related diseases. Because zinc turnover is mediated by metallothioneins, these proteins are the key to understanding the role of NK functions in aging and longevity (Mocchegiani *et al*., 2000).

## NKT cell biology

NKT cells represent a unique and heterogeneous T-cell population that shares some functional and phenotypical characteristics with NK cells (Emoto & Kaufmann, 2003).

The coexpression of the NK receptors NK1.1 (a type II membrane glycoprotein encoded by a member of the NKRP1 gene family) and of TCRs is commonly used to define NKT cells, but recent studies have shown that some NKT cells lack NK1.1 expression (Pellicci *et al*., 2002). In fact, NK1.1 expression depends on maturity, activation state, tissue location and expression of other receptors. Although a precise definition of NKT cells remains problematic, these cells are commonly divided into two groups: 'classical' and 'non-classical'. 'Classical' NKT cells are CD1d-restricted, express $CD4^+$ or are double negative (DN) and have a strong response to the glycosphingolipid α-galactosyl ceramide (α-GalCer). 'Non-classical' NKT cells are CD1d-unrestricted (the majority depend on classical MHC class I), and express CD8a, but not CD8b, or are DN (Emoto & Kaufman, 2003). Taking into account that some NKT cells do not express the NK1.1 receptor, the specificity of α-GalCer for classical NKT cells is the most reliable tool for testing 'classical' NKT cell function (Taniguchi *et al*., 2003a). Both 'classical' and 'non-classical' NKT cells express TCRαβ, whereas the expression of TCRγδ has been attributed only to 'non-classical' NKT cells (Emoto & Kaufmann, 2003). NKT cells bearing TCRαβ can express different kinds of TCRs. However, the best known category of these cells, referred to as Vα14i in mice and Vα24i in humans, use Vβ8.2 (Vβ11 in humans), Vβ7 or Vβ2 gene products and the invariant complementarity-determining region Vα14i (Vα24i in humans) for their TCRα/β rearrangement (Bendelac *et al*., 1997; Biron & Brossay, 2001). By contrast, 'non-classical' NKT cells bearing TCRγδ use Vγ2/Vδ6 or Vγ9/Vδ2 gene products for their TCRγδ rearrangement, in mice (Ohteki & MacDonald, 1994) and in humans (Poccia *et al*., 1998), respectively.

NKT cells have been found in the thymus, liver, spleen and bone morrow. Despite the existence of a thymus-independent differentiation pathway located in the liver for the NKT cell lineage, as demonstrated in athymic nude mice, the thymus seems to be a site for NKT development, and the liver for NKT homing (Emoto & Kaufmann, 2003).

As reported for NK cells, the same lytic mechanisms have been observed in NKT cells, but it seems that the Fas/Fas ligand is the preferred system (Inui *et al*., 2002). Moreover, NKT cells stimulated with anti-TCR or anti-CD3 produce IL-4, whereas, through the NKR-P1 molecule (CD161), they induce the production of IFN-γ. The production of IL-4 and IFN-γ occurs simultaneously, and therefore these cells may be estimated as Th0 (Abo *et al*., 2000). By analogy with NK cells, the secretion of cytokines by NKT is also induced by IL-12 (Biron & Brossay, 2001). Several effector functions, originally attributed to NK cells, have been recently ascribed to NKT cells and vice versa, suggesting that NK and NKT cells are functionally linked *in vivo*. Indeed, through α-GalCer stimulation, it has been shown that the communication between NK and NKT cells is mediated by IFN-γ, which is produced by activated NKT cells, despite the involvement of additional cytokines, and/or surface receptors required in order to achieve a more satisfactory activation of NK cells (Biron & Brossay, 2001).

## NKT cell functions and aging

Although the thymus is the site for NKT cell maturation, differentiation and their TCR rearrangement (Pear *et al*., 2004), these cells may directly migrate from the bone marrow to extrathymic sites (liver) for their development (Abo *et al*., 2000). Indeed, the liver contains c-kit+ pluripotent stem cells, which give rise to multiple cell lineages, including NKT cells (Watanabe *et al*., 1996). NKT cells, also developed within the thymus, migrate to the liver (Emoto & Kaufmann, 2003). Therefore, the liver is a focus for immune surveillance by NKT cells. However, nude (athymic) and thymectomized mice display a large concentration of NKT cells in the liver with a good performance of innate and adaptive immunity (Abo *et al*., 2000). In addition during aging, the thymus is atrophic. Thus, the extrathymic function of the liver becomes prominent in order to compensate for thymic failure during aging (Abo *et al*., 2000).

It has been reported that the number of liver NKT cells with invariant Vα14 in mice and Vα24 in humans decreases in old age, coupled with their impaired cytotoxicity and cytokine production (Tsukahara *et al*., 1997; DelaRosa *et al*., 2002). Because

NKT cells may be 'classical' or 'non-classical', strictly dependent on whether they are CD1d restricted or unrestricted, respectively (Emoto & Kaufman, 2003), the NKT cells mainly studied in this last decade are those classified as 'classical', bearing TCRαβ and being CD1d restricted. Under stimulation with IL-12, 'classical' NKT cells are cytotoxic and play an important immunoregulatory role through the production of Th1 (IFN-γ) and Th2 (IL-4) cytokines (Emoto & Kaufmann, 2003). A dysregulation in IL-4 production by classical NKT cells leads to pathology, as occurs during chronic inflammation and autoimmune diseases (Araujo *et al*., 2004). However, the main task of 'classical' NKT cells is to produce IFN-γ with a pivotal role in the anti-tumour cytotoxic response (Cui *et al*., 1997). In old mice, 'classical' NKT cells bearing TCRαβ display low cytotoxicity and impaired IFN-γ production both in basal conditions and after IL-12 stimulation (Mocchegiani *et al*., 2004a,b). This phenomenon also occurs in old humans, especially when referring to $CD57^+$ T cells expressing more Vα24 invariance compared with $CD57^-$ T cells (Miyaji *et al*., 2000). Other than the reduced cytotoxicity, perhaps due to a diminished number of perforin granules (Watanabe *et al*., 1995), the impaired IFN-γ production by NKT cells leads also to inefficient NK cell cytotoxicity because NKT cell response can 'cross-talk' to activate NK cells (Fig. 1A,B) (Biron & Brossay, 2001). This pathway linking NKT and NK cell functions is important under conditions of low innate cytokine induction (i.e of IL-12 and/or IFNα/β) during viral or bacterial infections. For this reason, NKT cells are considered the first sentinels for host defence from early life (Emoto & Kaufmann, 2003). The lack of this peculiar NKT cell function, as occurs in aging, provokes a cascade of decreased innate and adaptive immune responses with the subsequent appearance of age-related diseases (Fig. 1B). Indeed, very old mice and human centenarians display a satisfactory number of 'classical' NKT cells bearing TCRαβ and $CD57^+$ T cells as well as good performances regarding cytotoxicity and INF-γ production (Fig. 1A) (Miyaji *et al*., 2000; Mocchegiani *et al*., 2004a,b). In this context, liver NKT cells bearing TCRγδ also play a peculiar role. This NKT cell subset is classified as 'non-classical' because it is CD1d unrestricted, expresses neither CD4 nor CD8 (i.e is DN), expresses the NK1.1 marker, displays cytotoxicity and produces IFN-γ but not IL-4 after IL-12 stimulation (Emoto & Kaufman, 2003). In newborn mice, the NKT cells bearing TCRγδ increase numerically before those bearing TCRαβ, represent a substantial population in the liver in early life (Emoto *et al*., 2001) and remain in the liver throughout later life (Watanabe *et al*., 1995). Thus in the liver the TCRγδ cells predominate with respect to NKT cells bearing TCRαβ, the latter tending to be of thymic origin (Tsukahara *et al*., 1997). Although the number of liver NKT cells bearing TCRγδ is limited (2–4% of total NKT cells), they play a relevant role in host defence from early life (Emoto *et al*., 2001). Consistent data exist supporting the role played by the γδT cells during ontogeny and aging and for their particular importance in human centenarians. It has been reported that γδT cells (in particular the Vδ2 subset), being DN cells, display MHC-unrestricted cytotoxicity and produce IFN-γ under stimulation (Argentati *et al*., 2002). Their functions decrease in aging; however, they are preserved in human centenarians because they present the activation marker CD69 and do not undergo apoptosis via Fas (Colonna-Romano *et al*., 2002). With regard to liver NKT cells bearing TCRγδ, the role played by these cells in aging and successful aging, in particular in old and very old mice during the circadian cycle, has been recently reported for the first time. It has been shown that the number, cytotoxicity and IFN-γ production by liver NKT cells bearing TCRγδ decrease in old mice, but all their functions are preserved and increased in very old mice. The increment is evident during the dark period of the circadian cycle (Mocchegiani *et al*., 2004a,b). By contrast, the functions of liver NKT cells bearing TCRαβ are still preserved in very old mice, but they do not increase during the dark with respect to liver NKT cells bearing TCRγδ (Mocchegiani *et al*., 2004a). Although further studies are required in humans, these findings clearly demonstrate that liver NKT cells bearing TCRγδ are more capable than TCRαβ cells of remodelling innate and adaptive immune response in aging, leading to successful aging. As reported for NK cell function, the mechanism involved in the remodelling of liver NKT cell function in very old age is strictly related to zinc ion bioavailability, via metallothioneins homeostatasis (Fig. 1A,B) (Mocchegiani *et al*., 2004a).

## Concluding remarks and future directions

Immunosenescence, the deterioration of the immune response associated with aging, is characterized not only by a defective T-cell response, but also by changes in the number and function of other cells of the innate immune system. NK and NKT cells play a significant role in host defence against infections and tumours as a consequence of both their cytotoxic capacity and the cytokines produced, in particular IFN-γ. With regard to NK cells, despite their increased numbers in aging, cytotoxicity is impaired as is the production of cytokines and chemokines by activated NK cells. This leads to an impaired adaptive immune response and the subsequent appearance of age-related diseases. The causes of decreased NK innate immunity may be related to telomere shortening, reduced perforin granule production, diminished transduction of negative signals, an impaired neuroendocrine–immune network and a lack of zinc ion bioavailability. The restoration of NK cell cytotoxicity in the elderly and reduction of 50% in infection relapses in old infected patients after zinc supplementation may be consistent with this last interpretation. With regard to NKT cell functions, the different TCR rearrangement and the immunological site of their possible development, such as the liver, need to be considered. Liver NKT cells bearing TCRαβ or γδ can be classified as 'classical' or 'non-classical' dependent on whether they are CD1d restricted or unrestricted, respectively. The functions (cytotoxicity and IFN-γ production) of liver NKT cells bearing TCRαβ decrease in aging but are preserved in very old age. The same functions of liver NKT cells bearing TCRγδ are not only preserved but are strongly increased in very old mice. This discovery leads to the conclusion that the liver NKT cells bearing

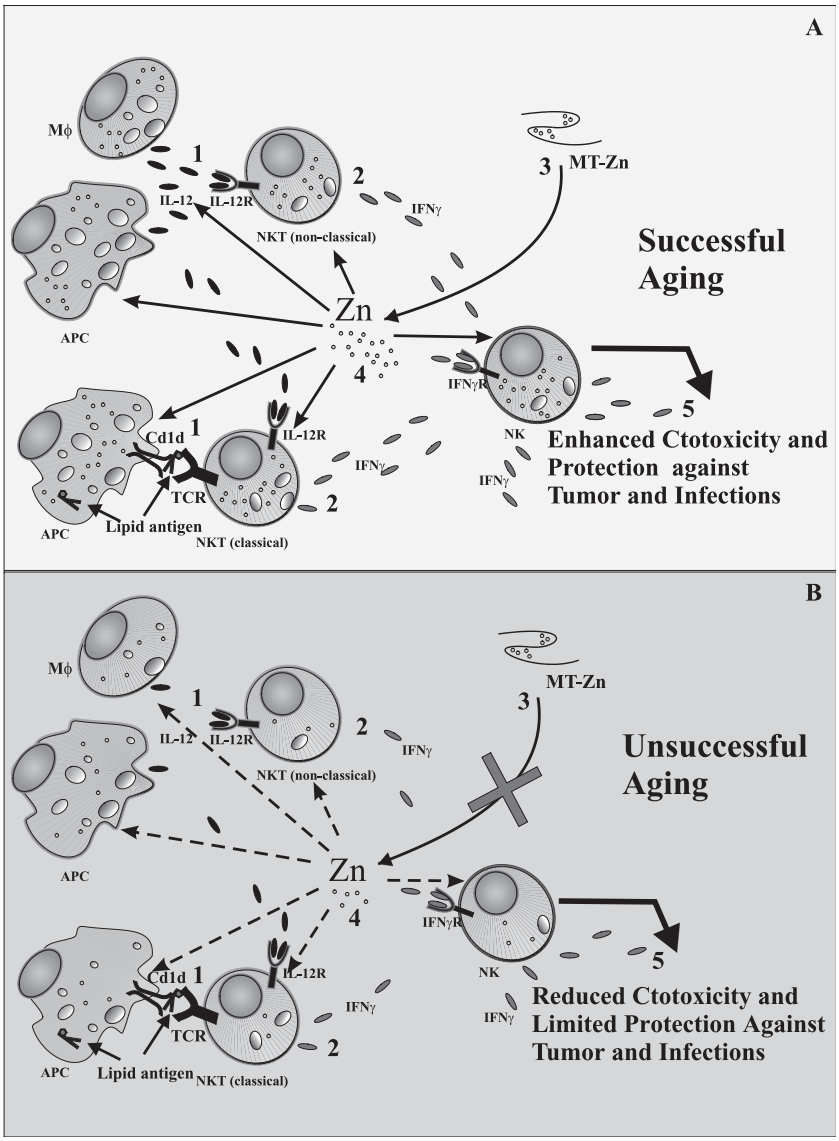


**Fig. 1** Schematic representation of the 'cross-talk' between NK and NKT cell functions in successful aging (A) and in aging (unsuccessful aging) (B) in relation to IFN-γ production (see number 2 in the figure) from activated NKT cells by IL-12 and/or lipid antigen (see number 1 in the figure). The central role played by zinc (see number 4 in the figure), via metallothionein homeostasis (see number 3 in the figure), is reported. The dashed lines (B) represent the low zinc ion bioavailability due to no release of zinc by metallothionein (Mocchegiani *et al*., 2000) with subsequent impaired production of cytokines, decreased cytotoxicity and appearance of tumour and infections (see number 5 in the figure). The symbol X in panel B indicates no zinc release by Zn-MT with subsequent low zinc ion bioavailability, as occurs in aging (Mocchegiani *et al*., 2000). Abbreviations: Mϕ = macrophage; APC = antigen presenting cell; MT-Zn = zinc-bound metallothionein; TCR = T cell receptor; CD1d = group-2 member of CD1 family of major histocompatibility (MHC)-like glycoproteins; IL2R = IL-2 receptor; IFN-γR = INF-γ receptor.

TCRγδ are involved in maintaining innate and adaptive immune response in aging leading to successful aging. As reported for NK cells, zinc ion bioavailability, via metallothionein homeostasis, is crucial in maintaining the functions of liver NKT cells bearing TCRγδ during aging. Although vitamin A increases the functions of 'classical' NKT cells in aged rats (Dawson & Ross, 1999), zinc ions are vital for the increased functions of both NKT cells ('classical and non-classical') because NKT cells, through the production of IFN-γ, affect NK cell cytotoxicity; in addition, NK cell cytotoxicity and IFN-γ production are controlled by zinc ion bioavailability (Fig. 1A,B) (Mocchegiani *et al*., 1998). Therefore, future directions can be sought in the interrelationship between NK/NKT cell functions and zinc ion bioavailability in the elderly and centenarians at the genetic and molecular level. This suggestion is supported by the additional involvement of the zinc pool in the maintenance of the neuroendocrine–immune network, which in turn affects innate and adaptive immune response in aging.

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