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THE IMMUNE SYSTEM IN AGING: ROLES OF CYTOKINES, T CELLS AND NK CELLS

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1. ABSTRACT

Aging is characterized by a proinflammatory state that contributes to the onset of disability and age-related diseases. Proinflammatory cytokines play a central role in mediating cellular and physiological responses. The levels of these cytokines may reflect immune system effectiveness. Studies of the effects of aging on inflammatory response show interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha) and interleukin–1beta (IL-1beta) to be important. In this review, age-related changes in inflammatory cytokines, T and NK cells, and the biology of IL-6 and TNF-alpha and their relevance to senescence are considered.

2. INTRODUCTION

For living organisms, aging is the consequence of time (1). Since Hayflick (2) assessed it in human diploid fibroblasts in 1961, the concept of senescence has been

Table 1. Other inflammatory mediators associated with aging

Inflammatory mediators	Group	Name
Cytokines	Type 1	IL-2
		IFN-?
		IL-12
	Type 2	IL-4
		IL-5
		IL-10
	Other	GM-CSF
		IL-1ß
		IL-7
		IL-13
		IL-17
		GCSF
Chemokines	CXCa	IL-8
		MCP-1
		MIP-1a
		MIP-1ß

extended to endothelial cells, T lymphocytes, epidermal keratinocytes, adrenocortical cells, smooth muscle cells, glial cells, lens epithelial cells and, recently, human pancreatic β-cells – all of which undergo senescence (3). T cell and Natural Killer (NK) cell senescence is also associated with changes in function (4).

Cytokines are central to immune cell communication. Therefore, age-related changes in cytokine profiles contribute to many changes in the immune system (5). Inflammatory cytokines, primarily macrophage products (first identified as acute phase responses to bacterial infection - but also associated with other conditions common in old age) (6) – have multisystem effects (7).

Chronic inflammation is a pathophysiological mechanism leading to age-associated diseases and declines in physical function (8, 9). The paradigm of immune dysregulation involving cytokines has been applied to the concept of frailty, a physiological decline occuring during aging, and the associated dysregulation of systems (10).

Aging is associated with an elevation in proinflammatory cytokines, such as IL-6 and TNF-a. Other cytokines/chemokines (see Table 1) are also associated with advanced age (11-20). Age-associated changes in these cytokines/chemokines are complex so it will not be practical to review them in depth. We focus on IL-6 and TNF-a (and their effects on age-associated diseases) and on T and NK cell changes.

3. IMMUNE SYSTEM AND AGING

The deterioration of immune function with age is called immune senescence, which reduces resistance to infection and, possibly, to cancer (21). Chronic activation of the immune system (e.g., autoimmune diseases, cancer, HIV, and other chronic infections) induces changes in immune response paralleling those in elderly individuals (22). Classically, although "immunosenescence" is an age-related decline in immune response, dysregulation (rather than simple "decline") is more characteristic.

Because chronic inflammatory activity is associated with aging (23), there have been efforts to determine the mechanisms of age-related immune dysregulation and chronic inflammation (10, 24). Age-associated changes in the immune system (25) include reduced *in vitro* responsiveness and impaired response to vaccination and acute infection (e.g., the influenza vaccine is only 30-40% effective in frail elderly people). Infectious disease ranks eighth among causes of deaths in the USA overall, but fourth in persons over age 65. Cancer incidence, partly related to ineffective surveillance by NK cells, increases after age 30 (1).

Cytokines, which affect hematopoiesis, inflammation, and wound healing, are soluble proteins secreted by cells of the immune system. These proteins alter the behavior and properties of different T cell types (26). In addition to being cell-to-cell communicators, cytokines have systemic effects (such as fever, intravascular coagulation, and shock). These conditions are often due to the production of cytokines in infectious, inflammatory, and neoplastic states (27). Cytokines often function as complexes, with one inducing another in a cascade (28).

3.1. IL-6 and aging

IL-6 is the prototypic member of a family of cytokines that includes leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), and interleukin-11 (IL-11) (29, 30); members of this family are termed "interleukin-6 type cytokines." They are involved in immunity, bone metabolism, reproduction, arthritis, neoplasia, and aging; and they are regulated by a variety of factors, including steroid hormones, in transcription, and post-transcription. IL-6 operates through a ligand-specific IL-6 receptor (IL-6R). Unlike most other cytokine receptors, the IL-6R is active in both membrane bound and soluble forms (31).

The major role of IL-6 is to mediate acute inflammatory responses. These include inflammatory cell, lymphocyte activation, and hepatocellular stimulation of acute phase protein synthesis (7). Evidence relates IL-6 increases to an age-associated loss of T cell immunoregulation and menopausal loss of estrogen; and these increases produce changes typical of chronic inflammation (4). Thus, elevated IL-6 serum levels are associated with disease, disability, and mortality in the elderly (32-34).

Genetic studies have identified two polymorphisms in the promoter region of the IL-6 gene: -174G/C and -572G/C (35, 36). A variable number of tandem repeat (VNTR) polymorphisms were found in the 3' flanking region of the IL-6 gene (C allele) (37, 38). In humans, a polymorphism in the IL-6 promoter (a/c polymorphism at position -174) altered IL-6 gene transcription rates *in vitro* and IL-6 levels *in vivo* (35, 36). Genetic variation in the IL-6 gene involved in immune response is an attractive focus in elucidating the molecular mechanisms involved with immunosenescence.

Table 2. Interleukin 6 and aging

IL-6 Results	Specimens	Species	Stumulus	Time	Assay	Author	Publish	Ref
?	Serum	MRL/lpr Mice	FCS	48h	RIA	Tang *et al*	1991	49
?	Plasma	Fischer 344 rats	LPS	90 min	RIA	Foster *et al*	1992	50
?	Supernateants (B cell hybridoma 7TD1)	GH treated C57BL/6 male Mice	ConA	48h	RIA	Goya *et al*	1992	51
?	Plasma	Humans	none	none	RIA	Wei *et al*	1992	52
?	Spleen cells and lymph nodes	BALB/c and 3H/HeN Mice	Nutridoma-SR	24h	ELISA	Daynes *et al*	1993	53
?	Serum	Humans	Nutridoma-SR	24h	ELISA	Daynes *et al*	1993	53
?	Serum soluble IL-6R	MRL/lpr Mice	none	none	ELISA	Suzuki *et al*	1993	54
?	Spleen cells	C57BL/6J Mice	ConA or LPS	24-72h	RIA	Zhou *et al*	1993	55
?	Plasma	Humans	Mitogen	96h	ELISA	Sindermann *et al*	1993	56
?	PBMC	Humans	PMA and PMA	24-72h	ELISA	Fagiolo *et al*	1993	32
?	Plasma	Rhesus monkeys	none	none	RIA	Ershler *et al*	1993	7
?	Plasma	Humans	none	none	RIA	Ershler *et al*	1993	7
=	Serum	Humans	none	none	RIA	Peterson *et al*	1994	57
?	Plasma	Humans	none	none	ELISA	Kania *et al*	1995	48
?	Plasma	Humans	none	none	ELISA	James *et al*	1997	47
?	PBMC	Humans	LPS	90min	ELISA	Cakman *et al*	1997	58
?	Plasma	Humans	none	none	ELISA	Cohen	1997	59
?	PBMC	Humans	ConA and PWM	72-120h	ELISA	Bernstein *et al*	1998	60
=	PBMC	Humans	PHA and ConA	48h	ELISA	Beharka *et al*	2001	61
=	Peritoneal macrophages	C57BL/6NCrlBR Mice	PHA and ConA	48h	ELISA	Beharka *et al*	2001	61

?: IL-6 level increased; = IL-6 level no change

3.1.1. IL-6 structure and function

The human IL-6 gene is found on chromosome 7p21 (39) and has 5 exons and 4 introns (40, 41). Human IL-6 contains 212 amino acids, including a hydrophobic signal sequence of 28 amino acids and two N-glycosylation sites. Murine IL-6 has 211 amino acids with a signal sequence of 24 residues. It has no N-glycosylation, but several O-glycosylation, sites (42).

3.1.2. IL-6 receptor structure and function

IL-6R was first cloned from a human NK–like cell line, YT (43), followed by cloning from a hepatoma cell line, HepG2 (44). The IL-6 Receptor (IL-6R, also known as gp80 or IL-6Rα subunit) is an 80-kDa protein with 467 amino acids. Located on chromosome 1 band q21 (45), the IL-6R gene encodes for a 5-kb mRNA (43). The IL-6R has a binding alpha chain and a signal transducer, gp130, shared by receptors of the IL-6 cytokine subfamily. Sharing a receptor subunit is a feature of cytokine receptors and is the molecular basis for the functional redundancy of cytokines (46).

3.1.3. IL-6 and "normal" aging.

IL-6 is called "a cytokine for gerontologists" (4) because age is associated with increased production. While initial studies supported this contention (47, 48, 4), once the individual's health was considered, it was accepted that changes in IL-6 levels reflected the inflammatory processes associated with aging – not aging itself.

High levels of IL-6 have been reported in aged mice and humans *in vivo* (see Table 2) (49-56, 32, 7, 57, 48, 47, 58-61). Epidemiological studies found increased circulating IL-6 in healthy elderly—including centenarians (62-64, 53) and indicated that IL-6 levels increase with age (65). It is detected in culture supernatants of unstimulated lymphoid cells from aged donors. Increased IL-6 released by isolated monocytes from the elderly is consistent with increased serum IL-6 levels and with production by peripheral mononuclear cells (53, 7, 66, 67). This suggests that dysregulation of IL-6 production is due to aging.

Bonafe (68) studied IL-6 promoter genetic variability at the -174 C/G locus and its effect on IL-6 levels in 700 people aged 60 to 110 years, including 323 centenarians. The proportion of homozygotes for the G allele at the -174 locus decreased in centenarian males – but not females. Only males homozygous for the G allele have higher IL-6 serum levels than C allele carriers. This suggests that a gender-dependent genetic predisposition to produce high levels of IL-6 reduces longevity.

3.1.4. IL-6 and Frailty or Disability

Frailty is associated with chronic inflammation and activation of coagulation pathways. Proteins (e.g., IL-6) in these pathways are biomarkers of frailty and disability. Therefore, these pathways may be involved in the age progression of chronic disability and death (69, 70, 24). They could be targets for "regenerative medicine," which is directed toward increasing function in elderly patients (71). Regenerative medicine intervenes at a fundamental biological level (e.g., altering production of tissue-specific growth factors) that may reverse age-related functional loss.

Population studies identified IL-6 levels as a predictor of disability (and mortality) in the elderly (72). Ferrucci (8) tested the hypothesis that, in older persons with no disability, high levels of IL-6 predict a physical decline. Cases with the highest IL-6 levels were 1.76 (95% CI, 1.17-2.64) times more likely to develop mobility-disability and 1.62 (95% CI, 1.02-2.60) times more likely to develop mobility plus Activities of Daily Living (ADL)-disability (compared to the lowest IL-6). This was little changed by adjusting for confounders.

The increased risk of mobility-disability had a non-linear relation to IL-6 levels, with risk rising rapidly beyond levels of 2.5 pg/ml. Some diseases that are characterized by chronic inflammation and high levels of IL-6 accelerate the catabolism of proteins and muscle-wasting (73, 74). This may be due to the effect of IL-6 on muscle tissue and/or to the pathophysiologic role played by IL-6. Other studies found increased levels of this protein associated with age and impairment of basic and instrumental activities of daily living (IADL) (34). Cohen (59) showed that this situation ocurred independent of disease and suggested an age-related dysregulatory phenomenon. In the Women's Health and Aging Study (75), high IL-6 levels were an independent risk factor for an accelerated decline in physical function. Ferrucci (76) found that older women with high IL-6 levels have a greater risk of disability – and a faster decline in walking ability – than those with lower levels. These effects are partly explained by a decline in muscle strength.

3.1.5. IL-6 and age-related diseases

IL-6 dysregulation is involved in age-related diseases such as cancer, lymphoma, cardiovascular disease (CVD), osteoporosis, Alzheimer's disease (AD), diabetes, and atherosclerosis (77-80).

3.1.5.1. IL-6 and cancer

In humans, IL-6 dysregulation is implicated in lymphoma (81), myeloma (82-85), and non-lymphoid tumors (86, 87).

For tumors without IL-6 receptors, IL-6 showed anti-tumor activity (due either to T cell-enhancing effects [88] or to non-specific anti-inflammatory effects [89]). In most cancers, however, IL-6 accelerates cancer cell growth. Increased production of IL-6 in the elderly may promote the proliferation and survival of malignant myelomas and may induce cell resistance against therapy-stimulating apoptosis (90). Increased IL-6 may cause tumor progression by action on cellular motility and adhesion (91) and may cause cell proliferation and expression of tumor-specific antigens (92). Melanomas (93), renal cell carcinoma (94), prostate (95) and ovarian (96) cancer are stimulated by IL-6. In many tumors, an increase in IL-6 receptors also induces proliferation (31). Elevated serum levels suggest an important role for IL-6 in cholangiocarcinoma. Inhibition of IL-6 signaling blocks anchorage independent growth of a malignant cholangiocyte cell line (97).

Increased levels of IL-6 may affect lymphoma risk in old mice (98). IL-6 stimulates B cell maturation and proliferation, and overproduction is found in many B-cell malignancies (e.g., multiple myeloma [82, 83, 99], non-hodgkins lymphoma [100, 101], and chronic lymphocytic leukemia [102]). Other lymphoproliferative diseases, including Castleman's disease (103, 104), Hodgkin's disease (101,105), angioimmunoblastic lymphadenopathy with dysproteinemia (106), and certain non-B cell lymphomas (107) are also associated with high IL-6 levels.

3.1.5.2. IL-6 and CVD

IL-6–mediated inflammation is involved in CVD. IL-6 levels may predict CVD events. Elevated levels of IL-6 are associated with increased risk of CVD death in elderly people (72, 108, 109) and myocardial infarction (MI) in middle-aged men (108). Included conditions are coronary artery disease (110-113), stroke (111), and congestive heart failure (CHF) (114).

Using data from Framingham, Roubenoff (66) found production of IL-6 by peripheral blood mononuclear cells to be higher in older non-disabled persons. This was due to overproduction of cytokines associated with chronic medical conditions that are prevalent in older persons (such as angina pectoris, MI, CHF, diabetes mellitus, and cancer). There is evidence of dysregulation in the mechanisms modulating cytokine response (76). Lindmark *et al* (115) reported that elevated IL-6 predicts CVD mortality and is an independent marker of increased mortality in unstable coronary artery disease (CAD) identifying patients eligible for invasive management.

There is controversy about whether or not the -174 G/C polymorphism modulates IL-6 levels; one study found that the C/C genotype is associated with higher IL-6 levels in patients who have an abdominal aortic aneurysm (116). In three other studies, the –174 C allele predicted higher coronary heart disease (CHD) mortality (116-118). However, the Ludwigshafen Risk and Cardiovascular Health (LURIC) study indicated that the IL-6 G (-174) C promoter polymorphism is not associated with CAD or MI and does not explain CVD risk (119). Jenny *et al* (120) assessed IL-6 levels and the -174G/C genotype in a case-control study of men and women (average age 73 years) in the Cardiovascular Health Study. IL-6 levels differentiated those with sub-clinical CVD from those without. Although the -174C allele was not associated with incident events, associations of the genotype with inflammation and MI, combined with the IL-6 level, suggest that IL-6 predisposes patients to atherosclerosis. The –174 C and –572 C genotypes likely have biological significance in atherosclerosis, thrombosis, and CHD. The -174 C polymorphism is important since IL-6 levels are influenced by the G/C genotype, which was correlated with higher IL-6 in Caucasians who were undergoing coronary revascularization (35, 121).

3.1.5.3. IL-6 and Osteoporosis

IL-6 and IL-6R contribute to bone remodeling (122-124). The parathyroid hormone, an inducer of bone resorption, stimulates IL-6 (125-127), which is produced by osteoblasts, monocytes, and T cells and which promotes osteoclast differentiation and activation (128). IL-6 also

stimulates osteoclasts and affects bone loss associated with estrogen deficiency (85, 81). Giuliani *et al* (129) examined 220 women (25 to 104 years of age) to examine changes in the levels of IL-6 and soluble receptors (sIL-6R and gp130). IL-6, sIL-6R, and sgp130 showed different age- and menopause-related changes. Hence, IL-6 activity may increase with age and affect osteoporosis. Ferrari *et al* (130) found that IL-6 polymorphisms -572 and -174 G→C regulating gene expression are associated with C-reactive protein (CRP) levels and bone resorption in postmenopausal women. Findings suggest that IL-6 levels can identify individuals genetically predisposed to osteoporosis (131-133).

3.1.5.4. IL-6 and Alzheimer's disease (AD)

Chronic inflammation and astrocytosis are histopathological AD features (134). IL-6 is produced primarily by astrocytes and microglia, but also by neurons (135, 136). It affects CNS development (137, 138) and acute inflammatory response (139). Inflammation is central to AD degenerative changes (140-143). Weaver (144) suggests a relation between elevated IL-6 and a decline in cognition. This is consistent with a relation of brain inflammation, as measured by elevated IL-6, to neuropathology. Transgenic mice over expressing IL-6 in the CNS exhibited neuropathology (145). IL-6 was also detected in the CNS of human patients with neurodegeneration, including Multiple Sclerosis (MS) and Acquired Immune Deficiency Syndrome (AIDS). IL-6 inhibited long-term potentiation in hippocampal neurons (146), and progressive neuropathological manifestations of IL-6 expression were also related to deficits in avoidance learning (147).

The association of AD with the -174G/C and -572G/C polymorphisms of the IL-6 gene promoter is controversial. Pola *et al* (148) evaluated the association of AD with -174G/C polymorphism in Italians. Frequency of the G/G genotype was higher in patients with AD, while people with the C/C genotype had reduced risk. Using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), Shibata *et al* (149) examined the C allele of the "IL-6 variable number of tandem repeat polymorphism" (IL-6vntr) and the G/C allele of the IL-6 gene promoter region (IL-6prom) polymorphisms in 128 Japanese AD cases and 83 controls. Frequency of the IL-6prom G allele was significantly increased in AD cases, while the IL-6vntr polymorphism was not. IL-6 levels in AD cases were significantly higher than in controls. IL-6prom G allele-positive AD patients had higher IL-6. Several studies also reported an association of IL-6vntr polymorphisms in the 3'flanking region of the IL-6 gene (C allele) with AD (37, 38, 150). Boojak *et al* (151) screened late-onset AD cases and age-matched controls to evaluate IL-6 –174 polymorphisms, but found no significant association. The G/C polymorphism at position -174 was examined in 102 AD patients and in control groups of 191 healthy and 160 depressed patients. There was no evidence of association between the IL-6 promoter polymorphism and earlier age of AD onset. Analysis showed a strong link to disequilibrium by comparing haplotype frequencies under the hypothesis of no allelic association and of allelic association between IL-6vntr and IL-6prom and interaction of IL-6vntr and IL-6prom modifying AD risk (37).

The role of IL-6 in aging is still a source of contention: Is it a result of inflammatory processes associated with aging, or with aging itself? This is a challenge for researchers.

3.2. TNF-a and aging

Aging is associated with increased TNF-a. TNF is a multifunctional proinflammatory cytokine produced by various cell types (including activated macrophages and lymphocytes) that affect lipid metabolism, coagulation, insulin resistance, and endothelial function. It stimulates the proliferation of normal cells, exerts cytolytic or cytostatic activity against tumor cells, and causes inflammatory, antiviral, and immunoregulatory effects (152, 153). TNF-a is a monocyte-derived tumor necrosis factor; TNF-ß is a lymphocyte-derived tumor necrosis factor.

3.2.1. TNF-a structure and function

Gearing *et al* (154) reported that TNF-a is initially expressed as a 233-amino-acid membrane-anchored precursor proteolytically processed to yield the 157-amino-acid cytokine. Processing of the TNF-a precursor is dependent on at least one matrix metalloproteinase-like enzyme, inhibition of which represents a therapeutic mechanism for interfering with TNF-a production. Interest in TNF was based on its potential as an anti-cancer drug. With the availability of purified TNF, it became evident that TNF not only induces necrosis of tumor tissue, but it also produces systemic endotoxic activity leading to fever, hypotension, and shock (155). TNF is a potent inflammatory mediator and is central in inflammatory reactions of innate immune systems (including induction of cytokine production, activation or expression of adhesion molecules, and growth stimulation [156]).

3.2.2. TNF receptor: structure and function

Different forms of TNF signals through two cell surface receptors: – TNF-R1(p55/p60) and TNF-R2 (p75/p80) (157-159). TNF-R1 initiates most of TNF's biological activity. Binding of TNF to TNF-R1 triggers intracellular events activating two transcription factors: nuclear factor ?B (NF-?B) and c-Jun. These induce expression of genes that is important for a variety of biological processes, including cell growth and death; development; oncogenesis; and immune, inflammatory, and stress responses (160).

3.2.3. TNF-a and Mortality

TNF levels in elderly institutionalized patients may predict early mortality (161). Bruunsgarrd (162) hypothesized that TNF-a predicts mortality. To investigate if TNF-a levels were associated with mortality - independent of IL-6- in 333 healthy 80-year-olds, Cox regression was used to explore TNF-a and IL-6 effects on survival over 6 years. TNF-a was associated with mortality in men, but not women. IL-6 elevation was associated with mortality in both sexes. TNF-a explained only 7% of the

variability in IL-6. The effects of the two cytokines were independent of each other – and other risk factors (such as smoking, blood pressure, exercise, cholesterol, co-morbidity, body mass index [BMI], and the use of anti-inflammatory drugs). This suggests different biological effects of the cytokines in age-associated mortality (162).

TNF-a levels relate to the level of inflammation, CVD, dementia, and frailty in very old people (163). TNF-a was associated with mortality, independent of CVD. It was also associated with mortality in centenarians, independent of comorbidity, although comorbidity affected the hazard. Elevated levels of TNF-a, but not IL-6, were associated with mortality in Danish centenarians (164). Although high TNF-a levels strongly predicted mortality in centenarians with dementia, TNF-a was associated with mortality in a Cox regression model including dementia (165). Low-grade elevations of TNF-a are independent risk factors of morbidity and mortality in the elderly. (166).

3.2.4. TNF-a and age-related diseases

TNF-a initiates the inflammatory cascade and is linked to age-associated disorders (162). Increased levels of TNF-a were reported in aged humans (167-169). TNF-a has been associated with the development of AD (164), CVD (e.g.,CHD), CHF (170), Type 2 diabetes mellitus (164), Rheumatoid arthritis (171) and cancer (172, 173). TNF-a levels increase with age, providing a unifying basis for these disorders (169).

3.2.4.1. TNF-a and AD

Cytokines, especially TNF-a, are related to neurotoxicity (174, 175). Increased levels of TNF-a have been reported in cerebrospinal fluid, cortex (176), and glial cell cultures from AD patients (177). It is suggested that amyloidogenesis may be caused by an acute-phase response in the brain (mediated by proinflammatory cytokines [178]).

High levels of TNF-a were associated with a high prevalence of AD in older individuals. In 126 Danish centenarians the highest TNF-a levels were in individuals with moderate to severe dementia, as measured by the clinical dementia rating scale (179). The association remained when centenarians with other medical disorders were excluded, indicating that an association of TNF-a and dementia. Associations between TNF-a levels and dementia in centenarians may reflect a whole-body increase in concentrations of proinflammatory cytokines. Damage to brain tissue may be an effect of increased peripheral concentrations of cytokines, such as TNF-a (180).

3.2.4.2. TNF-a and CVD

TNF-a affects CVD risk factors such as insulin resistance (181), dyslipidemia (182), endothelial dysfunction (183), and endothelial upregulation of cellular adhesion molecules (CAMs) (184). Circulating levels of cytokines were associated with a low (less than 0.9) ankle-brachial blood pressure index (ABI) that is indicative of generalized atherosclerosis (185-188), increased risk for CVD (189), and death (190, 191). In octogenarians, high levels of TNF-a correlated with high levels of triglycerides and a low rate of atherogenesis and thromboembolic complications. Elevated TNF-a levels were associated with early-onset atherosclerosis in apparently healthy middle-aged men (192). High levels of TNF-a after MI were associated with risk of recurrent coronary events (193).

Endothelial dysfunction may be an early warning sign of atherosclerosis (194). Bruunsgaard found that TNF-a impairs endothelium-dependent relaxation in humans (183) and causes endothelial up-regulation of CAMs, mediating attachment and transmigraton of leukocytes through the endothelium (184). One hundred thirty home-living octogenarians were divided into groups with low, intermediate, and high levels of TNF-α. In those with high TNF-a, a significantly larger population of patients had a history of atherosclerostic CVD.

To investigate the predictive value of inflammatory markers on the incidence of cardiovascular events in well-functioning older persons, 2,225 persons aged 70 to 79 years, without baseline CVD, were selected for the Health, Aging, and Body Composition study. Incident CHD, stroke, and CHF were detected during an average follow-up of 3.6 years. Results showed that IL-6 and CRP, TNF-a levels were independent predictors of CVD events (170). This suggests that increased levels of TNF-a promote atherogenesis and may be a marker of its severity.

3.2.4.3. TNF-a and Type 2 diabetes mellitus

Because of a decline in insulin action, aging is frequently associated with impaired glucose handling (195-197). High TNF-a levels also correlated with body fat and insulin insensitivity (169). TNF-a levels rise with age and might be related to metabolic control in Type 2 diabetes mellitus. Lechleitner *et al* (198) studied the relation of TNF-a levels to glycemic control in elderly patients with Type 2 diabetes over two years. TNF-a levels increased - a possible factor for the reduction of glycemic control in Type 2 diabetes.

Clausell *et al* (199) demonstrated that TNF-a expression was significantly increased in type 2 diabetic atherosclerotic lesions (compared with non-diabetic lesions). A minimal degree of immunostaining for this cytokine was apparent in the majority of non-diabetic lesions. Increased accumulation of TNF-a levels distinguished the two types of lesions. TNF-a induced obesity-linked insulin resistance, endothelial dysfunction, and dyslipidemia. TNF-a may also reflect the enhanced inflammatory activity associated with the development of vascular lesions in type 2 diabetic patients and with the impairment of insulin action with age (169).

In conclusion, studies show that circulating levels of TNF-a are the best predictor of morbidity and mortality in the elderly. Lifestyle factors and co-morbidities modulate these levels.

3.3. T cells and aging

Immune system alterations during aging are complex and pleiotropic, suggestive of remodeling, or

altered regulation, not deficiency. The most dramatic changes with age occur in the T cell compartment and are consistent with increased incidence and severity of infection and cancer in the elderly (200).

3.3.1. Thymic involution

The thymus is important in T cell development and the effect of T cells on aging. It provides a microenvironment in which bone-marrow-derived progenitors proliferate, mature, and undergo selection to create a population of major histocompatibility complex (MHC)-restricted, self-tolerant T cells (201).

3.3.1.1. Thymic aging

Thymic involution is the most prominent age-associated change in the immune system. In mice, this process occurs between the ages 4 and 9 months. It can be observed in mice and humans as the loss of thymus size, mass, cortical T cells and also as the loss of epithelial cells and stroma (202).

Gross anatomical thymic changes with age were recognized before its immune function was discovered (1, 203). As the thymus atrophies with age, there are fewer naive T cells to respond to new pathogens and neoantigens, with accumulation of oligoclonally expanded memory $CD8^+CD28^-CD57^+$T cells.

T cells influence the thymus by providing survival signals for the medullary microenvironment (204). As production of naive T lymphocytes decreases, cell-mediated and humoral immunity depending on T lymphocytes declines. Production of B lymphocytes by bone marrow in mammals also declines with age. The extent, rate, nature, and reversibility of the age-related decline in thymic function in humans are debated (205-207). Aging is associated with decreased thymic epithelial space. The decrease begins in late adolescence and continues throughout life. The etiology of involution is not clear; but, because young thymic cells can be repopulated in aging hosts (208), it appears to involve genetic programs. In centenarians thymic involution may shield the T cell system from genetic instability (209) because centenarians show DNA stability – and well-preserved immune function (210).

Histological studies of the thymus in mice and humans suggest that, although organ size may decrease (in mice) or the lymphoid compartment be replaced by fat (in humans), there remains tissue with youthful cortical and medullary architecture. Analysis of the proportions of different T cell subsets in the murine thymus suggests that the earliest (CD44+ CD25-) thymocyte population is preserved, while later populations decline (211). In fetal thymic organ cultures, CD44+ CD25- cells isolated from old mice generate mature thymocyte populations as efficiently as those from young mice. This suggests an age-related decline in thymic stromal function, as does administration of IL-7 to aged mice (increasing thymic subsets and the proportion of peripheral naïve-phenotype CD4+ T cells [203]). IL-7 may affect the peripheral naïve T cell pool or extra-thymic sources of T cells (e.g., the intestine).

3.3.1.2. Recent thymic emigrants (RTEs) and T cell receptor excision circles (TRECs)

$CD4^+$ and $CD8^+$ T cells progress through several stages in their lifespan. Mature $CD4^+$ and $CD8^+$ thymocytes emigrate from the thymus to the periphery as recent thymic emigrants (RTEs) (212). After RTEs mature, they are classified as naive T cells that circulate through blood and lymphoid tissues. The rate of RTE production by the thymus contributes to the peripheral T cell pool. It is important to monitor thymic production under conditions influencing T cell depletion and reconstitution (e.g., human immunodeficiency virus [HIV]-1 infection, bone marrow transplantation, and immunosuppressive therapy [213]). The property of immunity most sensitive to aging is the production and export of T cells from the thymus. This is manifested as a decrease in the peripheral levels of naive RTEs with age (206, 212), which (if a proportion of RTEs were tumor specific) could influence the precursor frequency of the functional cytotoxic T lymphocyte (CTL) .

T cell receptor excision circles (TRECs) are used to measure RTE levels, to assess thymic output in aging and disease (207, 214), and to study age changes in the frequency of RTEs in HIV-1 infection (206). TREC levels change with age (206, 215-220). It is unclear whether TREC concentrations are affected by thymic output or peripheral T cell events. Douek *et al* (206) and Patel *et al* (207) found a sharp decline in TREC levels with age; on the other hand, Zhang *et al* (217) found a gradual decline, with stable output in the first 5 years of life. Steffens (221) determined that $CD45RA^+CD45RO^-$ can be enriched for TRECs in adults, providing evidence of intact thymic function in adults.

3.3.2. Effect of age on peripheral T lymphocytes

The peripheral immune system changes with age. Previous studies noted age-associated changes in T lymphocyte subsets (222-225). Many studies report alterations in the distribution of subsets (e.g., increased CD45RO "memory" and NK cells (226) and a decrease of CD45RA naïve T cells (227). T-lymphocytes display reduced proliferative capacity, increases of $CD8^+$, and decreases of $CD19^+$ cells (228). The proportion of perforin-expressing $CD8^+$ T cells decreases (229), as does the secretory capacity of transforming growth factor-β (TGF-β), which alters regulation (230).

3.3.2.1. Naïve phenotype cells

Most T cells in children resemble naïve cells freshly emigrated from the thymus to peripheral immune organs. Aging causes a shift from naive cells[1] to an increase in antigen-experienced memory subsets (231-233). In mice, the shift accounts for age-related declines in response to mitogen *in vitro* (233) and contributes to declines with age of *in vivo* responsiveness to new antigens.

The frequency of naive T cells[2] declines with age, as does functional capacity, shown by decreased numbers of sequential replications in response to stimulation. Variability in the capacity of naive cells suggests a mosaic of reactive and anergic cells. (Anergy is related to microenvironmental and intrinsic cell properties.)

Microenvironmental effects (such as increased membrane-cholesterol-to-phospholipid ratio reducing membrane fluidity) on the proliferation of peripheral blood lymphocytes might be related to aging, but are potentially reversible by decreasing the proportion of lipids [234]). Other molecular changes in naive T cells include alterations in phosphorylating enzymes. That enzymes are altered, rather than simply declining (235), suggests age-related remodeling.

3.3.2.2. Memory phenotype cells

An increased population of memory T cells in old humans and mice exemplifies age-related immune alterations. The shift from naive T cells reflects cumulative exposure to pathogens. It might also represent a compensatory homeostatic response to reduced numbers of naive T cells generated in the thymus, intrinsic cellular differential sensitivities to apoptosis, and the effects of the environment – all of which promote the appearance and dominance of memory cells (236).

Most studies of memory T cells were done on whole spleen or peripheral blood lymphocyte populations. Because the frequency of memory cells in the aged is increased, it is unknown if alterations in cytokine production by the T cells from elderly individuals are linked to changes in the frequencies of naïve/memory T cell subsets. According to Pawelec et al (237), many studies compared the behavior of predominantly naive cells from young organisms with that of predominantly memory cells from old ones (238). The results indicate significant decreases in naïve T cells and increases in memory T cells in the elderly. Defects in TH1 and TH2 cytokine production were not significantly correlated with frequencies of naïve/memory T cells. Elderly people with normal frequencies of naïve/memory T cells exhibited decreases in cytokine production comparable to reductions observed for elderly donors. They also exhibited changes in the frequencies of naïve/memory T cells (239). Whether accumulation of memory T cells in the elderly is a biological marker of changes in T cell biochemical or of functional properties is yet to be determined (239, 240).

3.3.3. Effect of aging on signal transduction related to T cell activation

Changes in signal transduction may be responsible for the impairment of T cell function as people age. T cell activation is initiated when an antigenic peptide is recognized by the T cell receptor. This activates signaling molecules (such as kinases, phosphates, and adaptor proteins) that couple stimulatory signals from the T cell receptor (TCR) to intracellular signaling pathways. Activation of signaling stimulates transcription factors and the expression of genes that is crucial to regulating T cell function (241).

Studies of early activation reveal that T cells from aged mice show abnormalities soon after stimulation, including reduced activation of Raf-1/MEK/ERK kinases, JNK protein kinase, and an influx of calcium (242). Despite a three-fold decline with age in the tyrosine phosphorylation of CD3 ?, the Zap-70 kinase associated with the CD3? chain doubles with age in resting CD4 T cells. There is no effect of aging on the Zap-70 kinase function in activated T cells *in vitro*. Age-related impairment of the translocation of PKC? from cytoplasm to the site of T cell interaction with antigen-presenting cells may underlie downstream defects in activation (235).

In humans, CD3-stimulated T cells from 50% of old subjects showed reduced mitogen-activated-protein-kinase (MAPK) activation (243). Stimulation with phorbol ester with calcium ionophore activated MAPK in old cells, but less than in young cells (243) suggesting signaling deficits between TCR and inducers of MAPK. Similar findings were reported in mice using CD3/CD4-mAb-stimulation of T cells (244). Other signaling pathways may also be affected by aging. Liu et al (245) reported that extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) were diminished in CD3/PMA-stimulated T cells from elderly humans. ERK and JNK were accompanied by decreased Raf-1 kinase activation. ERK2 activation correlated with IL-2 production and may be a rate-limiting step for production by old T cells (245). Similar findings apply to rat (246) and mouse T cells (247, 248).

Signal transduction initiates physiological signals for growth and proliferation and for programmed cell death and is important for T cell development and function. Studies suggest that changes in signal transduction cause age-related declines in T cell function. Alterations in early signaling events (e.g., calcium mobilization, tyrosine phosphorylation, retrovirus-associated sequence [RAS], and MAPK activation) are linked to age decreases in IL-2 expression and T cell proliferation. Impairment of signaling molecules in the cell membrane or cytoplasmic level may contribute to the secondary defects of downstream nuclear events such as transcription. Although much is known about how signaling pathways lead to T cell growth and function, an understanding of how aging alters activation of signaling molecules (diminishing T cell responsiveness) is not yet complete (249, 250).

Activation of the transcription factors of NF-?B is regulated by alterations in intracellular reduction-oxidation (redox). NF-?B regulates the genes that are necessary for T cell responses (IL-2, IL-6, IL-8, and T cell surface receptors). Diminished NF-?B activity occurs in T cells with aging (251), suggesting impaired activation of NF-?B in cellular senescence. Disruption of NF-?B activity is implicated in diseases involving immune or inflammatory processes (e.g., atherosclerosis [252, 253], aging [254, 255], and HIV-1 infection [256, 257]). The role of reactive oxygen species (ROS) as secondary messengers for divergent T cell signals is complicated because T cell lines and peripheral blood T cells, which differ in NF-?B, are induced by ROS. The pathways of NF-?B activation are based on indirect evidence provided by experiments using antioxidants to inhibit NF-?B. Activation of T cells requires signals, stimulating increases in intracellular calcium and in enzymatic processes including kinases (258).

3.3.4. Effect of aging on T cell apoptosis

Apoptosis tends to increase with age (259-262), but it is unclear if alterations in T cell apoptosis are due to aging. Loss of the ability to regulate apoptosis may impact life span and normal aging (For example, cancer incidence and the onset of many neurodegenerative diseases are associated with aging). How diseases are initiated is unknown, but it is known that changes in apoptotic behavior contribute (263).

Animal models suggest that age-associated immune dysfunction correlates with defects in T cell apoptosis. An increased apoptosis was observed in the splenocytes and thymocytes from aged rats (264). Old lymphocytes are susceptible to activation-induced apoptosis (265); and aging humans demonstrated increased apoptosis of T cells ($CD3^+$ $CD45RO^-$) compared to younger ones in *in vitro* cultures of lymphocytes (266). Age modulates expression of cell death genes. An increased expression of Fas and Fas ligand and a decreased expression of Bcl-2 were observed in the memory cells of $CD4^+$ and $CD8^+$ T subsets from elderly people as compared with young controls. An increased proportion of $CD4^+$ and $CD8^+$ T subsets underwent apoptosis following anti-Fas Ab treatment in the elderly (261). Increased apoptosis is also responsible for lymphopenia and T cell deficiency in humans (267).

Prior to clonal deletion by apoptosis, $CD4^+$ T cell clones manifest an age-increasing susceptibility to activation-induced cell death (AICD), possibly related to decreased CD28 expression (and co-stimulators) and IL-2 secretion (268). When they are mediated by fas/fas-ligand interactions - even with exogenous IL-2 - these T cell clones show susceptibility to AICD. Clones show an age increase in fas-ligand mRNA, rather than an age increase in CD95 (fas) density (young clones express large amounts of fas), which may enhance susceptibility to AICD (269, 21). The situation *in vivo* is not clear. While studies report increased susceptibility to apoptosis of $CD4^+$ cells, there is disagreement on CD8.

In the senescent immune system, age-related biochemical alterations may trigger apoptosis (265). Transmembrane signaling defects (270-272), differential expression of genes in cell cycle control (273, 274), diminished activity of transcription factors regulating lymphokine gene expression (271), poor IL-2 production (275), defective IL-2 receptor expression (275, 276), and inappropriate activation of the sphingomyelin-ceramide pathway (277) may lead to untimely and/or excessive cell death and age-related immune dysfunction.

3.3.5. Effect of aging on Telomerase and Telomeres

Telomeres are composed of simple repetitive DNA. In mammals this is (TTAGGG)n. Repeats are elongated by telomerase, a ribonucleoprotein enzyme that extends the 3' end of telomeres. In immortal eukaryotic cells, telomerase balances telomere loss with *de novo* synthesis of DNA (278). Blasco (279), using a murine model with genetically engineered telomerase deficiency, found telomere shortening in successive generations, resulting in germ cell failure and sterility.

In telomerase-deficient mice, the immune response is compromised. This implies a role for maintenance of telomerase-dependent telomere length in sustaining germ cell formation and T cell-dependent B cell antibody responses (280). Successive generations of the telomerase knockout mouse, Terc-/-, show telomere dysfunction with critically short telomeres and end-to-end chromosomal fusion. An analysis of telomerase-deficient mice points to immunological defects as causes of poor health and death.

Telomere shortening during *in vitro* aging of human somatic cells may play a role in cellular senescence. Weng *et al* (281) reported that mean telomere lengths of naïve and memory human T cells shorten 0.3 population doublings each year. More study on T cell subsets and heterogeneity is needed to determine if the T cell pool contains outliers with potential for self–renewal. Activation-induced apoptosis of T cells has been reported to either increase (282) or decrease (283) with age. Old transgenic mice in which T cells express apoptosis-promoting the Fas ligand retain the T cell proliferation and cytokine production of youthful mice (283). This could yield insight into the pathways that regulate the functional capacity of age-sensitive T cell subsets.

In fibroblasts from centenarians, telomere length is indistinguishable from that of fibroblasts from young donors. Replications of dermal fibroblasts in *in vivo* aging and in the skin of centenarians are lower than that of (most) lymphocytes. Old and very old people have a mosaic of cells with different replicative potential. This has consequences for cell aging and age-related diseases. A mosaic represents localized heterogeneity (284). Cawthon *et al* (285) assessed the relation of telomere length to mortality in 143 individuals over age 60. Those with shorter telomeres had a poorer survival rate, with a 3.18-fold higher heart disease mortality (95% CI 1.36–7.45, P=0.0079) and 8.54-fold higher infectious disease mortality (1.52–47.9, P=0.015).

Introducing telomerase catalytic protein into normal human cells resulted in telomerase activity. Normal human cells expressing transfected telomerase can maintain telomere length and exceed maximum lifespan by fivefold. The longevity-determination mechanism of telomere shortening in human cells thus can be circumvented — evidence for telomere shortening in cell senescence and of telomerase expression in cell immortality (286). Mondello (287) analyzed the mean length of terminal restriction fragments (TRF) in fibroblasts from 4 healthy centenarians in cells aged *in vivo* and from 11 individuals of different ages. No correlation between mean TRF length and donor age was found. Telomere shortening was detected during *in vitro* propagation of centenarian fibroblasts (suggesting that in fibroblasts aged *in vivo*, telomeres can be far from a critical length). Chromosome analysis did not show the presence of telomeric associations in early passage centenarian fibroblasts. In blood cells, an inverse correlation of mean TRF length and donor age was found. Substantial differences (2 kb) between the telomere lengths of the two cell types were observed in centenarians.

Expression analysis of three senescence-induced genes (i.e., fibronectin, apolipoprotein J, and p21) revealed a positive correlation with donor age for fibronectin. Mondello (287) suggests that: (1) telomere shortening plays different roles in different T cell types and (2) the characteristics of fibroblasts aged *in vitro* might not represent what occurs *in vivo*.

3.3.6. Effect of aging on co-stimulatory pathways

Following the engagement of TCR, CD28 molecules provide a co-stimulatory signal (288). Studies show a population of $CD28^-$ cells in the elderly. This is correlated with an increased expression of CD95 in $CD28^-$ cells (289). Down-regulation is associated with shorter telomeres in CD28 cells (290, 291); shorter telomere are susceptible to apoptosis (292).

Studies shed light on CD28 co-stimulation – not only in T cell proliferation, but in survival (293). A CD28–related receptor, thymphocyte-associated antigen 4 (CTLA-4), may be involved in deleting activated cells. Leng *et al* (294) measured the intracellular levels of CTLA-4, a negative regulator of T cells, in 53 healthy individuals (aged 18-94). They found a significant correlation between age and the percentage of $CTLA\text{-}4^+CD4^+$ cells (r=0.6, P<0.001) and between age and the mean fluorescence intensities of CTLA-4 (i.e., the number of molecules, r=0.61, P<0.001). The CTLA-4 levels correlated with immune activation, as determined by levels of $HLA\text{-}DR^+CD3^+$ cells (r=0.55, P<0.001). It is postulated that immune senescence is caused partly by chronic immune activation decreasing CD28 co-stimulatory molecules and increasing inhibitory CTLA-4 molecules. In the T memory compartment there is an increase in cells that lack CD28 (295) and have short telomeres. *In vitro* studies show that senescing T cell clones lose CD28 (296) and that up-regulation of telomerase is co-stimulation dependent (297). $CD28^-$ cells in the elderly may be reaching the end of their lifespan and no longer divide.

$CD28^{null}$ T cells indicate immunoincompetence in humans. Loss of CD28 is due to an inoperative transcriptional initiator (INR), consisting of non-overlapping a and ß motifs with protein-binding profiles functioning as a unit (298). Although CD28 is expressed on all T cells, $CD28^{null}$ T cells are found in the immune system of the elderly, in $CD8^+$ (299, 300), and $CD4^+$ (301). $CD28^{null}$ cells have short telomeres indicating a long replicative history (302). These cells are highly oligoclonal (300, 303), occurring at large clonal sizes and contributing to reduced T cell diversity. Because of the limited replicative lifespan of T cells (304), $CD28^{null}$ cells indicate immunosenescence.

$CD28^{null}$ T cells are also found in patients with rheumatoid arthritis (RA) (304, 305). These patients have abnormalities in the T cell pool, which has reduced receptor diversity and large clonal populations. Clonally expanded $CD4^+$ T cells lose expression of CD28 and express perforin and granzyme. The profile of expanded $CD4^+CD28^{null}$ T cells is changed and shifted toward tissue-injurious capabilities. $CD4^+CD28^{null}$ T cells are important in patients with extra-auricular manifestations of RA, where they produce vascular injury, suggesting either that $CD8^+$ T cells have a higher turnover than $CD4^+$ T cells or that $CD8^+$ T cells have a high propensity for losing CD28. Cultures of $CD4^+$ and $CD8^+$ T cells resulted in a more rapid decline in CD28 expression on $CD8^+$ than on $CD4^+$ T cells (306).

Understanding the loss of T cell diversity and the emergence of $CD4^+CD28^{null}$ T cell clonotypes has implications for autoimmune syndromes (307). To describe the loss of CD28 in T cells in centenarians, when Effros (299) compared the expression of CD28 in T cell cultures, the result was a range of values for their *in vitro* proliferative activity. Senescence was accompanied by reducted CD28. This is in contrast to an undiminished expression of other T cell markers (CD2, CD3, CD29, CD11a, CD44, CD45RO) (308). The loss of CD28 expression in cultures reaching proliferative senescence suggests that $CD28^-$ cells in centenarians have undergone an increased number of cell divisions. Both peripheral blood T lymphocytes of elderly individuals and cultures of senescent T cells show declines in the percentage of cells expressing CD28. High frequencies of $CD28^{null}$ T cells *in vivo* are correlated with hypo-responsiveness to vaccination. Goronzy (309) suggests that such studies allow molecular dissection of pathway(s) leading to T cell senescence (297).

3.3.7. Effect of aging on cytokine secretion

T cell aging is characterized by the loss of autocrine proliferation, often with many population doublings (PD) before replicative senescence. T cell clones (TCCs) cannot secrete IL-2 in large amounts, but can be maintained with exogenous IL-2 (237). Old cells from TCC retain antigen-specific TCR function, as shown by their ability to secrete cytokines such as IFN-γ, and IL-10. Decreased IL-2 secretion with increased IL-10 and constant IFN-γ production reflects consensus on cytokine secretion (310). Argentati *et al* (311) demonstrated that γδT cells change in aging, with a reduced representation and an altered cytokine production. γδT deterioration may represent "immunosenescence"; therefore, old and very old subjects have little protection against infections and cancers.

3.3.8. The remodeling theory of immunoscenscence

Healthy centenarians illustrate successful physiological aging (210), escaping major age-associated diseases. Many are in good mental and physical condition. Franceschi (210) discussed the fact that immune parameters are preserved, suggesting that immune parameters remodel, rather than deteriorate, with age. Three paradoxes emerged when old people and centenarians were studied: (i) a relatively high number of T cells with an involuted thymus (312); (ii) absence of organ-specific autoantibodies and an increase of non organ-specific autoantibodies, with a ten-fold decrease of circulating B cells and higher IgG and IgA levels (312, 313); and (iii) increased proinflammatory cytokines, such as IL-6 and chemokines (Rantes, MCP-1), and soluble receptors for proinflammatory cytokines (TNFRI, TNFRII) (32).

In healthy elderly and centenarians, memory T cells ($CD45RO^+$, $CD95^+$) (314, 315) and cytotoxic effector T cells ($CD28^-$) expanded (315). This is more evident in $CD8^+$ than in $CD4^+$ T lymphocytes (227, 315). Age-related increases of memory cells occur with the expansion of T cell clones - mostly memory, but also naive – and shrinkage of the T cell repertoire (316). This occurs earlier in $CD8^+$ than $CD4^+$ cells. The paradoxical increase in circulating T cells in healthy centenarians is due to an expansion in peripheral T cells, rather than to naïve T cell development in the thymus.

T cell function is altered *in vivo* and *in vitro* in the elderly (311, 317), reflecting the deterioration of immune function. Changes in the T-lymphocyte compartment are the most critical component of immunological aging.

3.4. NK cell and aging

Immunosenescence affects all compartments of the immune system. Age changes were demonstrated in T lymphocytes and in innate immunity (including NK cells). An increase in NK cells showing a mature phenotype was found in healthy elderly donors who have an NK-cytotoxic capacity of total peripheral blood lymphocytes preserved (318). NK-cell killing of K562 is impaired on a per-cell basis (K562 is an erythroleukemia cell line derived from a chronic myeloid leukemia patient in blast crisis). NK cells from elderly people show a decreased proliferative response to IL-2 and an impaired expression of CD69 activation antigen. Therefore, phenotypic and functional alterations are found in the NK cells of healthy elderly people. Changes are compatible with the expansion of a mature NK subset (318).

3.4.1. NK cell number and phenotype in the elderly

Normal aging is associated with increased circulation of NK cells (CD16, CD56) and NK/T cells (CD16, CD56, or CD57), an increased proportion of T cells co-expressing NK cell markers (319-321), and decreased endogeneous and lymphokine-induced NK cell lytic activities (322). NK cells, with their cytokine and cytolytic activity, are critical to host defense against invading organisms and cancer. Using cell surface markers, NK cells divide into $CD16^+$ $CD56^{dim}$ NK cells and $CD16^-$ $CD56^{bright}$ NK cells (323-326). The two subsets of NK cells have different functions: $CD16^+CD56^{dim}$ cells are effectors of cytotoxicity and antibody-dependent T cell-mediated cytotoxicity (ADCC); $CD16^-CD56^{bright}$ cells have immunomodulatory function through cytokine secretion (327).

Based on the known functions of NK cells, the association of decreased NK activity with mortality and the incidence of severe infections in the elderly may be causal (226). Sansoni (312) showed that, while CD16, CD56, or CD57 NK numbers did not decline with age, their proportions increased. NK function on a per-cell basis was either stable to age 70 (328) or declined with age (319). Centenarians had higher NK and redirected killing activity than middle-aged donors (312).

CD56 expression in the healthy aging of NK subpopulations did not reveal phenotypic changes in the $CD56^{bright}$ subset. $CD56^{dim}$ NK cells from elderly donors expressed higher levels of HLA-DR and CD95 (Apo1/fas) and lower levels of CD69 than $CD56^{dim}$ NK cells from young donors (321). In the elderly, an increase in the of number NK cells is due to increases in the $CD56^{dim}$ subset. Because $CD56^{dim}$ represents a mature NK cell subset (329), this suggests that a phenotypic and functional shift in NK cell maturity occurs in aging (330).

An age-dependent functional decline of NK-cells is due to: 1) a decreased response to positive modulators (IFN-α, IL-2, IL-12) (331), 2) an increased response to ATP (332), and 3) increased microviscosity of the cell membrane.

3.4.2. NK cell cytotoxicity in the elderly

NK cells are important to the innate immune response against viral infections and cancer. Age-dependent modulation of NK cell activity may alter cell immunological protection and anti-tumor activity (318). Because non-MHC–restricted killing of target T cells is characteristic of NK cells, their cytotoxic capacity has been analyzed. NK cytotoxicity of total peripheral blood lymphocytes is not significantly affected in NK cells from healthy elderly people (333-335, 312). Facchini *et al* (319) and Mariani *et al* (336) showed that circulating NK cells from elderly people have decreased cytotoxic function – both after sorting freshly isolated cells and after limiting dilution and cloning of NK cell precursors. Neither binding of effector target T cells nor perforin content, distribution and utilization in lytic assays were different in old and young (337) organisms. Defective signal transduction may be important for decreased NK cytotoxic activity. Defective NK cell cytotoxicity is associated with the decreased capacity of NK cells to release IP3 after interacting with target T cells and with delayed hydrolysis of PIP2; indicating that PKC dependent pathways are affected by aging (338).

The CD16 molecule (IgG FcγRIIIA receptor) characteristic of NK cells (339) is a transmembrane protein with a cytoplasmic domain (340). FcγRIIIA links cellular and humoral immunity by linking antibody specificity and cytotoxic effector cell function - leading to ADCC, which is important in defending against infectious diseases and cancer (341). ADCC of peripheral blood lymphocytes is comparable in young and old (342, 343). The $CD16^-$ $CD56^{bright}$ NK subset lacks this function (323). The PI-3-kinase pathway triggered by CD16 crosslinking is not significantly affected in NK cells from elderly people, indicating that transduction pathways in natural or CD16-dependent NK cytotoxicity are differentially affected by aging (338).

3.4.3. NK cell response to cytokines in the elderly

NK cells express cytokine and chemokine receptors (and other immunoregulatory cytokines and chemokines supporting regulation of immune responses by promoting downstream adaptive, Th1-mediated responses to infection). NK cell activation by IL-2 enhances

cytotoxicity and induces the proliferation, production, and release of cytokines. The overlapping effects of IL-2, IL-12, IFN-? on human NK cells were mediated by differential utilization of the signal transducer and the transcription activator. IL-12 activates cytotoxicity; ADCC does not. Anti-tumor lytic activity of NK-cells is stimulated by factors such as IFN-γ, IL-2 and inhibitory factors (e.g., prostaglandins [344]).

In vitro activation of NK cells with IL-2 or other cytokines enhances the killing of NK-sensitive (i.e., K562) and NK-resistant target T cells (318). The response of NK cells from elderly individuals to IL-2 or other cytokines shows decreased proliferation, expression of CD69, and killing of NK-resistant T cell lines. Early IFN-γ and chemokine production in response to IL-2 or IL-12 is also decreased. Aging does not alter other NK cell functions (such as TNF-*a* production or perforin induction) in response to IL-2.

3.4.4. Altered NK cell cytokine production in the elderly

Reduced IFN-? production by leukocytes from the elderly is associated with a decrease in an IFN-?-producing cell population ($CD45RO^+/CD8^+$ T cells [345]). There are conflicting results regarding this population (32, 346-350). This may be the result of *in vivo* preactivation of cells or cell subsets that are not in circulation (due to recruitment). This is important because the number of NK cells increases with aging (312, 346, 350), and NK cells produce IFN-? (351); cytotoxic activity does not increase (352).

3.4.5. NK cell activity as a biomarker of healthy aging

NK cell activity changes with age in humans and mice. The mechanisms responsible for age declines of NK cell responses have been examined in many studies. The responses of NK cells to poly (I: C), Con A, and LPS have been examined in young and aged animals. Through an impaired production of chemokines, the decreased activity of NK cells may be involved in an increased incidence of infections and cancers in the elderly.

Due to differences in the selection criteria for subjects and study size, there are conflicting reports about NK cell activity in elderly people (312, 353-357, 320). The SENIEUR protocol standardized subject selection for immunological studies (358). In Ogata's study (359), although NK cell activity (lytic unit [LU]) did not show an age-related decrease, absolute NK cell activity (ALU) *in vivo* and NK cell activity on a per-cell basis showed an age-related decrease, suggesting that NK cells are not exempt from the effects of aging. Hsueh *et al* (360) observed that basal and induced NK cell activity declined with age. NK cell activity is important in human longevity (312) and predicts mortality in humans (359) and mice (361). Evidence shows that low NK cell function is related to a greater risk of infections in elderly subjects (359).

In conclusion, the increase in the number of "classical" mature NK and NK/T cells in aging is associated with the defective functional capacity of NK cells. Low NK cell numbers or function in elderly individuals is associated with increased mortality and incidence of severe infections (226).

4. SUMMARY

Aging is associated with a decline in immune function, increased inflammatory activity, and increased levels of proinflammatory cytokines (e.g., IL-6, TNF-a). While the data are not unanimous, IL-6 and TNF-a are believed to be associated with morbidity and mortality in the elderly, and IL-6 is the strongest risk marker in healthy elderly people. Circulating levels of TNF-a are the best predictor of mortality in frail elderly people (166). This indicates that, in healthy old populations, increases in circulating IL-6 represent a systemic response to local proinflammatory activities; when age-related inflammatory diseases progress, TNF-a increases and becomes a stronger risk marker than IL-6. Chronically elevated TNF-a and IL-6 have different biological functions that trigger age-associated pathology and mortality (166).

Alterations in the cells of the adaptive and innate immune system contribute to age-associated morbidity and mortality, determining the relative roles of these immune pathways. As we better understand age-associated inflammatory cytokine and cellular changes, we may develop immune-based therapies to combat the morbidity and mortality that are associated with aging. Aging is accompanied by alterations in most physiological functions - especially declines in cellular and humoral immunity. T cells appear more sensitive to aging than other immune cells. Changes in the T lymphocyte compartment is the most critical component of immunological aging. Significant changes in the functional and the phenotypic profiles of T cells have been documented both in aging humans and in rodents. NK cells play a significant role in defending against infections (due to their cytotoxicity and the cytokines produced - in particular IFN-*?*). Phenotypic and functional alterations are found in the NK cells of healthy elderly people.

A better understanding of the cellular and molecular basis of age-associated T cell and NK cell alterations will open new possibilities to manipulate the immune system in the elderly and will enhance innate and adaptive immune responses against infectious diseases.

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Footnote: 1**.** naïve: An undifferentiated immune system cell. Naive cells are "uncommitted" and respond to general antigenic stimulation (i.e., not only to a specific antigen). 2. naive t-cell: a subset of CD4 and CD8 cells, which proliferate rapidly when exposed to new antigens. A naive T-cell is not yet "programmed" to stimulate either a cell-mediated (Th1) or a humoral (Th2) response.

Key Words: Inflammatory Cytokines, Interleukin–6, IL-6, Tumor Necrosis Factor-alpha, TNF- a, Aging, T cells, NK cells, Review

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