The clinical impact of systemic low-level inflammation in elderly populations

With special reference to cardiovascular disease, dementia and mortality

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1. INTRODUCTION

Old-age survival has improved substantially since 1950 in developed countries. As a result the number of octogenarians has increased four-fold, the number of nonagenarians eight-fold, and the number of centenarians twenty-fold [1]. Whether the added years at the end of the life cycle are healthy, enjoyable, and productive depends upon preventing and controlling a number of chronic diseases and conditions.

It has become increasingly clear during the last decade that inflammatory processes are central parts of the pathology in nearly all causes of morbidity in populations aged 65+ years, e.g., atherosclerosis, Alzheimer's disease (AD), type 2 diabetes, pulmonary diseases, osteoporosis, and osteoarthritis. Furthermore, we begin to understand the geriatric syndrome of frailty (defined largely by wasting and functional disability) that appears in part to be characterized by inflammatory mechanisms [2].

Investigations of age-related inflammatory activity originated in studies of the aged immune system. Immunosenescence is defined as an age-related deterioration of immune function [3]. Until recently immunogerontological studies focused mainly on cross-sectional studies of small groups of healthy, elderly subjects selected by "The SENIEUR protocol", which attempted to separate the influence of disease from physiological aging [4]. This type of investigations lead to the paradigm that enhanced activity in the innate immune system reflected "successful" aging that counteracted decreased adaptive immunity in elderly people [5]. However, "The SENIEUR protocol" excludes up to 90% of old nursing home residents and the protocol misses that the evaluated parameters may be associated with subclinical disease. Furthermore, immunosenescence represents probably a continuum of changes that are related to age-related pathology and attempts to make a strict separation are, in my opinion, of academic rather than of clinical importance.

In the light of these considerations, we initiated investigations of the immune function in 1996 in well-described cohorts of old people. Furthermore, dynamic aspects of the immune system during stress situations were studied *in vivo* in smaller groups of patients and well-characterized volunteers. The main purpose was initially to characterize immune function in approximations to normal old populations. The research focus was subsequently directed towards the origin and the clinical impact of the systemic inflammatory burden. This orientation was highly stimulated by the appearance of new and more sensitive commercial assays for pro-inflammatory products that demonstrated increased risk among persons who were previously all thought to have values within the normal range.

1.1. HYPOTHESES

The purpose of the present thesis was to test the following hypotheses:

1. Aging is associated with a dysregulated acute phase response due to enhanced production of proinflammatory cytokines that contributes to increased morbidity and mortality from infections and a chronic proinflammatory state.
2. Systemic low-level inflammation defined as 2-4 fold increases in circulating levels of inflammatory mediators predict high mortality risk due to a causal relation to age-related disorders such as cardiovascular disease (CVD) and dementia. Tumor necrosis factor (TNF)-α and interleukin (IL)-6 have important biological functions although different clinical effects as the two cytokines provide central links between the innate immune system, the metabolism, endocrine systems, and the brain. TNF-α is considered to be a risk factor because it acts proatherosclerotic, catabolic, and neurotoxic. The common assumption of IL-6 as an overall harmful proinflammatory cytokine is challenged because IL-6 has also anti-inflammatory properties and counteracts TNF-α induced pathology.
3. Functional cytokine polymorphisms are risk factors in age-associated diseases and predictors of mortality risk.

The following investigations tested these hypotheses:

Age related differences in the production of proinflammatory and anti-inflammatory cytokines in response to an acute challenge were studied *in vitro* (III) and *in vivo* (VI) following lipopolysaccharide (LPS) stimulation and *in vivo* in patients with pneumoccocal infections (II) in order to test the hypothesis that aging is associated with a dysregulated acute phase response.

Associations between inflammatory markers in the blood, cytokine polymorphisms, morbidity and mortality were evaluated in:

A. Centenarians from the Danish Centenarian Study at Aging Research Center, University of Southern Denmark, Odense, Denmark (I, VII, X). Participants were examined at home in 1995-1996 and represented 75% of Danes (N = 207) who celebrated their 100th birthday during the study period [6]. Not all subjects agreed to a blood test and a full physical examination and accordingly, inflammatory parameters in plasma were only analyzed in 126 centenarians. There was no difference in the housing situation, the cognitive function or the ankle-brachial arterial blood pressure index (ABI) in these 126 participants and the remaining part of the cohort.
B. 80-year-old people (N = 333), constituting the 1914-population in Glostrup population studies at Research Centre for Prevention and Health, Glostrup University Hospital, Denmark (IV, V, VIII, IX). Participants were examined at Glostrup Hospital in 1996 [7]. A subset of 174 subjects managed an extra visit at Department of Infectious Diseases, Rigshospitalet, making it possible to measure leukocyte subsets and to perform cellular assays. These people represent probably the healthiest part of the 1914-population.

Danish centenarians represented the ultimate clinical end state [6] whereas octogenarians were relatively healthy [7] and constituted to a higher degree a population at risk. The evaluated inflammatory markers included plasma levels of cytokines in both cohorts. This thesis addresses mainly TNF-α and IL-6 although other cytokines and acute phase proteins may be relevant in this context as well. However, this focus is chosen because TNF-α and IL-6 are pleio-

tropic cytokines (see the second hypothesis). An initial study of centenarians (I) made us believe that TNF-α was an important player in age-related morbidity. However, the rest of the world has focused on IL-6, which has been called "a cytokine for gerontologists" [8]. The clinical impact of the TNF-308G/A promoter polymorphism was studied in centenarians because TNF-α protein in plasma was the strongest risk factor as well as disease marker within the oldest old. The IL6 –174G/C promoter polymorphism was studied in octogenarians, as circulating IL-6 protein possessed the best predictive value among old elderly. Neutrophils and NK cells were only studied in the subgroup of the 1914-population that visited Department of Infectious Diseases, Rigshospitalet (V).

The thesis will mainly discuss human studies.

2. INFLAMMATORY ACTIVITY IN ELDERLY POPULATIONS

It has been recognized for a long time that aging is associated with changes in immune function characterized by an increased number of natural killer (NK) cells concomitant with a decreased adaptive immune response and an altered capacity of cytokine production in old people [9; 10]. The purpose of the present chapter is to review data on age-related changes in inflammatory mediators and innate immunity and to discuss the hypothesis that aging is associated with a dysregulated acute phase response due to an altered capacity of proinflammatory cytokine production, which contribute to a pro-inflammatory profile as well as increased morbidity and mortality from infections.

2.1. THE INFLAMMATORY RESPONSE

Most information about inflammatory processes comes from studies on acute infections and tissue injury. Thus, in response to tissue damage elicited by trauma or infection the acute phase response set in, constituting a complex network of molecular and cellular interactions with the purpose to clear infectious agents, to aid tissue repair, and to facilitate a return to physiological homeostasis [11]. The acute phase response is composed of local events as well as a systemic activation, Figure 1.

Cytokines are defined as chemical mediators released by cells that affect the behaviour of other cells [12]. TNF-α and IL-1β are classical proinflammatory cytokines that are produced in large amounts by macrophages at the local inflammatory site [13]. They induce a second wave of cytokines including IL-6 and chemokines (e.g., IL-8 and macrophage inflammatory proteins) [11]. Among many activities, IL-6 induces the synthesis of acute phase proteins in the liver such as C reactive protein (CRP), serum amyloid A (SAA), and fibrinogen [14]. TNF-α and IL-1β are also able to stimulate CRP production independently of IL-6. Chemokines regulate the influx of leukocytes to the site of infection [11]. Cells of innate immunity appears in the initial phase of the inflammatory response including neutrophils followed by macrophages, which mature from their precursor monocytes, and NK cells. These cells perform phagocytic and cytotoxic activities and are defined as inflammatory cells [12]. Beside their early control of infections they play a central part in the initiation and subsequent direction of adaptive immune responses by their production of cytokines.

The balance between proinflammatory and anti-inflammatory cytokines and the magnitude of the inflammatory response are crucial. It has recently been suggested that insufficient proinflammatory responses can lead to increased susceptibility to infections and cancer whereas excessive responses cause morbidity and mortality in diseases such as atherosclerosis, diabetes, AD, and autoimmune diseases or shock during acute infections [15].

IL-6 represents a key point in the regulation of the acute phase response. This important cytokine is often classified as a proinflammatory cytokine but it has also many anti-inflammatory and immunosuppressive effects: IL-6 stimulates the pituitary-adrenal axis, inhibits the synthesis of TNF-α, and stimulates the production of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (Ra) that binds to IL-1 receptors in competition with IL-1 [16]. Furthermore, it induces the shedding of TNF receptors by neutrophils. Soluble TNFRs (sTNFR) secondly bind circulating TNF-α, attenuating the bioactivity or serving as slow-release reservoirs [16]. Plasma levels of TNF-α and sTNFRs are strongly correlated but sTNFRs are more stable in the circulation and it has been suggested that they act as long-term markers of TNF-α [17; 18].

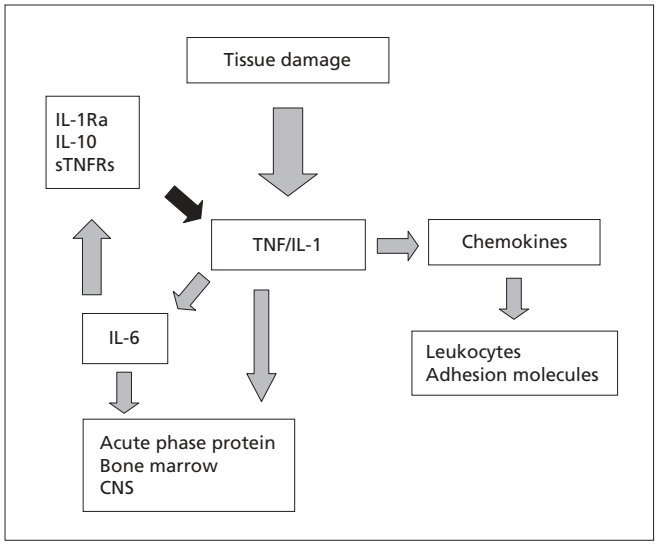


Figure 1. The acute phase response. Grey arrows mark positive stimulation. Black arrow marks inhibition.

It was believed until recently that cytokines had mainly immunoregulatory effects and immune cells were believed to be the main source of their origin. However, consistent with the new paradigm that the major, chronic age-related diseases are inflammatory diseases it has also been recognized that cytokines such as TNF-α and IL-6 possess powerful metabolic and endocrine effects (see section 3). Moreover, a wide range of cells outside the immune system contribute also importantly to their production including fat cells, endothelial cells, muscle cells, osteoclasts, and cells in the central nervous system (CNS) [19], Figure 2. Depending on the cellular source, cytokines are categorized as monokines, lymphokines, adipokines, and etcetera.

It is not surprising that plasma levels of inflammatory mediators are often correlated, considering their production is tightly linked. This considerable covariance makes it difficult to separate their effects from each other in epidemiological designs and in some experimental studies.

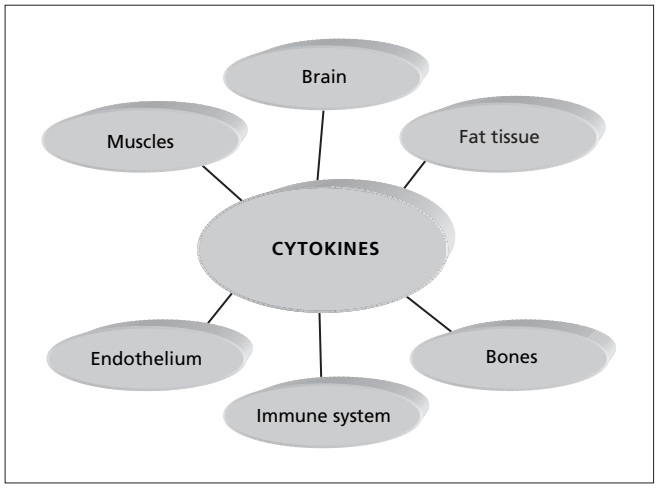


Figure 2. Sources of cytokines. A wide range of organs and different cell types contributes to the production of cytokines such as TNF-α and IL-6, which have local as well as endocrine activities and act as important regulators of the metabolism and immune functions.

2.2. AGE-RELATED CHANGES IN CIRCULATING LEVELS OF INFLAMMATORY MEDIATORS

In a study of centenarians compared to octogenarians, middle-aged people and young, healthy controls, an age-related increases in plasma levels of TNF-α, sTNFR-II and IL-6 were demonstrated (I). This observation resulted in the conclusion that aging was associated with a proinflammatory state. Plasma levels of TNF-α, IL-6, sTNFR-II, and CRP were correlated in centenarians, suggesting an activation of the entire inflammatory cascade in the oldest old. Increases in inflammatory markers were, however, only 2-4 fold and thus far from increases observed during acute infections.

Consistent with our findings of a systemic low-level pro-inflammatory state in old humans (I), other studies have reported an age-related increase in circulating levels of TNF-α [20], IL-1β [21], IL-6 [22-28], sTNFRs [29; 30], IL-1Ra [29], and acute phase proteins such as CRP and SAA [31; 32] and we have confirmed our results in later studies as well (IV)[33]. However, it is still an on-going debate how circulating levels of single inflammatory mediators are affected in old populations. Thus, some studies have failed to demonstrate age-related increases in TNF-α [34; 35] and IL-6 [34; 36]. Discrepancies probably relate to variations in sensitivity of the used assays, to lack of power in some studies, to differences in age of the study populations, and especially to differences in the health status. For instance, IL-6 was already increased in middle-aged humans compared to young controls whereas elevated TNF-α was only detected in 80-year-olds and centenarians (I), Figure 3. This likely explains why most immunogerontological studies have focused on IL-6. Plasma levels of IL-6 were, moreover, higher in randomly selected subjects compared to very healthy elderly individuals selected in accordance with the SENIEUR protocol [37], demonstrating a strong influence of health status. However, "successful ageing" (defined as aging without comorbidity) was still associated with low-level increases in plasma levels of TNF-α and IL-6 in a study of 20 very healthy, elderly subjects aged 65-80 years compared with 16 elderly patients with type 2 diabetes and young controls aged 20-35 years [33]. The latter finding may reflect an association to subclinical disease, different body compositions, different life style factors, or an age-related change in the production/clearance of inflammatory cytokines.

Accordingly, it appears that the process of aging with or without the accompaniment by age-related disorders is associated with an increasing activation of the entire inflammatory cascade. I find it most likely that failures of detecting TNF-α often reflect that this cytokine is mainly produced and works locally. Moreover, TNF-α has a limited half-life, making it difficult to detect in the circulation unless large amounts are produced. However, a minor local and/or systemic production of TNF-α is probably sufficient to induce a systemic anti-inflammatory response including increased circulating levels of IL-6. It is thus likely that the evaluation of sTNFRs and IL-6 in plasma gives us a better picture of local TNF-α production than circulating TNF-α protein in healthy, younger elderly with less extensive pathological processes.

2.3. AGE-RELATED CHANGES IN COUNTS AND CYTOTOXIC FUNCTIONS OF INFLAMMATORY CELLS

It is expectable by intuition that a chronic proinflammatory state mobilises inflammatory cells in the blood (neutrophils, monocytes, and NK cells) and affects their cytokine production as well as cytotoxic activities. The extensive amount of data on monokine production is reviewed in section 2.4. The available information in the literature is, however, limited regarding age-related effects on counts and cytotoxicity in relation to neutrophils/monocytes/macrophages. Octogenarians from the 1914-cohort had a minor increase in the number of neutrophils in the blood compared to young controls in their twenties (mean 3.2×10^9 cells/l versus 2.6×10^9 cells/l) and the number of monocytes was elevated in elderly men but not in elderly women (III). The number of monocytes was also enhanced in

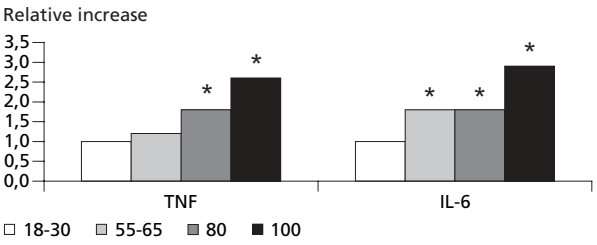


Figure 3. Age-associated increases in plasma levels of cytokines. Relative increases in plasma levels of cytokines in young elderly (55-65 years), old elderly (80 years) and centenarians (100 years) compared to young humans (18-30 years). Based on geometric means from Table 2 in I. * = denotes $p<0.5$ compared to young controls.

healthy Danes aged 61-69 years (VI). In accordance with this, others have reported that the neutrophil count is enhanced [38] or unaltered [39] in apparently healthy, elderly humans. Furthermore, the respiratory burst and the production of reactive nitrogen intermediates are impaired, whereas phagocytosis and chemotaxis are either moderately impaired or unaltered (reviewed in [40; 41]). In contrast to the sparse data about early inflammatory cells, a large number of studies have evaluated age-related changes in NK cells. Most investigations report either increased (V)[42-45] or unaltered [46; 47] counts of NK cells in healthy old humans and decreased cytotoxicity per NK cell in short-term assays (V)[46; 48-52]. Accordingly, it appears that elderly humans have enhanced counts but attenuated cytotoxic functions of cells in the innate immune system.

There is some evidence from smaller studies that decreased NK cell mediated cytotoxicity is associated with frailty in elderly populations. Thus, lack of functional independence in daily activity was associated with a low number of NK cells [53] and poor NK cell cytotoxicity was accompanied by a history of severe infections [46]. Middle-aged humans had decreased NK cell activity compared to young controls, whereas NK cell activity of centenarians was within the range of young controls [54]. The latter study did not compensate for an age-related increase in the percentage of NK cells among blood mononuclear cells (BMNC) as a consequence of decreased numbers of T lymphocytes. Nevertheless, it formed the basis for the hypothesis that well-preserved NK cell activity was important for successful aging and increased NK cell counts in healthy, elderly people represented a reshaping of immune function with the purpose to compensate for decreased cytotoxicity per NK cells as well as attenuated adaptive immunity [5]. However, the potential of natural cytotoxicity was not preserved in octogenarians from the 1914-cohort compared to young controls when an index was calculated including cytotoxicity per NK cells and the number of NK cells in blood (V). On one hand, this might reflect that octogenarians represented a population-based cohort, which did not fulfil the criteria of the SENIOUR protocol. On the other, I find it plausible that chronic immune activation induces mobilisation and anergy of inflammatory cells. Thus, chronic immune activation is associated with decreased NK cell mediated cytotoxicity in other clinical situations such as early HIV infection [55]. Furthermore, plasma levels of TNF-α were weekly correlated to the total white blood cell (WBC) count in octogenarians (IV) and in Finnish men aged 49-70 years [56], and infusion of IL-6 in physiological doses (corresponding to levels obtained during intense exercise) resulted in mild neutrocytosis and lymphopenia [57].

2.4. AGE-RELATED CHANGES IN THE PRODUCTION OF PROINFLAMMATORY CYTOKINES

Increased mortality in old rodents has been related to decreased ability to down-regulate excessive proinflammatory cytokine release in septic models [58; 59]. However, clinical studies of acute serious illness indicate that aged humans are less likely to develop fever and leukocytosis and are more likely to die from the infection than younger age groups [60-62], suggesting a paucity of inflammatory

signs. This section will focus on TNF-α, IL-1β, and IL-6 because the first two cytokines represent classical proinflammatory cytokines whereas the latter has proinflammatory activities but is also considered to switch on the anti-inflammatory response (see section 2.1).

Escherichia coli (*E. coli*) LPS is a wall constituent of gram negative bacteria that induces a strong stimulation of monocytes and macrophages. Most studies report the *in vitro* production of TNF-α, IL-1β and IL-6 to be attenuated in old rodents in response to LPS [63-68]. Consistent with this, levels of TNF-α and IL-1β were decreased in whole blood supernatants following 24 hours of *in vitro* LPS stimulation in 168 octogenarians compared with 91 younger controls whereas no difference was observed with regard to IL-6 (III). Subsequent analyses revealed that the cytokine production in the old group was only attenuated compared to young men but not compared to young women, suggesting an age-related decline as well as a suppressive effect of estrogens in young women and/or a selection of individuals with a low TNF-α and IL-1β production for longevity (III). Other human *in vitro* studies have reported extremely conflicting results [69-75], see Table 1 for an overview. Most studies include a low number of subjects including both men and women, which is problematic due to the large interpersonal variances and the strong influence of sex as demonstrated in the study of octogenarians (III). Moreover, reverse results may be obtained from cultures of BMNC versus whole blood [75]. Whole blood is a more dynamic culture system and peak levels depend largely on the dilution and occur at an earlier time point compared to BMNC culture [76]. Cultures with a fixed number of isolated BMNC favour a higher number of monocytes from aged people than from young controls because the number of monocytes is unaltered or increased in elderly people whereas the number of lymphocytes is decreased. Most studies measure also the cytokine production only at one time point. The choice of this will largely affect the conclusion as reflected in the following pilot study: LPS stimulation for 4, 6, 12, 24, 48 hours resulted in more rapid increases but no difference in peak levels of TNF-α in whole blood cultures (diluted 1:4) from 8 healthy elderly people aged 77-81 years compared with 8 young controls aged 20-30 years [77], Figure 4. The study by Born et al [71] reported an age-related increase in levels of TNF-α and IL-1β after LPS stimulation for 48 hours in undiluted blood but our pilot study (Figure 4) showed that peak levels were much earlier (12-24 hours) and pronounced decreases had probably occurred after 48 hours, especially as undiluted whole blood cultures were used. Thus, the 48 hours assay [71] reflected probably age-related prolonged proinflammatory activity rather than differences in peak values. In the light of these considerations, I conclude that most studies have demonstrated

Table 1. *In vitro* production of TNF-α, IL-1β and IL-6 in elderly populations.

References	Design	Stimulation	Cell type	Time	Elderly versus young
Rudd and Banerjee, 1989 [69]	Elderly (>70 y): N = 33 Elderly with infections, N = 40 Young (<40 y): N = 40	LPS	Monocytes	24 h culture	No difference in IL-1β
Riancho et al., 1994 [70]	Elderly (>55 y): N = 15 Young (<55 y): N = 18	LPS	BMNC	24 h culture	Increased IL-1β No difference in TNF-α
Born et al., 1995 [71]	Elderly (mean 80 y): N = 16 Young (mean 25 y): N = 16	LPS	Undiluted whole blood	48 h culture	Increased TNF-α and IL-1β
McLachlan et al., 1995 [72]	Elderly (>65 y): N = 25 Young: N = 25	LPS	Monocytes	16 h culture	Decreased IL-1β
Gon et al., 1996 [73]	Elderly (>80 y): N = 10 Young (<39 y): N = 10	LPS	Monocytes	24 h culture	Decreased TNF-α and IL-1β
Roubenoff et al., 1998 [74]	Elderly (mean 79 y): N = 742 Young (mean 39 y): N = 21	(a) LPS and *S. epidermidis* (b) PHA	(a) BMNC (b) BMNC	(a) 22 h culture (b) 22 h culture	(a) No difference in TNF-α and IL-1β (b) No difference in IL-6
Bruunsgaard et al., 1999 [III]	80 y: N = 168 18-30 y: N = 91	LPS	Whole blood diluted 1:4	24 h culture	Decreased production of IL-1β and TNF-α. No difference in IL-6
Gabriel et al., 2002 [75]	Elderly (mean 73 y): N = 16 Young (mean 28 y): N = 16	(a) LPS (b) LPS	(a) Whole blood diluted 1:9 (b) BMNC	(a) 24 h and 72 h culture (b) 24 h culture	(a) Increased IL-1β and IL-6 after 24 hours. No difference in TNF-α (b) Decreased IL-1β and IL-6
Fagiolo et al., 1993 [35]	Elderly (mean 81 y): N = 13 Young (mean 27 y); N = 13	PMA + PHA	BMNC	24 h, 48 h and 72 h culture	Increased production of TNF-α, IL-1β and IL-6
O'Mahony et al., 1998 [79]	Elderly (mean 73 y): N = 9 Young (mean 29 y): N = 10	PMA	BMNC	24 h, 48 h and 72h culture	Increased percentage of TNF^+ $CD3^+$ and $IL6^+$ $CD3^+$ cells. No significant difference in TNF, IL-1β and IL-6 producing monocytes. No difference in TNF-α, IL-1β or IL-6 in culture supernatants (72 h)
Saurwein-Teissl et al., 2000 [80]	Elderly (>65 y): N = 31 Young (<35 y): N = 29	Influenza virus	BMNC	7 days	Increased TNF-α production
Ahluwalia et al., 2001 [39]	Elderly (62-88 y): N = 44 Young (20-40 y): N = 26	PHA	BMNC	48 h culture	No difference in IL-1β and IL-6
Beharka et al., 2001 [36]	Elderly (65-85 y): N = 26 Young (20-30 y): N = 21	(a) PHA (b) ConA	(a) BMNC (b) BMNC	(a) 48 h culture (b) 48 h culture	(a) No difference in TNF, IL-1β, and IL-6 producing monocytes (b) Decreased IL-6 production
McNerlan et al., 2002 [78]	Elderly (mean 92 y): N = 13 Young (mean 24 y): N = 6	PMA + Ionomycin	Whole blood diluted 1:1	4 h culture	Increased percentage of TNF^+ $CD3^+$ cells
Sandmand et al., 2003 [81]	100 y: N = 25 80 y: N = 14 18-30 y: N = 28	PMA + ionomycin	BMNC	4 h culture	Increased percentage of TNF^+ $CD3^+$ cells

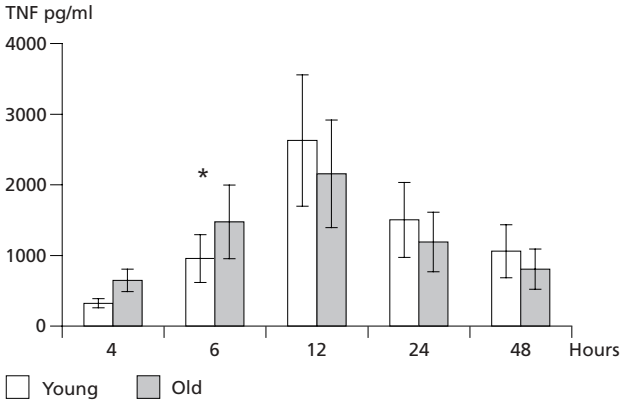


Figure 4. *In vitro* LPS-stimulated TNF-α production in whole blood from old versus young humans. Whole blood (diluted 1:4) was stimulated by *in vitro* LPS for 4, 6, 12, 24, 48 hours in 8 healthy elderly people aged 77-81 years and 8 young controls aged 20-30 years. The level of TNF-α was evaluated in culture supernatants. Mean and SE is shown. * = p<0.05. From ref. [77].

decreased or unaltered peak values of TNF-α, IL-1β and IL-6 in response to *in vitro* LPS stimulation of blood derived cell cultures. However, it also appears that elderly humans have a more rapid response following LPS stimulation and maybe a prolonged production. Further well-conducted studies with several time points and calculations of the area under curve are needed to really understand the capacity of cytokine production in aged monocytes. Furthermore, it is important to realize that LPS stimulated *in vitro* production of cytokines are in general poorly correlated with circulating plasma levels, e.g., plasma levels of TNF-α were not correlated with *in vitro* LPS-stimulated TNF-α levels in whole blood supernatants in octogenarians (III). Thus, stimulated *in vitro* cultures reflect probably the capacity of cytokine production in the blood whereas plasma levels reflect the sum of ongoing inflammatory processes in the whole body (the inflammatory burden).

With regard to culture systems including other types of stimulation such as PHA, PMA, and influenza antigen, which all include activation of T lymphocytes, most studies find an increased production of TNF-α and IL-6 both on a single cell level as well as in culture supernatants from aged adults [35; 78-81] although no difference has also been reported [36; 39], Table 1. It is probable that increased production of inflammatory cytokines by T lymphocytes from elderly people is related to an altered phenotype (increased number and percentage of $CD45R0^+$ and $CD28^-$ cells) and a shift in the balance between type 1 and type 2 cytokines [82]. However, there was no difference between the proportions of T lymphocytes producing TNF-α following PMA+ionomycin stimulation in centenarians compared with young controls whereas the maximal TNF-α levels measured in culture supernatants were severely decreased in the oldest old, indicating that T cells were not responsible for high plasma levels of TNF-α in the very old [81].

Unstimulated short-term cultures reflect to some extent priming of the cytokine profile *in vivo.* An age-related increased production of IL-6 and IL-1Ra was reported in unstimulated cultures of BMNC from 711 elderly participants from the Framingham Study and 21 healthy, young volunteers whereas the production of TNF-α and IL-1β was equal [74]. Considering that IL-6 has also anti-inflammatory effects [16; 57; 83], this finding suggests an anti-inflammatory priming of BMNC and indicates that mainly cells outside the blood are responsible for the increased production of early proinflammatory mediators.

Conclusions from *in vitro* studies are limited as cells outside the blood also produce proinflammatory cytokines during *in vivo* situations, Figure 2. For instance, arterial walls of aged rats displayed an increase in IL-6 and TNF-α production in response to LPS compared to young animals [84]. Few studies have evaluated *in vivo* cytokine production in old versus young humans. High age was associated with a slower normalisation of circulating levels of TNF-α and sTNFR-I as well as a prolonged increase in the TNF-α/IL-10 ratio in patients with severe pneumococcal infections (II). This finding could reflect decreased ability to control the infection and/or a dysregulated down-regulation of activity in the TNF system. This aspect was further investigated in a human *in vivo* sepsis model in which *E. coli* endotoxin was injected intravenously to nine very healthy, elderly volunteers aged 61 to 69 years and 8 young controls (VI). The elderly demonstrated more rapid increases in TNF-α and sTNFR-I in plasma and a slower normalization of TNF-α, sTNFR-I and CRP although there was no age-related difference in peak levels, Figure 5. Furthermore, elderly humans were capable of producing a fever response equal to that observed in the young controls, but they had a slower normalization of the body temperature (VI), supporting the hypothesis of an age-related dysregulated acute phase response.

There was no significant difference in peak values of cytokines in VI whereas IL-6, but no other inflammatory mediators, was lower in old patients with pneumoccocal infections on admission to hospital in II. In contrast, circulating TNF-α was significantly higher in the oldest patients at enrolment in a study of 930 patients with septic shock [85] whereas levels of cytokines in serum were lower in 15 old patients compared with 22 younger patients with pneumonia [73]. These discrepancies are likely to reflect that many clinical factors vary on admission to the hospital. Accordingly, *in vivo* models point towards a more rapid response and a delayed termination of activity in the TNF system, which are largely in accordance with observations in LPS *in vitro* experiments. The more excessive production of TNF-α in the early response may be caused by pre activation due to systemic low-level inflammation or a contribution from other cellular sources such as endothelial cells and macrophages within atherosclerotic plaques. Prolonged proinflammatory activity in the recovery period indicates an age-related dysregulated down-regulation of TNF-α production. Circulating levels of IL-6, IL-10, or IL-1Ra were neither decreased in the recovery phase in patients with pneumococcal infections (II) nor in the sepsis model (VI). Thus, a defect anti-inflammatory response did not seem to explain the prolonged proinflammatory activity. Other possible explainations of this

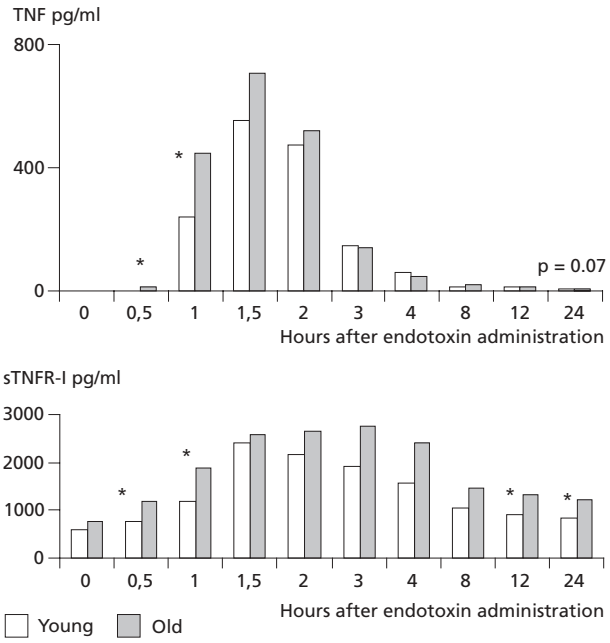


Figure 5. Activation of the TNF system following LPS administration *in vivo*. Geometric means are shown. * = P<0.05. Old: 9 elderly volunteers aged 61-69 years; Young: 8 young controls aged 20-40 years. Based on Figure 1 in VI.

phenomenon could be insensitivity to feedback mechanisms or changes in the interaction of the neuro-endocrine-immune network but these hypotheses need further investigations. I think it is plausible that a delayed down-regulation of proinflammatory activity contributes to a worse outcome from severe infections in old populations but a causal relation remains to be demonstrated. Furthermore, I find it likely that an important determinant of circulating cytokine levels is not simply the peak value after an inflammatory stimulus but rather the time taken for activity to return to basal levels after stimulation. Accordingly, prolonged proinflammatory activity in response to triggers may contribute to systemic low-level inflammation.

2.5. CONCLUSION AND COMMENTS

Aging as associated with a low-grade activation of the entire inflammatory cascade. I suggest that increased counts of neutrophils, monocytes and NK cells as well as attenuated natural cytotoxicity are related to systemic low-level inflammation rather than it reflects successful aging due to a compensation for decreased adaptive immunity. Studies of age-related differences in the production of proinflammatory cytokines in response to acute stimulations *in vitro* have yielded inconsistent results with the extent and even the direction of the aging effect being dependent on variations in stimulus, culture systems, and culture duration. *In vivo* infectious models point towards a more rapid response and prolonged activity in the TNF system, suggesting a delayed down-regulation of proinflammatory activity that may contribute to systemic low-level inflammation. The clinical significance of this phenomenon remains unclear in relation to severe infections. However, elderly people maintain their ability to generate fever and afebrile bacteraemia in older humans is thus connected to the presence of comorbidity or limited to the very old.

3. SYSTEMIC LOW-LEVEL INFLAMMATION AND CHRONIC DISEASES

It became recently clear that local inflammatory processes were characteristic parts of the pathology in almost all chronic, age-associated diseases. This discovery made us wonder if systemic low-level inflammation marked subjects at risk in cohort studies (I, IV, V). We also speculated if individual mediators in the cytokine network were causal related to age-related pathology or merely passive disease markers. These considerations lead us to the hypothesis that TNF-α was an important risk factor whereas IL-6 was a disease marker and acted as a surrogate marker of TNF-α in some epidemiological studies due to their tight regulated production (see section 2.1.) although the main role of IL-6 was really to counteract TNF-α induced pathology. Furthermore, genetic polymorphisms, which determined the rate of TNF-α and IL-6 production, were also expected to be important risk factors if TNF-α and/or IL-6 were causal related to age-associated pathology and/or possessed protective effects (IX, X). Other inflammatory mediators will only be shortly mentioned as I consider them mainly to be secondary to enhanced production of TNF-α and IL-6.

The focus is directed towards dementia, ischaemic CVD, and the syndrome of frailty as these disorders have a high prevalence in population-based studies of old people. Thus, the longitudinal study of Danish centenarians has demonstrated that the extreme life span is accompanied by multi-morbidity and a high prevalence of CVD (>70%) and dementia (>50%) [6]. CVD is also the most common single cause of morbidity and mortality in younger elderly (65+). Thus, among relatively healthy octogenarians, at least 18% suffer from a diagnosis within this category (VIII).

3.1. COGNITIVE FUNCTION

In this section the discussion will cover associations between cognitive function and inflammatory mediators in the blood, effects of local cytokines in neurodegeneration, and how local pathological processes in CNS and systemic low-level inflammation are related in elderly humans.

3.1.1. Inflammatory mediators in the peripheral blood and senile dementia

The prevalence of senile dementia increases exponentially until the age of 95 years [86]. We found that plasma levels of TNF-α were increased in centenarians who were moderate to severe demented compared to cognitive intact centenarians (I), Figure 6. The association persisted after exclusions of centenarians with a history of cancer, inflammatory diseases, acute infections, infectious diseases, intakes of anti-inflammatory drugs, or a severely screwed biochemical profile. This indicated a specific association between TNF-α and senile dementia. It is difficult to distinguish between AD and vascular dementia (VaD) in centenarians [87]. Despite this, it was attempted to separate AD from vascular dementia (VaD) by excluding centenarians if they had a history of stroke, transient cerebral ischemia/amarosis fugax or an ABI below 0.9 (I). TNF-α levels were still increased in the remaining demented centenarians who probably suffered from AD. Similar associations were found with regard to sTNFR-II. Although TNF-α was correlated with IL-6 and CRP in centenarians, no associations were found between dementia and the latter parameters, leading to the hypothesis that the TNF system had a specific effect in age-associated cognitive decline whereas the elevation in other inflammatory parameters was a bystander phenomenon to TNF-α. In accordance with the centenarian study, elevated plasma levels of TNF-α was described in patients with AD [88] as well as in patients with VaD [21].

In contrast to the findings in centenarians (I), other studies have demonstrated that plasma levels of IL-6 acts as a marker of the cognitive function in elderly populations. Thus, high plasma levels of IL-6 and CRP were associated with poor cognitive performance at baseline and with a greater risk of cognitive decline over two years of follow-up in 3031 well-functioning Americans aged 70-70 years from the Health, Aging, and Body Composition (ABC) Study [89]. In the latter study no similar associations were found with regard to TNF-α. Furthermore, IL-6, CRP and α1-antichymotrypsin were associated with increased risk of dementia in a case-control study of a sub cohort in the Rotterdam Study [90]. Increased plasma IL-6 has also been reported in patients with AD [91; 92]. It is thus a bit surprising that IL-6 and CRP in plasma were not associated with dementia in centenarians (I). The discrepancy may reflect a cohort effect, differences in age (young elderly versus oldest old), and differences in the prevalence of comorbidity (well-functioning elderly versus frail centenarians). Thus, it is possible that TNF-α is the strongest disease marker in frail, very old populations whereas IL-6 may be a better predictor of cognitive decline in younger elderly (see discussion in section 4.1.).

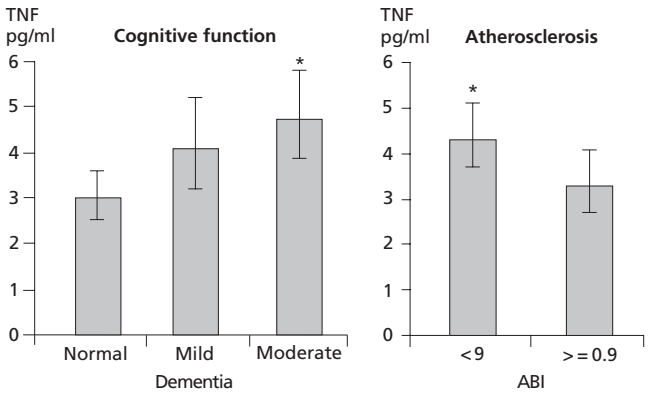


Figure 6. Plasma TNF-α, prevalent dementia, and the ankle-brachial blood pressure index (ABI) in centenarians. Medians and interquartile range (25^{th}-75^{th} percentile) are shown. * = $p<0.05$ compared to normal. Based on Table 3 and Table 5 in I.

It has also been reported that AD patients have high levels of IL-1β in plasma [92], increased production of TNF-α, IL-1β and IL-6 in LPS-stimulated whole blood [93], enhanced production of interferon gamma, IL-2, and IL-6 in response to PHA-stimulation of BMNC [94-96], and increased natural killer cell activity [97].

Accordingly, there is strong evidence that dementia is accompanied by a general immune activation in the peripheral blood but it is still unclear if and, in particular, which specific inflammatory mediators in the peripheral blood that affect local processes in CNS or vice versa.

3.1.2. Effects of cytokines in neurodegeneration

All cell types in CNS are able to produce cytokines including neurons, glia and endothelial cells [98]. AD and VaD constitute the major categories within age-related dementia and the prevalence of mixed dementia, defined as the coexistence of Alzheimer disease (AD) and vascular dementia (VaD), increase as the population ages [99]. In the AD brain, damaged neurons, amyloid-β peptide deposits and neurofibrillary tangles are accompanied by a local upregulation of cytokines, acute phase reactants, complement, and other inflammatory mediators [100]. Moreover, atherosclerosis, which is the underlying cause of VaD, has been recognized as an inflammatory disease within the arterial wall (see section 3.2.). It is still put into question if local inflammatory mechanisms cause damage in CNS or if they are present to remove the detritus from other, more primary pathological processes. A recent review and meta-analysis pointed towards a minor protective effect of long-term administrated non-steroid anti-inflammatory drugs in AD patients [101], supporting the hypothesis of an overall detrimental effect of inflammatory activity. Nevertheless, a later meta-analysis has concluded that the reported beneficial effects of NSAIDs may result from various forms of bias [102].

3.1.2.1. TNF-α

TNF-α was found in the local pathological processes of AD [100] and both AD and VaD have been associated with increased levels of TNF-α in the cerebrospinal fluid [103; 104]. TNF-α has prothrombotic and atherogenic effects (see section 3.2) that may play a detrimental part at least in the pathogenesis of VaD. It is controversial if endogenous TNF-α is neurotoxic (based largely on acute interventions) or neuroprotective (based largely on studies on genetically modified animals) during inflammatory processes in CNS and it is probable that TNF-α can both enhance and inhibit neuronal injury depending on the time course and extent of expression [98]. Chronic overexpression of TNF-α in transgenic mice resulted in severe neurodegeneration [105; 106]. However, in an animal model of multiple sclerosis inactivation of the TNF gene converted disease-resistant mice to a state of high susceptibility and treatment with TNF dramatically reduced disease severity in both TNF-/- mice and in TNF+/+ mice, demonstrating that TNF-α limited the extent and duration of severe, chronic CNS pathology [107]. The functional outcomes in TNF-α knockout mice were improved early after acute brain injury compared with wild type mice but TNF-α deficient mice showed greater neurological dysfunction at later times [108]. The latter study indicated that TNF-α contributed to early neuronal injury, but could improve recovery. The role of TNF-α in CNS repair is not yet well described [109].

Genetic polymorphisms that determine the rate of cytokines provide a tool to investigate the role of cytokines in health and disease in human epidemiological studies. At least nine polymorphisms and five microsatelittes in the TNF locus have been characterized [110]. The TNF –308G/A promoter polymorphism is relative common (Figure 7). It has been demonstrated that the –308A variant is a stronger transcriptional activator than the more common G allele but the effect is dependent on the cell line and the stimulus [111]. The –308A variant has been associated with increased LPS stimulated TNF-α production in whole blood [112] although other

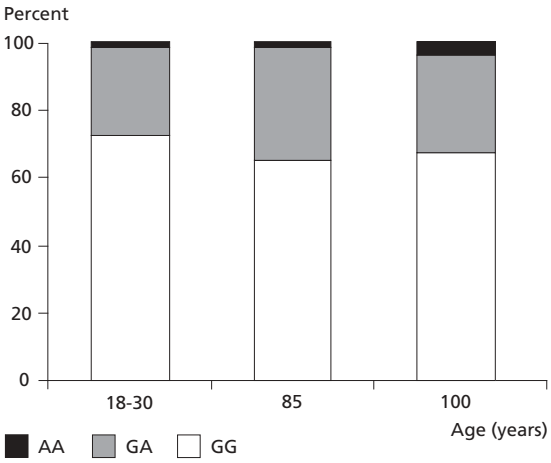


Figure 7. The distribution of the TNF-308G>A promoter polymorphism in different age groups. Based on Table 1 in X.

studies have not been able to confirm this finding [113; 114]. Furthermore, the –308A variant has been associated with decreased prevalence of leprosy, in which high circulating levels of TNF-α play a protective role [115], and increased risk of diabetes [116], cerebral malaria and autoimmune diseases [117], in which TNF-α has a detrimental effect.

With the purpose to test the hypothesis that TNF-α was a risk factor in senile dementia it was investigated if the TNF –308G/A promoter polymorphism was associated with the prevalence of dementia in centenarians (X). Carriers of the AA genotype tended to have higher plasma levels of TNF-α. However, contrary to our hypothesis, the GA genotype was associated with a decreased prevalence of dementia compared to the GG genotype (GG: 55%, N = 83; GA: 34%, N = 35; AA: 60%, N = 5). This result suggested that a balanced or strong TNF-α response possessed the optimal inflammatory response against age-related degenerative processes in CNS. It was difficult to make conclusions about the AA genotype due to the low N-value.

A few other studies have investigated if there is a relation between the TNF –308 G/A promoter polymorphism and dementia. The –308A variant in combination with the TNF –238G promoter polymorphism (wild type G has increased transcriptional activity compared to mutant A) and microsatellite TNF-a2 (associated with high TNF production) was associated with a low prevalence of dementia in a cohort of 235 post-mortem confirmed AD cases and 130 controls [118], suggesting a protective role of TNF-α in accordance with the centenarian data (X). In contrast, in a family study the same haplotype was a risk factor in AD [119]. It has been suggested [118] that this discrepancy might result from different pathological pathways driven by the strong genetic loadings in the cohort with the familiar component versus community acquired AD. In another study of patients with AD the –308A variant was associated with a mean age of onset three years younger than wild type carriers but there was no difference in the prevalence of different genotypes in patients versus healthy controls [120]. The latter study [120] was a case-control study designed to detect differences between patients and controls rather than to evaluate the onset age of symptoms within patients. Patients with AD displayed higher intrathecal levels of TNF-α than healthy controls but levels were not related to the TNF –308G/A polymorphism [103]. The TNF –850C/T polymorphism has also been associated with sporadic AD as well as VaD [121] but later studies have not been able to confirm these results [122; 123].

3.1.2.2. IL-6

Like TNF-α, IL-6 is detected in CNS in patients with VaD and in the pathological plaques of AD [100] and IL-6 can both enhance and

inhibit neuronal injury in animal studies [98]. With regard to polymorphism studies, most attention has been centered on the –174G/C promoter polymorphism. The –174C variant was initially associated with lower promoter activity [124] but subsequent studies have detected increased activity of the –174C variant in the absence of 17β-estradiol but not with 17β-estradiol present [125]. Moreover, results in AD patients are contradictory, showing both a decreased incidence of the C allele in AD [126; 127], no difference between AD patients and controls [128] as well as an increased incidence of the C allele in AD [129]. This apparent incongruity seems to be based on different distributions of the C allele in the control populations whereas the distribution in the AD population is quite similar from study to study [130]. There is, accordingly, a need for further and larger studies.

3.1.2.3 IL-1

Although IL-1 has been shown to exert neuroregulatory roles and to be of importance for intact neurological function in animals [131] IL-1 has also in particular been related to AD. Thus, animal studies point unambiguously to a direct role of IL-1β in neurodegeneration [98] and this finding is supported by associations between AD and polymorphisms in the IL-1β gene [132; 133] and a meta-analysis of IL-1α polymorphism studies [134].

3.1.3. Cross-talk between CNS and the peripheral immune system

It remains to be discussed how peripheral inflammatory activity is related to the brain function. At least TNF-α is able to cross the blood-brain barrier by specific transport systems in mice [135; 136]. Peripheral cytokines interact, moreover, with CNS by afferent neurons whereas stimulation of the efferent vagus nerve inhibits the production of TNF-α in liver, spleen and heart, and attenuates serum concentrations of TNF during endotoxaemia [15]. The function of the blood-brain-barrier decreases also with age [137], in AD [138], and in response to TNF-α, IL-1β and IL-6 [139], making a passive diffusion of cytokines possible. It is well known that peripheral inflammation activates the hypothalamic-pituitary-adrenal axis and induce sickness behavior [15]. However, it has also been shown that CNS releases IL-6 and TNF-α to the peripheral blood in patients with meningitis (Møller K, submitted data) and IL-6 is released during prolonged exercise [140]. Thus, it is either possible that low-level inflammation in the peripheral blood triggers cognitive decay or peripheral inflammation represents spillover from inflammatory processes in CNS and perhaps both hypotheses hold true.

3.1.4 Conclusion and comments of section 3.1

Increased levels of inflammatory markers in the peripheral blood are associated with cognitive decline in elderly populations and it is plausible that systemic low-level inflammation with advancing age interacts with cognitive aging and vice versa. Conflicting reports from polymorphism studies probably reflect a complex role of TNF-α and IL-6 in neurodegeneration as indicated by animal studies. At this time, the findings of the lowest dementia prevalence among centenarians with the TNF –308GA genotype (X) and among community acquired AD patients with a haplotype related to high promoter activity of the TNF-α gene indicates that the aspect of TNF-α associated repair mechanisms in CNS is important in the protection against age-related cognitive decline rather than acute neurotoxic effects.

3.2. ISCHAEMIC CARDIOVASCULAR DISEASE (CVD)

Atherosclerosis is the principal contributor to ischaemic CVD. The process of atherogenesis was formerly considered to consist largely of the accumulation of lipids within the artery wall. Advances in basic and experimental methods have illuminated that lesions of atherosclerosis contain cytokines, smooth muscle cells, activated T lymphocytes, and monocyte-derived macrophages and atherosclerosis can be defined as an age-related inflammatory disease [141]. It has widely been assumed that inflammation occurred in the arterial wall as a response to injury, lipid peroxidation, and perhaps infection and systemic low-level inflammation in CVD was derived from inflammation within atheromatous lesions and reflected their extent and severity. A shift in this paradigm has occurred towards understanding the pathology of atherosclerosis also as a consequence of systemic low-level inflammation and it has been recognized that inflammation has a fundamental role in mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications [142].

This section discuss studies of associations between CVD and inflammatory mediators in the blood, current knowledge in support of a causative role of systemic inflammation in CVD, and experimental and epidemiological studies that concern effects of TNF-α versus IL-6 in the pathogenesis of CVD.

3.2.1. Systemic low-level inflammation and CVD

During the last 5-10 years a rapidly increasing number of epidemiological and clinical studies have shown strong and consistent relationships between markers of inflammation, the prevalence of subclinical as well as manifest CVD, and the risk of future CV events.

High plasma levels of TNF-α (Figure 6), sTNFR-II and CRP were associated with a low ABI index (marker of universal atherosclerosis and CVD) independently of dementia in centenarians (I). Furthermore, high plasma levels of TNF-α were associated with increased prevalence of CVD compared with medium and low levels of TNF-α in 130 octogenarians from the 1914-cohort (IV), Figure 8. Consistent with these findings, elevated plasma levels of TNF-α were associated with degrees of early atherosclerosis in healthy middle-aged men [143] and cardiovascular as well as subclinical cardiovascular disease in cross-sectional and longitudinal studies of participants aged 70-79 years from the Health ABC Study [144; 145]. Moreover, high TNF-α levels were also associated with increased risk of recurrent coronary events in the stable phase after myocardial ischaemia [146] and patients with peripheral vascular disease or a history of myocardial infarction had increased levels of TNF-α as well as sTNFR-II compared to healthy age-matched controls [147]. High levels of sTNFRs were also associated with increased risk of coronary heart disease in young to middle-aged women from Nurses' Health Study [148]. A high LPS-induced TNF-α production together with a low IL-10 production in whole blood were associated with an elevated risk of death from a cardiovascular event in 311 Dutch women aged 85 years from the Leiden Study [149], demonstrating that the balance between the capacity of proinflammatory and anti-inflammatory activity is important.

A high plasma level of IL-6 was a marker of subclinical CVD in a case-control study of people aged 65+ from the Cardiovascular

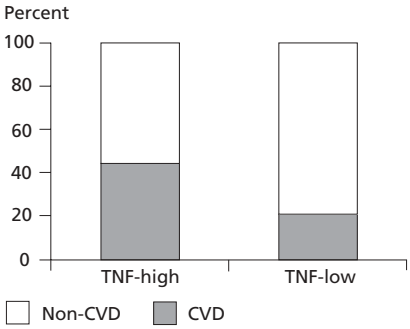


Figure 8. Plasma levels of TNF-α and prevalent cardiovascular diseases in 130 octogenarians. The population was divided by tertiles into groups with low, intermediate and high (TNF-high) levels of TNF-α. The groups with low and intermediate TNF-α levels were pooled due to the low number of people with prevalent CVD (TNF-low). * = $p<0.05$. Based on Figure 2 in IV.

Health Study [150] and subclinical as well as manifest CVD in the Health ABC Study [144; 145]. IL-6 was also a predictor of mortality related to CVD in relatively healthy participants aged 65+ from the Women's Health and Aging Study [151] and cardiovascular events in healthy middle-aged men [152], postmenopausal women [153], and young to middle-aged health professionals [148].

CRP predicts coronary events in a very large number of studies (reviewed in [154]) and in meta-analyses [155; 156] and it is currently considered to be the most reliable and accessible inflammatory marker for clinical use [157]. Furthermore, CRP has provided an additional measure to the risk of coronary heart disease beyond that afforded by the Framingham risk score (age, prevalent hypertension and diabetes, smoking status, and ratio of total to HDL cholesterol) in several studies [148; 158-160].

High total WBC counts is an old, well-known risk factor in CVD (e.g., [161; 162]). In octogenarians from the 1914-population an ABI <0.9 was associated with an increased neutrophil count, decreased cytotoxicity per NK cell, and a trend towards an enhanced NK cell count ($p = 0.08$)(V). In accordance with this, high counts of neutrophils and monocytes were associated with CVD independently of age, sex, smoking and BMI in a relatively healthy English population aged 75+ years [163] and neutrophils were independently associated with ischaemic events in a high-risk population of 18 558 patients with ischaemic stroke, myocardial infarction, or peripheral arterial disease [162].

3.2.2. Inflammatory cytokines and risk factors in CVD

Inflammatory mediators interact with the endothelium, markers of coagulation/fibrinolysis, the glucose metabolism, the lipid metabolism, the renin-angiotensin system, and the hypothalamic–pituitary axis, Figure 9. TNF-α and IL-6 have in particular been related to a wide range of risk factors in CVD.

3.2.2.1. Endothelial dysfunction

Endothelial dysfunction is considered to be one of the first steps in atherosclerosis [141]. Activated endothelial cells are known to be targets as well as sources of inflammatory cytokines and chemokines that induce the upregulation of adhesion molecules and the attraction of leukocytes, promoting altogether a migration across the endothelium. Consistent with this, circulating levels of cytokines are correlated with levels of soluble cellular adhesion molecules in several studies, e.g., [143]. It has been demonstrated that TNF-α and IL-1β, but not IL-6, impairs the endothelium dependent relaxation in humans [164] and TNF-α and IL-1β, but not IL-6, causes directly endothelial upregulation of cellular adhesion molecules, mediating the attachment and transmigration of leukocytes through the endothelium [165].

3.2.2.2. Smoking

Smokers had higher circulating IL-6 levels compared to non-smokers among octogenarians (VIII). In adjusted analyses, CRP, IL-6, soluble intercellular adhesion molecule-1 (sICAM-1) and endothelial-leukocyte adhesion molecule-1 (E-selectin) were all independently associated with smoking status in 340 apparently healthy women, suggesting that smoking caused vascular inflammation [166].

3.2.2.3. The metabolic syndrome

The metabolic syndrome refers to the presence of at least three of the following: Abdominal obesity, hypertension, a reduced level of HDL-C, elevated triglycerides, and high fasting glucose [157].

Adipose cells secrete high amounts of TNF-α and IL-6 [167; 168] and circulating levels of the two cytokines have been related to fat mass in a large number of studies, e.g., [33]. Up to a third of circulating plasma IL-6 are derived from fat tissue [169].

TNF-α and IL-6 are potent regulators of the lipid metabolism. TNF-α stimulates hepatic lipogenesis and cholesterol synthesis that are paralleled by elevated serum triglycerides and total cholesterol [170]. IL-6 induces lipolysis in adipose tissue and whole body fat oxidation in humans [171]. Mice with chronic elevated IL-6 had a minor increase in plasma levels of triglycerides, decreased HDL-C, decreased fat mass, and increased liver production of cholesterol and fatty acids that was considered necessary to maintain hepatic triglyceride secretion and support increased hepatocyt proliferation, [172]. In octogenarians, circulating levels of TNF-α were weakly correlated with serum triglycerides and a low HDL/total cholesterol ratio (IV). Correlations between TNF-α and IL-6 on one hand and high levels of triglycerides and low HDL on the other hand are confirmed in a large number of recent studies, e.g., [152; 173].

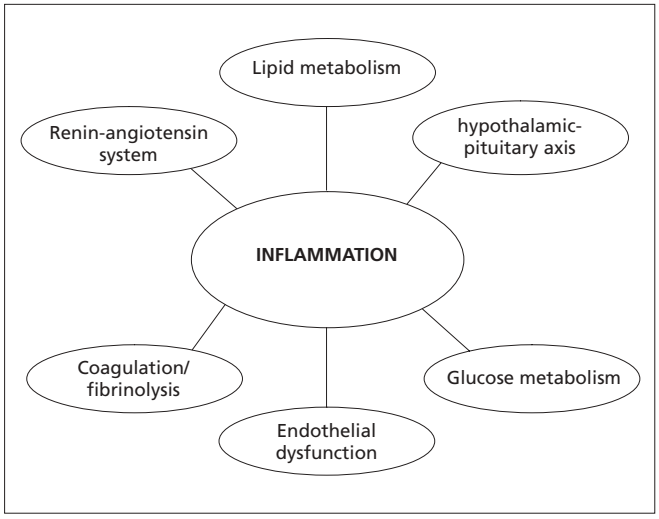


Figure 9. Inflammatory mediators interact with the metabolism and endocrine systems. See text for further details.

It has been demonstrated that patients with essential hypertension had increased production of TNF-α and IL-1β [174] and spontaneously hypertensive rats had increased circulating levels of TNF-α [175]. It is unclear at this time whether increased production of pro-inflammatory cytokines is a bystander phenomenon or whether it represents a causing factor, triggering hypertension. Angiotensin II causes monocyte activation [176] and renin-angiotensin inhibitors have anti-inflammatory properties [177], supporting the first hypothesis. In octogenarians, the diastolic blood pressure was weakly correlated with IL-6 in an unadjusted analysis (VIII).

TNF-α and IL-6 are associated with insulin resistance in elderly populations [171; 178]. It is well established that TNF-α downregulates GLUT-4 and inhibits insulin receptor activity [179] whereas the role of IL-6 is still debated. IL-6 induced insulin resistance in mice [180] but IL-6 knockout mice developed obesity and impaired glucose tolerance that was reverted by IL-6 [181] whereas mice with IL-6 producing tumors had hypoglycemia and lost fat mass [172].

Accordingly, there is good evidence that TNF-α has the potential to be an important driver in the metabolic syndrome, considering that TNF-α is produced by fat tissue and induces dyslipidaemia and insulin resistance, Figure 10. The role of IL-6 in insulin resistance is unclear. Data from IL-6 knockout mice and mice with chronic high expression of IL-6 support the hypothesis that IL-6 act as a surrogate marker of TNF-α in some epidemiological studies.

3.2.2.4. Physical inactivity

Physical activity offers protection against CVD and physical training is effective in the treatment. It has been shown that in response to a low local glycogen content working muscles release high amounts of IL-6 (but not TNF-α) to the circulation, resulting in 100-fold increases in plasma levels [171]. It has been suggested that exercise-

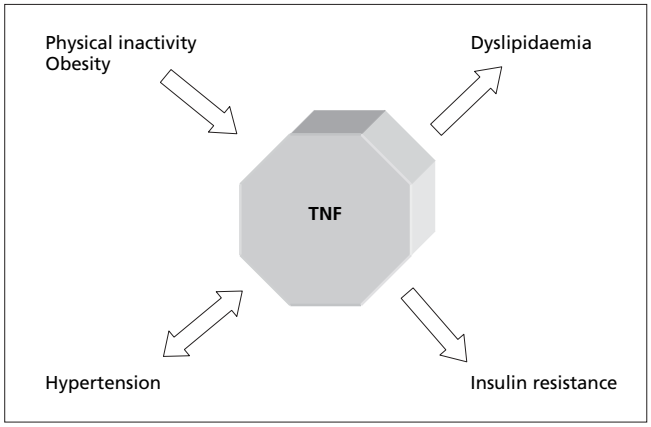


Figure 10. TNF-α as a driver in the metabolic syndrome. Fat tissue produces TNF-α and circulating levels of TNF-α is correlated with the fat mass. Muscle contractions inhibit TNF-α production in vivo and physical inactivity is associated with high circulating levels of TNF-α. Hypertension may induce increased production of TNF-α and vice versa. TNF-α induces insulin resistance. See text for further details.

induced acute elevations in plasma IL-6 mediate some of the health beneficial effects in exercise including lipolysis in fat tissue, improvement of insulin resistance, and the induction of an anti-inflammatory response [171] as exercise as well as physiological infusions of IL-6 inhibited the TNF-production elicited by low-level endotoxemia [182] and induced the production of IL-1Ra, IL-10 and cortisol [57; 183]. It has been suggested that acute elevations in IL-6 provide a mechanism as to why physical exercise either reduces the susceptibility to or improves the symptoms associated with low-level inflammation in the metabolic syndrome and CVD [171]. This hypothesis strongly challenges the common assumption that IL-6 is a causative factor in these disorders. Thus, I suggest a differentiated role of acute, pronounced, short-term elevations versus chronic, low-level increases in systemic IL-6.

High circulating levels of TNF-α and IL-6 were associated with physical inactivity in Danish octogenarians (VIII), Figure 11, and in the Health ABC Study of Americans aged 70-79 years [184]. An inverse relation between physical activity and fat mass explained a part, but not all, of this association [184]. The InCHIANTI study demonstrated that high levels of IL-6, CRP, and IL-1Ra were associated with poor physical performance and low muscle strength in Italians aged 65+ years [185]. Considering that large amounts of IL-6 are released to the circulation in relation to muscle contractions without muscle damage, it is unexpected that chronic low-level increases in IL-6 are associated with physical inactivity. However, if the hypothesis is true that low-level increases in systemic IL-6 is largely secondary to increased TNF-α activity in relation to physical inactivity and obesity this would fully explain the discrepancy.

3.2.2.5. Coagulation

IL-6 induces directly procoagulant changes by increasing the production of fibrinogen, tissue factor, factor VIII, von Willebrand Factor and platelets [186] and CRP induces the expression of tissue factor by monocytes [157]. Consistent with this, low-level increases in circulating TNF-α, IL-6 and CRP are often strongly correlated with fibrinogen in epidemiological studies, e.g., [56].

3.2.2.6. Infections

Chronic infections such as urinary infections, dental infections, and infections caused by *Chlamydia pneumoniae* and *Helicobacter pylori* are seen with high prevalence in elderly populations and have been implicated in the pathogenesis of atherosclerosis with inflammation as the pathophysiological link [187]. Frail, elderly patients with asymptomatic bacteriuria had low-level elevations in sTNFR-I and higher neutrophil counts in the blood compared to patients matched with regard to age, morbidity and medical intakes but with negative urine culture [188]. C. pneumoniae infection spreads from the respiratory tract to other organs by the blood stream via infected monocytes [189] and it is able to survive within vascular smooth muscle cells, endothelial cells, and macrophages as well [190]. This pathogen has been demonstrated in atherosclerotic plaques. It augments the expression of endothelial cell activation markers, induces endothelial dysfunction, and induces systemic immune activation by stimulating the production of cytokines including TNF-α, IL-1β, and IL-6 [191-195]. The IgA antibody titer against *C. pneumoniae* is considered as the best marker of chronic infection [196]. Plasma levels of TNF-α were elevated in centenarians with high IgA antibody titers against *C. pneumoniae* compared to centenarians with low titers although this parameter only explained a small part of the inflammatory burden in this population [197].

It has recently been demonstrated that acute infections such as respiratory tract infections and urinary tract infections are associated with a transient increase in the risk of vascular events including stroke and myocardial infarction [198], supporting the hypothesis of a link between infections, inflammation, and CVD.

3.2.3. Studies of cytokine polymorphisms in CVD

The –308A variant in the TNF-α gene is associated with increased promoter activity (se section 3.1.2.1). This polymorphism has been associated with unstable angina [199], insulin resistance [116; 200-202] and increased risk of coronary heart disease in patients with type 2 diabetes [203], supporting the hypothesis of TNF-α as an important driver of the metabolic syndrome and a role in the elevated risk of CVD that is associated with type 2 diabetes and hypertension. Other studies have yet failed to detect an association between the polymorphism and a history of coronary arterial disease or myocardial infarction [204; 205].

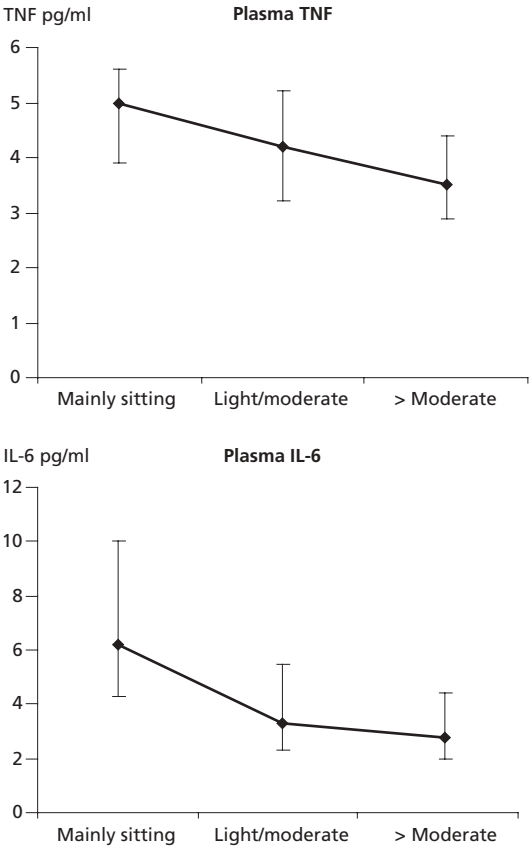


Figure 11. Plasma levels of TNF-α and IL-6 in relation to self-reported physical activity in 333 octogenarians from the 1914-population. Medians and interquartile range (25th-75th percentile) are shown. Mainly sitting, N = 25; Light/Moderate exercise, N = 248; > Moderate exercise, N = 60. Based on data from Table 1 in VIII.

Most epidemiological studies have focused on the IL6 –174G/C promoter polymorphism, Table 2. Large, well-described cohorts have reported that the C allele was associated with CVD [206; 207], increased carotid intimal-medial wall thickness in older subjects [208] and other related risk factors such as endothelial dysfunction [209], a high systolic blood pressure [206], elevated levels of fibrinogen [150], type 2 diabetes [116], and high white cell counts [210]. Consistent with this, CC-carrier status was associated with a high prevalence of CVD in octogenarians from the 1914-cohort: GG = 14/90 (16%); GC = 27/171 (16%); CC = 18/63 (29%) (IX). However, no association with CVD [211] and even the opposite effect of the polymorphism [212; 213] has also been reported in smaller studies with a case-control design. Discrepancies may reflect that smaller cross-sectional studies enrolled pre-existing cases, having the risk to examine associations between the genetic factor and disease progression/severity rather than the risk of disease development [214]. In my opinion, most well conducted epidemiological studies with high n-values point towards the –174C variant as a risk factor in CVD. It remains, however, to be a major question if this reflects a harmful or a protective effect of IL-6 in CVD.

The –174C variant was associated with low promoter activity in LPS or IL-1 stimulated HeLA cells and decreased plasma levels of IL-6 in 102 healthy subjects aged 40-75 years [124]. In the light of these data, the association between the C allele and CVD pointed towards a protective role of IL-6. However, the interpretation in the literature has widely been the opposite because the C variant was associated with high plasma levels of IL-6 in several elderly cohorts [150; 215](IX) and in patients with small abdominal aortic aneurisms [207] and, moreover, the C variant was associated with increased levels of CRP [150; 206; 216] although the opposite relation between plasma IL-6 and the –174C variant [124; 217; 218], no association [211; 219], or an association in newborns, but not in adults [220] have also been reported, Table 2. It is likely that associations between cytokine polymorphisms, low-level inflammation, and morbidity are blurred by the accumulation of a wide range of other contributing factors in old populations. Moreover, high plasma levels of IL-6 have probably a very poor correlation with the capacity of IL-6 production in the elderly or in patients with atherosclerosis. Thus, a weak counteracting IL-6 response to local TNF-α activities could result in higher chronic circulating IL-6 levels due

Table 2. The IL6 –174G/C promoter polymorphism, circulating IL-6, cardiovascular disease (CVD), and mortality in studies of elderly populations (>65 years).

Study	Design	Frequencies GG / GC / CC (%)	Circulating IL-6	CVD	Survival
Wang et al., 2001 [258]	*Cross-sectional study:* 400 younger controls 250 nonagenarians (Finnish population)	 30 / 50 / 20 23 / 52 / 25			No difference across age groups
Bonafe et al., 2001 [218]	*Cross-sectional study:* 93 60-80-year-old men 57 81-99-year-old men 68 male centenarians	*GG / C-allele* 58% / 42% 58% / 42% 38% / 62%	IL-6 increased in GG		C-variant associated with longevity in men
	101 60-80-year-old women 126 81-99-year-old women 255 female centenarians (Italian population)	54% / 46% 52% / 48% 51% / 49%			No difference across women age-groups
Jenny et al., 2002 [150]	*Case-control study >65-year-olds:* 1857 white Americans 344 African Americans	40% / 44% / 16% 85% / 15% / 0%	No significant difference in IL-6	Non-smoking men with C-variant have increased risk of CVD	No difference across age groups in women
Rea et al., 2003 [215]	*Cross-sectional study:* 193 octogenarians/nonagenarians 182 controls (Irish population)	 29 / 54 / 17 38 / 47 / 15	IL-6 increased in CC		C-variant associated with longevity
Bruunsgaard et al., 2003 [IX]	*Longitudinal study:* 333 octogenarians (234 non-smokers) Follow-up: 5-6 years (Danish population)	 28 / 53 / 19	IL-6 increased in GC/CC	CC has increased risk of CVD	C-variant associated with increased mortality in non-smokers
Chapman et al., 2003 [208]	*Cross-sectional study:* 22-77-year-olds: N = 1109 (Australian population)	*GG / C-allele:* 59 / 41		C-variant associated with carotid plaque formation (whole population) C-variant associated with increased carotid IMT in subjects > 53 years	
Christiansen et al., 2004 [263]	Cross-sectional study: <50-year-olds: N = 182 50-59-year-olds: N = 292 60-69-year-olds: N = 94 70-79-year-olds: N = 302 80-89-year-olds: N = 81 90-95-year-olds: N = 581 100-year-olds: N = 178 (Danish population)	 25 / 54 / 21 24 / 54 / 22 25 / 50 / 25 28 / 50 / 22 30 / 53 / 17 30 / 49 / 21 32 / 47 / 21			GG associated with longevity
Hurme et al., 2005 [264]	*Longitudinal study:* 285 nonagenarians Follow-up: 5 years Survivors: N = 114 Non-survivors: N = 171 (Finnish population)	 21 / 48 / 31 29 / 52 / 19			C-variant associated with increased mortality

to an increased inflammatory burden, contrasting the intuitive thought of a direct association between low promoter activity and low plasma levels. In accordance with this hypothesis, the investigators of the initial study of promoter activity in HeLA cells [124] have subsequently expressed reservations about the interpretation of their assay [206] and it has been suggested that the important determinant of plasma IL-6 concentration is not simply the peak value after an inflammatory stimulus but rather the time taken for activity to return to basal levels after stimulation [150; 207]. Therefore, in subjects with chronic inflammatory activity such as patients with CVD or elderly populations the association between IL-6 genotype and circulating IL-6 may be the converse of that observed in young healthy populations or middle-aged populations without inflammation [150; 207; 219]. Further complexity in the interaction between the polymorphism and the production of protein has been reported as BMNC production of IL-6 increased with age in carriers with the –174C variant whereas this phenomenon was not observed among carriers with the GG genotype [217]. Moreover, it has been reported in later reporter studies that the –174C variant was associated with increased PMA stimulated promoter activity in the absence of 17β-estradiol but not with 17β-estradiol present in a human endometrial adenocarcinoma cell line [125]. This finding would favour that elderly women with the C variant had enhanced IL-6 promoter activity compared with younger age groups but it also indicate a differentiated promoter sensitivity in response to different stimulations (PMA versus LPS and IL-1) and in different cell lines (adenocarcinoma cell line versus HeLA cells). Accordingly, there is a large need for further experimental research studies in order to elucidate the relation between the IL6 –174G/C promoter polymorphism and the production of IL-6 protein.

Although polymorphisms in the CRP gene are associated with CRP levels in the blood [221] it is not a risk factor of arterial thrombosis [222], supporting my view that CRP is a surrogate marker of local cytokine production.

3.2.4. Medical treatment of inflammation in CVD

Aspirin reduced the risk of first CV events and the magnitude of the relative risk reduction was greatest in persons with the highest levels of CRP and declined in direct relation to CRP levels [223].

In addition to lowering lipids, several studies have described reduced CRP-levels in subjects treated with statins (reviewed in [157]). Furthermore, reduced serum-TNF [224] and reduced monocyte expression of TNF-α and IL-2 [225] have been described after treatment with statins for 3 months respectively 8 weeks. Although the magnitude of risk reduction associated with statin use appears to be largest for those with the highest serum levels of CRP, whether CRP reduction per se lowers cardiovascular risk is unknown [157].

3.2.5. Conclusion and comments of section 3.2

It is well documented that systemic low-level inflammation is associated with subclinical as well as clinical CVD in elderly populations. Based on experimental studies, epidemiological polymorphism studies, and intervention studies there is good evidence that inflammation plays a causative role in CVD. TNF-α seems to be a strong risk factor due to the induction of endothelial dysfunction and especially as a potential driver of the metabolic syndrome. The effect of IL-6 is still unclear. On the one hand, IL-6 seems to mediate the health beneficial effects of physical exercise and it may counteract some of the pathological effects of TNF-α especially in relation to the metabolic syndrome. On the other hand, chronic elevations of IL-6 induce dyslipidaemia and a procoagulant state. Studies of the IL6 –174G/C promoter polymorphism are confusing and have not elucidated this aspect further.

3.3. THE SYNDROME OF FRAILTY

Sarcopenia is a central part of the geriatric syndrome of frailty that has been defined as a wasting syndrome characterized by an age-related decline in lean body mass, decreased muscle strength, endurance, balance and walking performance, low activity and weight loss accompanied by a high risk of disability, incident falls, hospitalisation, and mortality [226].

It has been suggested that frailty in geriatric patients reflects a metabolic imbalance caused by overproduction of catabolic cytokines such as TNF-α and by diminished availability or action of anabolic hormones, resulting from aging itself and the presence of associated chronic conditions [227]. Thus, TNF-α was identified as cachectin in the 80'ies. It is known to cause increased basal energy expenditure, anorexia, and loss of muscle and bone mass [228-230] and it has been associated with wasting/cachexia in chronic inflammatory disorders such as HIV infection [231], rheumatoid arthritis [232], and cancer [229]. Consistent with this, muscle protein synthesis was inversely related to local levels of TNF-α protein in muscles in a study of frail, very elderly humans [233]. Furthermore, circulating levels of TNF-α and IL-6 were inversely related to the muscle mass [33] as well as muscle strength [185; 234]. IL-6 was also a risk marker of functional disability [235] that turned out to be related to decreased muscle strength [236] in elderly populations and CRP was a marker of frailty in 65+ year-old participants from the Cardiovascular Health Study [2]. The literature on other inflammatory mediators is sparse but it has been reported that the NK cell number is inversely related to functional disability in 90+ year-olds [53].

Considering the catabolic activities, TNF-α has the potential to be a driver in the frailty syndrome. IL-6 causes also increased basal energy expenditure and anorexia but the lean body mass was preserved in mice with IL-6 producing tumours [172]. Furthermore, handgrip strength was a powerful predictor of CVD-specific and total mortality but this association was independent of IL-6 and CRP in old disabled women from The Women's Health and Aging Study [237]. Accordingly, IL-6 may act as surrogate marker of TNF-α in studies of age-related wasting and sarcopenia.

Studies in human liver cell cultures, mice, and human volunteers indicate that IL-6 induces the iron regulatory peptide hormone, hepcidin during inflammation and that the IL-6-hepcidin axis is responsible for the hypoferremia of inflammation [238]. This is of major impact in the understanding of age-related anaemia that occurs in 10-20% of community dwelling people over the age of 65 years and in as much as 50% of nursing home patients [239].

3.4. INFLAMMATION AS A COMMON LINK BETWEEN AGE-RELATED DISEASES

The syndrome of frailty was associated with clinical and subclinical CVD measured by carotid ultrasound and ABI, left ventricular hypertrophy by ECG and echocardiography as well as infarct-like lesions in the brain on magnet resonance [240]. Furthermore, age-associated cognitive decline and AD has been related to CVD [241] and related risk factors such as the metabolic syndrome [240; 242; 243]. These findings support the existence of common pathological pathways that is likely to involve inflammatory mediators. In support of this hypothesis, the metabolic syndrome was associated with cognitive impairment in elderly Americans, but primarily in those with high levels of inflammation [240].

4. SYSTEMIC LOW-LEVEL INFLAMMATION AND MORTALITY

Systemic low-level inflammation has been suggested to be causal related to several age-associated diseases such as the metabolic syndrome, CVD and the syndrome of frailty as described in chapter 3. If this postulation is true it is expectable that inflammatory mediators as well as cytokine polymorphisms are independent predictors of all-cause mortality in elderly populations. Moreover, if TNF-α and IL-6 have different biological activities in age-associated morbidity they are expected to have separate effects in statistical models.

4.1. MORTALITY AND INFLAMMATORY MEDIATORS IN BLOOD

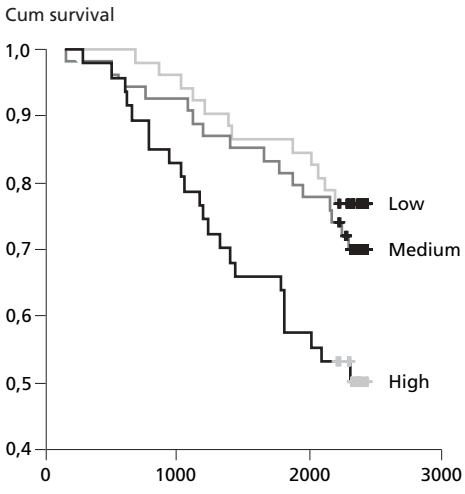
Associations between all-cause mortality and low-level increases in circulating levels of cytokines and acute phase proteins have been demonstrated in several elderly populations (VII, VIII) [151; 161; 244-250], Table 3. The neutrophil count was also associated with all-cause mortality in octogenarians (Figure 12) whereas the NK cell count, NK cell mediated cytotoxicity, and the monocyte count did not affect the survival function (Bruunsgaard et al, unpublished data). With regard to cause specific mortality, IL-6 [151], CRP [151] and WBC [161] have been related to mortality from CVD in relatively healthy elderly people. Moreover poor NK cell mediated cytotoxicity was accompanied by the development of infections and high mortality in a smaller study of 108 elderly subjects [251].

Associations between inflammatory parameters and mortality are independent of prevalent morbidity and other known risk factors (e.g., sex, hypertension, cholesterol, physical activity, and BMI) in most studies with high n-values, supporting the hypothesis that low-level inflammation is a causative risk factor in elderly populations.

Low serum albumin and low total cholesterol is associated with poor nutritional status but they act as acute-phase reactants as well. Albumin and total cholesterol have been inversely associated with

Table 3. Low-level inflammation and mortality in elderly populations.

Study	Design	Follow-up time	Parameters	Mortality
Mooradian et al., 1991 [247]	129 nursing home patients (mean age 89 years)	4 and 13 months. Survivors compared with non-survivors	Detectable TNF-α and IL-1 in serum	TNF-α was associated with increased all-cause mortality in univariate analyses
Klonoff et al., 1992 [252]	2342 healthy, home-living men and women aged 50-89 years	3 years	Serum albumin	Low albumin predicted increased all-cause mortality in multivariate analyses
Weijenberg et al., 1996 [161]	884 randomly selected men aged 64-84 years. The Zutphen Elderly Study	5 years	Total WBC count	WBC count predicted increased all-cause and CVD related mortality in multivariate analyses
Weijenberg et al., 1997 [253]	820 randomly selected men aged 64-84 years. The Zutphen Elderly Study	5 years	Serum albumin	Low albumin predicted all-cause and CVD related mortality
Weverling-Rijnsburger et al., 1997 [254]	724 participants aged 85-103 years. Leiden study	10 years	Cholesterol	Low cholesterol predicted high all-cause, cancer related and infection related mortality
Rosenthal et al., 1997 [248]	72 male patients aged >60 years admitted to a geriatric rehabilitation unit	1 year	sIL-2R, CRP and albumin in serum	sIL-2R predicted all-cause mortality in multivariate analyses. CRP and albumin were only associated with mortality in univariate analyses
Harris et al., 1999 [151]	1293 healthy participants, Iowa 65 + Rural Health Study	4.6 years	IL-6 and CRP in serum	IL-6 and CRP predicted increased all-cause and CVD related mortality in multivariate analyses
Ogata et al., 2001 [251]	108 nursing home patients (mean age 81 years)	12 months	NK cell activity	Low NK cell activity predicted high mortality risk due to infection in multivariate analyses
Volpato et al., 2001 [244]	620 frail women >65 years. Women's Health and Aging study	3 years	IL-6 in serum	Increased all-cause mortality in women with prevalent CVD in multivariate analyses
Reuben et al., 2002 [245]	870 healthy participants aged 70-79 years. MacArthur Studies of Successful Aging	3 years and 7 years	Albumin, cholesterol, IL-6 and CRP in serum	>3 markers predicted increased all-cause mortality in multivariate analyses
Bruunsgaard et al., 2003, VII	126 100-year-old participants, Danish Centenarian Study	5-6 years	TNF-α, IL-6, IL-8, and CRP in plasma	TNF-α predicted increased all-cause mortality in multivariate analyses CRP predicted increased all-cause mortality in a univariate analysis
Bruunsgaard et al., 2003, VIII	333 80-year-old participants, the 1914-cohort	5-6 years	TNF-α and IL-6 in serum	TNF-α in men and IL-6 in the whole cohort predicted all-cause mortality in multivariate analyses
Hu et al., 2003 [255]	870 healthy participants aged 70-79 years. MacArthur Studies of Successful Aging	3 years and 7 years	Cholesterol	Low cholesterol predicted increased all-cause mortality in univariate analyses but in multivariate analyses
Roubenoff et al., 2003 [246]	525 free-living participants aged 72-92 years. Framingham Heart Study	4 years	IL-6 and IGF-1 in serum TNF-α, IL-1β, IL-6 and IL-1Ra production by unstimulated PMNC	Serum IL-6, IGF-1 (low) and cellular production of TNF-α predicted all-cause mortality in multivariate analyses
Cappola et al., 2003 [249]	718 women >65 years. Women's Health and Aging Study	3 years	IL-6 and IGF-1 in serum	High IL-6 together with low IGF-1 predicted high all-cause mortality in multivariate analyses
Yeh et al., 2004 [250]	66 geriatric cachectic patients	>4 years	IL-6, sTNFR-I, sTNFR-II, sIL-2R, CRP in serum and WBC	IL-6, sTNFR-II, neutrophils, and albumin (low) predicted increased all-cause mortality in multivariate analyses sTNFR-I predicted increased all-cause mortality in univariate analysis



Cox regression

	Hazard ratio (95% CI)	P
Low	1.0	–
Medium	1.2 (0.6-2.6)	0.6
High	2.5 (1.2-4.9)	0.009

Figure 12. Neutrophils and mortality in 80-year-olds. Octogenarians are divided by tertiles (high, medium, low) in neutrophil counts. Follow-up time is 5-6 years. CI = confidence interval. (*Bruunsgaard et al.,* unpublished data).

mortality risk in several elderly cohorts (VIII) [245; 252-254], Table 3. Cholesterol was inversely correlated with serum IL-6 in octogenarians from the 1914-cohort and it had no significant effect in survival analyses when IL-6 was also included in the statistical model (VIII). Furthermore, cholesterol was not an independent risk factor in the MacArthur Studies of Successful Aging but in this population it was concluded that low cholesterol was related to common cardiovascular risk factors, rather than underlying inflammation or undernutrition [255].

Only a few studies have simultaneously tested the role of different inflammatory mediators as predictors of mortality in elderly populations. TNF-α was found to be a predictor of imminent mortality risk in centenarians independently of dementia and a history of CVD, suggesting that TNF-α was a marker of frailty (VII), Figure 13. IL-6 and IL-8 did not affect the survival function and CRP had an effect that disappeared when TNF-α was included in the analysis. In contrast to the centenarian study, IL-6 was strongly associated with all-cause mortality in octogenarians whereas TNF-α was only associated with mortality in men in this cohort (VIII), Figure 14. When the increased mortality risk in men is taken into consideration, TNF-α could have acted as a marker of frailty within men only but it was also possible that the interaction between TNF-α and sex was due to a power problem in the long-living women. In octogenarians, effects of TNF-α and IL-6 were independent of each other as well as of other traditional risk factors for death such as smoking, blood pressure, physical exercise, total cholesterol, co-morbidity, BMI, and intake of anti-inflammatory drugs, pointing

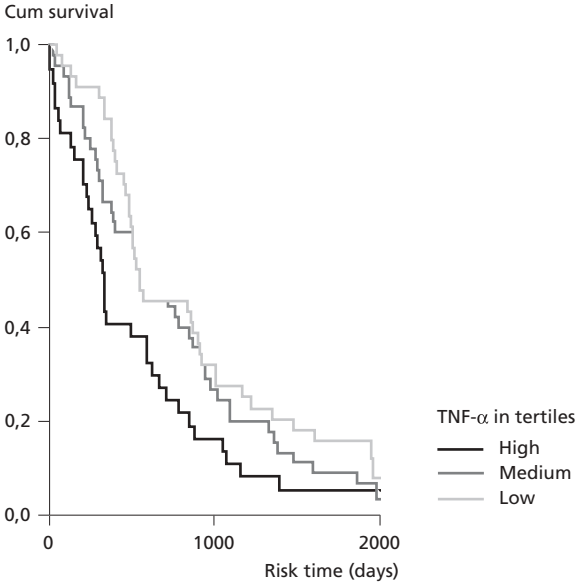


Figure 13. TNF-α and imminent mortality in centenarians. Centenarians are divided by tertiles based on TNF-α levels in plasma. Based on data from VII.

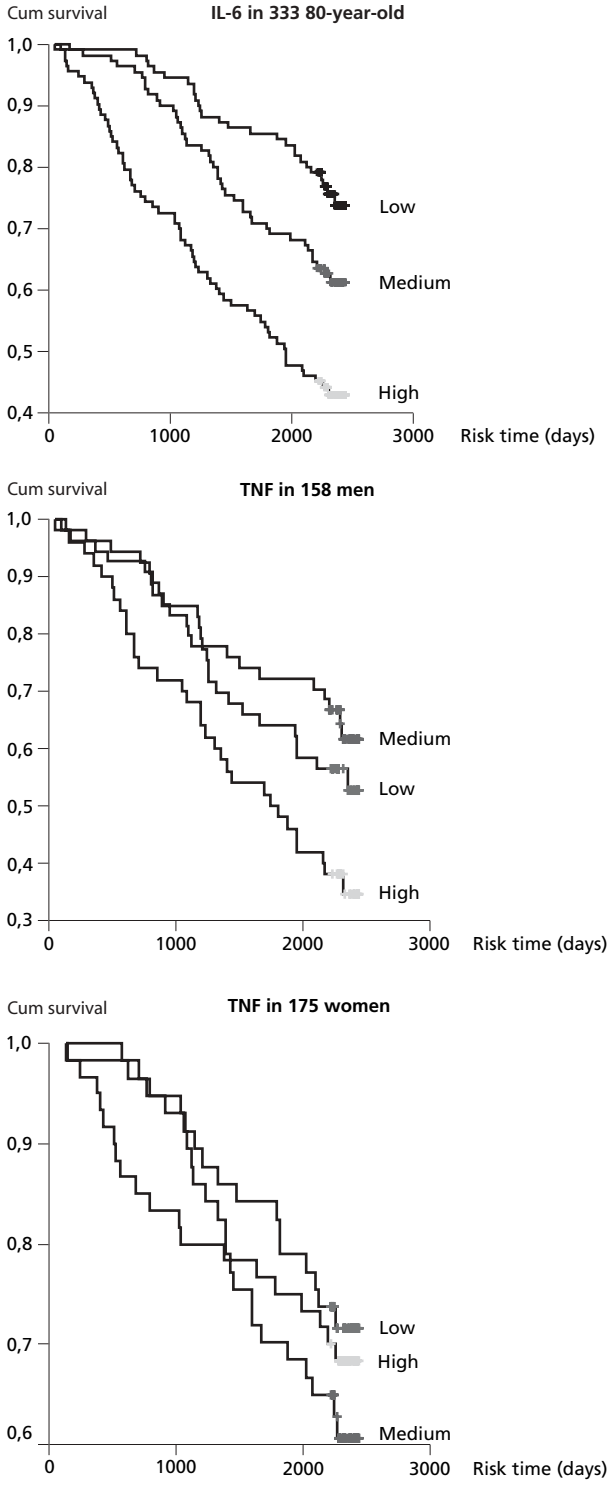


Figure 14. TNF-α, IL-6, and mortality in 80-year-olds. Octogenarians are divided by tertiles (high, medium, low) in serum levels of IL-6 and TNF-α. Follow-up time is 5-6 years. Based on data from VIII.

towards causative roles as well as separate effects of the two cytokines in morbidity and mortality (VIII). Study VII and VIII together suggest also differentiated roles of TNF-α and IL-6 with different clinical impact in octogenarians versus centenarians although a power problem or a cohort effect cannot be ruled out. I speculate if increased circulating levels of IL-6 in relatively healthy, old populations represent a systemic anti-inflammatory response to local proinflammatory activities and is a marker of the overall inflammatory burden. When age-related inflammatory pathology progresses increasing levels of TNF-α also appear in the circulation and this gradually becomes a stronger risk marker. Furthermore, TNF-α may reflect the syndrome of frailty and risk in relation to the metabolic syndrome whereas chronic elevations in IL-6 may constitute a risk of thromboembolic complications in middle-aged and in relatively healthy old people that could be of less importance in frail populations and in centenarians who have already survived to advanced age despite considerable comorbidity. Consistent with this hypothesis, IL-6 predicted mortality in frail women with prevalent CVD but not in frail women without CVD in the Women's Health and Aging study [244].

Low-level increases in inflammatory mediators downstream in the inflammatory cascade are likely secondary to increased levels of TNF-α and IL-6. Consistent with this theory, IL-6 was a stronger predictor of mortality than CRP in the Iowa 65+ Rural Health Study [151] and the effect of CRP disappeared in survival analysis of Danish centenarians when TNF-α was also included in the statistical model (VIII). Nevertheless, it is possible to isolate harmful effects of chronic low-level increases in most inflammatory mediators, e.g., CRP determines the uptake of LDL by macrophages, affects coagulation and induces the expression of cellular adhesion molecules [157]. The strong covariance of inflammatory mediators makes it difficult to isolate their clinical impact from each other as mentioned previously, and it is possible that their effects should merely be considered as parallel phenomena (se section 5).

In the Leiden 85+ study participants who at age 85-years produced low levels of LPS-induced IL-1β, IL-6, TNF-α IL-1Ra, and IL-10, were found to have a more than 2-fold elevated overall mortality risk, independent of comorbidity compared to peers with a higher production [256]. Consistent with this, low levels of LPS-induced IL-1β were associated with morbidity among 80-year-old Danes from the 1914-cohort (III). Fading peak values in the cytokine production following *in vitro* stimulation seem to accompany systemic low-level inflammation in aging (see section 2.4) as well as other chronic inflammatory diseases, e.g., HIV infection [257]. However, in general there is a poor direct correlation between *in vitro* LPS-stimulated cytokine production and circulating levels of cytokines *in vivo* (III).

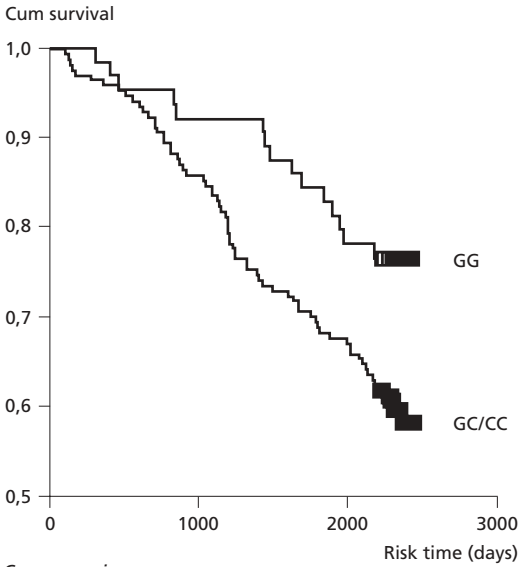
4.3. MORTALITY AND POLYMORPHISM STUDIES

The distribution of the TNF –308G/A promoter polymorphism did not vary across different age groups in Finnish [258], Italian [259], and Danish (X) populations, Figure 7. Based on the finding that the GA genotype was associated with a low prevalence of senile dementia compared with GG in centenarians and the few centenarians with AA carrier status had increased mortality risk and tended to show higher plasma levels of TNF-α, one may speculate if the TNF –308A allele is maintained during aging because subjects who are heterozygous for this polymorphism posses the optimal inflammatory response with regard to protection against age-related neurodegeneration, (X). Further cohort studies are needed to illuminate this hypothesis further.

An evaluation of combinations of TNF –308G/A and IL10 –1082 G/A (G is associated with high IL-10 production) in Italians demonstrated an increase of the anti-inflammatory IL10 –1082GG/TNF –308GG haplotype in male centenarians compared with younger men whereas a similar association was not found among women [259]. This finding indicates once more that the balance between proinflammatory and anti-inflammatory activity is crucial and it underlines the importance of a strong innate ability to resolve proinflammatory activity in chronic disease. The frequency of the IL10 –1082GG genotype alone was also increased in Italian centenarian men compared with younger men [260; 261] but these findings were not confirmed in Finnish nonagenarians [258] or in centenarians from Sardinia [262] compared with younger age groups.

Most studies of associations between cytokine polymorphisms and survival/longevity in elderly populations have focused on the IL6 –174G>C promoter polymorphism (see Table 2 for an overview). This polymorphism in relation to mortality risk was studied prospectively in octogenarians from the 1914-cohort with the aim to further characterize the effect of IL-6 (IX). The polymorphism showed a strong interaction with the smoking status and the C-allele was associated with all-cause mortality in non-smokers independently of sex, body mass index, prevalent comorbidity, and low-level elevations in serum IL-6 compared to the GG genotype (Figure 15). Due to a low number of smokers, conclusions cannot be made about this subgroup. Unfortunately, it is difficult to extrapolate from these data to the effect of IL-6 in the elderly for the reasons already outlined in section 3.2.3. Thus, at this time it is difficult to conclude whether the –174C variant is associated with increased [125] or decreased promoter activity [124] and it is probable that the response depends on the stimulant and the cell line. Accordingly, it is still unclear if high transcriptional activity of the IL-6 gene was actually an advantage or a disadvantage in longevity. Since the –174C variant and circulating IL-6 were independent risk factors in all-cause mortality (Figure 15) the two variables seemed to represent different biological effects in non-smoking octogenarians, e.g., peak values in response to triggers versus the actual inflammatory burden *in vivo* (IX).

In a recent study of 1710 Danes in different age groups a modest, but significant increase was detected in the frequency of the –174GG genotype that was mainly due to the disappearance of the GC genotype [263] in accordance with data in IX. Moreover, consistent with the findings in IX, a very recent Finnish study reported that –174C



Cox regression

	Hazard ratio	P
C-variant	2.04	0.02
IL-6 pg/ml	1.07	0.02

GG is reference for the C-variant (GC and CC). Adjusted for the effect of gender, BMI, CVD, and cancer.

Figure 15. The IL6 –174G>C promoter polymorphism and mortality in 234 non-smoking 80-year-olds. Follow-up time is 5-6 years. GG is reference for the C-allele. IL-6 = serum levels (continuous variable). Based on Figure 2 and Table 1 from IX.

variant was associated with increased mortality from 90 to 95 years of age in a cohort of 285 nonagenarians [264]. However, in an earlier cross-sectional study the same Finnish octogenarians at the age of 90 years were compared with 400 healthy younger blood donors but no difference in the genotype or the C-allele frequency were detected in this study [258], suggesting an effect restricted to very old age. Consistent with this, the frequency of the different IL6 –174G/C genotypes was unaltered in a small study of Italian octogenarians and nonagenarians compared to younger age groups in cross-sectional designs [218]. Nevertheless, disturbingly incoherent the frequency of the C-variant was significantly enhanced among Irish octogenarians/nonagenarians [215] and Italian male centenarians [218] compared to younger age groups. It is likely that these discrepancies result from cohort effects, population stratification, small sample sizes, complex interactions between life style and genetic factors together with cultural and genetic differences across countries. Furthermore, it has been suggested that the association between the C-variant, CVD, and mortality risk represent a bell-shaped or inverted "U" shaped curve across increasing age groups so that the C-variant is associated with increased mortality risk until the age of 80-90 years and then become a survival advantage in men beyond this age [265]. However, this hypothesis fits poorly Finnish and Danish data sets (IX)[263; 264].

A recent study has confirmed that the effect of the IL6 –174G/C promoter polymorphism interacts with life style factors such as smoking and physical fitness [210]. Furthermore, non-smoking C-allele carriers had an increased odds ratio for CVD in a case-control study of adults aged 65+ years whereas such an association was not found among smokers [150]. Accordingly, it appears that the C-variant is a risk factor for mortality in old populations but the effect is complex and interacts with life style factors and perhaps with gender.

Polymorphisms in the IL-1 gene have not been associated with longevity [258; 266] to my knowledge.

4.4. CONCLUSION AND COMMENTS

Systemic low-level inflammation is an independent predictor of mortality in elderly populations, supporting the hypothesis of a causal relation between inflammatory mediators and age-associated diseases. Furthermore, simultaneously analyses of TNF-α and IL-6 in survival models have demonstrated that the two cytokines have separate effects that appear to have different clinical impact across various age groups. Thus, contrary to the initial hypothesis, IL-6 seems to be a strong, independent risk factor rather than a surrogate marker of plasma TNF-α. However, it is still unclear to which extent plasma IL-6 reflects local TNF-α activity and the inflammatory burden versus an independent effect as a biological driver in age-related pathology. Cytokine polymorphism studies have the potential to elucidate this aspect further. Unfortunately, these studies have not added much more evidence and conclusions are severely limited by the confusion with regard to the effect of the IL6 –174G/C promoter polymorphism on the production of IL-6 protein in elderly populations.

5. THE ORIGIN OF THE LOW-LEVEL INFLAMMATORY STATE IN ELDERLY POPULATIONS

When systemic low-level inflammation is a causative factor in age-related morbidity and all-cause mortality the reflection arises if one single factor is able to trigger the inflammatory wobble in old populations.

Low-level inflammation has the potential to be a cause as well as consequence in age-related pathological processes, providing a self-enhancing cascade, Figure 16. Beyond this, a wide range of other factors seems also to add to low-level inflammation such as subclinical infections, the body composition and different life style factors including smoking and physical inactivity (see section 3.2.2.), Figure 17. I speculate that the age-related relative increase in adipose tissue is not counteracted by the anti-inflammatory effect of muscle contractions due to the common age-related decrease in physical activity. As a consequence, a shift in the balance between fat tissue (proinflammatory profile) and the working muscle mass (anti-inflammatory effect) may push the old organism towards a proinflammatory state. It is also expectable that genetic factors could make a different contribution to the variation in elderly versus younger age groups: On one hand, it is probable that the surplus and variety of triggers present in later age may decrease the importance of genetic variation but on the other, it is also possible that functional genetic variations in the promoter region of the genes contribute more in the elderly because there are more triggers. A recent study of elderly twins demonstrated that genetic factors accounted for 20-55% of the variation in plasma levels of inflammatory variables including TNF-α, IL-6, CRP, sICAM-1 and fibrinogen but genetic polymorphisms (IL6 –174G/C, TNF –238G/A, CRP –1059G/C, fibrinogen β-455G/A, ICAM K/E469) explained only a minor part of the inheritability, pointing to a need for further understanding of the genetic regulation [267]. Furthermore, it has been suggested that the loss of anti-inflammatory sex steroids lead to increased production of proinflammatory cytokines [268]. It is still unclear if an altered capacity of cytokine production by immune cells contributes to chronic low-level inflammation. It is likely that a delayed down-regulation of proinflammatory activity in response to triggers (II, VI) rather than increased peak values contribute to chronic low-level inflammation in old adults (see section 2.3). Further unravelling of the source of low-level inflammation awaits the identification of tissue-specific markers of inflammation as well as a better understanding of the local and distant roles of peripheral tissues such as CNS, adipose tissue, muscles, and bones.

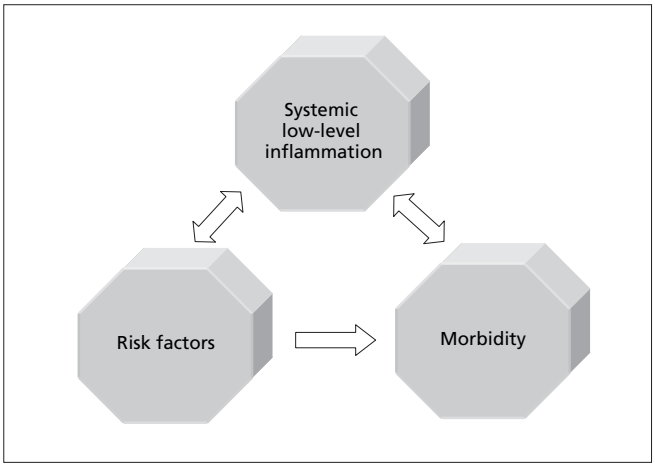


Figure 16. Low-level inflammation, age-related risk factors and morbidity. Inflammatory mediators such as TNF-α and IL-6 is probable both a cause and a consequence of age-related morbidity and associated risk factors, providing a self-enhancing cascade (see text).

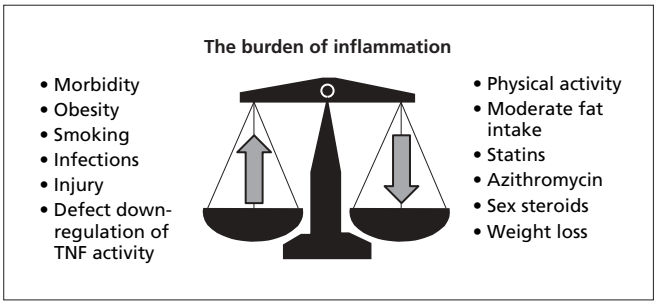


Figure 17. The origin of the inflammatory burden in elderly populations. A wide range of factors contributes to systemic low-level inflammation in the elderly. Counteracting factors are only at the beginning to be understood.

Markers of inflammation, coagulation/fibrinolysis, glucose metabolism, lipid metabolism, the renin-angiotensin system, the hypothalamic–pituitary axis and others are interconnected, as already discussed, and they exhibit considerable covariance in population studies, making causal analyses difficult, Figure 9. "The inflammation hypothesis of aging" has recently suggested that aging is the accumulation of damage that results from the chronic activity of these systems because the organism trades short-term benefit for long-term damage through the constant utilization of these interfacial response mechanisms (RP Tracy, oral communication, NIA, Workshop on Inflammation, Inflammatory mediators and Aging, 2004). Preceding this hypothesis, the concept of "Inflamm'aging" was introduced at the millennium, defining a global reduction in the capacity to cope with stressors and a concomitant progressive increase in proinflammatory status provoked by a continuous antigenic load and stress [269]. Finally, it has lately been demonstrated that there exists strong associations between early-age mortality and subsequent mortality in the same cohorts and this observation has given rise to the hypothesis that chronic inflammatory mechanisms drive much of the influence of early-life infections on later morbidity and mortality (the hypothesis of a "cohort morbidity phenotype") and, as a consequence, improved public health conditions and medical interventions resulting in decreased inflammation during early life has also led directly to a decrease in morbidity and mortality resulting from chronic conditions in old age [270]. All these hypotheses are strongly inspired by the hypothesis that adaptive responses to short-term infections or injury can become maladaptive in the long term – a double-edged sword that evolutionary biologists refer to as antagonistic pleiotropy [271]. In accordance with this hypothesis, a LPS-induced cytokine profile with high TNF-α and low IL-10 predicted resistance to fatal infectious diseases but decreased reproductive success and increased mortality from cardiovascular events in old women [149].

In conclusion, a wide range of different parameters rather than one single factor are likely contributors to age-associated systemic low-level inflammation (Figure 17), involving integrated and parallel processes in the innate immune system, the metabolism, endocrine systems, and the nervous system (Figure 9).

6. TREATING SYSTEMIC LOW-LEVEL INFLAMMATION IN ELDERLY POPULATIONS

Among 2232 apparently healthy Europeans aged 70 to 90 years adherence to physical activity, non-smoking, a Mediterranean diet, and moderate alcohol use was associated with a more than 50% lower rate of all-cause and cause-specific mortality [272]. Considering that inflammation is involved in nearly all age-related disorders it is not surprising that these life style factors have also been associated with inflammatory factors. Accordingly, interventions focused on life style factors are likely candidates in order to lower the inflammatory burden whereas the anti-inflammatory effect of pharmacological interventions constitutes still a rather unexplored area in gerontology.

6.1. EXERCISE

Physical activity offers protection and is effective in the treatment of a large number of medical disorders including the metabolic syndrome, CVD, and age-related sarcopenia partly mediated through an improved lipid profile, elevated insulin sensitivity and a lower blood pressure [171]. IL-6 in relation to exercise has been suggested to mediate central, health beneficial, metabolic and physiological effects and to inhibit low-grade TNF-α production (see section 3.2.2.4). In theory, elderly people may benefit from exercise induced anti-inflammatory activities that provide a possible, but yet unexplored pathway to reduce systemic low-level inflammation [273]. Nine months of endurance training reduced CRP in younger subjects preparing for a marathon [274] but the relevance of this observation in relation to older people is questionable.

With regard to inflammatory parameters in relation to patients with the metabolic syndrome and CVD, 3 months of treadmill exercise reduced CRP in patients with intermittent claudication [275]. However, in a study of obese/overweight sedentary men and women aged 60+ years, 18 months of combined weight training and walking for 1 hour three times a week did not affect circulating levels of IL-6, sTNFR-I or CRP [276].

Low physical activity is associated with age-related cognitive decline [277-279] but to my knowledge the role of inflammation has not been evaluated in this context.

It has become clear that an important part of age-related sarcopenia and disability is due to physical inactivity and progressive resistance training of both healthy and frail nonagenarians appears to be an effective way of improving muscle strength and perhaps activities of daily living [280]. Thus, at present high intensity resistance training for a short duration is considered to be the state of art when physical training of the oldest old is planned and performed. TNF-α levels in muscles decreased after resistance exercise for 3 months in frail, old adults [233]. However, 12 weeks of resistance training did not affect plasma levels of TNF-α, IL-6, or sTNFR-I in frail nonagenarians [281] although a lack of power did not allow a final conclusion in this study. Furthermore, it is possible that global muscle training rather than training of isolated muscle groups is necessary in order to mount a detectable systemic anti-inflammatory response.

In order to evaluate if old populations benefit from the anti-inflammatory effects of exercise there is a need for further large studies to address the necessary duration of the training program, intensities of the exercise, how many and which muscle groups should be involved, and if long-term indurance training is more effective with regard to anti-inflammatory activities than short-term, high-intensity resistance training.

6.2. DIET

Instructions in a Mediterranean-style diet for 2½ years resulted in reduced serum concentrations of CRP, IL-6, IL-7, IL-18 as well as decreased insulin resistance and improved endothelial function score whereas these parameters remained stable in the control group in an intervention study of patients with the metabolic syndrome [282]. Consistent with this, dietary fatty acids modulated plasma levels of IL-6, CRP, and fibrinogen in a study of 50 men who consumed controlled diets in a randomised crossover design [283]. Furthermore, a moderate alcohol intake was associated with lower levels of inflammatory markers (WBC count and fibrinogen) in 5865 older adults free of CVD in the Cardiovascular Health Study [284]. A diet-induced weight loss resulted in greater reductions in circulating CRP, IL-6, and sTNFR-I than did no weight-loss treatment and changes in sTNFR-I correlated with changes in body weight in a study of overweight/obese sedentary older men and women aged 60+ years [276]. These studies are likely to be relevant in young elderly but probably not in the frail, oldest old.

6.3. ANTIOXIDANTS

An intake of antioxidants was associated with low levels of CRP and IL-6 (borderline) in the Health ABC study [184]. Vitamin E and vitamin C are considered two of the most important dietary antioxidants and moreover, vitamin E decreases the *in vitro* production of TNF-α [285-287], IL-1β [286-289] and IL-6 [290] by BMNC and reduces levels of CRP [290]. Although a combined supplementation of vitamin E+C for three years retarded the progression of common carotid atherosclerosis in healthy men aged 45-69 years with hypercholesterolemia compared with a placebo group in the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study [291] this cocktail did not affect circulating levels of TNF-α, IL-6, or CRP [292]. Furthermore, supplementation with vitamins E+C attenuated the IL-6 release from contracting skeletal muscles [293] that is considered health beneficial. Recent intervention studies have also

failed to show a beneficial effect of anti-oxidant treatment in CVD [294-296].

6.4. ANTIBIOTICS

Chronic *C. pneumonia* infection has been implicated as a potential factor in activation of the vascular endothelium in atherosclerosis (see section 3.2.2.6), providing the rational for testing if treatment with anti-chlamydial macrolide antibiotics might improve the outcome of patients who survived an acute coronary syndrome. Initial randomised placebo-controlled trial reported a cardioprotective effect in patients allocated to antibiotic treatment but data from later trials conclude that there is no good evidence to justify the use of such a strategy in the prevention of CVD [297]. Nevertheless, in patients with clinical coronary artery disease 6 months with azithromycin resulted in significant decreases in IL-6 and CRP [298] and a brief course of treatment reduced levels of sICAM-1 [195], suggesting that azitromycin had an anti-inflammatory effect.

With regard to asymptomatic bacteriuria, the common recommendation is not to treat with antibiotics because the treatment is often associated with adverse drug reactions, development of resistant bacterial isolates, and no improvement of survival [299].

Thus, at this time it is not the clinical practice to treat asymptomatic infections in old patients but it remains unexplored if treatments of chronic and/or asymptomatic infections during a lifetime lowers the inflammatory burden at old age.

6.5. STATINS

Statins have anti-inflammatory effects and are effective in the treatment of CVD (see section 3.2.4.). It remains to be tested whether reduction in low-level inflammation per se lovers cardiovascular risk. Furthermore, the potential role in the treatment of other patient categories such as HIV, bacteraemia and geriatric patients are still under investigation.

6.6. CYTOKINE ANTAGONISTS

Anti-TNF IgG (infliximab), sTNFR-II (etanercept) and IL-1Ra (anakira) are effective in the treatment of rheumatoid arthritis and inflammatory bowel diseases but their use has not yet been extended to other patient categories.

6.7. CONCLUSION AND COMMENTS

Interventions that drive risk factors in elderly populations towards more beneficial profiles also tend to be anti-inflammatory, e.g., physical activity, statins, modulation of fat contents in the diet, and diet-induced weight loss in overweight elderly, Figure 17. Understanding the precise effects of different inflammatory mediators in age-associated pathology is important when we evaluate future intervention strategies in old populations. If TNF-α is a driver in the syndrome of frailty and/or the metabolic syndrome intervention strategies with the purpose to reduce the production of TNF-α may be effective in these disorders. This could include treatment with TNF-α antagonists or IL-6 if it is confirmed that the latter cytokine is important in the counteracting response to TNF-α induced pathology and if effects on the coagulation system and the lipid metabolism turns out to be minor when IL-6 is administrated with a similar pattern to what is observed during physical exercise rather than during systemic low-level inflammation.

7. CONCLUDING REMARKS AND PERSPECTIVES

Systemic low-level inflammation is a risk factor as well as a disease marker in old adults and provides a joint link between several age-related disorders. Thus, there is good evidence that systemic low-level inflammation is causal related to the metabolic syndrome, CVD, frailty and all-cause mortality. Enhanced counts of inflammatory cells in the innate immune system is rather a part of systemic low-level inflammation than it reflects "successful" aging as it is associated with markers of atherosclerosis and all-cause mortality.

Looking over the existing literature has emphasized that TNF-α and IL-6 are central players in a network consisting of the innate immune system, the metabolism, the endocrine system, the nervous system, and the coagulation system. Moreover, simultaneously analyses of the two cytokines in survival models have supported the hypothesis that the two cytokines have separate effects with different clinical impacts. Experimental as well as epidemiological polymorphism studies suggest that TNF-α is causal related to the metabolic syndrome, endothelial dysfunction, and maybe to the syndrome of frailty. Given TNF-α works mainly locally, TNF-α transcription may not always be reflected by enhanced protein levels in the circulation. Rather TNF-α may stimulate IL-6 production and subsequent mediators in the inflammatory cascade. In my opinion, chronic low-level increases in IL-6, CRP, IL-1Ra, sTNFRs, and inflammatory cells are thus likely to reflect on-going TNF-α production. IL-6 constitutes a strong risk factor in epidemiological studies of elderly populations but this does not necessarily reflect that it is also a biological driver, e.g., the effect of circulating IL-6 may partly reflect local TNF-α production in statistical models. Large amounts of IL-6 are released to the circulation during exercise that is considered health beneficial. Considering that IL-6 has strong anti-inflammatory activities and inhibits directly as well as indirectly the production of TNF-α it is possible that the balance between TNF-α and IL-6 is important in age-related morbidity. However, chronic elevations in IL-6 cause increased energy expenditure, dyslipidaemia, a procoagulant state, and anaemia. Thus, IL-6 may act as a double-edged sword in the old.

Polymorphism studies have the potential to add important knowledge to the unravelling of the causal versus responsive effects of TNF and IL-6 as well as other individual inflammatory mediators. Unfortunately, the new information from this research area has so far been limited. Maybe we sometimes confuse ourselves in the interpretation of data as we forget that high promoter activity may not always be accompanied by high plasma levels of protein and vice versa just as we often forget that stimulated cytokine production *in vitro* and circulating levels of cytokines represent different biological parameters.

The role of systemic low-level inflammation in age-related cognitive decline is unclear. High plasma levels of TNF-α was a marker of dementia in centenarians but, surprisingly, polymorphism studies in the same population pointed towards a protective effect, suggesting that TNF-α is upregulated to counteract neurodegeneration. At this time, investigations of CNS repair processes are almost unexplored.

A wide range of factors is likely to trigger systemic low-level inflammation in old populations including life style factors, body composition, hormonal changes, and etcetera. *In vivo* studies and some *in vitro* studies have demonstrated age-related changes in the acute phase response including a more rapid response as well as prolonged proinflammatory activity, suggesting a dysregulated down-regulation of proinflammatory activity. It remains to be demonstrated if this is causal related to systemic low-level inflammation and the clinical outcome of acute infections. However, there is no evidence of enhanced peak values in the cytokine production by circulating monocytes and T lymphocytes seem to play a minor role. Furthermore, cytotoxic activities by inflammatory cells in the blood may be exhausted by chronic activation in the inflammatory environment.

It is likely that high proinflammatory and low anti-inflammatory activities are beneficial by increasing the protection against fatal infections early in life whereas these responses become harmful and increase the risk to die from chronic inflammatory diseases such as CVD late in life [149]. Moreover, I speculate if TNF-α is first upregulated in CNS in order to repair age-related neurodegeneration but this induces secondly systemic low-level inflammation accompanied by the metabolic syndrome, CVD, and wasting, which become the prize for limiting the progressive CNS damage. This fits

the observations that the metabolic syndrome and dementia is associated with each other in epidemiological studies; circulating TNF-α protein is a marker of both disorders; the TNF –308A variant is maintained at very old age, indicating that TNF-α is not solely a harmful cytokine; and TNF–308A is related to the metabolic syndrome and related CVD whereas preliminary data suggest it is a protective factor in dementia. A high prevalence of the metabolic syndrome and related CVD in the western part of the world may thus result from the increased life expectancy in relation to enhanced survival from severe, acute infections but with the continuous presence of low-virulent pathogens, ongoing repair of a progressing neurodegeneration, and a life style that induces a response similar to what is observed during chronic infections.

It represents a new paradigm that cytokines are not only regulators of immune function but also central parts in the metabolism, the endocrine system, the nervous system, and the coagulation system, providing central links in this supernetwork. Furthermore, we have probably only seen the top of the iceberg in the understanding of inflammatory processes as new cytokines are discovered all the time (e.g., leptin, adiponectin, resistin). This thesis has mainly focused on effects of single cytokines but it is very possible that balances in the cytokine network and combinations of different cytokine polymorphisms are more important for the clinical outcome. Moreover, the last decade has taught us that the adipose tissue is an inflammatory organ with the identification of "adipokines". Given that skeletal muscle mass is the largest organ in the body, the discovery of working muscles as a cytokine producing organ (e.g., IL-6, IL-8, IL-15) opens also up for another new paradigm: Skeletal muscles as an endocrine organ that by contractions stimulates the production and release of "myokines", which can influence the metabolism and modify cytokine production in other tissues and organs. Furthermore, we are only on the threshold to explore effects of cytokines, adipokines and myokines on the brain function and to address the production of "neurokines".

Epidemiological studies have so far given us important although limited information about inflammatory mediators as players in the network consisting of the immune system, the nervous system, the endocrine system, and the metabolism. In the future we shall determine regulatory pathways for known monokines, lymphokines, adipokines, myokines, and etcetera; try to understand interactions and regulatory roles for the presently known cytokines; and apply array and proteomic techniques in the search for new inflammatory mediators with possible potentials to be new targets of interventions. There is a need for a new research concept, linking a molecular approach to the whole body *in vivo* metabolism in the intact human being by a synchronous research of inflammation/metabolism in cell cultures, animal models, and human integrative physiological models with classic and genetic epidemiology as a base.

8. SUMMARY

Aging is associated with systemic low-level inflammation defined as 2-4 fold increases in levels of circulating proinflammatory and anti-inflammatory cytokines, acute phase proteins, and minor elevations in counts of inflammatory cells (neutrophils, monocytes, and natural killer (NK) cells). The aim of the current thesis was to investigate if systemic low-level inflammation was an independent risk factor in age-associated morbidity and mortality and to test if the cytokines tumor necrosis factor (TNF)-α and interleukin(IL)-6 had different clinical effects. Furthermore, it was investigated if an altered acute phase response was a potential contributing factor to low-level inflammation in old populations.

High plasma levels of TNF-α were independently associated with both dementia and atherosclerosis in centenarians. Moreover, TNF-α was an independent risk factor of imminent mortality. No similar associations were found with regard to IL-6, suggesting a specific effect of TNF-α. The association between TNF-α and atherosclerotic cardiovascular disease was confirmed in octogenarians. However, TNF-α was only associated with mortality in men in this population at follow-up after 5 years. In contrast, IL-6 was a strong prognostic risk factor independently of TNF-α and other known risk factors. Furthermore, high counts of neutrophils and NK cells as well as attenuated cytotoxicity per NK cell were also associated with atherosclerosis and the neutrophil count was associated with mortality in octogenarians. Low-level inflammation was also correlated with smoking, cholesterol, triglycerides, body mass index, hypertension, and physical inactivity.

It was tried to further characterize whether inflammatory mediators represented causal risk factors or passive disease markers by investigating effects of common, functional cytokine promoter polymorphisms. The effect of the TNF –308G/A promoter polymorphism was tested in centenarians in relation to dementia and mortality. The A variant has increased promoter activity. The GA genotype was associated with decreased prevalence of dementia compared to GG. No difference in the frequency of different genotypes was detected in centenarians compared to younger age groups. However, the few centenarians with the AA genotype had increased mortality risk and tended to show higher circulating levels of TNF-α protein. Accordingly, it was possible that the TNF –308A allele was maintained during aging because subjects heterozygous for this polymorphism possessed the optimal inflammatory response with regard to age-related neurodegeneration. The IL6 –174G/C promoter polymorphism was investigated in octogenarians. The C-variant was associated with increased prevalence of ischaemic cardiovascular disease and it was an independent risk factor of mortality in non-smokers. However, at this time it is unclear if this reflected an enhanced or decreased production of IL-6 protein in response to triggers, as the interpretation of data in experimental studies has been controversial.

Studies of age-related differences in the production of proinflammatory cytokines in response to acute stimulations *in vitro* have yielded inconsistent results with the extent and even the direction of the aging effect being dependent on variations in sex, stimulus, culture systems, and culture duration. *In vivo* infectious models including patients with pneumococcal infections and a human sepsis model showed a delay in the down-regulation of proinflammatory activity in elderly subjects, supporting the hypothesis of an age-related dysregulated cytokine response that may contribute to systemic low-level inflammation and perhaps to increased morbidity and mortality during acute infections. However, a causal relationship remains to be demonstrated.

In conclusion, aging was associated with an altered acute phase response. Furthermore, systemic low-level inflammation was associated with chronic, age-related diseases such as atherosclerosis and dementia and it was an independent risk factor in all-cause mortality. Simultaneously analyses of TNF-α and IL-6 indicated that the two cytokines were active players but with separate clinical activities that in some contexts were causal and in others protective in age-related morbidity. Thus, the effect of TNF-α and IL-6 were independent of each other and other known risk factors and comorbidity in survival analyses, and, moreover, functional cytokine promoter polymorphisms were also associated with morbidity and mortality.

THIS THESIS IS BASED ON THE FOLLOWING ARTICLES:

I. Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhøj P, Pedersen BK. A high plasma concentration of TNF-? is associated with dementia in centenarians. J Gerontol A Biol Sci Med Sci 1999;54A(7):M357-M364.

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IV. Bruunsgaard H, Skinhøj P, Pedersen AN, Schroll M, Pedersen BK. Ageing, TNF-? and atherosclerosis. Clin Exp Immunol 2000;121:255-260.

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X. Bruunsgaard H, Benfield TL, Andersen-Ranberg K, Hjelmborg Jv.B., Pedersen AN, Schroll M, Pedersen BK, Jeune B. The TNF-α –308G>A polymorphism is associated with dementia in the oldest old. JAGS 2004;52(8):1361-1366.

Data in manuscripts I-III were included in the PhD-thesis "Ageing and immune function" by Bruunsgaard H (University of Copenhagen, 2000). Manuscripts IV-X have not previously been submitted with the aim of obtaining an academic degree.

ABBREVATIONS

ABI	Ankle-brachial arterial blood pressure index
AD	Alzheimer's Disease
BMNC	Blood mononuclear cells
CVD	Cardiovascular disease
IL	Interleukin
LPS	Lipopolysaccharide
NK	Natural killer
RA	Receptor antagonist
STNFR	Soluble TNF receptors
TNF	Tumor necrosis factor
VaD	Vascular dementia
WBC	White blood cells

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