Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Gerontology 39 (2004) 687–699
Experimental Gerontology
www.elsevier.com/locate/expgero

Mini–Review

# Inflammatory mediators in the elderly

Karen Suárez Krabbe*, Maria Pedersen, Helle Bruunsgaard

*Department of Infectious Diseases, H:S, Rigshospitalet, University of Copenhagen, M7641, Blegdamsvej 9, DK-2100 Copenhagen, Denmark*

Received 15 October 2003; received in revised form 16 January 2004; accepted 21 January 2004

**Abstract**

Ageing is accompanied by 2–4-fold increases in plasma/serum levels of inflammatory mediators such as cytokines and acute phase proteins. A wide range of factors seems to contribute to this low-grade inflammation, including an increased amount of fat tissue, decreased production of sex steroids, smoking, subclinical infections (e.g. asymptomatic bacteriuria), and chronic disorders such as cardiovascular diseases and Alzheimer's disease. Furthermore, there is some evidence that ageing is associated with a dysregulated cytokine response following stimulation. Several inflammatory mediators such as tumour necrosis factor-α and interleukin-6 have the potential to induce/aggravate risk factors in age-associated pathology, providing a positive feedback mechanism. Thus, it is possible that inflammatory mediators constitute a link between life style factors, infections and physiological changes in the process of ageing on the one hand and risk factors for age-associated diseases on the other. Consistent with this, inflammatory mediators are strong predictors of mortality independently of other known risk factors and co-morbidity in elderly cohorts. A direct pathogenetic role of inflammatory mediators would be highly likely if longevity was shown to be associated with cytokine polymorphisms regulating cytokine production. Several studies support indeed this hypothesis but, unfortunately, findings in this area are conflicting, which probably reflects the complexity of the effect of cytokine polymorphisms and their interaction with the lifestyle and sex.


*Keywords:* Low-grade inflammation; Aging; Tumour necrosis factor; Interleukin-6; Atherosclerosis; Sarcopenia; Cognition

Increased inflammatory activity accompanies ageing and is a characteristic part of the pathological processes in cardiovascular diseases (CVDs) and senile dementia, which represent the major medical disorders and causes of mortality at the very end stage of the human life span. Moreover, a dysregulated acute phase response may be an important contributor to an increased susceptibility, an altered clinical presentation, and a high mortality risk in elderly patients with infections.

In this article we address aspects of the association between an excessive inflammatory response, morbidity, and mortality in elderly populations and we suggest that inflammatory mediators provide a common link between ageing and life style factors on the one hand and age-related diseases on the other. To this end, we focus mainly on human studies.

## 1. The ageing immune system

The term immunosenescence usually refers to the notion that there is an age-related dysfunction of the immune system. The number of studies in this area is large and, unfortunately, the results are often contradictory. Many investigators compare donors of different ages in cross-sectional studies and these populations differ in their genetic background, nutritional and environmental influences, co-morbidity, selection criteria, and in the techniques applied to measure immune parameters across different studies. In our opinion, it is important to perform longitudinal studies of epidemiologically well-described cohorts, representing approximations to normal populations in order to assess the clinical relevance of the aged immune system. The effect of ageing cannot be completely separated from the contributions of co-morbidity, medication, or malnutrition in such studies. However, it is possible to test whether these parameters act independently of each other in statistical analyses of large cohort studies. Furthermore, we believe

* Corresponding author. Tel.: +45-35-45-7377; fax: +45-35-45-7644.
*E-mail address:* krabbe@rh.dk (K.S. Krabbe).

0531-5565/$ - see front matter © 2004 Published by Elsevier Inc.
doi:10.1016/j.exger.2004.01.009

that immunosenescence represents a continuum of changes that are related to age-associated pathology and that attempts to make a strict separation of the effects of ageing versus co-morbidity are of academic interest rather than of clinical importance.

## 2. Ageing is associated with increased levels of circulating cytokines

Levels of circulating inflammatory mediators and their clinical importance have not been explored in population-based samples of elderly people until recently. Most studies have focused on interleukin (IL)-6, which has been called a cytokine for gerontologists (Ershler, 1993) and most studies report increased plasma/serum levels of this cytokine with advancing age (Wei et al., 1992; Ershler et al., 1993; Bruunsgaard et al., 1999a; Hager et al., 1994) although some studies have found no changes (Beharka et al., 2001; Peterson et al., 1994). Similarly, some studies find that plasma levels of tumour necrosis factor (TNF)-α are increased in elderly populations (Bruunsgaard et al., 1999a; Paolisso et al., 1998) whereas other studies are not able to detect this difference (Fagiolo et al., 1993; Peterson et al., 1994). Discrepancies probably relate to variations in sensitivity of the used assays, to lack of power in some studies, to the health status of the old participants, and to differences in their age. For instance, we have previously reported that IL-6, but not TNF-α is increased in middle-aged humans whereas both cytokines are elevated in octogenarians (Bruunsgaard et al., 1999a). Moreover, it has been demonstrated that inflammatory mediators act as disease markers (Bruunsgaard et al., 1999a; Di Iorio et al., 2003; Ferrucci et al., 1999) and levels are higher in randomly selected subjects compared to very healthy elderly individuals selected in accordance with the so-called SENIEUR protocol (Baggio et al., 1998). However, 'successful ageing' (ageing without co-morbidity) is still associated with low-grade inflammatory activity in vivo. Considering the fact that elevated plasma IL-6 acts as a marker of subclinical CVD (Jenny et al., 2002), we find it difficult to separate an isolated ageing effect from an effect of disease on levels of inflammatory mediators. With regard to anti-inflammatory mediators (e.g. IL-1 receptor antagonist (ra) and sTNFR), acute phase proteins (e.g. C-reactive protein (CRP) and serum amyloid A (SAA)), sIL-2R and neutrophils, there seems to be a consensus that circulating levels/numbers are increased in elderly populations (Ballou et al., 1996; Cakman et al., 1997; Caswell et al., 1993; Catania et al., 1997; Gerli et al., 2000; Rea et al., 1996). Increases in inflammatory markers are, however, only 2–4-fold and thus far from the increases observed during acute infections. Nonetheless, mortality studies demonstrate the clinical relevance of chronic low-grade inflammation among the elderly.

### *2.1. Levels of circulating cytokines and mortality in elderly populations*

Low-grade increases in the levels of circulating TNF-α, IL-6, sIL-2R, and CRP and low levels of albumin and cholesterol, which also act as inflammatory markers, are strong predictors of all-cause mortality risk in longitudinal studies of several elderly cohorts (Bruunsgaard et al., 2003a, c; Klonoff-Cohen et al., 1992; Mooradian et al., 1991; Rosenthal et al., 1997; Weijenberg et al., 1997; Weverling-Rijnsburger et al., 1997; Harris et al., 1999; Reuben et al., 2002; Volpato et al., 2001). The effects of inflammatory mediators are independent of pre-existing morbidity and of other traditional risk factors for death (smoking, blood pressure, physical exercise, total cholesterol, co-morbidity, BMI, and intake of anti-inflammatory drugs) in survival analyses (Bruunsgaard et al., 2003a,c; Harris et al., 1999; Reuben et al., 2002; Volpato et al., 2001), suggesting that inflammatory cytokines possess specific biological activities.

Only a few studies have investigated whether the different inflammatory mediators have distinct effects in elderly populations. Detectable serum levels of TNF-α, but not IL-1, were associated with mortality in a study of old nursing home patients, indicating that TNF-α has specific effects in frail, old people (Mooradian et al., 1991). In a cohort of 80-year-old people, serum levels of IL-6 at baseline were strong predictors of all-cause mortality during the following 6 years (Bruunsgaard et al., 2003c). In this population, TNF-α was only associated with mortality in men but the effect was independent of IL-6. In contrast to these data, TNF-α was a strong predictor of mortality in Danish centenarians whereas IL-6 had no similar effect although the two cytokines were strongly intercorrelated. Furthermore, CRP had an effect that disappeared when TNF-α was included in the survival analysis (Bruunsgaard et al., 2003a). IL-6 was also a stronger predictor of mortality than CRP in relatively healthy elderly Americans from the Iowa 65 + Rural Health Study (Harris et al., 1999).

These findings lead us to suggest that TNF-α and IL-6 form independent risk factors with distinctive biological effects on old populations and with different clinical importance during the process of ageing whereas CRP acts as a surrogate marker of the two cytokines in survival analyses, reflecting that the production of CRP is induced by TNF-α and/or IL-6. In accordance with this, we have previously suggested that IL-6 is a strong predictor of thromboembolic complications that are of major clinical importance in young elderly but is of less importance in centenarians who have already survived to advanced age despite considerable morbidity. TNF-α, however, is a strong prognostic marker in very old populations and acts as a marker of the frailty syndrome (Bruunsgaard et al., 2003a). This hypothesis needs of course to be confirmed in other cohort studies.

### 2.2. Cytokine polymorphisms and survival in elderly cohorts

A direct pathogenetic role of inflammatory mediators would be highly likely if longevity was shown to be associated with cytokine polymorphisms regulating cytokine production.

In an Italian study, the frequency of the GG allele of the −1082 IL-10 promoter polymorphism was increased in male centenarians as compared to younger men. This genotype has been associated with increased production of the anti-inflammatory cytokine IL-10 (Lio et al., 2002). However, this finding was not confirmed in a study of a Finnish population (Wang et al., 2001). Furthermore, the latter study found no association between human longevity and polymorphisms of IL-1 cluster and TNF-α-308G > A genes among others. Consistent with this there was no difference in the TNF-α-308G > A promoter polymorphism between Danish octogenarians, centenarians and young controls (Bruunsgaard et al., 2004a).

Most studies have yet focused on the 174G > C promoter polymorphism in the IL-6 gene but unfortunately, data are very conflicting (Table 1): in a longitudinal study of 80-year-old Danes, the C allele was an independent risk marker of all-cause mortality for non-smokers in the following 6 years (Bruunsgaard et al., 2003b). Consistent with this, the C allele was also associated with high plasma levels of IL-6 in octogenarians (Bruunsgaard et al., 2003b), in 65 + years old Americans from the Cardiovascular Health Study (Jenny et al., 2002), in patients with small abdominal aortic aneurisms (Jones et al., 2001), and in octogenarians/non-agenarians from the Belfast Elderly Longitudinal Ageing Study (Rea et al., 2003). Furthermore, the C allele was accompanied by increased levels of CRP (Humphries et al., 2001; Jenny et al., 2002; Vickers et al., 2002). However, some studies find no difference in the frequency of the IL-6-174G > C polymorphism across different age groups (Wang et al., 2001), no association between the polymorphism and plasma levels of IL-6 (Basso et al., 2002), or an association in newborns, but not in adults (Kilpinen et al., 2001). Furthermore, it has also been reported that the frequency of the GG genotype was significantly lower among 80 + year old Irish people (Rea et al., 2003) and Italian male centenarians (Bonafe et al., 2001) compared to younger age groups in cross-sectional

Table 1
The clinical effects of the IL-6-174G > C promoter polymorphism in studies of elderly populations (+65 years)

| Study | Design | Frequencies GG/GC/CC | Outcome | | |
|---|---|---|---|---|---|
| | | | Longevity/survival | Plasma IL-6 | Cardiovascular diseases (CVD) |
| Wang et al. (2001) | Cross-sectional study: (Finnish population) | | | | |
| | 250 nonagenarians | 23%/52%/25% | No difference across age groups | | |
| | 400 controls (age 18–60 yrs) | 30%/50%/20% | | | |
| Bonafe et al. (2001) | Cross-sectional study: (Italian population) | GG/C-allele | | | |
| | 93 60–80-year-old men | 58%/42% | GG decreased in male centenarians | Increased in GG (Male group) | |
| | 57 81–99-year-old men | 58%/42% | | | |
| | 68 male centenarians | 38%/62% | | | |
| | 101 60–80-year-old women | 54%/46% | No difference across women age-groups | | |
| | 126 81–99-year-old women | 52%/48% | | | |
| | 255 female centenarians | 51%/49% | | | |
| Jenny et al. (2002) | Case-control study of >65-year-olds: | | | | |
| | 1857 white Americans | 40%/44%/16% | | No significant difference | Increased in non-smoking men with GC/CC |
| | 344 African Americans | 85%/15%/0% | | | |
| Rea et al. (2003) | Cross-sectional study: (Irish population) | | | | |
| | 193 octogenarians/nonagenarians | 29%/54%/17% | GG decreased in the elderly | Increased in CC | |
| | 182 controls (age 20–45 yrs) | 38%/47%/15% | | | |
| Bruunsgaard et al. (2003b) | Longitudinal study: (Danish population) | | | | |
| | 333 octogenarians (234 non-smokers) | 28%/53%/19% | Increased mortality in non-smokers with GC/CC | Increased in non-smokers with GC/CC | Increased in CC |

studies; and the GG genotype has been associated with higher levels of IL-6 compared to C allele carriers (Bonafe et al., 2001; Fishman et al., 1998; Olivieri et al., 2002).

It is very possible that discrepancies result from the complex interaction between life style and genetic factors together with cultural and genetic differences across countries. For instance, the genotype had a stronger association with inflammation, blood pressure and CVD in Americans aged $\geq 65$ years who were non-smokers and without obesity compared to the general population (Jenny et al., 2002), and a strong interaction between genotype and smoking status was detected in survival analyses of octogenarians (Bruunsgaard et al., 2003b). Furthermore, some studies report an effect of gender (Bonafe et al., 2001) that has not been confirmed in other studies (Bruunsgaard et al., 2003b; Wang et al., 2001). It is also possible that studies with a cross-sectional design detect a cohort effect rather than an effect of an age-related frailty gene. For instance, it is somewhat contradictory that longevity was associated with a decreased frequency of the GG genotype, which was also associated with the lowest plasma levels IL-6 in the Belfast study (Rea et al., 2003), considering that high plasma levels of IL-6 consistently act as a predictor of high mortality risk in several elderly cohorts. It has also been suggested that the $-174G > C$ polymorphism exhibits different effects in older individuals compared with younger healthier people (Jenny et al., 2002). In vitro studies in HeLa cells have shown that the $-174C$ allele was associated with lower promoter activity (Fishman et al., 1998). The authors of the latter study (Fishman et al., 1998) have subsequently expressed reservations about the interpretation of this assay. Thus, they have stated that it is likely that the simple determination of reporter gene expression using only parts of the IL-6 upstream regulatory sequence, and in a cell line which may not have all the necessary cellular receptors to respond appropriately, will at best be an approximation to the in vivo functional effects of a promoter variant (Humphries et al., 2001). Furthermore, 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 (Jenny et al., 2002; Jones et al., 2001). 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 (Basso et al., 2002; Jenny et al., 2002; Jones et al., 2001). Consistent with this, the IL-6 production of peripheral blood mononuclear cells (PMNC) from C allele carriers increased with age whereas this phenomenon was not detected in the GG genotype (Olivieri et al., 2002).

It is difficult to make any clear-cut conclusions about polymorphism studies due to the conflicting data and there is a great need for more longitudinal studies. However, several studies indicate that cytokine polymorphisms are associated with mortality in elderly populations although the effect is complex and interacts with life style and sex.

## 3. Cytokine production in vitro versus in vivo in elderly humans

A wide range of different factors is likely to contribute to increased low-grade inflammatory activity in elderly populations including decreased production of sex steroids, smoking, subclinical disorders such as atherosclerosis and asymptomatic bacteriuria, and a higher relative/absolute amount of fat tissue, which has been demonstrated to produce cytokines such as TNF-α and IL-6. Furthermore, increased levels of circulating inflammatory mediators may result from a constant, low-grade activation of cytokine producing cells or a dysregulated cytokine response following stimulation.

Several studies have investigated the in vitro production of a wide range of cytokines by PMNC and in whole blood. This review will focus on TNF-α, IL-1β and IL-6 (Table 2). Conflicting results have been published on the response to LPS, which activates mainly monocytes and macrophages. Thus, in elderly humans decreased production of these cytokines has been reported (Gon et al., 1996; McLachlan et al., 1995), as well as unaltered production (Roubenoff et al., 1998; Rudd and Banerjee, 1989), and an increased production (Born et al., 1995; Riancho et al., 1994). Most studies measure the cytokine production only at one point in time, which significantly affects the conclusion. For instance, TNF-α levels were evaluated following in vitro LPS stimulation for 4, 6, 12, 24, and 48 h in whole blood (diluted 1:4) from eight healthy elderly people aged 77–81 years and eight young control persons aged 20–30 years. In this study on the kinetics of TNF production, the elderly group showed larger initial increases in the production of TNF-α, whereas there was no difference between age groups in peak levels of TNF-α. IL-6 peaks later than TNF-α and IL-1β and therefore, when studying the production of several cytokines, it is crucial to bear in mind the kinetics of cytokine production, as measuring at only one point in time is not optimal. Conflicting results are also obtained when studying cultures of PMNC as compared to whole blood (Gabriel et al., 2002). The number of monocytes is unaltered or increased in aged people whereas the lymphocyte counts decrease with age and therefore, cultures of isolated PMNC from aged people contain higher amounts of monocytes relative to lymphocytes than cultures obtained from young control subjects. This will affect the number of effector cells in assays that are based on a fixed number of cells. Moreover, whole blood is a more dynamic culture system and peak levels depend largely on the dilution and occur at an earlier point in time compared to PMNC cultures. Furthermore, gender and health status of the donors affect the results: in a study of 80-year-old Danes compared to

Table 2
In vitro studies of the stimulated production of TNF-α, IL-1β and IL-6 in elderly populations

| Study | Design | Stimulation | | | Cytokine production in ageing |
|---|---|---|---|---|---|
| | | Agent | Cell type | time | |
| Gon et al. (1996) | 10 > 80-year-olds<br>10 < 39-year-olds | LPS | Monocytes | 24 h culture | Decreased TNF-α and IL-1β |
| McLachlan et al. (1995) | 25 > 65-year-olds<br>25 young controls | LPS | Monocytes | 16 h culture | Decreased IL-1β |
| Rudd and Banerjee (1989) | 33 > 70-year-olds<br>40 elderly with infections<br>40 < 40-year-olds | LPS | Monocytes | 24 h culture | No difference in IL-1β |
| Roubenoff et al. (1998) | 742 elderly (mean 79 yr)<br>21 young (mean 39 yr) | (a) LPS and *S. epidermidis*<br>(b) PHA | PMNC | 22 h culture | (a) No difference in TNF-α and IL-1β<br>(b) No difference in IL-6 |
| Riancho et al. (1994) | 15 elderly > 55 yr<br>18 young < 55 yr | LPS | PMNC | 24 h culture | Increased IL-1β. No difference in TNF-α |
| Born et al. (1995) | 16 elderly (mean 80 yr)<br>16 young (mean 25 yr) | LPS | Undiluted whole blood | 48 h culture | Increased TNF-α and IL-1β |
| Gabriel et al. (2002) | 16 elderly (mean 73 yr)<br>16 young (mean 28 yr) | LPS | (a) Whole blood diluted 1:9<br>(b) PMNC | (a) 24 and 72 h culture<br>(b) 24 h culture | (a) Increased IL-1β and IL-6 after 24 h. No difference in TNF-α<br>(b) Decreased IL-1β and IL-6 |
| Bruunsgaard et al. (1999b) | 168 80-year-olds<br>91 young (mean 25 yr) | LPS | Whole blood diluted 1:4 | 24 h culture | Decreased production of IL-1β and TNF-α compared to young men but not compared to young women. No difference in IL-6 |
| Fagiolo et al. (1993) | 13 elderly (mean 81 yr)<br>13 young (mean 27 yr) | PMA + PHA | PMNC | 24, 48 and 72 h culture | Increased production of TNF-α, IL-1β and IL-6 |
| Saurwein-Teissl et al. (2000) | 31 > 65-year-olds<br>29 < 35-year-olds | Influenza virus | PMNC | | Increased TNF-α production |
| O'Mahony et al. (1998) | 9 > 62-year-olds (mean 73 yr)<br>10 young (mean 29 yr) | PMA | PMNC | 24, 48 and 72 h culture | Increased percentage of TNF + CD3 + cells and IL-6 + CD3 + cells. No significant difference in TNF, IL-1β and IL-6 producing monocytes. No difference in TNF-α, IL-1β or IL-6 or culture supernatants (72 h) |
| McNerlan et al. (2002) | 13 elderly (mean 92 yr)<br>6 young (mean 24 yr) | PMA + ionomycin | Whole blood diluted 1:1 | 4 h culture | Increased percentage of TNF + CD3 + cells |
| Sandmand et al. (2003) | 25 centenarians<br>14 80-year-olds<br>28 young (mean 23 yr) | PMA + ionomycin | PMNC | 4 h culture | Increased percentage of TNF + CD3 + cells |
| Beharka et al. (2001) | 26 elderly (65–85 yr)<br>21 young (20–30 yr) | (a) PHA<br>(b) ConA | PMNC | 48 h culture | (a) No difference in IL-6<br>(b) Decreased IL-6 production |

young controls, elderly men and women had decreased production of TNF-α and IL-1β compared to young men but not compared to young women (Bruunsgaard et al., 1999b). Moreover, IL-1β production was significantly lower in octogenarians with chronic diseases. With regard to culture systems including other types of stimulation such as PHA, PMA, and influenza antigen, which all activate T lymphocytes, the findings seem to be more consistent than regarding findings for monocytes and macrophages. Thus, most studies find an increased production of inflammatory cytokines both on a single cell level as well as in culture supernatants in cultures from aged subjects (Fagiolo et al., 1993; McNerlan et al., 2002; O'Mahony et al., 1998; Sandmand et al., 2003; Saurwein-Teissl et al., 2000) although the opposite has also been reported (Beharka et al., 2001). It is likely that increased production of inflammatory cytokines by T lymphocytes from elderly people is related to an altered phenotype/chronic activation and to a shift in the balance between type 1 and type 2 cytokines in ageing.

Only a few studies have evaluated the capacity of cytokine production in vivo. In a large study of 930 patients with septic shock, levels of TNF-α were significantly higher in the oldest patients at the time of enrolment (Marik and Zaloga, 2001). In contrast, levels of cytokines in serum were lower in 15 old patients compared with 22 younger patients with pneumonia (Gon et al., 1996). This discrepancy probably reflects that many clinical factors vary on admission to hospital. In a human sepsis model an intravenous bolus of *Escherichia coli* LPS was given to young and elderly healthy volunteers in order to test the hypothesis that ageing was associated with an altered acute phase response (Krabbe et al., 2001). In accordance with in vitro data, this study demonstrated an age-related pre-activation of monocytes reflected by a more pronounced initial production of TNF-α by the elderly subjects. No difference in peak cytokine levels or body temperature was found between age groups. Furthermore, the elderly participants had a prolonged inflammatory response as indicated by a slower normalisation of sTNFRs, CRP, and body temperature, thus reflecting an age-related dysregulated cytokine response (Fig. 1). Consistent with this finding, levels of TNF-α and sTNFR-I were still increased in elderly patients with pneumococcal infections after sufficient antibiotic treatment for 1 week, and moreover declines in sTNFR and in the TNF-α/IL-10 ratio from day 0 to day 7 were correlated with age (Bruunsgaard et al., 1999c). Based on these data, we suggest that that an age-related defect termination of inflammatory activity in vivo contributes to a pre-activation of cytokine producing cells. However, there is a great need for more in vivo studies in order to confirm this hypothesis.

Stimulated cultures of PMNC reflect the cytokine-producing capacity of peripheral blood whereas unstimulated short-term cultures may reflect in vivo priming/activation. In a study of unstimulated cultures of PMNC from 711 elderly participants and 21 healthy young volunteers, increased production of IL-6 and IL-1ra was reported whereas no age-related difference was detected with regard to the production of TNF-α and IL-1β (Roubenoff et al., 1998). Although IL-6 is often classified as a proinflammatory cytokine, it has also very important anti-inflammatory properties (Starkie et al., 2003) including inhibition of the transcription of the TNF-α gene, stimulation of the production of anti-inflammatory cytokines, and the shedding of TNF-receptors that bind TNF-α with high affinity. One possible interpretation of the data by Roubenoff et al. (1998), is thus that increased production of IL-6 and mediators downstream in the inflammatory cascade by unstimulated PMNC from elderly people represents a systemic anti-inflammatory response to local proinflammatory activity associated with pathological processes.

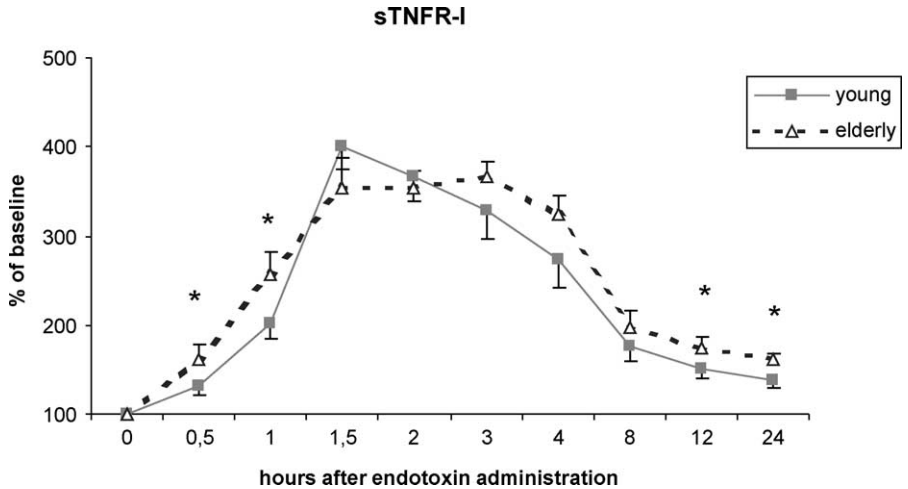


Fig. 1. Plasma levels of sTNFR-I following in vivo administration of *E. coli* endotoxin (2 ng/kg BW) to healthy humans (young were aged 20–29, $n = 8$; elderly were aged 60–68, $n = 8$), means and SE are shown, asterisk denotes significant difference between age groups (adapted from Krabbe et al. (2001)).

In continuation of this hypothesis it appears that mainly cells outside the blood such as adipocytes, endothelial cells and macrophage-derived cells in peripheral tissue and the central nervous system (CNS) are responsible for the increased production of early inflammatory mediators including TNF-α. It is thus likely that levels of circulating IL-6 represent a better marker of the sum of ongoing inflammation in the body than systemic levels of TNF-α in younger age groups and healthy, old populations because a local production of TNF-α may not escape into the blood stream. Then, along with the progression of pathological processes, increasing levels of TNF-α also appear in the circulation and this gradually becomes a stronger risk marker. This hypothesis needs to be confirmed in other cohort studies but it provides an explanation for the finding that IL-6 is a strong predictor of mortality risk in cohorts of healthy elderly whereas TNF-α predicts mortality in very old and frail populations as already discussed.

## 4. Effects of inflammatory mediators in age-associated chronic diseases

The strong association between low-grade elevations in levels of circulating inflammatory mediators and high mortality risk independently of pre-existing morbidity, suggests that cytokines trigger/exaggerate pathological processes or act as very sensitive markers of subclinical disorders in elderly populations.

The longitudinal study of Danish centenarians has demonstrated that the extreme lifespan is accompanied by a high prevalence of CVD (>70%) and dementia (>50%) (Andersen-Ranberg et al., 2001). CVD is directly linked to atherosclerosis, which represents an inflammatory disease (Ross, 1999). Inflammation also occurs in the pathologically vulnerable regions of Alzheimer's disease (AD), which together with vascular dementia (VAD) represents the major categories of age-related dementia (Akiyama et al., 2000). Markers of atherosclerosis are indeed associated with both subtypes of dementia (Hofman et al., 1997), indicating that atherosclerosis results in accelerated brain ageing. Moreover, CVDs have been associated with the syndrome of frailty in which age-related sarcopenia is a central part (Fried et al., 2001) and recent research suggests that in addition to loss of anabolic signals to the ageing muscle there is also an increased catabolic signal driven by inflammation (Roubenoff, 2003).

Age-related multi-factorial conditions such as atherosclerosis, sarcopenia, and cognitive decline seem, accordingly, to share some aetiological factors in which inflammatory mechanisms provide a possible common basis. On the other hand, it is still controversial whether inflammatory mediators have primarily causal or counter-regulatory functions and how systemic low-grade inflammation is related to local pathology in peripheral tissues and in the CNS. These aspects will be discussed in the following sections.

### 4.1. Atherosclerosis

Atherosclerotic plaques contain smooth muscle cells, activated T lymphocytes, and monocyte-derived macrophages. It is commonly accepted that this pathology results from an inflammatory response to vessel wall injury and endothelial dysfunction caused by various factors such as oxidised low-density lipoprotein (LDL), hypertension, diabetes mellitus, infectious agents, free radicals induced by smoking, and combinations of these or other factors. Activated endothelial cells are known to be targets as well as sources of inflammatory cytokines and chemokines, which induce upregulation of adhesion molecules and attract leucocytes, promoting a migration across the endothelium.

Several epidemiological studies have linked systemic low-grade inflammation in elderly populations to the prevalence and prognosis of CVD. IL-1β serum levels were associated with congestive heart failure, angina and a history of dyslipidaemia in 1292 participants from the InCHIANTI project (Di Iorio et al., 2003). Plasma levels of TNF-α have been correlated with dyslipidaemia and a high prevalence of CVD in 80-year-old people (Bruunsgaard et al., 2000) and a low ankle-brachial blood pressure index (marker of CVD) in centenarians (Bruunsgaard et al., 1999a). Consistent with this, TNF-α was also correlated with the blood pressure, insulin resistance, levels of soluble cellular adhesion molecules, and common carotid intima-media thickness in healthy middle-aged men (Skoog et al., 2002). Levels of circulating IL-6 acted as a marker of subclinical CVD in a case-control study of elderly people (Jenny et al., 2002) and was a predictor of mortality related to CVD in relatively healthy people aged 65 + years (Harris et al., 1999) as well as of mortality risk in disabled older women with a history of CVD. Accordingly, IL-6 was a predictor of myocardial infarcts in healthy middle-aged men (Ridker et al., 2000). As mentioned previously, IL-1β, TNF-α and IL-6 induce the production of CRP, which has turned out to be a strong and consistent predictor of coronary events in a very large number of studies (reviewed in Pepys and Hirschfield (2003)).

It has been widely assumed that systemic low-grade inflammation in CVD arises from inflammation within atheromatous lesions and reflects their extent and/or severity but a shift in this paradigm has occurred towards understanding the pathology of atherosclerosis as a consequence of systemic low-grade inflammation. Thus, chronic systemic non-vascular inflammation is known to be proatherogenic in general, and acute systemic inflammatory episodes are strongly associated with atherothrombotic events. A direct pathogenetic role of inflammatory mediators in CVD has been made more likely by the finding that the IL-6-174G > C promoter polymorphism is

associated with levels of circulating IL-6 and with CVD (Bruunsgaard et al., 2003b; Humphries et al., 2001; Jenny et al., 2002; Jones et al., 2001). Moreover, clinical intervention studies suggest that aspirin and statins reduce the risk of future events of CVD through their anti-inflammatory effects, although there still persists a need for randomised trials in this area (reviewed in Meir et al. (2003)). Finally, experimental studies show that systemic low-grade inflammation has the potential to induce several risk factors in CVD and to have a central role in the development of the metabolic syndrome: TNF-α induces insulin resistance and endothelial dysfunction including impairment in the endothelium dependent relaxation and upregulation of cellular adhesion molecules. Furthermore, TNF-α as well as IL-6 affect the coagulation system and metabolism of lipids, causing a procoagulant state and dyslipidaemia (reviewed in Bruunsgaard and Pedersen (2003)). CRP stimulates tissue factor production by monocytes, binds to oxidised LDL and to partly degraded LDL and then activates complement (Pepys and Hirschfield, 2003).

Considering that low-grade inflammation is also associated with parameters such as obesity, smoking, and physical inactivity (Bruunsgaard et al., 2003c,d; Pedersen et al., 2003) we suggest that in elderly populations, inflammatory mediators constitute a link between life style factors, infections and physiological changes in the process of ageing on the one hand and risk factors for CVD on the other.

### 4.2. Sarcopenia and the syndrome of frailty

Frailty has been defined as 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 (Fried et al., 2001).

It has been suggested that this syndrome reflects a metabolic imbalance caused by overproduction of catabolic cytokines and by the diminished availability or action of anabolic hormones, resulting from ageing itself and the presence of associated chronic conditions (Hamerman, 1999). In accordance with this hypothesis, plasma levels of TNF-α were strongly associated with impendent death independently of dementia and CVD in centenarians, supporting the hypothesis that TNF-α has specific biological effects and is a marker of the frailty syndrome in the oldest old (Bruunsgaard et al., 2003a).

Sarcopenia is obviously a central part of the frailty syndrome. Furthermore, systemic low-grade inflammation has been associated with decreased muscle mass (Ferrucci et al., 2002; Pedersen et al., 2003; Visser et al., 2002) as well as the development of functional disability in elderly populations (Ferrucci et al., 1999). Associations between inflammatory mediators, sarcopenia, and functional disability may simply reflect the fact that levels of circulating cytokines can act as markers of underlying medical disorders. On the other hand, especially TNF-α has effects that may contribute directly to sarcopenia, e.g. studies of culture muscle cells indicate that TNF-α disrupts the differentiation process and can promote catabolism in mature muscle cells and that TNF-α induces apoptosis through the triggering of death domain receptors that are up-regulated in aged muscle cells (Roubenoff, 2003). TNF-α also causes increased basal energy expenditure, anorexia, and loss of muscle and bone mass in vivo and has been associated with wasting/cachexia in chronic inflammatory disorders such as reumatoid artritis, HIV and cancer. Consistent with this, muscle protein synthesis was inversely related to local levels of TNF-α protein in skeletal muscles in a study of frail, very elderly humans (Greiwe et al., 2001).

The role of IL-6 in sarcopenia is controversial. Although epidemiological studies have reported that IL-6 is strongly associated with functional disability and loss of muscle mass, experimental studies have not been able to link IL-6 to sarcopenia. It is very possible that IL-6 acts as surrogate marker of TNF-α in some epidemiological studies because the production of the two cytokines is closely related: e.g. TNF-α stimulates the production of IL-6 and, in return, IL-6 inhibits the transcription of TNF-α, stimulates the production of anti-inflammatory cytokines, and the shedding of TNFR that bind TNF-α with high affinity.

Age-related sarcopenia is partly reversed by exercise. Muscle contractions induce production and release of IL-6 into the blood stream and it has been suggested that muscle-derived IL-6 contributes to the beneficial metabolic effects of exercise and this may be partly mediated by a decreased production of TNF-α (Starkie et al., 2003). Consistent with this, resistance training of frail very elderly people resulted in a decreased level of TNF-α in skeletal muscles (Greiwe et al., 2001). In a recent study of nursing home residents aged 85–96 years, systemic low-grade activation of the TNF system at baseline was inversely correlated to muscle strength after resistance training for 12 weeks, demonstrating that TNF-α could also be a limiting factor for training-induced improvement in muscle strength in very old people (Bruunsgaard et al., 2004b).

### 4.3. Cognitive impairment

Inflammatory mechanisms have been linked to age-associated cognitive decline, including both AD and VAD (Hofman et al., 1997) and to symptoms of depression in aged subjects (Penninx et al., 2003). This might seem somewhat paradoxical, as several cytokines including IL-1, IL-6 and TNF-α have been shown to exert neuroregulatory roles and to be of importance for intact neurological function in animals (Avital et al., 2003). On the other hand, epidemiological studies show an increasing body of evidence on the deleterious association between chronic peripheral cytokine elevations found in aged subjects and cognitive functions.

Thus, as a part of the Health ABC study, in a large cohort of 3031 well-functioning white and black Americans aged 70–79, high baseline plasma levels of IL-6 and CRP were associated with poorer cognitive performance at baseline and with a greater risk of cognitive decline over 2 years of follow-up (Yaffe et al., 2003). In this age group, no association was found between plasma TNF-α levels and cognitive performance. In a study of Danish centenarians, plasma TNF-α was shown to be associated with dementia, also when centenarians with severe medical disorders and with a history of stroke or transient cerebral ischemia were excluded, indicating a specific role for TNF-α in AD in centenarians. Increased blood levels of IL-1β have also been reported in patients with AD (Licastro et al., 2000a) and late onset AD (Alvarez et al., 1996). A recent meta-analysis on the effects of the use of non-steroidal anti-inflammatory drugs (NSAIDs) has yielded results pointing towards a protective role of long-term use of NSAID against AD (Etminan et al., 2003), which underlines the role of inflammation in the development of cognitive decline.

The association between peripheral cytokines and VAD has not been studied directly, but cytokines exert both prothrombotic and atherogenic effects, indicating a possible role of inflammatory mediators in the pathogenesis of VAD. The association between peripheral cytokine levels and atherosclerosis in general has already been discussed.

There is an ongoing debate as to whether peripheral cytokine elevations found in relation to cognitive impairment are a causative agent to cognitive decay, as cytokines are known to be able to cross the blood–brain barrier and to interact with the CNS via receptors in afferent neurons or whether they represent spillover from inflammatory processes in the CNS. The possibility that both hypotheses hold true is also present.

Studies on autopsy specimens report a marked over-expression of IL-1 (Griffin et al., 1989), and IL-6 (Bauer et al., 1991) in the brains of patients with AD. Furthermore, Tarkowski et al. have found levels of TNF-α being 25-fold higher in the cerebrospinal fluid (CSF) of patients with AD compared to controls pointing towards the intracerebral production of TNF. Patients with VAD also displayed significantly higher levels of TNF-α in the CSF compared to control individuals but neither of these patient groups displayed significant elevations in plasma levels of TNF-α (Tarkowski et al., 2000, 2003). These findings point to the CNS as the major site of production of TNF-α in overt dementia. Another study has shown that even in healthy young subjects there can be an efflux of IL-6 from the CNS (Nybo et al., 2002). Thus, it seems to be clear that the CNS not only is able to produce cytokines, but it can also contribute to the pool of peripheral cytokines. In this context, it is worth mentioning that the question of whether central cytokine elevations found among patients suffering from AD (and VAD) are actually causing damage or simply represent the presence of reparatory processes in order to counteract other, more primary pathologic processes is unanswered.

On the other hand, although not many studies have assessed the direct influence of peripheral cytokines on the human brain with regard to cognitive functions, there is an increasing body of studies showing that peripherally administered (or produced) cytokines do affect human brain functions. Thus, peripherally produced cytokines are responsible for sickness behaviour in animals. In humans, several studies have shown that peripheral cytokines affect sleep functions (for a review, see Pollmacher et al. 2002). As for cognitive parameters, a transient increase in proinflammatory cytokines following a subclinical dose of endotoxin resulted in negative effects on memory functions and depression score in young subjects (Reichenberg et al., 2001).

Quite a number of studies have looked into the association of cytokine polymorphisms with the development of dementia, particularly AD. The findings are contradictory even when confined to Caucasian populations.

For IL-1, most consistency has been found regarding the IL-1A and IL-B T < C polymorphism (Grimaldi et al., 2000; Licastro et al., 2000b; Sciacca et al., 2003), pointing towards a detrimental association between the T alleles of both polymorphisms and the development of AD. The IL-1A 2 allele has been reported to be associated with both an increased risk for AD (Du et al., 2000; Nicoll et al., 2000), and with no effect (Green et al., 2002). A recent meta-analysis (Combarros et al., 2003) concludes that there is an association of early onset AD only (age of onset <65) with the IL-1A 2 allele. On the other hand, a follow-up study on 130 patients (both early and late onset) found that homozygotes for the IL-1A 1 allele declined significantly more rapidly on MMSE than others.

For IL-6, most attention has been centred on the −174G > C promoter polymorphism. Again the results are contradictory, showing both decreased incidence of the C allele in AD (Faltraco et al., 2003; Pola et al., 2002), no difference between AD patients and controls (Bhojak et al., 2000) and an increased incidence of the C allele in AD (Licastro et al., 2003). This apparent incongruity seems to be based on a different distribution of the C allele in the control populations, whereas the distribution in the AD populations is quite similar from study to study (Table 3).

For TNF-α, two different sites have been in focus. McCusker et al. (2001) reported that possession of the TNF-α 850 T-allele significantly increases the risk of VAD in an Irish population. Furthermore, they found an interaction between ApoE and TNF-α polymorphisms in AD; a finding that has not been reproduced in Spanish or Italian populations (Infante et al., 2002; Terreni et al., 2003). Recently, an association was described between the TNF-308G > A polymorphism and senile dementia in centenarians, the GA genotype being associated with a decreased prevalence of senile dementia compared to the GG genotype (Bruunsgaard et al., 2004a). In contrast, in a cross-sectional study on

Table 3
Distribution of the IL-6-174G > C promoter polymorphism in patients with AD and non-demented controls

| Genotype | AD | | | | Controls | | | |
|---|---|---|---|---|---|---|---|---|
| | Pola et al. (2002) ($n = 124$) | Faltraco et al. (2003) ($n = 101$) | Licastro et al. (2003) ($n = 332$) | Bhojak et al. (2000) ($n = 531$) | Pola et al. (2002) ($n = 134$) | Faltraco et al. (2003) ($n = 133$) | Licastro et al. (2003) ($n = 393$) | Bhojak et al. (2000) ($n = 248$) |
| GG (%) | 45.2 | 44 | 41.3 | 38.4 | 21.6 | 32 | 53.2 | 37.4 |
| GC (%) | 41.1 | 46 | 48.5 | 47.6 | 43.3 | 53 | 42 | 46.0 |
| CC (%) | 13.7 | 10 | 10.2 | 14 | 35.1 | 15 | 4.8 | 16.6 |

patients with late onset AD (aged 60–97) and age-matched healthy control persons, an association between the TNF-α 308G < A polymorphism and the onset-age of AD was found, in that carriers of the A allele had a mean age of onset 3 years younger than non-carriers (Alvarez et al., 2002). This difference in results can well reflect differences in the age of the participants and in the study design.

It is evident that there exist tight links between age-related chronic low-grade inflammation and cognitive parameters; not only in disease states like AD and VAD but in general. Further research is needed in order to clearly establish the nature of these links. At the moment, there is no direct experimental data available on whether small chronic increases in levels of cytokines (as seen with advancing age) affect human brain function. It is important to bear in mind that acute and chronic cytokine increases may exert different effects on cognition, but also that there might be a dose-dependent relationship between peripheral cytokine levels and cognitive function. Such a relationship might turn out not to be linear, as small amounts of cytokines seem to be necessary for normal neurological function.

## 5. Concluding remarks

Ageing is associated with low-grade elevations in levels of circulating inflammatory mediators, which act as predictors of mortality independent of pre-existing morbidity. Moreover, there is strong evidence that the development of age-related multi-factorial conditions such as atherosclerosis, cognitive decline and the syndrome of frailty are associated to these elevations. The nature of these associations remains controversial, as it is unclear whether inflammatory mediators have primarily causal or whether they have counter-regulatory functions. Furthermore, there is an increasing body of evidence showing that low-grade inflammation is also associated with parameters such as obesity, smoking, and physical inactivity. It is thus possible that in elderly populations, inflammatory mediators constitute a link between life style factors, infections and physiological changes in the process of ageing on the one hand and risk factors for age-associated diseases on the other.

Quite a number of in vitro studies have been performed with the purpose of shedding some light on the age-related changes in the production of inflammatory mediators at a cellular level. Due to differences in their design, these studies do not yield a clear picture, although they seem to point toward a pre-activation of circulating cytokine producing cells, which is underscored by in vivo findings. Current knowledge of how systemic low-grade inflammation is related to local pathology in peripheral tissues and in the CNS is limited. None the less there is currently little doubt that atherosclerosis is an inflammatory disease or that inflammatory mediators are present and play an important role in the development of dementia, including AD or that the TNF system is closely associated to sarcopenia and the syndrome of frailty. Some studies further point to the different inflammatory mediators in circulation having distinct biological effects on different age groups. We believe it would be worthwhile to explore the possible separate biological effects of different inflammatory mediators in young and elderly populations.

Much interest has recently been given to the study of a possible association between polymorphisms in genes encoding inflammatory mediators, the levels of these mediators in the blood stream, and longevity. In this emerging research area, several polymorphisms have been studied and so far results are inconsistent. It is very possible that discrepancies result from the complex interaction between life style and genetic factors together with cultural and genetic differences across countries and gender-based differences. Furthermore, it is possible that a certain genetic configuration has different effects on aged (with low-grade inflammation) vs. young individuals (without low-grade inflammation). It is therefore of utmost importance that all these factors are taken into account in future studies.

Finally, there is a need to test different intervention trials in order to evaluate if systemic low-grade inflammation represents a reversible state. Such studies will constitute an important contribution to the development of new strategies in preventing and controlling chronic diseases in the growing number of old humans.

## References


Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., Finch, C.E., Frautschy, S., Griffin, W.S., Hampel, H., Hull, M., Landreth, G., Lue, L., Mrak, R., Mackenzie, I.R., McGeer, P.L., O'Banion, M.K., Pachter, J., Pasinetti, G., Plata-Salaman, C., Rogers, J., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F.L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., Wyss-Coray, T., 2000. Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383–421.

Alvarez, X.A., Franco, A., Fernandez-Novoa, L., Cacabelos, R., 1996. Blood levels of histamine, IL-1 beta, and TNF-alpha in patients with mild to moderate Alzheimer disease. Mol. Chem. Neuropathol. 29, 237–252.

Alvarez, V., Mata, I.F., Gonzalez, P., Lahoz, C.H., Martinez, C., Pena, J., Guisasola, L.M., Coto, E., 2002. Association between the TNFalpha-308 A/G polymorphism and the onset-age of Alzheimer disease. Am. J. Med. Genet. 114, 574–577.

Andersen-Ranberg, K., Schroll, M., Jeune, B., 2001. Healthy centenarians do not exist, but autonomous centenarians do: a population-based study of morbidity among Danish centenarians. J. Am. Geriatr. Soc. 49, 900–908.

Avital, A., Goshen, I., Kamsler, A., Segal, M., Iverfeldt, K., Richter-Levin, G., Yirmiya, R., 2003. Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity. Hippocampus 13, 826–834.

Baggio, G., Donazzan, S., Monti, D., Mari, D., Martini, S., Gabelli, C., DallaVestra, M., Previato, L., Guido, M., Pigozzo, S., Cortella, I., Crepaldi, G., Franceschi, C., 1998. Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors. FASEB J. 12, 433–437.

Ballou, S.P., Lozanski, F.B., Hodder, S., Rzewnicki, D.L., Mion, L.C., Sipe, J.D., Ford, A.B., Kushner, I., 1996. Quantitative and qualitative alterations of acute-phase proteins in healthy elderly persons. Age Ageing 25, 224–230.

Basso, F., Lowe, G.D., Rumley, A., McMahon, A.D., Humphries, S.E., 2002. Interleukin-6-174G > C polymorphism and risk of coronary heart disease in West of Scotland coronary prevention study (WOSCOPS). Arterioscler. Thromb. Vasc. Biol. 22, 599–604.

Bauer, J., Strauss, S., Schreiter-Gasser, U., Ganter, U., Schlegel, P., Witt, I., Yolk, B., Berger, M., 1991. Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. FEBS Lett. 285, 111–114.

Beharka, A.A., Meydani, M., Wu, D., Leka, L.S., Meydani, A., Meydani, S.N., 2001. Interleukin-6 production does not increase with age. J. Gerontol. A Biol. Sci. Med. Sci. 56, B81–B88.

Bhojak, T.J., DeKosky, S.T., Ganguli, M., Kamboh, M.I., 2000. Genetic polymorphisms in the cathespin D and interleukin-6 genes and the risk of Alzheimer's disease. Neurosci. Lett. 288, 21–24.

Bonafe, M., Olivieri, F., Cavallone, L., Giovagnetti, S., Mayegiani, F., Cardelli, M., Pieri, C., Marra, M., Antonicelli, R., Lisa, R., Rizzo, M.R., Paolisso, G., Monti, D., Franceschi, C., 2001. A gender-dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. Eur. J. Immunol. 31, 2357–2361.

Born, J., Uthgenannt, D., Dodt, C., Nunninghoff, D., Ringvolt, E., Wagner, T., Fehm, H.L., 1995. Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep. Mech. Ageing Dev. 84, 113–126.

Bruunsgaard, H., Pedersen, B.K., 2003. Age-related inflammatory cytokines and disease. Immunol. Allergy Clin. North Am. 23, 15–39.

Bruunsgaard, H., Andersen-Ranberg, K., Jeune, B., Pedersen, A.N., Skinhoj, P., Pedersen, B.K., 1999a. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. J. Gerontol. A Biol. Sci. Med. Sci. 54, M357–M364.

Bruunsgaard, H., Pedersen, A.N., Schroll, M., Skinhoj, P., Pedersen, B.K., 1999b. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. Clin. Exp. Immunol. 118, 235–241.

Bruunsgaard, H., Skinhoj, P., Qvist, J., Pedersen, B.K., 1999c. Elderly humans show prolonged in vivo inflammatory activity during pneumococcal infections. J. Infect. Dis. 180, 551–554.

Bruunsgaard, H., Skinhoj, P., Pedersen, A.N., Schroll, M., Pedersen, B.K., 2000. Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. Clin. Exp. Immunol. 121, 255–260.

Bruunsgaard, H., Andersen-Ranberg, K., Hjelmborg, J.B., Pedersen, B.K., Jeune, B., 2003a. Elevated levels of tumor necrosis factor alpha and mortality in centenarians. Am. J. Med. 115, 278–283.

Bruunsgaard, H., Christiansen, L., Pedersen, A.N., Schroll, M., Jørgensen, T., Pedersen, B.K., 2003b. The IL6-174G > C polymorphism is associated with cardiovascular diseases and mortality in 80-year-old humans. Exp. Gerontol. 39, 255–261.

Bruunsgaard, H., Ladelund, S., Pedersen, A.N., Schroll, M., Jørgensen, T., Pedersen, B.K., 2003c. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. Clin. Exp. Immunol. 132, 24–31.

Bruunsgaard, H., Poulsen, H.E., Pedersen, B.K., Nyyssonen, K., Kaikkonen, J., Salonen, J.T., 2003d. Circulating levels of TNF-alpha, IL-6, and CRP following antioxidant supplementation in the Atherosclerosis Prevention Study (ASAP). J. Nutr., 1170–1173.

Bruunsgaard, H., Benfield, T., Andersen-Ranberg, K., 2004a. The TNF-308G > A polymorphism is associated with dementia in the oldest old. Neurobiol. Aging.

Bruunsgaard, H., Bjerregaard, E., Schroll, M., Pedersen, B.K., 2004b. Muscle strength following resistance training is inversely correlated with baseline levels of soluble tumor necrosis Factor receptors in the oldest old. J. Am. Geriatr. Soc. 52, 237–241.

Cakman, I., Kirchner, H., Rink, L., 1997. Zinc supplementation reconstitutes the production of interferon-alpha by leukocytes from elderly persons. J. Interferon Cytokine Res. 17, 469–472.

Caswell, M., Pike, L.A., Bull, B.S., Stuart, J., 1993. Effect of patient age on tests of the acute-phase response. Arch. Pathol. Lab. Med. 117, 906–910.

Catania, A., Airaghi, L., Motta, P., Manfredi, M.G., Annoni, G., Pettenati, C., Brambilla, F., Lipton, J.M., 1997. Cytokine antagonists in aged subjects and their relation with cellular immunity. J. Gerontol. A Biol. Sci. Med. Sci. 52, B93–B97.

Combarros, O., Llorca, J., Sanchez-Guerra, M., Infante, J., Berciano, J., 2003. Age-dependent association between interleukin-1A (−889) genetic polymorphism and sporadic Alzheimer's disease. A meta-analysis. J. Neurol. 250, 987–989.

Di Iorio, A., Ferrucci, L., Sparvieri, E., Cherubini, A., Volpato, S., Corsi, A., Bonafe, M., Franceschi, C., Abate, G., Paganelli, R., 2003. Serum IL-1beta levels in health and disease: a population-based study. The InCHIANTI study. Cytokine 22, 198–205.

Du, Y., Dodel, R.C., Eastwood, B.J., Bales, K.R., Gao, F., Lohmuller, F., Muller, U., Kurz, A., Zimmer, R., Evans, R.M., Hake, A., Gasser, T., Oertel, W.H., Griffin, W.S., Paul, S.M., Farlow, M.R., 2000. Association of an interleukin 1 alpha polymorphism with Alzheimer's disease. Neurology 55, 480–483.

Ershler, W.B., 1993. Interleukin-6: a cytokine for gerontologists. J. Am. Geriatr. Soc. 41, 176–181.

Ershler, W.B., Sun, W.H., Binkley, N., Gravenstein, S., Volk, M.J., Kamoske, G., Klopp, R.G., Roecker, E.B., Daynes, R.A., Weindruch, R., 1993. Interleukin-6 and aging: blood levels and mononuclear cell production increase with advancing age and in vitro production is modifiable by dietary restriction. Lymphokine Cytokine Res. 12, 225–230.

Etminan, M., Gill, S., Samii, A., 2003. Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. BMJ 19 (327), 128–132.

Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C., Paganelli, R., 1993. Increased cytokine production in mononuclear cells of healthy elderly people. Eur. J. Immunol. 23, 2375–2378.

Faltraco, F., Burger, K., Zill, P., Teipel, S.J., Moller, H.J., Hampel, H., Bondy, B., Ackenheil, M., 2003. Interleukin-6-174G/C promoter gene polymorphism C allele reduces Alzheimer's disease risk. J. Am. Geriatr. Soc. 51, 578–579.

Ferrucci, L., Harris, T.B., Guralnik, J.M., Tracy, R.P., Corti, M.C., Cohen, H.J., Penninx, B., Pahor, M., Wallace, R., Havlik, R.J., 1999. Serum IL-6 level and the development of disability in older persons. J. Am. Geriatr. Soc. 47, 639–646.

Ferrucci, L., Penninx, B.W., Volpato, S., Harris, T.B., Bandeen-Roche, K., Balfour, J., Leveille, S.G., Fried, L.P., Md, J.M., 2002. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J. Am. Geriatr. Soc. 50, 1947–1954.

Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J.S., Humphries, S., Woo, P., 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J. Clin. Invest. 102, 1369–1376.

Fried, L.P., Tangen, C.M., Walston, J., Newman, A.B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W.J., Burke, G., McBurnie, M.A., 2001. Frailty in older adults: evidence for a phenotype. J. Gerontol. A Biol. Sci. Med. Sci. 56, M146–M156.

Gabriel, P., Cakman, I., Rink, L., 2002. Overproduction of monokines by leukocytes after stimulation with lipopolysaccharide in the elderly. Exp. Gerontol. 37, 235–247.

Gerli, R., Monti, D., Bistoni, O., Mazzone, A.M., Peri, G., Cossarizza, A., Di Gioacchino, M., Cesarotti, M.E., Doni, A., Mantovani, A., Franceschi, C., Paganelli, R., 2000. Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians. Mech. Ageing Dev. 20 (121), 37–46.

Gon, Y., Hashimoto, S., Hayashi, S., Koura, T., Matsumoto, K., Horie, T., 1996. Lower serum concentrations of cytokines in elderly patients with pneumonia and the impaired production of cytokines by peripheral blood monocytes in the elderly. Clin. Exp. Immunol. 106, 120–126.

Green, E.K., Harris, J.M., Lemmon, H., Lambert, J.C., Chartier-Harlin, M.C., St Clair, D., Mann, D.M., Iwatsubo, T., Lendon, C.L., 2002. Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? Neurology 58, 1566–1568.

Greiwe, J.S., Cheng, B., Rubin, D.C., Yarasheski, K.E., Semenkovich, C.F., 2001. Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. FASEB J. 15, 475–482.

Griffin, W.S., Stanley, L.C., Ling, C., White, L., MacLeod, V., Perrot, L.J., White, C.L. III, Araoz, C., 1989. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc. Natl Acad. Sci. USA 86, 7611–7615.

Grimaldi, L.M., Casadei, V.M., Ferri, C., Veglia, F., Licastro, F., Annoni, G., Biunno, I., De Bellis, G., Sorbi, S., Mariani, C., Canal, N., Griffin, W.S., Franceschi, M., 2000. Association of early-onset Alzheimer's disease with an interleukin-1 alpha gene polymorphism. Ann. Neurol. 47, 361–365.

Hager, K., Machein, U., Krieger, S., Platt, D., Seefried, G., Bauer, J., 1994. Interleukin-6 and selected plasma proteins in healthy persons of different ages. Neurobiol. Aging 15, 771–772.

Hamerman, D., 1999. Toward an understanding of frailty. Ann. Intern. Med. 130, 945–950.

Harris, T.B., Ferrucci, L., Tracy, R.P., Corti, M.C., Wacholder, S., Ettinger, W.H. Jr., Heimovitz, H., Cohen, H.J., Wallace, R., 1999. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am. J. Med. 106, 506–512.

Hofman, A., Ott, A., Breteler, M.M., Bots, M.L., Slooter, A.J., van Harskamp, F., van Duijn, C.N., Van Broeckhoven, C., Grobbee, D.E., 1997. Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam study. Lancet 349, 151–154.

Humphries, S.E., Luong, L.A., Ogg, M.S., Hawe, E., Miller, G.J., 2001. The interleukin-6-174G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. Eur. Heart J. 22, 2243–2252.

Infante, J., Llorca, J., Berciano, J., Combarros, O., 2002. No synergistic effect between −850 tumor necrosis factor-alpha promoter polymorphism and apolipoprotein E epsilon 4 allele in Alzheimer's disease. Neurosci. Lett. 328, 71–73.

Jenny, N.S., Tracy, R.P., Ogg, M.S., Luong, L.A., Kuller, L.H., Arnold, A.M., Sharrett, A.R., Humphries, S.E., 2002. In the elderly, interleukin-6 plasma levels and the −174G > C polymorphism are associated with the development of cardiovascular disease. Arterioscler. Thromb. Vasc. Biol. 22, 2066–2071.

Jones, K.G., Brull, D.J., Brown, L.C., Sian, M., Greenhalgh, R.M., Humphries, S.E., Powell, J.T., 2001. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 103, 2260–2265.

Kilpinen, S., Hulkkonen, J., Wang, X.Y., Hurme, M., 2001. The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults. Eur. Cytokine Networks 12, 62–68.

Klonoff-Cohen, H., Barrett-Connor, E.L., Edelstein, S.L., 1992. Albumin levels as a predictor of mortality in the healthy elderly. J. Clin. Epidemiol. 45, 207–212.

Krabbe, K.S., Bruunsgaard, H., Hansen, C.M., Moller, K., Fonsmark, L., Qvist, J., Madsen, P.L., Kronborg, G., Andersen, H.O., Skinhoj, P., Pedersen, B.K., 2001. Ageing is associated with a prolonged fever response in human endotoxemia. Clin. Diagn. Lab. Immunol. 8, 333–338.

Licastro, F., Pedrini, S., Caputo, L., Annoni, G., Davis, L.J., Ferri, C., Casadei, V., Grimaldi, L.M., 2000a. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? J. Neuroimmunol. 103, 97–102.

Licastro, F., Pedrini, S., Ferri, C., Casadei, V., Govoni, M., Pession, A., Sciacca, F.L., Veglia, F., Annoni, G., Bonafe, M., Olivieri, F., Franceschi, C., Grimaldi, L.M., 2000b. Gene polymorphism affecting alpha1-antichymotrypsin and interleukin-1 plasma levels increases Alzheimer's disease risk. Ann. Neurol. 48, 388–391.

Licastro, F., Grimaldi, L.M., Bonafe, M., Martina, C., Olivieri, F., Cavallone, L., Giovanietti, S., Masliah, E., Franceschi, C., 2003. Interleukin-6 gene alleles affect the risk of Alzheimer's disease and levels of the cytokine in blood and brain. Neurobiol. Aging 24, 921–926.

Lio, D., Scola, L., Crivello, A., Colonna-Romano, G., Candore, G., Bonafe, M., Cavallone, L., Franceschi, C., Caruso, C., 2002. Gender-specific association between -1082 IL-10 promoter polymorphism and longevity. Genes Immun., 3, 30–33.

Marik, P.E., Zaloga, G.P., 2001. The effect of aging on circulating levels of proinflammatory cytokines during septic shock. Norasept II study investigators. J. Am. Geriatr. Soc. 49, 5–9.

McCusker, S.M., Curran, M.D., Dynan, K.B., McCullagh, C.D., Urquhart, D.D., Middleton, D., Patterson, C.C., McIlroy, S.P., Passmore, A.P., 2001. Association between polymorphism in regulatory region of gene encoding tumour necrosis factor alpha and risk of Alzheimer's disease and vascular dementia: a case-control study. Lancet 357, 436–439.

McLachlan, J.A., Serkin, C.D., Morrey, K.M., Bakouche, O., 1995. Antitumoral properties of aged human monocytes. J. Immunol. 154, 832–843.

McNerlan, S.E., Rea, I.M., Alexander, H.D., 2002. A whole blood method for measurement of intracellular TNF-alpha, IFN-gamma and IL-2 expression in stimulated CD3 + lymphocytes: differences between young and elderly subjects. Exp. Gerontol. 37, 227–234.

Meir, K., Leitersdorf, E., Hennekens, C.H., 2003. Inflammation in atherosclerosis: causal or casual? The need for randomized trials. Am. Heart J. 146, 199–202.

Mooradian, A.D., Reed, R.L., Osterweil, D., Scuderi, P., 1991. Detectable serum levels of tumor necrosis factor alpha may predict early mortality in elderly institutionalized patients. J. Am. Geriatr. Soc. 39, 891–894.

Nicoll, J.A., Mrak, R.E., Graham, D.I., Stewart, J., Wilcock, G., MacGowan, S., Esiri, M.M., Murray, L.S., Dewar, D., Love, S., Moss, T., Griffin, W.S., 2000. Association of interleukin-1 gene polymorphisms with Alzheimer's disease. Ann. Neurol. 47, 365–368.

Nybo, L., Nielsen, B., Pedersen, B.K., Moller, K., Secher, N.H., 2002. Interleukin-6 release from the human brain during prolonged exercise. J Physiol, 542, 991–995.

Olivieri, F., Bonafe, M., Cavallone, L., Giovagnetti, S., Marchegiani, F., Cardelli, M., Mugianesi, E., Giampieri, C., Moresi, R., Stecconi, R., Lisa, R., Franceschi, C., 2002. The −174 C/G locus affects in vitro/in vivo IL-6 production during aging. Exp. Gerontol. 37, 309–314.

O'Mahony, L., Holland, J., Jackson, J., Feighery, C., Hennessy, T.P., Mealy, K., 1998. Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin. Exp. Immunol. 113, 213–219.

Paolisso, G., Rizzo, M.R., Mazziotti, G., Tagliamonte, M.R., Gambardella, A., Rotondi, M., Carella, C., Giugliano, D., Varricchio, M., DOnofrio, F., 1998. Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha. Am. J. Physiol. Endocrinol. Metabolism 38, E294–E299.

Pedersen, M., Bruunsgaard, H., Weis, N., Hendel, H.W., Andreassen, B.U., Eldrup, E., Dela, F., Pedersen, B.K., 2003. Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. Mech. Ageing Dev. 124, 495–502.

Penninx, B.W., Kritchevsky, S.B., Yaffe, K., Newman, A.B., Simonsick, E.M., Rubin, S., Ferrucci, L., Harris, T., Pahor, M., 2003. Inflammatory markers and depressed mood in older persons: results from the health, aging and body composition study. Biol. Psychiatry 54, 566–572.

Pepys, M.B., Hirschfield, G.M., 2003. C-reactive protein: a critical update. J. Clin. Invest. 111, 1805–1812.

Peterson, P.K., Chao, C.C., Carson, P., Hu, S., Nichol, K., Janoff, E.N., 1994. Levels of tumor necrosis factor alpha, interleukin 6, interleukin 10, and transforming growth factor beta are normal in the serum of the healthy elderly. Clin. Infect. Dis. 19, 1158–1159.

Pola, R., Flex, A., Gaetani, E., Lago, A.D., Gerardino, L., Pola, P., Bernabei, R., 2002. The −174 G/C polymorphism of the interleukin-6 gene promoter is associated with Alzheimer's disease in an Italian population [corrected]. Neuroreport 13, 1645–1647.

Pollmacher, T., Haack, M., Schuld, A., Reichenberg, A., Yirmiya, R., 2002. Low levels of circulating inflammatory cytokines—Do they affect human brain functions? Brain Behav. Immunity 16, 525–532.

Rea, I.M., Stewart, M., Campbell, P., Alexander, H.D., Crockard, A.D., Morris, T.C., 1996. Changes in lymphocyte subsets, interleukin 2, and soluble interleukin 2 receptor in old and very old age. Gerontology 42, 69–78.

Rea, I.M., Ross, O.A., Armstrong, M., McNerlan, S., Alexander, D.H., Curran, M.D., Middleton, D., 2003. Interleukin-6-gene C/G 174 polymorphism in nonagenarian and octogenarian subjects in the BELFAST study. Reciprocal effects on IL-6, soluble IL-6 receptor and for IL-10 in serum and monocyte supernatants. Mech. Ageing Dev. 124, 555–561.

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., Pollmacher, T., 2001. Cytokine-associated emotional and cognitive disturbances in humans. Arch. Gen. Psychiatry 58, 445–452.

Reuben, D.B., Cheh, A.I., Harris, T.B., Ferrucci, L., Rowe, J.W., Tracy, R.P., Seeman, T.E., 2002. Peripheral blood markers of inflammation predict mortality and functional decline in high-functioning community-dwelling older persons. J. Am. Geriatr. Soc. 50, 638–644.

Riancho, J.A., Zarrabeitia, M.T., Amado, J.A., Olmos, J.M., Gonzalez, M.J., 1994. Age-related differences in cytokine secretion. Gerontology 40, 8–12.

Ridker, P.M., Rifai, N., Stampfer, M.J., Hennekens, C.H., 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 101, 1767–1772.

Rosenthal, A.J., McMurtry, C.T., Sanders, K.M., Jacobs, M., Thompson, D., Adler, R.A., 1997. The soluble interleukin-2 receptor predicts mortality in older hospitalized men. J. Am. Geriatr. Soc. 45, 1362–1364.

Ross, R., 1999. Atherosclerosis—an inflammatory disease. N. Engl. J. Med. 340, 115–126.

Roubenoff, R., 2003. Catabolism of aging: is it an inflammatory process? Curr. Opin. Clin. Nutr. Metab. Care 6, 295–299.

Roubenoff, R., Harris, T.B., Abad, L.W., Wilson, P.F., Dallal, G.E., Dinarello, C.A., 1998. Monocyte cytokine production in an elderly population: effect of age and inflammation. J. Gerontol. Med. Sci. 53, M20–M26.

Rudd, A.G., Banerjee, D.K., 1989. Interleukin-1 production by human monocytes in ageing and disease. Age Ageing 18, 43–46.

Sandmand, M., Bruunsgaard, H., Kemp, K., Andersen-Ranberg, K., Schroll, M., Jeune, B., 2003. High circulating levels of tumor necrosis factor-alpha in centenarians are not associated with increased production in T lymphocytes. Gerontology 49, 155–160.

Saurwein-Teissl, M., Blasko, I., Zisterer, K., Neuman, B., Lang, B., Grubeck-Loebenstein, B., 2000. An imbalance between pro- and anti-inflammatory cytokines, a characteristic feature of old age. Cytokine 12, 1160–1161.

Sciacca, F.L., Ferri, C., Licastro, F., Veglia, F., Biunno, I., Gavazzi, A., Calabrese, E., Martinelli, B.F., Sorbi, S., Mariani, C., Franceschi, M., Grimaldi, L.M., 2003. Interleukin-1B polymorphism is associated with age at onset of Alzheimer's disease. Neurobiol. Aging 24, 927–931.

Skoog, T., Dichtl, W., Boquist, S., Skoglund-Andersson, C., Karpe, F., Tang, R., Bond, M.G., de Faire, U., Nilsson, J., Eriksson, P., Hamsten, A., 2002. Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. Eur. Heart J. 23, 376–383.

Starkie, R., Ostrowski, S.R., Jauffred, S., Febbraio, M., Pedersen, B.K., 2003. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J. 17, 884–886.

Tarkowski, E., Liljeroth, A.M., Nilsson, A., Ricksten, A., Davidsson, P., Minthon, L., Blennow, K., 2000. TNF gene polymorphism and its relation to intracerebral production of TNFalpha and TNFbeta in AD. Neurology 54, 2077–2081.

Tarkowski, E., Liljeroth, A.M., Minthon, L., Tarkowski, A., Wallin, A., Blennow, K., 2003. Cerebral pattern of pro- and anti-inflammatory cytokines in dementias. Brain Res. Bull. 61, 255–260.

Terreni, L., Fogliarino, S., Quadri, P., Ruggieri, R.M., Piccoli, F., Tettamanti, M., Lucca, U., Forloni, G., 2003. Tumor necrosis factor alpha polymorphism C-850T is not associated with Alzheimer's disease and vascular dementia in an Italian population. Neurosci. Lett. 344, 135–137.

Vickers, M.A., Green, F.R., Terry, C., Mayosi, B.M., Julier, C., Lathrop, M., Ratcliffe, P.J., Watkins, H.C., Keavney, B., 2002. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc. Res. 53, 1029–1034.

Visser, M., Pahor, M., Taaffe, D.R., Goodpaster, B.H., Simonsick, E.M., Newman, A.B., Nevitt, M., Harris, T.B., 2002. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC study. J. Gerontol. A Biol. Sci. Med. Sci. 57, M326–M332.

Volpato, S., Guralnik, J.M., Ferrucci, L., Balfour, J., Chaves, P., Fried, L.P., Harris, T.B., 2001. Cardiovascular disease, interleukin-6, and risk of mortality in older women: the women's health and aging study. Circulation 20 (103), 947–953.

Wang, X.Y., Hurme, M., Jylha, M., Hervonen, A., 2001. Lack of association between human longevity and polymorphisms of IL-1 cluster, IL-6, IL-10 and TNF-alpha genes in Finnish nonagenarians. Mech. Ageing Dev. 123, 29–38.

Wei, J., Xu, H., Davies, J.L., Hemmings, G.P., 1992. Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci. 51, 1953–1956.

Weijenberg, M.P., Feskens, E.J., Souverijn, J.H., Kromhout, D., 1997. Serum albumin, coronary heart disease risk, and mortality in an elderly cohort. Epidemiology 8, 87–92.

Weverling-Rijnsburger, A.W., Blauw, G.J., Lagaay, A.M., Knook, D.L., Meinders, A.E., Westendorp, R.G., 1997. Total cholesterol and risk of mortality in the oldest old. Lancet 350, 1119–1123.

Yaffe, K., Lindquist, K., Penninx, B.W., Simonsick, E.M., Pahor, M., Kritchevsky, S., Launer, L., Kuller, L., Rubin, S., Harris, T., 2003. Inflammatory markers and cognition in well-functioning African–American and white elders. Neurology 61, 76–80.