A High Plasma Concentration of TNF-α Is Associated With Dementia in Centenarians

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Background. Inflammatory mechanisms and immune activation have been hypothesized to play a role in the pathogenesis of age-associated diseases such as dementia and atherosclerosis. The purpose of this study was to evaluate the plasma concentration of tumor necrosis factor (TNF)-α in a large cohort of centenarians and to look for its possible associations with cognitive function, atherosclerosis, and general health status. Furthermore, we investigated whether the concentration of TNF-α was correlated with the blood concentration of leucocyte subsets or the plasma concentrations of interleukin (IL)-6, soluble TNF receptor (sTNFR)-II (75 kDa) and C-reactive protein (CRP).

Methods. Plasma TNF-α was measured by ELISA in 126 centenarians, 45 subjects aged 81 years, 23 subjects aged 55-65 years, and 38 subjects aged 18-30 years. Atherosclerosis was evaluated by the ankle-brachial blood pressure index, and general health status was evaluated by the body mass index and the number of diagnoses present.

Results. The concentration of TNF-α was significantly increased in 126 centenarians compared to younger control groups, and a high concentration of TNF-α was associated with both Alzheimer’s disease and generalized atherosclerosis in the centenarians. The concentration of TNF-α was positively correlated with the concentrations of plasma IL-6, sTNFR-II, and CRP. No associations were found with increased leucocyte subsets or the body mass index.

Conclusion. This study demonstrates that, even in apparently healthy subjects, age-associated immune activation indicated by raised levels of pro-inflammatory cytokines may reflect age-associated pathological processes that develop over decades.

Aging is associated with immune activation and changes in the balance of cytokine secretion patterns in blood mononuclear cells (BMNC) (1-3). An age-related increase in serum/plasma concentrations of interleukin (IL)-6 (4-9), IL-1 receptor antagonist, and soluble tumor necrosis factor receptor (sTNFR)-I (55 kDa) (10) has been reported, whereas others have found unaltered or undetectable IL-6 (11), tumor necrosis factor (TNF)-α (2,10-12), and IL-1 (10,12). Inflammatory mechanisms and immune activation have been hypothesized to play a role in the pathogenesis of age-associated diseases such as dementia, atherosclerosis, and osteoporosis (2,13-19). TNF-α has been shown to predict mortality in elderly institutionalized patients (20). Associations between increased immune activation in the peripheral circulation and cognitive decline due to Alzheimer’s disease (AD) have been reported. Thus, raised serum levels of IL-6, increased TNFR-I (55 kDa) and TNFR-II (75 kDa) expression on lymphocytes, increased IL-6 production by BMNC, and increased histamine in serum have been related to dementia (13,21-23). To our knowledge, the concentration of TNF-α in plasma and its relation to age-associated diseases have never been evaluated in large cohorts of centenarians representing the extreme limit of human life. Centenarians have been described as a homogenous, relatively healthy, independent group (24), whereas Danish centenarians have been found to be heterogeneous and characterized by multimorbidity (25). Centenarians have a high incidence of dementia. It has been reported in the range of 30%-70% (25-29).

TNF-α is an early acting mediator in the acute phase response, having a prominent role in the initiation of the inflammatory cascade including the induction of IL-6 and liver production of acute phase proteins such as C-reactive protein (CRP), serum amyloid A protein (SAA), and serum amyloid P (SAP) (30). Soluble TNF receptors prevent ligand binding of TNF to its surface receptors and thus represent naturally occurring inhibitors.

The purpose of this study was to investigate the plasma concentration of TNF-α in a large cohort of centenarians and to look for a possible association with cognitive function. Furthermore, other possible associations between the clinical state and increased concentrations of TNF-α in plasma were tested. Atherosclerosis was evaluated by the ankle-brachial blood pressure (BP) index (31-33); general health status was evaluated by the number of diagnoses present and the body mass index (BMI) (34). Also, we examined whether the concentration of TNF-α was correlated with the total blood leucocyte number, concentrations of individual leucocyte subsets, or plasma concentrations of IL-6, sTNFR-II, and CRP.

Materials and Methods

Subjects
One-hundred twenty-six centenarians from the Danish Centenarian Study, including 96 women, participated in this study. The Danish Centenarian Study includes 207 out of 276 Danes who celebrated their 100th birthday from 1995 to 1996 and who
consented to participate with an interview, a physical examination, and a review of medical records. Subject characteristics are given in Table 1. All centenarians were examined within 5 months of their 100th birthday. In the nonparticipant group, 13 died after their 100th anniversary but before contact was made. Fifty-six subjects declined the invitation to take part in the study. One hundred fifty subjects agreed to a blood test. For some subjects the amount of plasma available was too small to perform cytokine analyses. Furthermore, not all subjects agreed to a full physical examination. The distribution of gender did not differ significantly between the 126 subjects who had their plasma TNF-α measured and (a) the 150 subjects accounting for the rest of the 276 subjects or (b) the 81 subjects who accepted an examination but did not have plasma TNF-α measured (chi-squared test). The proportion of subjects living in their own home was significantly lower among the subjects who had their plasma TNF-α measured compared to the remaining 150 subjects (chi-squared test = 6.25, 2 df, \( p < .05 \)). This indicated that the first group might be less independent and perhaps less healthy than the rest of the cohort. However, in the group of deceased centenarians the proportion of persons living in their own home at the time of death was also high.

There was no significant difference in the distribution of housing situation between the 126 subjects and the 81 subjects who accepted a visit but did not have their TNF-α level measured. Among the 207 who accepted a visit, the cognitive status was categorized in 196 subjects (men/women: \( n = 45/151 \)). No significant difference was found in the distribution of cognitive rating or gender between the 125 centenarians with known cognitive state and with measured plasma TNF-α concentration and the remaining 71 centenarians of the 196 subjects (chi-squared test). Furthermore, 124 centenarians agreed to have their ankle-brachial BP index measured (men/women: \( n = 33/91 \)). There was no significant difference between the distribution of the ankle-brachial index or gender in the 97 subjects with known TNF-α level and the remaining 27 of the 124 subjects.

Three control groups were formed from the following: (a) 45 subjects aged 81 years, of whom 24 were women. The 45 subjects were chosen randomly out of 180 who had agreed to have blood samples taken in 1995–1996 as a part of a cohort study called the 1914-population in Glostrup, Denmark (35); (b) 23 healthy volunteers aged 55–65 years, 10 of whom were women; and (c) 38 healthy volunteers aged 18–30 years, 21 of whom were women.

### Dementia Diagnosis and Rating

The diagnosis of dementia was achieved by using the World Health Organization's criteria of ICD-10 (36), based on results from a Mini-Mental State Examination [MMSE; (37)], Lawton’s Instrumental Activity of Daily Living (IADL) scale (38), and caregivers’ information about ability in decision making, judgment, orientation, short-term memory and long-term memory. Depressive symptoms were sought through interview questions suitable for very old people, in order to exclude pseudodementia. A rating of dementia was done by using the Clinical Dementia Rating scale [CDR; (39)]. According to this, the centenarians could be divided into three groups: Group I consisted of cognitively intact centenarians with no memory loss or only slight, inconstant forgetfulness, no difficulties in judgment or problem solving, and with restraints in IADL and personal care caused only by physical disabilities. Group II consisted of centenarians with possible and mild dementia according to the criteria of the CDR (e.g., mild, consistent forgetfulness or moderate memory loss mostly related to recent events, which may interfere with everyday activities; some difficulty in time orientation; moderate difficulty in solving problems or handling complex problems; mild impairment in IADLs), whereas personal care was done without any problems apart from occasional prompting. Group III consisted of centenarians with moderate to severe dementia according to the criteria of the CDR (e.g., severe memory loss; only fragments of orientation left, if any; severely impaired ability, if any, in mak-

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### Table 1. Subject Characteristics in Participants and Nonparticipants in the Danish Centenarian Study

<table>
<thead>
<tr>
<th></th>
<th>Participants (( n = 207 ))</th>
<th>Nonparticipants (( n = 69 ))</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF-α Measured ( (n = 126) )</td>
<td>TNF-α Not Measured ( (n = 81) )</td>
<td>Declined to Participate ( (n = 56) )</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>30 (24%)</td>
<td>15 (19%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Women</td>
<td>96 (76%)</td>
<td>66 (81%)</td>
<td>50 (89%)</td>
</tr>
<tr>
<td>Housing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At home</td>
<td>41 (33%)</td>
<td>29 (36%)</td>
<td>27 (48%)</td>
</tr>
<tr>
<td>Sheltered house</td>
<td>19 (15%)</td>
<td>4 (5%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Nursing home</td>
<td>66 (52%)</td>
<td>48 (59%)</td>
<td>23 (41%)</td>
</tr>
<tr>
<td>Cognitive status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondemented</td>
<td>54 (43%)</td>
<td>22 (27%)</td>
<td>—</td>
</tr>
<tr>
<td>Mildly demented</td>
<td>31 (24%)</td>
<td>19 (23%)</td>
<td>—</td>
</tr>
<tr>
<td>Severely demented</td>
<td>40 (32%)</td>
<td>30 (38%)</td>
<td>—</td>
</tr>
<tr>
<td>Not categorized</td>
<td>1 (1%)</td>
<td>10 (12%)</td>
<td>—</td>
</tr>
<tr>
<td>Ankle-Arm BP index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.9</td>
<td>54 (43%)</td>
<td>16 (20%)</td>
<td>—</td>
</tr>
<tr>
<td>≥0.9</td>
<td>43 (34%)</td>
<td>11 (14%)</td>
<td>—</td>
</tr>
<tr>
<td>Not measured</td>
<td>29 (23%)</td>
<td>54 (67%)</td>
<td>—</td>
</tr>
</tbody>
</table>
The nutritional status for the centenarians was evaluated as weight divided by height squared. In old age, spinal deformity and thinning of the intervertebral discs may produce a false decrease in the height. Furthermore, many centenarians are not able to stand. Long-bone length bears a close correlation with the height of an individual, and age-related changes in height are not accompanied by corresponding changes in the length of long bones. The total arm length with the subject supine has been suggested as a better predictor of total height (40). The total arm length (from the tip of the acromial process to the end of the styloid process of the ulna) was measured, and the height was calculated using the formula:

\[ \text{Height (cm)} = 90.55 + (5.26 \times \text{Sex}) + (1.24 \times \text{length of arm}), \quad \text{man} = 1 \text{ and woman} = 0. \]

**Cytokines.**—Cytokines were measured in plasma. Ethylenediaminetetraacetate (EDTA) was used as an anticoagulant. Plasma was stored at −80°C until analyzed by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine High Sensitivity, catalog numbers: HSTA50, HS600, and DRT200, R & D Systems, Minneapolis, MN). The TNF-α and IL-6 immunoassays measure the total amount of free TNF-α or IL-6 plus the amount bound to soluble receptors. The sTNFR-II immunoassay measures the total amount of free receptors plus the total amount of receptors bound to TNF.

According to the manufacturer’s manual, the detection limit was <180 femtograms/ml, the coefficient of variance (CV) for the intraassay precision was 6%, for the interassay precision it was 9%, and for the recovery it was 96% regarding TNF-α. For IL-6 the detection limit was 0.094 pg/ml, and CV was 7% (intraassay precision), 15% (interassay precision), and 103% (recovery), and for sTNFR-II the detection limit was <10 pg/ml, and CV was 2% (intraassay precision), 4% (interassay precision), and 102% (recovery). All samples and standards were run as duplicates, and the mean of duplicates was used in the statistical analyses.

**Clinical chemistry tests.**—Standard laboratory procedures were employed.

**Statistics.**—Statistical calculations were performed using SYSTAT statistical software 7.0 (SYSTAT, Evanston, IL). Initial analyses revealed that the TNF-α data were not normally distributed. Therefore, TNF-α was log_{10} transformed in the further statistical analyses, and data are presented as geometric means and the empirical 2.5%, 97.5% centiles (95% central range).

Analyses of variance (ANOVAs) were used to test for differences in the concentration of TNF-α across groups. Sex had no influence on TNF-α and was left out in the final analyses. If a significant effect (p < .05) was found in the ANOVA, Tukey’s test for pairwise comparisons was performed to localize differences. Furthermore, linear dependencies between parameters were detected by simple linear regression or by multiple linear regression (forward stepwise regression).

**RESULTS**

**Concentrations**

The concentrations of cytokines in the centenarians compared to younger control groups.—The levels of TNF-α, IL-6, and sTNFR-II increased with age and were significantly higher in the centenarians compared to the younger age groups (Table 2).

**Table 2.** The Plasma Level of TNF-α, IL-6, and sTNFR-II in Centenarians Compared to Younger Control Groups

<table>
<thead>
<tr>
<th>Age</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>sTNFR-II (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–30 yrs</td>
<td>1.4 (0.4–5.5)</td>
<td>2.1 (0.3–17.0)</td>
<td>1.8 (1.1–3.1)</td>
</tr>
<tr>
<td>n=38</td>
<td>n=38</td>
<td>n=38</td>
<td>n=38</td>
</tr>
<tr>
<td>55–65 yrs</td>
<td>1.7 (0.6–5.5)</td>
<td>3.8 (0.7–19.4)*</td>
<td>2.1 (1.0–4.0)</td>
</tr>
<tr>
<td>n=23</td>
<td>n=23</td>
<td>n=23</td>
<td>n=23</td>
</tr>
<tr>
<td>80 yrs</td>
<td>2.5 (0.5–11.2)*</td>
<td>3.8 (0.7–20.4)</td>
<td>2.8 (1.4–5.6)††</td>
</tr>
<tr>
<td>n=45</td>
<td>n=45</td>
<td>n=45</td>
<td>n=45</td>
</tr>
<tr>
<td>Centenarians</td>
<td>3.7 (1.0–14.2)* † †</td>
<td>6.1 (1.5–25.3)* † †</td>
<td>5.0 (2.5–10.2)* † †</td>
</tr>
<tr>
<td>n=126</td>
<td>n=103</td>
<td>n=121</td>
<td></td>
</tr>
</tbody>
</table>

Note: Geometric means (95% central range) are shown. *Significantly different from 18–30 yr-old subjects (p < .05). †Significantly different from 55–65-yr-old subjects (p < .05). ††Significantly different from 80-yr-old subjects (p < .05).
of centenarians with disorders suspected to influence TNF-α, moderately to severely demented centenarians still had a significantly higher plasma concentration of TNF-α compared to cognitively intact subjects (Group B) (Table 3).

### Table 3. The Plasma Concentration of TNF-α (pg/ml) and the Cognitive Function in Centenarians

<table>
<thead>
<tr>
<th>Group</th>
<th>Ankle index &lt; 0.9</th>
<th>Ankle index ≥ 0.9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>4.5 (1.3–14.1)</td>
<td>3.2 (0.8–13.1)</td>
<td>3.2 (0.8–13.1)</td>
</tr>
<tr>
<td>Group B</td>
<td>4.5 (1.3–15.4)</td>
<td>3.3 (0.8–13.1)</td>
<td>3.3 (0.8–13.1)</td>
</tr>
<tr>
<td>Group C</td>
<td>4.3 (1.4–13.4)</td>
<td>3.2 (0.9–11.4)</td>
<td>3.2 (0.9–11.4)</td>
</tr>
<tr>
<td>Group D</td>
<td>4.1 (1.5–11.4)</td>
<td>2.2 (0.5–8.7)</td>
<td>2.2 (0.5–8.7)</td>
</tr>
</tbody>
</table>

**Notes:** Group I: normal cognitive function; Group II: mild dementia; Group III: moderate to severe dementia. A multiple linear regression model showed no linear dependency between TNF-α and BMI, an ANOVA test showed no differences in the TNF-α level between groups based on BMI. The concentration of TNF-α was significantly higher in subjects with an ankle-brachial BP index below 0.9 compared to subjects with an index of 0.9 or higher, when nondemented centenarians with an ankle-brachial index below 0.9 were compared to nondemented subjects with an index of 0.9 or higher, defined as Group A, the TNF-α concentration was significantly higher in the former group. Geometrical means (95% central range) are shown.

In order to separate vascular dementia (VD) from AD, subjects with previously clinically diagnosed strokes, transient cerebral ischemia (TCI) or amaurosis fugax were excluded. In regard to the remaining subjects (Group C), moderately to severely demented centenarians were still found to have a significantly higher plasma concentration of TNF-α compared to cognitively intact subjects (Table 3). This observation remained unchanged when excluding subjects with previous atherosclerotic manifestations affecting the brain as well as subjects having an ankle-brachial BP index below 0.9 (Group D, Table 3). No association was found between generalized atherosclerosis (ankle-brachial BP index < 0.9) and the cognitive state when using a chi-squared test (Table 4).

### Table 4. The Distribution of Cognitive Function and Generalized Atherosclerosis in Centenarians

<table>
<thead>
<tr>
<th>Cognitive status</th>
<th>Ankle index &lt; 0.9</th>
<th>Ankle index ≥ 0.9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>21</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>Group II</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Group III</td>
<td>20</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>43</td>
<td>96</td>
</tr>
</tbody>
</table>

**Notes:** Group I: normal cognitive function; Group II: mild dementia; Group III: moderate to severe dementia. An ankle-arm BP index below 0.9 was considered as a marker for generalized peripheral atherosclerosis. Chi-squared test: $\chi^2 = 2$; $p = 0.7$.

The concentration of TNF-α and the number of clinical diagnoses.—The centenarians were divided into two groups based on their ankle-brachial BP index (< 0.9 and ≥ 0.9). The concentration of TNF-α was significantly higher in subjects with an ankle-brachial BP index below 0.9 compared to subjects with an index of 0.9 or higher, defined as Group B, TNF-α was still found to be significantly higher in subjects with a low ankle-brachial BP index (Table 5, Group B). When moderately to severely demented centenarians without generalized atherosclerosis were excluded, TNF-α was significantly higher in the former group: geometrical means (95% central range) were 5.2 pg/ml (2.3–11.6), $n = 13$ versus 2.2 (0.5–8.7), $n = 19$, $p = .001$. When nondemented centenarians with an ankle-brachial index below 0.9 were compared with nondemented subjects with an ankle-brachial index of 0.9 or more, the former group was found to have a significantly higher TNF-α concentration (Table 5, Group E).

### Table 5. The Plasma Concentration of TNF-α (pg/ml) and Generalized Atherosclerosis in Centenarians

<table>
<thead>
<tr>
<th>Group</th>
<th>Ankle index &lt; 0.9</th>
<th>Ankle index ≥ 0.9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>4.5 (1.3–14.1)</td>
<td>3.2 (0.8–13.1)</td>
<td>3.2 (0.8–13.1)</td>
</tr>
<tr>
<td>Group B</td>
<td>4.5 (1.3–15.4)</td>
<td>3.3 (0.8–13.1)</td>
<td>3.3 (0.8–13.1)</td>
</tr>
<tr>
<td>Group C</td>
<td>4.3 (1.4–13.4)</td>
<td>3.2 (0.9–11.4)</td>
<td>3.2 (0.9–11.4)</td>
</tr>
<tr>
<td>Group D</td>
<td>4.1 (1.5–11.4)</td>
<td>2.2 (0.5–8.7)</td>
<td>2.2 (0.5–8.7)</td>
</tr>
</tbody>
</table>

**Notes:** An ankle-arm BP index below 0.9 was considered as a marker for generalized peripheral atherosclerosis. Group A: all centenarians; Group B: centenarians without disorders known or suspected to influence TNF-α; Group C: centenarians without previous strokes, transient cerebral ischemia (TCI), or amaurosis fugax; Group D: centenarians without previous strokes, TCI, amaurosis fugax, or generalized atherosclerosis (ankle-brachial BP index below 0.9). Geometrical means (95% central range) are shown.

In order to separate vascular dementia (VD) from AD, subjects with previously clinically diagnosed strokes, transient cerebral ischemia (TCI) or amaurosis fugax were excluded. In regard to the remaining subjects (Group C), moderately to severely demented centenarians were still found to have a significantly higher plasma concentration of TNF-α compared to cognitively intact subjects (Table 3). This observation remained unchanged when excluding subjects with previous atherosclerotic manifestations affecting the brain as well as subjects having an ankle-brachial BP index below 0.9 (Group D, Table 3). No association was found between generalized atherosclerosis (ankle-brachial BP index < 0.9) and the cognitive state when using a chi-squared test (Table 4).

### Table 4. The Distribution of Cognitive Function and Generalized Atherosclerosis in Centenarians

<table>
<thead>
<tr>
<th>Cognitive status</th>
<th>Ankle index &lt; 0.9</th>
<th>Ankle index ≥ 0.9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>21</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>Group II</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Group III</td>
<td>20</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>43</td>
<td>96</td>
</tr>
</tbody>
</table>

**Notes:** Group I: normal cognitive function; Group II: mild dementia; Group III: moderate to severe dementia. An ankle-arm BP index below 0.9 was considered as a marker for generalized peripheral atherosclerosis. Chi-squared test: $\chi^2 = 2$; $p = 0.7$.

The concentration of TNF-α and the number of clinical diagnoses.—The centenarians were divided into three groups based on the number of present diagnoses: (Group 1) 0–2, $n = 44$; (Group 2) 3–4, $n = 53$; (Group 3) 5 or more, $n = 29$. No differences in plasma TNF-α were found between groups (data not shown).

### The concentration of cytokines and the body mass index.—

In regard to BMI, the centenarians were divided into four groups based on quartiles. No differences in the concentration of TNF-α were found across groups (data not shown).

### Correlations between TNF-α and leucocyte subsets.—

Simple linear regression showed no linear dependency between TNF-α and the leucocyte number, and multiple linear regression revealed that the concentration of TNF-α was not positively dependent on any leucocyte subsets including the concentrations of lymphocytes, neutrophils, and monocytes (data not shown). Furthermore, an ANOVA test showed no differences in the TNF-α level between subjects with decreased levels, levels within the normal range (laboratory reference interval including 95% of a normal population), or increased levels with regard to total number of leucocytes or the concentrations of neutrophils, lymphocytes, or monocytes in the blood (Table 6). Exclusion of subjects with increased leucocyte levels due to leukemia ($n = 2$) did not change the above findings (data are not shown).
Correlations between IL-6, sTNFR-II, CRP, and TNF-α.

The concentrations of IL-6, sTNFR-II, and CRP were all positively dependent on TNF-α (Figure 1, A–C). IL-6 and CRP were not associated with cognitive state (data not shown), whereas CRP was significantly higher in subjects with an ankle-brachial BP index below 0.9: the geometrical mean (95% central range) was 4.0 (0.3–56), n = 54 versus 2.2 (0.2–32), n = 43, p = .04.

Table 6. The Plasma Concentration of TNF-α (pg/ml) in Centenarians With Leucocyte Subsets Below the Normal Range (Low), Within the Normal Range (Normal), and Higher Than the Normal Range (High)

<table>
<thead>
<tr>
<th></th>
<th>Low Concentration</th>
<th>Normal Concentration</th>
<th>High Concentration</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes (1)</td>
<td>4.4 (1.1–17.1)</td>
<td>3.7 (1.0–14.0)</td>
<td>4.4 (1.1–17.1)</td>
<td>0.6</td>
</tr>
<tr>
<td>Lymphocytes (2)</td>
<td>4.0 (1.2–13.6)</td>
<td>3.6 (0.8–15.4)</td>
<td>3.1 (0.9–10.6)</td>
<td>0.6</td>
</tr>
<tr>
<td>Neutrophils (3)</td>
<td>4.0 (1.2–13.3)</td>
<td>3.6 (0.9–14.7)</td>
<td>4.1 (1.2–13.9)</td>
<td>0.6</td>
</tr>
<tr>
<td>Monocytes (4)</td>
<td>—</td>
<td>3.7 (1.0–13.8)</td>
<td>3.7 (0.8–17.0)</td>
<td>1</td>
</tr>
</tbody>
</table>

Notes: Geometric means (95% central range) are shown.
(1): Normal range: 4.0–10.0 X 10^9/L.
(2): Normal range: 2.0–4.5 X 10^9/L.
(3): Normal range: 4.5–7.5 X 10^9/L.
(4): Normal range: <0.08 X 10^9/L.

DISCUSSION

The present study resulted in four major findings: (a) The plasma concentration of TNF-α increases with age and is significantly enhanced in a cohort of centenarians compared to younger control groups; (b) High plasma concentrations of TNF-α are associated with moderate or severe dementia, independent of atherosclerosis; (c) However, high concentrations of TNF-α are also associated with peripheral generalized, atherosclerosis defined as an ankle-brachial BP index below 0.9 independently of the cognitive status; (d) The concentration of TNF-α is positively correlated with the concentrations of plasma IL-6, sTNFR-II, and CRP. In agreement with the latter finding, it is commonly accepted that TNF-α, together with IL-1β, induces the production of IL-6; TNF-α and IL-1β together with IL-6 induce liver production of CRP; and stimuli that cause TNF levels to rise also induce shedding of TNF receptors (30).

To our knowledge, the present study is the first one to show an age-associated increase in plasma TNF-α and sTNFR-II. Other studies have not been able to detect significant age-related
differences in the serum levels of TNF-α (2,11), or TNF-α has been undetectable in the majority of plasma samples from both elderly and young humans (10,12). These discrepancies may be due to differences in TNF-α assays including high detection limits (10,12) and in the number of studied subjects (2,11). TNF-α acts at a low concentration and it is rapidly broken down. Thus, even small differences may be of physiological importance, and very sensitive assays should be used. Furthermore, a large number of subjects have to be studied in order to detect significant differences between different age groups. In the present study a high-sensitivity ELISA was used and TNF-α plasma levels were measured in a large number of individuals of all age groups. Fagiolo and colleagues (2) included only 13 individuals in each age group and Peterson and coworkers (11) only 26 elderly subjects and 13 young controls. In addition, in both studies the included subjects were selected in accordance with the guidelines described by the Sennieur Protocol (42), which might favor a very selected population of exceptional individuals. Monocytes are the most important producers of TNF-α in the blood (43,44), but the production of TNF-α was not found to be significantly increased in cultured BMNC from 711 elderly individuals compared to 21 young controls (45). Following LPS stimulation or mitogen stimulation, TNF-α production has been reported to be decreased in elderly humans in some studies (46) but increased in others (47,48), probably due to variation in stimulating agents, time of stimulation, and to different assays. However, it is important to keep in mind that in vitro studies may not mimic the in vivo situation in full. Thus, cells in tissues other than blood may contribute to the plasma level of TNF-α, for instance, macrophages within atherosclerotic plaques in the arterial vessel wall (15,49).

In the centenarians we studied the association between a high TNF-α level in the blood and dementia was present, regardless of whether subjects with other conditions potentially influencing the TNF-α level were excluded or not. Furthermore, the association was also present when excluding subjects with earlier clinical manifestations of atherosclerosis affecting the brain function (stroke, TCI, or amaurosis fugax) and/or presenting an ankle-brachial BP index below 0.9 indicating generalized peripheral atherosclerosis. Thus, the probability was high that the remaining subjects had AD and that high TNF-α concentrations were associated with AD.

The association between a high TNF-α concentration in the blood and generalized atherosclerosis was present whether subjects with other conditions potentially influencing the TNF-α level were excluded or not. The association was also present when demented subjects were left out of the analyses. Thus, TNF-α may be associated independently with both AD and atherosclerosis in centenarians.

Turning to AD, it has been suggested that an acute phase response mediated by pro-inflammatory cytokines could augment amyloidogenesis in AD (50). Cytokines, including TNF-α, are present in senile plaques in AD (21). TNF-α mediates myelin and oligodendrocyte damage and proliferation of astrocytes resulting in reactive gliosis besides stimulating the production of other cytokines and acute phase proteins (51). An age-related decrease in the blood brain barrier (BBB) has been reported (52), and local alterations of the BBB function in AD (53,54) may facilitate a local bidirectional passage of cytokines and acute phase proteins between the brain and the systemic circulation. If cytokines in senile plaques of AD are plasma derived, increased levels in the cerebrospinal fluid would be expected. In patients with AD, increased IL-6 in plasma has not been found to be accompanied by increased IL-6 in the cerebrospinal fluid (17). However, to what extent IL-6 and other cytokines cross the BBB in centenarians is not known. The acute phase protein SAP has been detected in plaques from patients with AD, but not in plaques from nondemented elderly humans (55). Hepatocyte production of this protein is induced by TNF-α, IL-1β, and IL-6, and a local synthesis in CNS has not been detectable. Thus, SAP in the plaques is likely to be plasma derived (56). The plasma concentration of SAP is significantly increased in severely demented centenarians compared with cognitively intact centenarians and younger controls (57). On the other hand, the increased plasma concentration of TNF-α associated with dementia may be a result of age-associated inflammatory processes in CNS. Following stimulation by insoluble aggregates of β-amyloid protein microglial production of TNF-α has been demonstrated (21). Injection of recombinant TNF-α intracerebroventricularly in mice resulted in increased serum levels of IL-6, demonstrating that the level of pro-inflammatory cytokines in the blood can be induced by inflammatory mediators in CNS (58).

The lesions in atherosclerosis result from an excessive inflammatory-fibroproliferative response to various forms of insults to the endothelium and smooth muscle of the artery wall (15). The finding of increased concentrations of cytokines related to inflammation in elderly people and especially in centenarians may be related to progressing atherosclerosis with age. Large numbers of macrophages and activated CD4+ T cells are present within inflammatory atherosclerotic plaques, and cytokines such as TNF-α, IL-1, IL-6, and IFNγ are secreted in the lesions in atherosclerosis resulting to the endothelium and smooth muscle of the artery wall (15,59). Furthermore, TNF and IL-6 production have been shown to be increased in supernatants from blood vessels from old mice compared to young mice (49). It has been reported that serum concentrations of CRP, which are induced by TNF-α, IL-1β, and IL-6, correlate with the presence and severity of coronary, cerebral, and peripheral arterial atherosclerosis (60). Accordingly, we found that CRP was higher in subjects with an ankle-brachial index below 0.9.

In the present study, the independent associations between a high concentration of TNF-α and AD and a high concentration of TNF-α and atherosclerosis may be due to a common initiating pathological pathway or they may reflect parallel ongoing processes. Furthermore, an elevated TNF-α level may be a causative factor as well as a result of underlying pathological processes.

As the purpose of the present study was to investigate associations between TNF-α and age-associated diseases in a cohort of centenarians, it was obviously important to include all subjects and not use the exclusion criteria defined by the Sennieur Protocol, which attempt to separate age-associated changes from the immune system from changes caused by age-associated diseases (42,61). However, the exclusion of patients with diseases, intakes of medications, or other conditions with known or suspected influence on immune function did not influence the conclusions regarding associations between the level of TNF-α and the cognitive state or generalized atherosclerosis. It is difficult to exclude a vascular component in the pathology of dementia in the very old before death. This is reflected in the different reports of the prevalence of AD and VD in centenarians (27–29). Because the ankle BP was only measured on one
side, the prevalence of atherosclerosis may be underestimated in the present study. Thus, the possibility that the association between dementia and TNF-α may have a vascular component cannot be ruled out completely. Furthermore, increased levels of TNF might have been due to subclinical infections such as asymptomatic bacteriuria (62,63), dental infections (64), or helicobacter pylori (65,66), which are seen with increasing incidence with age and which may cause only minor or no increases in the concentration of leucocyte subsets. When comparing different age groups we cannot exclude the possibility that the observed differences are due to a cohort effect.

The inflammatory effect of the increase in plasma TNF-α in elderly people may be masked by the increase in sTNFR-II, which acts as a TNF-α inhibitor. One might speculate that the subjects with exceptionally low levels of sTNFR-II may develop TNF-α-related inflammatory pathology. However, this is not likely to be the case, as the present study shows that low levels of sTNFR-II are associated with low levels of TNF-α, IL-6, and CRP. Furthermore, the subjects in the lowest quartile of sTNFR-II concentrations also had significantly lower concentrations of IL-6 and CRP compared to the rest of the centenarians, and the distribution of dementia and atherosclerosis did not differ either (data not shown).

In conclusion, the plasma concentration of TNF-α was higher in centenarians compared to younger control groups, and a high concentration was independently associated with both AD and generalized atherosclerosis in the centenarians. It cannot be concluded from this study whether a common pathological pathway is present or not. Increased plasma levels of TNF-α may reflect a general activation of the immune system resulting from underlying pathological processes, but they could also play a direct role in the pathogenesis of dementia and/or atherosclerosis. TNF-α was highly correlated with the levels of both sTNFR-II, IL-6, and CRP, whereas none of these parameters were associated with dementia. This finding supports the hypothesis that TNF-α itself plays an important role in the pathogenesis of dementia. Longitudinal cohort studies, case control studies, or animal studies including brain dissections are necessary to further elucidate this. The present study demonstrates that age-associated increased immune activation including raised levels of pro-inflammatory cytokines even in apparently healthy subjects may reflect age-associated pathological processes that develop over decades.

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