Plasma Cytokine Levels in a Population-Based Study: Relation to Age and Ethnicity

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Background. Aging is one factor believed to contribute to processes that underlie chronic low-grade inflammation in older adults. Moreover, more recent studies have suggested that cytokine levels are influenced by ethnicity.

Methods. In this study, we determined plasma cytokine profiles in a population-based sample (n = 1,411; aged 25–91 years) to determine the relationship between circulating cytokine levels, aging, and ethnicity. We measured interleukin-1 receptor antagonist (IL-1ra), interleukin (IL)-6, -10, C-reactive protein (CRP), and tumor necrosis factor-receptor 1 (TNF-r1).

Results. IL-6 and TNF-r1 significantly increased with age, whereas IL-1ra, IL-10, and CRP did not significantly increase with age. After adjusting for age, non-Hispanic whites had significantly higher levels of IL-1ra than Mexican Americans, whereas non-Hispanic blacks had significantly higher levels of IL-6 and CRP than Mexican Americans as well as non-Hispanic whites. CRP levels in non-Hispanic blacks were no longer significantly higher after adjusting for body mass index (BMI), indicating that BMI is an important predictor of this inflammatory marker.

Conclusions. These results demonstrate that cytokine levels are influenced by both age and ethnicity. Furthermore, these results show that inflammatory profiles for Mexican Americans are lower than non-Hispanic whites and non-Hispanic blacks.

Key Words: IL-6—IL-10—IL-1—TNF—CRP.

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INFLAMMATION is believed to contribute to the onset of many age-related diseases as evidenced by a variety of medical studies linking proinflammatory cytokines to Alzheimer’s disease (1), Parkinson’s disease (2), amyotrophic lateral sclerosis (3), and multiple sclerosis (4). Much attention has been paid to interleukin (IL)-6 because of its association with cardiovascular disease, the leading cause of death. IL-6 is associated with a broad spectrum of age-related illnesses, chronic stress, and functional disability in older adults (5,6). IL-6 is also a strong inducer of C-reactive protein (CRP) by the liver, and both IL-6 and CRP are important in the development of cardiovascular disease (7,8). IL-6 and CRP also play a pathogenic role in a number of diseases associated with disability in older adults, such as arthritis, osteoporosis, and depression among others (9).

Studies of older humans have reported age-related increases in proinflammatory cytokines, but the switch from the inflammatory burst that resolves following an infection or injury to the chronic elevation encountered in many older adults is not well understood (10). Several investigations have indicated that there is an age-related increase in circulating IL-6 (11–14), which has been called a “cytokine for gerontologists” (15). However, some studies have found no changes with age (16,17). Similarly, tumor necrosis factor (TNF)-α, a cytokine that is involved in septic shock, was reportedly increased in some studies (11,18) but not others (17,19).

Anti-inflammatory mediators, such as IL-10 and interleukin-1 receptor antagonist (IL-1ra), may also be important in the aging process because they counteract proinflammatory cytokines. With regards to IL-10, few studies have measured circulating levels of this cytokine, but there have been reports that indicated no change occurs with aging (17,20). Reports have also shown an age-related increase in the IL-1ra (21,22). Altogether, the discrepancies regarding these cytokines mostly likely relate to variations in age and sample size.

Besides age, cytokine levels may also be influenced by ethnicity. Plasma levels of IL-8 and granulocyte colony-stimulating factor were elevated in African Americans compared with Caucasians (23), and TNF-α has been reported to be higher in nonobese Mexican Americans compared with non-Hispanic whites (24). Because there is little other
information on circulating proinflammatory cytokine levels and ethnicity, our goal was to investigate plasma levels of circulating cytokines in relation to ethnicity as well as age in a large population-based study. We found age-related differences in proinflammatory cytokines as well as significant differences in circulating cytokine levels between Mexican Americans, non-Hispanic whites, and non-Hispanic blacks.

**Materials and Methods**

**Participants**

Respondents for this study come from the Texas City Stress and Health Study, an ongoing assessment of risk, coping, stress, and health in a tri-ethnic community living in Texas City, Texas. This study set out to explore sociobiological patterns with a focus on Hispanics (25). The sample was generated through a census of Hispanic households and a simple random sample of non-Hispanic households. We first selected three ethnic strata: Mexican Americans aged 25–64 years, Mexican Americans aged 65 years and older, and non-Hispanics. We next selected housing units (HUs) in each stratum, including all Hispanic HUs and one in eight non-Hispanic HUs. Finally, we randomly selected one adult per household among Mexican Americans aged 25–64 years and among non-Hispanics and all Mexican Americans aged 65 years and older. Listing, enumeration, and interviewing followed standard U.S. Census Bureau Current Population Survey methods with appropriate local modifications. Interview rates in the main study were 80%, yielding a sample size of 2,706. There were 102 respondents who did not self-identify as white, black, or of being of Mexican descent and were removed from the analyses. Thus, the final sample size was 2,604. Blood samples were received from 54% of the participants (n = 1,411).

Blood samples were drawn from participants between 8 and 11 AM. To measure biomarkers, blood was drawn into EDTA tubes. Plasma was obtained after centrifugation and stored in 1-mL aliquots at −70°C until testing. The institutional review board at the University of Texas Medical Branch approved the study protocol, and informed consent was obtained from all participants.

**Measurement of Plasma Cytokines**

Cytokines were assayed in batched samples using ELISA or enzyme immunoassay (EIA) assays. CRP (high sensitivity) was measured using commercially available kits according to the manufacturer’s instructions (Diagnostic Systems Laboratories, Webster, TX).

For measurement of IL-6, IL-1ra, IL-10, and tumor necrosis factor-receptor 1 (TNF-r1), OptEIA assay kits were purchased from BD Pharmingen (San Diego, CA) or R&D Systems (Minneapolis, MN). In general, the protocol for the analysis of each cytokine was similar. For each cytokine, all patient samples from each study period were analyzed simultaneously with an identical set of control samples. Briefly, all patient serum samples and reagents were brought to room temperature. First, standards were prepared from a stock solution. The appropriate dilutions were made to establish a standard curve. Fifty microliters of ELISA diluent was pipetted into each well of the 96-well plates. Then, 100 µL of the standards and samples (all done in duplicate) was pipetted into wells and mixed with the diluent. The plate was shaken gently and covered with a plate sealer, and the samples were incubated for 2 hours at room temperature. After incubation, the standards and samples were aspirated from the wells and washed with buffer using an Embla 96/384-well microplate washer (Molecular Devices, Menlo Park, CA). One hundred microliters of detection antibody (conjugated to avidin–horseradish peroxidase) was added. This was incubated for 1 hour at room temperature. The wells then were washed with buffer and 100 µL of 3,3',5,5'-tetramethylbenzidine subsequently was added. The plate then was incubated in the dark at room temperature for 20–30 minutes. Afterward, 50 µL of sulfuric acid (2N) was added. Absorbance was read at 450 nm using a SpectraMax Plus plated reader (Molecular Devices). Results initially were calculated by optical density and converted to picograms per milliliter by log–log computation with application according to the standard curve.

**Statistics**

Analysis of variance (ANOVA) was conducted to determine differences by age and ethnicity in cytokine levels. Age was divided into seven age groups: 25–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80 years and older (age range of sample 25–91). Ethnic groups included non-Hispanic white, non-Hispanic black, foreign-born Mexican American, and US-born Mexican American. In addition, we tested whether or not the relationships between age and cytokine levels were linear. All analyses were performed using SAS 9.2.

**Results**

Blood samples were received from 54% of participants (n = 1,411); the remainder of the participants declined to participate in the blood sampling. Compared with the entire study cohort, those providing blood samples were a higher percentage of females (p < .03), a lower percentage of non-Hispanic blacks (p < .01), lower mean perceived physical health (p < .001), and higher mean levels of chronic conditions (p < .01). The demographic data and cytokine levels for all respondents are shown in Table 1.

The unadjusted mean cytokine levels, grouped by decade of age, are shown in Table 2. No significant differences in IL-1ra, IL-10, or CRP were found. Two statistical tests were performed for each cytokine. An ANOVA assessed whether there were any significant differences in cytokine levels among the age categories, whereas a test for linear trend assessed whether cytokine values increased or decreased...
Cytokines in a population-based study

Table 1. Demographic and Cytokine Levels (in picograms per milliliter) for Respondents in the Texas City Stress and Health Study (n = 1,411)

<table>
<thead>
<tr>
<th>Demographic</th>
<th>IL-1ra</th>
<th>IL-6</th>
<th>IL-10</th>
<th>TNF-r1</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–29</td>
<td>182 ± 8</td>
<td>1.4 ± 0.5</td>
<td>4.6 ± 1.6</td>
<td>1461 ± 140</td>
<td>12.4 ± 1.6</td>
</tr>
<tr>
<td>30–39</td>
<td>122 ± 24</td>
<td>1.7 ± 0.3</td>
<td>5.0 ± 1.0</td>
<td>1529 ± 88</td>
<td>14.2 ± 1.0</td>
</tr>
<tr>
<td>40–49</td>
<td>180 ± 23</td>
<td>1.5 ± 0.3</td>
<td>6.7 ± 0.9</td>
<td>1466 ± 84</td>
<td>12.4 ± 1.0</td>
</tr>
<tr>
<td>50–59</td>
<td>213 ± 24</td>
<td>2.1 ± 0.3</td>
<td>4.3 ± 1.0</td>
<td>1819 ± 87</td>
<td>14.3 ± 1.0</td>
</tr>
<tr>
<td>60–69</td>
<td>145 ± 25</td>
<td>1.8 ± 0.3</td>
<td>3.9 ± 1.0</td>
<td>1947 ± 93</td>
<td>14.0 ± 1.1</td>
</tr>
<tr>
<td>70–79</td>
<td>184 ± 28</td>
<td>2.8 ± 0.3</td>
<td>4.0 ± 1.1</td>
<td>2494 ± 103</td>
<td>13.1 ± 1.2</td>
</tr>
<tr>
<td>80+</td>
<td>95 ± 49</td>
<td>3.3 ± 0.6</td>
<td>5.9 ± 2.0</td>
<td>2946 ± 182</td>
<td>15.7 ± 2.1</td>
</tr>
</tbody>
</table>

Note: CRP = C-reactive protein; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; TNF-r1 = tumor necrosis factor-receptor 1.

Discussion

Inflammatory cytokines represent important links between aging and associated diseases. Several prior studies have shown age-related increases in inflammatory cytokine levels, and our results are in agreement with these studies. The cytokine with the strongest association with age was TNF-r1. We analyzed TNF-r1 instead of TNF-α for two reasons: (a) it is detectible when TNF-α is not and (b) TNF-α has a short half-life (~15 minutes); as such, TNF-r1 has been proposed as a surrogate marker for TNF-α (26). Importantly, TNF-α has been implicated in obesity, diabetes, and the insulin resistance syndrome. TNF-α is also strongly associated with mortality in older adults (27) as well as with Alzheimer’s disease and atherosclerosis (11,28).

Consistent with prior studies (11–14), IL-6 increased with age. Our data, which includes a large population of adults over a wide range of ages, support prior studies demonstrating age-related increases in these proinflammatory markers (29,30). Notably, the age group older than 80 years had the highest levels of both IL-6 and CRP. This is important because increased levels of IL-6 and CRP are associated with atherosclerosis (31), coronary heart disease (32,33), congestive heart failure (34), and arthritis (35), well-known diseases that affect older adults. Overall, our results are in agreement with the belief that immunosenescence is associated with chronic low-grade inflammation.

We also measured the anti-inflammatory mediators IL-10 and IL-1ra in this study. Plasma concentrations of IL-10 were not increased with age. These results are in agreement with prior studies (17,20) that showed no changes in circulating

Table 2. Unadjusted Mean Cytokine Levels (in picograms per milliliter) by Age Group for Respondents in the Texas City Stress and Health Study (n = 1,411)

<table>
<thead>
<tr>
<th>Age Group (y)</th>
<th>n</th>
<th>IL-1ra</th>
<th>IL-6</th>
<th>IL-10</th>
<th>TNF-r1</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25–29</td>
<td>103</td>
<td>182 ± 8</td>
<td>1.4 ± 0.5</td>
<td>4.6 ± 1.6</td>
<td>1461 ± 140</td>
<td>12.4 ± 1.6</td>
</tr>
<tr>
<td>30–39</td>
<td>262</td>
<td>122 ± 24</td>
<td>1.7 ± 0.3</td>
<td>5.0 ± 1.0</td>
<td>1529 ± 88</td>
<td>14.2 ± 1.0</td>
</tr>
<tr>
<td>40–49</td>
<td>289</td>
<td>180 ± 23</td>
<td>1.5 ± 0.3</td>
<td>6.7 ± 0.9</td>
<td>1466 ± 84</td>
<td>12.4 ± 1.0</td>
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<td>50–59</td>
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<td>234</td>
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<td>193</td>
<td>184 ± 28</td>
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<tr>
<td>80+</td>
<td>61</td>
<td>95 ± 49</td>
<td>3.3 ± 0.6</td>
<td>5.9 ± 2.0</td>
<td>2946 ± 182</td>
<td>15.7 ± 2.1</td>
</tr>
</tbody>
</table>

Note: All data are expressed as means ± SE. ANOVA = analysis of variance; CRP = C-reactive protein; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; TNF-r1 = tumor necrosis factor-receptor 1.
IL-10. Our study, which included a larger number of participants \((n = 1,411)\) across a wide range of ages, confirms these findings. In contrast to prior studies \((21,22)\), we found no changes in plasma concentrations of IL-1ra. However, Furrucchi and coworkers \((30)\) found no correlation between age and IL-1ra after adjusting for cardiovascular risk factors. The lack of changes in these anti-inflammatory markers may have important health consequences given the increased levels of TNF-r1 and IL-6 as this may be reflective of an imbalance in the cytokine network in the elderly participants. Interestingly, the lowest levels of IL-1ra were found in the oldest group \((aged 80 years and older)\). As IL-1 is inhibited by circulating IL-1ra, it is tempting to speculate that lower IL-1ra levels reflect reduced production of IL-1. This would correlate with the lack of fever in the elderly participants, which is often absent or blunted \((35)\). It has been reported that high levels of plasma IL-1ra, along with IL-6 and CRP, were predictive of higher mortality \((36)\). Further research is needed to determine the mechanisms underlying the differences in cytokine profiles between foreign-born and US-born Hispanics.

One potential explanation for the ethnic variations in cytokine levels is differences in cytokine gene polymorphisms. Allelic variations in the regulatory regions of inflammatory cytokine genes have been shown to affect the expression of some cytokines. Several studies have focused on IL-6 because of its biological importance and have demonstrated that the G/G IL-6 genotype, which results in high IL-6 production, is predominantly found in blacks \((39,40)\). It has been hypothesized that the dissimilarities in cytokine gene polymorphisms may contribute to the differences in inflammatory responses and cancer incidence and mortality in blacks \((41)\). Additionally, obesity was a significant determinant of CRP levels in non-Hispanic blacks, and BMI was significantly higher in non-Hispanic whites than neither non-Hispanic whites nor Hispanics \((data not shown)\).

In summary, our results confirm and extend other studies demonstrating age-related increases in circulating proinflammatory cytokines. In addition, we have shown ethnic differences in cytokine levels, and to our knowledge, this is the first study demonstrating ethnic differences in proinflammatory and anti-inflammatory cytokine profiles in large population-based study. Future studies are needed to determine the epigenetic link between inflammation and ethnicity.

Table 3. Age-Adjusted Mean Cytokine Levels (in picograms per milliliter) by Ethnicity for Respondents in the Texas City Stress and Health Study \((n = 1,411)\)

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>n</th>
<th>IL-1ra</th>
<th>IL-6</th>
<th>IL-10</th>
<th>TNF-r1</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Hispanic white</td>
<td>535</td>
<td>208±17</td>
<td>1.7±0.2</td>
<td>5.3±0.7</td>
<td>1917±62</td>
<td>13.4±0.7</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>150</td>
<td>148±32</td>
<td>2.7±0.4</td>
<td>4.3±1.3</td>
<td>1668±117</td>
<td>17.2±1.3</td>
</tr>
<tr>
<td>Hispanic</td>
<td>726</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foreign born</td>
<td>181</td>
<td>119±29</td>
<td>1.8±0.4</td>
<td>4.5±1.2</td>
<td>1756±108</td>
<td>12.3±1.2</td>
</tr>
<tr>
<td>US born</td>
<td>545</td>
<td>147±17</td>
<td>2.1±0.2</td>
<td>4.8±0.7</td>
<td>1811±61</td>
<td>13.2±0.7</td>
</tr>
</tbody>
</table>

Notes: All data are expressed as means ± SE. CRP = C-reactive protein; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; TNF-r1 = tumor necrosis factor-receptor 1.

* Significantly \((p < .05)\) higher than US-born Hispanics.
† Significantly \((p < .05)\) higher than foreign-born Hispanics.
‡ Significantly \((p < .05)\) higher than whites.
CYTOKINES IN A POPULATION-BASED STUDY

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