CONCISE COMMUNICATIONS

Elderly Humans Show Prolonged In Vivo Inflammatory Activity during Pneumococcal Infections

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Levels of circulating cytokines were measured in 22 hospitalized patients with pneumococcal infections during the first week after admission, to test for age-associated differences. Twenty-two healthy age- and sex-matched subjects were included as controls. Concentrations of tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-6, IL-1 receptor antagonist, soluble TNF receptor 1 (sTNFR-I), and IL-10 were increased on admission (P < .05), but macrophage inflammatory protein (MIP)-1β was not. Whereas levels of cytokines were similar on admission, levels of TNF-α and sTNFR-I after 1 week were higher (P < .05) in elderly (68–91 years) than in young (37–55 years) patients. Furthermore, plasma levels of IL-10 and sTNFR-I after 1 week were positively correlated with age, and the declines in sTNFR-I and in the TNFα/IL-10 ratio from day 0 to day 7 were correlated with age. Thus, aging was associated with prolonged inflammatory activity. This may reflect decreased ability to control the infection or a dysregulated cytokine response.

The highest rates of bacteremic pneumococcal disease are seen in the elderly and in the very young [1]. Mortality is associated with old age but also with leukopenia and lack of fever [2], indicating an impaired acute-phase response.

Interleukin (IL)-1β and tumor necrosis factor (TNF)-α are the earliest mediators of the acute-phase response. Both cytokines induce a second wave of cytokines including IL-6 and chemokines, which are important mediators of chemotaxis. The bioactivity of TNF-α and IL-1β is inhibited by natural antagonists such as IL-1 receptor antagonist (IL-1RA), soluble TNF receptor (sTNFR), and the antiinflammatory cytokine IL-10 [3]. TNF-α, produced in the course of pneumococcal pneumonia, may be produced in host defense by increasing the number of neutrophils in the blood [4]. Antibodies to TNF-α and intranasal inoculation of IL-10 impair the host defense during pneumococcal pneumonia in mice [5, 6], and a high ratio of IL-10 to TNF-α has been associated with a fatal outcome in febrile patients with community-acquired infections [7].

Several observations indicate that mediators of the acute-phase response are severely altered in elderly humans. These include reports of blunted fever responses [8], decreased in vivo peak levels of circulating proinflammatory cytokines during pneumonia, decreased lipopolysaccharide (LPS)-induced in vitro production of proinflammatory cytokines in human subjects [9], and increased circulating levels of proinflammatory and antiinflammatory cytokines [10]. Increased mortality in old mice following LPS administration has been related to a decreased ability to down-regulate excessive cytokine release [11].

The present study measured levels and clearance of proinflammatory and antiinflammatory cytokines in the course of a Streptococcus pneumoniae infection, with emphasis on the importance of patient age. We measured TNF-α, IL-1β, and IL-6 as representatives of proinflammatory cytokines; macrophage inflammatory protein (MIP)-1β as a representative of chemokines; and IL-10, sTNFR-I (55 kDa), and IL-1RA as representatives of antiinflammatory and naturally occurring cytokine inhibitors.

Materials and Methods

Patients. The study was designed to test for differences based on age. However, patients constituted an age continuum in order to enroll as many as possible. Twenty-two consecutive patients (10 men, 12 women) with infections caused by S. pneumoniae were included. Median age was 68 years (range, 37–91). Eleven patients had an invasive infection (bacteremia and/or meningitis), but none had multiple organ failure. The remaining patients had noninvasive pneumonia. A microbiologic diagnosis of S. pneumoniae infection was obtained in 19 patients. The remaining patients were included because they had radiologically verified lobar pneumonia, high C-reactive protein (CRP) at admission, and an immediate clinical response to treatment with penicillin G. Median length of hospitalization was 13 days (range, 2–38).
In general, the included subjects were healthy prior to the infection. Among the younger patients (≤55 years), 1 was splenectomized. Elderly subjects suffered from congestive heart disease (n = 2), chronic obstructive lung disease (n = 2), arterial hypertension (n = 4), previous strokes (n = 2), and previous cancer (n = 4), and 1 was splenectomized. All patients survived the acute infection.

Blood samples were collected during the first 24 h after admission (day 0), after 3–4 days (day 3), and after 1 week (day 7). Only 13 patients were seen at a follow-up visit 2–9 months after discharge from the hospital (postinfection). Twenty-two healthy sex- and age-matched subjects served as a control group.

Clinical chemistry tests. Standard laboratory procedures were used. The concentration of leukocyte subsets was determined by a cell counter (Technicon H.I.; Miles, Tarrytown, NY).

Cytokines. IL-1β, TNF-α, and IL-6 were measured in serum. MIP-1β, IL-1RA, IL-10, and sTNFR-I were measured in plasma containing EDTA and trasylol. Commercially available ELISA kits (R&D Systems, Minneapolis) were used.

Statistics. Statistical calculations were performed with software (version 7.0; SYSTAT, Evanston, IL). Because initial analyses revealed that the cytokine data were not normally distributed, medians and quartiles/ranges are given. Independent groups were compared by Mann-Whitney test. Changes in the levels of cytokines or leukocyte subsets were evaluated by Friedman’s test. Wilcoxon signed rank test was used for pairwise comparisons. Spearman rank order correlation coefficients (Rs) were used to test for correlations. P < .05 was considered significant.

Results

Cytokines during acute infection in patients and controls. When data for all patients were pooled, concentrations of TNF-α, IL-6, sTNFR-I, IL-1RA, and IL-10 and the TNF-α/IL-10 ratio were increased, compared with those of the age-matched control group on admission and during the following week (data not shown). IL-1β was increased only the first 3 days, and MIP-1β did not change. TNF-α, IL-1β, IL-6, IL-1RA, and sTNFR-I declined during the first week. On admission, TNF-α was correlated with MIP-1β (Rs = 0.706, n = 21), sTNFR-I (Rs = 0.620, n = 17), IL-1RA (Rs = 0.596, n = 21), and IL-10 (Rs = 0.538, n = 14) and was borderline correlated (P = .06) with CRP (Rs = 0.445, n = 18, P = .06).

Cytokines and CRP were not correlated with the concentration of neutrophils on admission. However, the concentration of neutrophils on day 7 was significantly correlated with TNF-α (Rs = 0.544, n = 17), MIP-1β (Rs = 0.548, n = 18), sTNFR-I (Rs = 0.653, n = 14), IL-1RA (Rs = 0.631, n = 18), IL-10 (Rs = 0.566, n = 13), and with CRP (Rs = 0.668 n = 15) on day 0.

Postinfection blood levels of cytokines or leukocyte subsets in patients did not differ from those of controls.

Comparisons of patients with pneumonia (without bacteremia) and patients with bacteremia meningitis. On admission, subjects with bacteremia meningitis had increased levels of TNF-α, IL-10, and IL-1RA, compared with subjects with pneumonia alone (median [range]: TNF-α, 5.1 pg/mL (1.9–19.1), n = 10, versus 2.6 pg/mL (0.8–4.8), n = 11, P = .003; IL-10, 14.2 pg/mL (6.4–80.0), n = 9, versus 8.3 pg/mL (4.0–14.0), n = 6, P = .05; IL-1RA, 4.3 ng/mL (1.7–11.7), n = 11, versus 1.4 ng/mL (0.0–5.1), n = 11, P = .01. IL-1RA and IL-6 remained elevated in the group with invasive infection on day 3 (data not shown). The TNF-α/IL-10 ratio was higher in patients with invasive infections on days 3 and 7 (data not shown). The total leukocyte count was significantly higher on days 3 and 7 in subjects with bacteremia meningitis as a result of increased numbers of neutrophils (data not shown).

There was no difference in age distribution between patients with different clinical manifestations of S. pneumoniae infection. Accordingly, invasive and noninvasive infections were pooled in the following analyses.

Cytokines with emphasis on age. Patients were divided into young (ages 37–55 years) and elderly groups (ages 68–90 years) (figure 1). On admission, there was no difference in fever, total leukocyte count, or concentrations of neutrophils and lymphocytes or in levels of circulating TNF-α, IL-1β, IL-1RA, sTNFR-I, and IL-10 between the two groups. However, IL-6 was lower among the elderly. No differences were found on day 3. Concentrations of TNF-α and sTNFR-I were higher on day 7 in the elderly patients. No differences were found in other cytokines or in the neutrophil count. Plasma levels of sTNFR-I and IL-10 on day 7 were directly correlated with age (sTNFR-I, Rs = 0.575, n = 17, P = .02; IL-10, Rs = 0.546, n = 14, P = .05). The level of TNF-α on day 7 was borderline correlated with age (Rs = 0.416, n = 21, P = .06).

To test whether higher levels on day 7 were caused by higher levels of these cytokines in even apparently healthy, elderly subjects, the two age groups were compared with their respective age-matched control groups. TNF-α, IL-10, and IL-1RA on day 7 remained increased in the elderly patients, compared with age-matched controls, but had returned to normal levels in the younger patients. Levels of sTNFR-I, IL-6, and neutrophils were increased in both age groups, compared with age-matched controls, after 1 week. IL-1β had returned to normal levels in both groups. No difference was observed in MIP-1β.

Decreases in levels of sTNFR-I and MIP-1β from days 0 to 7 were less pronounced in the elderly than in the young group (P = .02), whereas the decrease in TNF-α did not reach significance (P = .06). In addition, ΔsTNFR-I and ΔTNF-α/IL-10 were correlated with age when all patients were pooled (ΔsTNFR-I, R = −0.573, n = 17, P = .02; ΔTNF-α/IL-10, R = 0.636, n = 13, P = .03).

The median number of days in hospital was 10 (range, 2–33) in the young and 15 (4–36) in the elderly (P = .3). There was no correlation between age and number of days in hospital (Rs = 0.283, n = 22, P = .2). The median number of days from admission until the body temperature was normalized (<37°C in the morning) was 7 (range, 1–17) in the young and 5 (range,
Figure 1. Levels of circulating proinflammatory and antiinflammatory cytokines during week 1 of *Streptococcus pneumoniae* infection and in healthy, age-matched controls. Medians and quartiles are shown. * Significant difference ($P<.05$) between young and elderly patients; † significantly increased levels ($P<.05$) in patients on day 7 vs. controls. Young group (ages 37–55 years): Tumor necrosis factor (TNF-α): days 0 and 3, $n=9$; day 7, $n=10$; age-matched controls, $n=10$. Interleukin (IL)-1β: day 0, $n=9$; day 3, $n=8$; day 7, $n=10$; age-matched controls, $n=10$. IL-6: day 0, $n=6$; day 3, $n=5$; day 7, $n=7$; age-matched controls, $n=10$. MIP-1β: day 0, $n=10$; day 3, $n=8$; day 7, $n=10$; age-matched controls, $n=10$. Soluble TNF receptor-1 (sTNFR-1): day 0, $n=10$; day 3, $n=5$; day 7, $n=7$; age-matched controls, $n=10$. IL-1 receptor antagonist (IL-1RA): day 0, $n=10$; day 3, $n=8$; day 7, $n=10$; age-matched controls, $n=10$. IL-10: day 0, $n=6$; day 3, $n=5$; day 7, $n=6$; age-matched controls, $n=9$. Neutrophils: day 0, $n=10$; day 3, $n=9$; day 7, $n=8$; age-matched controls, $n=10$. Elderly group (ages 68–90 years): TNF-α: day 0, $n=12$; days 3 and 7, $n=11$; age-matched controls, $n=12$. IL-1β: days 0 and 3, $n=12$; day 7, $n=11$; age-matched controls, $n=12$. IL-6: day 0, $n=11$; days 3 and 7, $n=10$; age-matched controls, $n=12$. MIP-1β: days 0 and 3, $n=12$; day 7, $n=11$; age-matched controls, $n=12$. sTNFR-1: days 0 and 3, $n=10$; day 7, $n=11$; age-matched controls, $n=12$. IL-1RA: days 0 and 3, $n=12$; day 7, $n=10$; age-matched controls, $n=12$. IL-10: days 0 and 3, $n=9$; day 7, $n=8$; age-matched controls, $n=10$. Neutrophils: day 0, $n=11$; day 3, $n=12$; day 7, $n=10$; age-matched controls, $n=12$. 

[Graphs showing cytokine levels for young and elderly groups with median and quartile bars, and significant difference markers (* and †).]
Discussion

The major findings of this study were that in adults with *S. pneumoniae* infection, regardless of age group, levels of TNF-α, IL-1β, IL-6, IL-1RA, IL-10, and sTNFR-I were markedly increased on day 0 and declined during subsequent days. Furthermore, we demonstrated a decreased rate of clearing inflammatory activity in the elderly patients: after 1 week of antibiotic treatment, circulating levels of TNF-α and sTNFR-I were increased in the elderly, compared with younger patients. TNF-α, IL-10, and IL-1RA had returned to normal levels after 1 week in younger patients but not in the elderly; the slopes of the decreases in sTNFR-I and in the TNF-α/IL-10 ratio from days 0 to 7 were correlated with patient age. Accordingly, aging is associated with prolonged inflammatory activity during the course of treatment for a bacterial infection. Possible explanations include that it takes longer for older persons to combat infection, regulatory/feedback inhibition pathways that control stimulated cytokine production become dysfunctional with age, and cytokine uptake of cells by receptors and cytokine breakdown in the blood by peptidases or elimination by the kidneys decrease with age.

Decreased ability to clear bacterial infections may be related to immunosenescence [12], as described in rodent models [13]. Alterations in cytokine production in the elderly may be a primary defect or secondary to age-associated changes in T lymphocyte subsets. Previous reports of increased production of proinflammatory cytokines after in vitro stimulation for 72 h [14, 15] are in accordance with the present findings of increased circulating levels of TNF-α and sTNFR-I in vivo after 1 week of treatment of a bacterial infection.

With regard to the initial levels of circulating mediators in the acute-phase response, serum concentrations of TNF-α, granulocyte-macrophage colony-stimulating factor (CSF), granulocyte CSF, IL-8, and MIP-1α were lower during the acute phase in elderly than in young persons with pneumonia caused by different bacterial species [9]. In the present study, TNF-α, IL-1β, MIP-1β, IL-1RA, sTNFR-I, and IL-10 were not significantly decreased on hospital admission, in contrast to IL-6. The reason for these discrepancies may be that more subjects were included and the age difference was larger between the young and the elderly groups in the study by Gon et al. [9].

Increased inflammatory activity after 1 week in elderly patients was not reflected by the clinical outcome measured as number of days in hospital or until normalized body temperature. However, the number of days in hospital may be an insensitive parameter affected by other factors in addition to the infection. It is also questionable whether clearance of fever is a good parameter in the present context, because it is possible that elderly persons have a blurred fever response.

In conclusion, aging is associated with prolonged inflammatory activity with regard to levels of circulating proinflammatory and antiinflammatory cytokines in humans during an infection with *S. pneumoniae*. This may reflect decreased ability to control the infection and/or dysregulation of the production/elimination of cytokines. Prolonged inflammatory activity may be of importance for the increased morbidity and mortality with increasing age from this type of infection.

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References