Cytokine Antagonists in Aged Subjects and Their Relation With Cellular Immunity

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Host responses to infectious and inflammatory stimuli are altered with aging. Because cytokines and their antagonists are significant factors in these host responses, the present research on aged subjects was designed to investigate plasma concentrations of the cytokines interleukin 1β (IL-1β) and tumor necrosis factor α (TNFα) and those of their antagonists IL-1 receptor antagonist (IL-1ra) and soluble TNF receptor (sTNFr). For this research, 122 apparently healthy aged subjects (79.6 ± 5.8 yr), 39 aged individuals with documented urinary tract infections (UTIs) (81.6 ± 6.3 yr), and 100 young controls (39.3 ± 11.2 yr) were included. Plasma IL-1β, TNFα, IL-1ra, sTNFr (55 kDa), and neopterin were measured using enzyme-linked immunosorbent assay techniques. In subsets of normal aged subjects and UTI patients, we investigated relations between plasma concentrations of cytokine antagonists and IL-2 production by phytohemagglutinin-stimulated peripheral blood mononuclear cells. The results show that plasma concentrations of both IL-1ra and sTNFr were greater in healthy aged subjects than in young controls. Plasma neopterin, a product of activated monocytes/macrophages, was likewise elevated in the aged. IL-1 and TNF were not detectable in the majority of plasma samples. There was a positive correlation between neopterin concentration and both IL-1ra and sTNFr. There was a significant negative correlation between plasma IL-1ra and IL-2 production by phytohemagglutinin-stimulated peripheral blood mononuclear cell in healthy aged subjects. IL-1ra and sTNFr concentrations were significantly greater in patients with UTI than in the healthy aged subjects. In UTI patients IL-2 production in vitro was lower than in healthy subjects, but there was no significant correlation with IL-1ra in plasma. Therefore, plasma concentrations of cytokine antagonists are increased in plasma of apparently healthy aged subjects. Elevated concentrations of neopterin suggest that this increase can be traced to monocyte activation. The negative correlation between plasma IL-1ra and IL-2 production in vitro suggests that enhancement of this cytokine antagonist can contribute to immunodepression of aging. We propose that unapparent infections in aged subjects cause monocyte activation and release of cytokine antagonists. These cytokine antagonists reduce IL-2 production and the capability of T cells to proliferate, thereby inhibiting immune responses in the elderly.

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(Seckinger et al., 1989). TNFα receptor shedding from cells is induced by several stimuli including TNF itself. Soluble TNF receptors bind TNF and counteract effects of the cytokine. They increase in the circulation in autoimmune, infectious, and chronic inflammatory states (Catania et al., 1994a).

The balance between cytokines and cytokine antagonists can be altered when either the cytokine or its agonist is generated in excess, as in patients with septic shock (Exley et al., 1990), cachexia (Beutler et al., 1988), cancer (Balkwill et al., 1987), long-term hemodialysis (Dinarello and Wolff, 1993), or AIDS (Lahevirta et al., 1988; Catania et al., 1994b).

The aim of the present research was to investigate plasma concentrations of IL-1β and TNFα and those of their antagonists IL-1ra and sTNFr in aged subjects. After we found that cytokine antagonists were elevated in plasma of apparently healthy aged subjects, the question was whether these molecules can interfere with immune responses. T lymphocyte proliferation is driven by interactions between cytokines and cells of the immune system. IL-1 induces production of IL-2 by CD4+ T lymphocytes and constitutes the first signal for T cell proliferation. IL-2 binds to specific receptors on T lymphocytes and sustains proliferation of these cells. Because IL-1ra reduces mitogen-stimulated T cell proliferation in vitro, we investigated relations between plasma concentration of this cytokine antagonist and IL-2 production by mitogen-stimulated peripheral blood mononuclear cells (PBMC). In view of the elevations in cytokine antagonists in the aged group we wondered if the values are maximal in the aged or if they can be increased further. To answer this question we measured IL-1ra and sTNFr in aged patients who had documented urinary tract infections (UTIs) and had, therefore, a naturally occurring inflammatory stimulus.

METHODS

Normal elderly subjects (n = 122) of mean age 79.6 ± 5.8 yr (46 males and 76 females) were included in the study. Two groups of 52 and 20 subjects were institutionalized, whereas a third group of 50 individuals was followed in an outpatient geriatric clinic. Admission criteria were based on SENIEUR protocol (Ligthart et al., 1984). There were no significant demographic differences among these groups.

To determine if plasma cytokine antagonist values were already maximal in aged subjects, patients with UTI (30 subjects: 20 females and 10 males, aged 82 ± 6.4 yr) were studied. UTI was identified on the basis of the clinical history and documented through urine culture. Renal function was normal as estimated from creatinine clearance (Cockcroft and Gault, 1976).

Control blood samples were obtained from 100 healthy blood donors (76 males and 24 females; 39.32 ± 11.2 yr). Blood (20 ml) was collected from an antecubital vein in vacutainer tubes containing EDTA at 0900 h ± 30 min after an overnight fast. The samples were immediately centrifuged, aliquoted, and stored at −70 °C.

IL-2 production in vitro. — We measured IL-2 production by PBMC in the last 16 of the 122 normal subjects and in a further 9 patients with UTI infection. PBMC were isolated from heparinized blood by density centrifugation through Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO). Cells were washed twice in sterile phosphate-buffered saline (PBS) and then incubated in polypropylene tubes (5 ml) at a density of 1 × 10⁶ cells/ml for 24 h in RPMI 1640 medium (GIBCO/BRL, Paisley, U.K.) supplemented with 10 mM Hepes (Sigma), 2 mM L-glutamine (GIBCO), 10% fetal calf serum (GIBCO), 100 units/ml penicillin (ICN Flow, Costa Mesa, CA), and 100 μg/ml streptomycin (ICN Flow). Cells were incubated in 5% CO₂ atmosphere at 37 °C in the presence or absence of 65 μg/ml phytohemagglutinin (PHA) (GIBCO). After a 24-h incubation, samples were centrifuged and supernatants separated and stored at −70 °C for IL-2 assay.

Assays. — IL-1β, IL-2, TNFα, IL-1ra, and sTNFr (55 kDa) were determined using enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Neopterin was also measured by ELISA (Henning Berlin GMBH, Berlin, Germany). Cortisol in unextracted plasma was determined using a solid phase radioimmunoassay kit (Sorin Biomedica, Saluggia, Italy); adrenocorticotropic hormone (ACTH) was measured using an immunoradiometric method (Nichols Institute, San Juan Capistrano, CA).

Statistical analysis. — We have performed an omnibus analysis of variance (ANOVA) including 10 parameters: age, IL-1ra, sTNFr, ACTH, cortisol, neopterin, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), α₂-globulin, and body temperature in aged healthy versus aged UTI patients. Further, we have performed an ANOVA for IL-1ra, sTNFr, ACTH, and cortisol to compare young controls, aged healthy, and aged UTI. ANOVA was followed by a Tukey’s protected t-test for comparisons of specific means (GB-STAT, Dynamic Microsystems, Silver Spring, MD). Pearson product–moment correlation or Spearman rank order correlation coefficients were calculated to determine the significance of relations among measures.

RESULTS

Plasma concentrations of IL-1ra and sTNFr were greater in aged subjects (Figure 1), and there was a significant correlation between concentrations of the two cytokine antagonists in old (r = .450; p < .01) but not in young (r = .077; p = 0.5) individuals. IL-1 and TNF were not detectable in plasma samples of either age group. Aged subjects had elevated plasma neopterin concentrations (3.17 ± 1.1 ng/ml, mean ± SE; normal 1.4 ± 0.6), and there was a significant correlation between neopterin and IL-1ra (Figure 2). Plasma ACTH and cortisol were similar in aged and young individuals (26.4 ± 1.9 vs 23.9 ± 1.3 pg/mI, and 15.6 ± 1.1 vs 15.9 ± 0.7 μg/dl, respectively), and there was no significant correlation between concentrations of cytokine antagonists and those of pituitary-adrenal hormones.

To investigate the significance of elevated concentrations of cytokine antagonists in aged subjects, we analyzed correlations between IL-2 production by PHA-stimulated PBMC and plasma concentrations of IL-1ra or sTNFr in 16 consecutive aged subjects. There was a significant negative correlation between production of IL-2 in vitro and plasma
Figure 1. Plasma concentrations of cytokine antagonists were greater in healthy aged subjects than in young controls. Bars denote mean ± SE; **p < .01.

Figure 2. There was a significant correlation between plasma IL-1ra and plasma neopterin in both normal aged subjects and UTI patients (Spearman rank order correlation coefficients: r = .73; p < .01 and r = .61; p < .01, respectively). There was a significant correlation between sTNFr and neopterin in UTI patients (r = .72; p < .01) but not in normal aged subjects (r = .004; p > .05).

Figure 3. There was a significant negative correlation between plasma concentration of IL-1ra and IL-2 production by PHA-stimulated PBMC in vitro whereas there was no statistical correlation between sTNFr and IL-2 production (Spearman rank order correlation coefficients: r = -.66; p < .01 and r = .09; p > .05, respectively).

Figure 4. Aged patients with bacterial infections had significantly greater concentrations of both IL-1ra and sTNFr than old healthy subjects who did not have demonstrated infections. In the UTI group, plasma neopterin was 5.9 ± 1.42 ng/ml; ACTH, 56.2 ± 15 pg/ml; and cortisol, 28.6 ± 5.4 μg/dl. Both IL-1ra and sTNFr were significantly correlated with plasma neopterin (Figure 2) but only sTNFr was correlated with ACTH (r = .804) and cortisol (r = .595; p < .01). sTNFr was likewise correlated with other markers of the acute phase response, including ESR (r = .465) and WBC (r = .581; p < .01). TNF and IL-1 were undetectable or near the limit of detection in the majority of patients with UTI. The values for the acute phase protein α2-globulin and ESR were higher in patients with UTI than in normal aged subjects (α2-globulin, 9.8 ± 0.6 g/L vs 6.2 ± 0.5; ESR, 44.4 ± 4.9 vs 22.4 ± 2.1 mm, respectively). To learn
whether there is a correlation between plasma IL-1ra and IL-2 production also during infection, we measured IL-2 production by PHA-stimulated PBMC in nine further patients with UTI infection. Although in UTI patients there was no significant correlation between plasma IL-1ra and IL-2 production in vitro (r = .256; p > .05), IL-2 production was lower compared to normal aged subjects (388 ± 105 pg/ml vs 908 ± 176; p = .03).

**DISCUSSION**

Plasma concentrations of the cytokine antagonists IL-1ra and sTNFr were elevated in apparently healthy aged subjects. Because there was a negative correlation between plasma concentrations of IL-1ra and IL-2 production by PBMC of aged subjects in vitro, it may be that elevation of this cytokine antagonist contributes to immunodepression of aging. Aged patients with UTI had even greater concentrations of cytokine antagonists, which indicates that infectious/inflammatory stimuli can further enhance production of these molecules.

An important question concerns the basis for the increased concentrations of cytokine antagonists in aged subjects. Because renal function was normal in all subjects, reduced clearance should not be involved. Rather, increased plasma neopterin suggests that there was an ongoing immune activation. Neopterin, produced and secreted by monocytes/macrophages activated by interferon-γ, is considered a marker of cell-mediated immunity (Fuchs et al., 1988). Increased neopterin concentrations occur in early stages of T cell/macrophage interactions. Its concentrations in plasma are used to predict disease progression in several conditions including human immunodeficiency virus (HIV) infection, cancer, and lymphoproliferative disorders (Diamondstone et al., 1994). Although clinical infections were excluded, it may be that repeated minor infections caused monocyte activation and increased production of cytokines/cytokine antagonists in the apparently healthy subjects.

Production of proinflammatory cytokines can be enhanced in aged subjects (Fagiolo et al., 1993). Mitogen-stimulated mononuclear cells of healthy aged subjects produced more IL-6, TNFα, and IL-1β, whereas spontaneous production and plasma concentrations of these cytokines did not differ from those of young controls (Fagiolo et al., 1993). Similarly, in the present research, plasma TNF was not increased in plasma samples of aged subjects. However, it should be noted that the currently available ELISA assays, including the one we used, cannot detect TNFα that is bound to sTNFr. Therefore, it may be that enhanced levels of TNF are buffered and masked by elevated sTNFr. In this regard, it is interesting that detectable TNF in plasma was a predictor of early mortality in elderly institutionalized patients (Mooran-dian et al., 1991). This further suggests that sTNFr protects against excessive TNF when TNF is not bound to its soluble receptor and it is detectable in the circulation, it can have detrimental effects.

Measurements of cytokine antagonists in aged subjects with UTI showed even greater plasma concentrations of these mediators. It appears, therefore, that production of these molecules in clinically normal aged people is not maximal but can be further increased by inflammatory stimuli. In patients with UTI, plasma concentrations of IL-1ra were correlated with neopterin levels. Thus, release of this cytokine antagonist appears to be commensurate with the degree of monocyte activation during infection. On the contrary, there was a significant correlation between sTNFr and markers of the acute phase response, including ESR, WBC, and pituitary/adrenal axis hormones. It appears that release of sTNFr is part of a general host response to infectious or inflammatory stimuli, much as other acute phase proteins.

There are several age-related defects in T cell activation and proliferation. Characteristically, T cell response to in vitro stimulation is diminished in the aged. This has been traced, at least in part, to a reduction in IL-2 secretion and high-affinity IL-2 receptor expression (Froelich et al., 1988). IL-1ra inhibits IL-2 production and T cell proliferation in vitro (Dinarello and Wolff, 1993). Further, experiments in mice have shown that IL-1ra decreases severity of graft-versus-host disease, improves survival of cardiac allografts, and reduces severity of experimental autoimmune encephalomyelitis. In the present research, subjects with the greatest concentrations of IL-1ra had lower production of IL-2 by mitogen-stimulated PBMC. These observations suggest that elevated concentrations of IL-1ra can have inhibitory influences on cell-mediated immunity.

In conclusion, the data show that plasma concentrations of cytokine antagonists are increased in plasma of apparently healthy aged subjects. Because there were also elevated concentrations of neopterin it is reasonable to believe that, despite lack of clinical infection, increased cytokine antagonists are associated with chronic monocyte activation. The negative correlation between plasma IL-1ra and IL-2 production in vitro suggests that enhancement of this cytokine
antagonist can contribute to immunodepression of aging. We propose that repeated, perhaps unapparent, infections in aged subjects cause monocyte activation and release of cytokine antagonists that inhibit IL-2 production and the capability of T cells to proliferate.

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