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Monocyte Cytokine Production in an Elderly Population: Effect of Age and Inflammation

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Objective. To determine the association among aging, inflammation, and cytokine production by peripheral blood mononuclear cells.

Population and Methods. We examined production of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-1 receptor antagonist (IL-1Ra), and IL-6 in 711 elderly participants in the Framingham Heart Study (mean age, 79 y) and 21 young healthy volunteers (mean age, 39 y). The elderly subjects were categorized by serum C-reactive protein (CRP) concentration, a marker of systemic inflammation.

Results. Production of IL-6 ($p < .00001$) and IL-1Ra ($p < .00001$) was higher in the elderly subjects than in the control group. IL-6 production increased with increasing CRP, whereas IL-1RA was uniformly elevated in elderly subjects regardless of CRP. However, we found no difference in the production of IL-1β or TNF-α between the young and elderly groups, regardless of CRP status. IL-6 population correlated with IL-1β ($r = .36$, $p < .0001$) and TNF-α production ($r = .25$, $p < .0001$), but IL-1Ra production did not.

Conclusion. Production of IL-6 and IL-1Ra — but not IL-1β or TNF-α — was increased in the elderly compared to healthy, young subjects. The increase in IL-6 also correlated with increased production of CRP, a marker of inflammation. However, IL-1Ra was increased in the elderly independently of CRP production. Although limited by the small control group, these data suggest that dysregulation of some inflammatory cytokines occurs with age, but the role of inflammation in aging remains unclear.

IT is unclear how aging affects the production of the mononuclear cell cytokines interleukin-1β (IL-1β), cachectin/tumor necrosis factor-α (TNF-α), IL-1 receptor antagonist (IL-1 Ra), or IL-6 (1–7). IL-1β and TNF-α, are of special interest in the elderly because they induce IL-6 production and because they have profound effects on body metabolism, body composition, and the acute phase response (8–13) — all of which are altered with age. These two cytokines are capable of causing many of the changes seen in aging, such as the increase in the erythrocyte sedimentation rate and other markers of the acute phase response (14–17), reduced serum albumin (18,19), and increased circulating IL-6 found in apparently healthy elderly subjects (15,20–23). We have shown that IL-1β and TNF-α regulate energy expenditure in humans during inflammation (24), and we and others have shown that both cytokines are associated with cachexia (reviewed in ref. 25). These effects are especially important in the elderly, where we and others have shown that low lean body mass (cachexia) is an important biomarker of physiologic status and a major predictor of survival, strength, and functional status (26–29).

The role of IL-6 in inflammation differs from that of IL-1β and TNF-α. On the one hand, IL-6 is elevated in a variety of inflammatory situations and can cause cachexia in animal models (30). IL-6 has also been shown to have an important effect on bone resorption and has been implicated in the development of osteoporosis in animal models, although human data are less clear (31–33). However, IL-6 does not cause hypotension or inflammatory symptoms when infused into humans at doses 10,000-fold greater than those of IL-1β or TNF-α (34). Instead, IL-6 induces high levels of IL-1Ra (35), suppresses IL-1β-induced cyclooxygenase (36), and suppresses gene expression and synthesis of inflammatory cytokines (37). Mice deficient in the IL-6 gene have higher levels of TNF-α than control mice and develop more prolonged arthritis in response to intraarticular IL-1β than do controls (38,39). IL-6 causes production of acute phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA), and along with IL-1β and TNF-α suppresses albumin gene transcription (40). IL-6 production is increased in the elderly (6,22) and is suppressed by both estrogen and androgens (41–43), suggesting that one explanation for the age-associated increase in IL-6 is the loss of inhibition by the sex steroids with age.

IL-1Ra is a member of the IL-1β gene family, which is structurally similar to IL-1β but has no agonist activity. IL-1Ra binds to IL-1β receptors without activating them, and it prevents IL-1β from binding (11). In healthy young subjects, IL-1Ra is not present in plasma or peripheral

blood mononuclear cells (PBMC), and injection of IL-1Ra at doses 1 million-fold greater than IL-1β leads to no agonist effects (43). IL-1Ra is produced in response to the same stimuli that lead to IL-1β production, such as endotoxin (44). Thus, IL-1Ra represents a potentially important counterregulatory response to inflammation.

Different studies have found that TNF-α and IL-1β are increased (5,6,20,22,23), decreased (1,6,7), or unchanged (2-4,33) with age. These studies differ in the species studied (rodent or human), the manner of cytokine measurement (bioassay, immunoassay, ELISA), and the matrix in which it is measured (urine, plasma, serum, or PBMC production). In addition, the human studies are all relatively small, ranging in size from 13 to 40 subjects. Finally, no study has examined the balance between cytokine agonists and their naturally occurring antagonists. To examine this issue further, we studied inflammatory cytokine production in ambulatory elderly participants in the Framingham Heart Study (FHS) and in healthy younger controls from Framingham, MA.

Methods

Study population. — The FHS began in 1948 with 5500 residents of Framingham, MA, who were recruited and followed with biennial examinations. Of the original cohort, approximately 1000 survive. Of these, the 862 (541 women and 321 men) who remain ambulatory donated blood for analysis in cycle 22 of the study. Sufficient blood was available for cytokine analysis in 711–742 subjects, depending on the cytokine and level of stimulation. Although nonambulatory and institutionalized subjects were contacted by home or nursing home visit, they are not included in the present data because blood could not be transported to the laboratory quickly enough for cell culture. In addition, 21 healthy adults under age 55 employed at FHS generously donated blood to act as controls. Control subjects were not taking any medications or dietary/vitamin supplements. The control subjects were studied over the 2 years of data collection from the FHS participants to avoid secular differences between elderly and control groups. The protocol was approved by the Institutional Review Board of the FHS and the Human Investigation Review Committee of Tufts University/New England Medical Center.

Cell isolation and culture. — Twenty milliliters of blood (17 mL in heparinized tubes and 3 mL for serum) were drawn from the Framingham subjects into heparinized tubes between noon and 1 p.m. Monday through Thursday and transported by courier from Framingham to Tufts Human Nutrition Research Center on Aging in downtown Boston. Subjects were not fasting prior to venipuncture. The mean time between drawing and beginning of PBMC isolation was 75 min (range, 35–120 min). PBMC were isolated by Ficoll-Hypaque centrifugation as described (24). Cells were washed three times in sterile, pyrogen-free saline (45,46). Cells were counted in a hemocytometer and suspended at 5×10^6/mL in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) which had been subjected to ultrafiltration (47) to remove cytokine-inducing substances. Ultrafiltered RPMI 1640 medium was supplemented with 100 μg/ml streptomycin and 100 U/mL penicillin (Sigma), and contained 1% autologous heat-inactivated serum and 1% L-glutamine (GIBCO, Grand Island, NY) (24,45). Cells were cultured in 96-well flat-bottom plates; each well contained 0.1 mL of cells plus 0.1 mL of one of the following: RPMI 1640 medium (unstimulated culture); *E. scherichia coli* lipopolysaccharide (LPS; B55:0, Sigma) at 1 or 100 ng/mL in RPMI 1640 medium; heat-killed *Staphylococcus epidermidis* in RPMI 1640 medium used at a 20:1 ratio of bacteria to target cells; or 100 μg/ml phytohemagglutinin (PHA, Sigma) in RPMI 1640 medium. After 22 h at 37 °C in 5% CO_2, the plates were frozen at −80 °C. Plates were thawed and frozen three times to lyse the cells.

Cytokine assays. — Measurement of total IL-1β, IL-1Ra, IL-6, and TNF-α synthesis was carried out in duplicate by specific, non-cross-reacting radioimmunoassays as described (45,48–50). The precursor form of IL-1β was measured using a commercially available ELISA according to the manufacture's instructions (Cistron Biotechnology, Pine Brook, NJ). Precursor IL-1β was measured only in cells stimulated with 1 ng/mL of LPS. Dilutions of the lysates from stimulated cells were made to obtain measurements in the 40–70% portion of the standard curves. Unstimulated cell lysates were assayed undiluted. The interassay variability for samples was <10%, and the intraassay variability was <5% for all cytokines. Lysates from elderly and control subjects' cells were measured in the same assays.

Other assays. — CRP was measured using an immunoprecipitation assay (Incstar Corp., Stillwater, MN) run on a COBAS FARA instrument (Roche Diagnostics, Nutley, NJ) according to the manufacturers' instructions. The sensitivity of the assay is 1.0 μg/dL, and the coefficient of variation is 9.2% within assay and 7.7% between assays. Data below the detection limit are reported as zero; detectable levels are reported to the nearest μg/dL. Complete blood counts were performed using a Serono-Baker 9000 cell counter (Serono Laboratories, Norwell, MA). Determination of standard serum chemistry measurements was performed using a COBAS FARA instrument. All assays were performed at the Nutrition Evaluation Laboratory of the Human Nutrition Research Center on Aging under established laboratory protocols.

Statistical analysis. — The data were examined graphically, and a logarithmic transformation was applied to positively skewed variables (IL-1β, TNF-α, and IL-6) prior to formal analysis in order to normalize their distribution. Data are reported as means and standard deviations. The elderly group was first compared *in toto* to the young controls and then divided into four groups based on serum CRP concentrations of undetectable (called zero, 66% of the population), 1 (7.9%), 2 (5.5%), or >2 mg/L (14.2%). Analysis of variance (ANOVA) techniques were used to assess differences between groups, and linear contrasts were used to test for trend. To test for an effect of age independent of inflammation, Student's *t*-test for independent samples was used to compare the elderly subjects with undetectable CRP to

the young subjects. Differences were considered statistically significant when the observed of two-tailed significance level (p-value) was $p < .05$. All calculations were performed using SYSTAT for Windows; version 5.03 (SPSS, Inc., Chicago, IL).

Results

Study population. — Sufficient blood was drawn for cytokine analysis in between 711 and 742 elderly subjects, depending on the cytokine and level of stimulation; the number of subjects analyzed for each endpoint is shown. The mean age of the elderly participants was 78 y (range, 69–93 y). Clinical laboratory parameters of the subjects are shown in Table 1.

Young, healthy control subjects from Framingham were also recruited (Table 1). These were 21 men and women employees of the Framingham Study, whose mean age was 39.3 y (range, 22–54 y). Although not members of the Framingham cohort, the control group is drawn from people who live in the same geographic area and have a similar racial, ethnic, and socioeconomic background as the elderly group. CRP levels were <1 mg/L in all control subjects.

IL-6 production. — Production of IL-6 by unstimulated cells was higher in the elderly subjects compared to controls [3.3 ± 2.4 ng/mL (n = 730) vs 2.0 ± 1.8 ng/mL, $p < .002$]. However, IL-6 production in response to ex vivo stimulation with PHA did not differ between elderly and young subjects [7.0 ± 2.0 ng/mL (n = 731) vs 5.8 ± 1.7 ng/mL, $p < .11$]. The increase in IL-6 after PHA stimulation was similar in all age and CRP groups (median change, 3.1–3.5 ng/mL, $p < .001$ vs zero change). In the elderly, unstimulated IL-6 production increased with increasing CRP (linear trend, $p < .0001$, Figure 1). In pairwise comparisons (adjusted for multiple comparisons) within the elderly population, IL-6 was higher in the two highest CRP groups compared to the group without detectable circulating CRP (Elderly/0 vs Elderly/1–2, $p < .044$; Elderly/0 vs Elderly/>2, $p < .003$, Tukey's HSD test). When the effect of age in the absence of laboratory evidence of systemic inflammation was tested by comparing the young control subjects with the elderly subjects who had no detectable circulating CRP (n = 499), the difference between these two groups was also significant ($p < .034$, Student's t-test). In contrast, there was no increase in PHA-stimulated IL-6 production with age or increasing CRP (Figure 1). Within the elderly subjects, unstimulated IL-6 production correlated with unstimulated IL-1 production (r = 36, $p < .0001$) and to a lesser extent with unstimulated TNF-α production (r = .25, $p < .0001$). Within the young subjects, similar but stronger correlations were seen between IL-6 production and IL-1β production (r = .69, $p < .001$) and TNF-α production (r = .78, $p < .0001$).

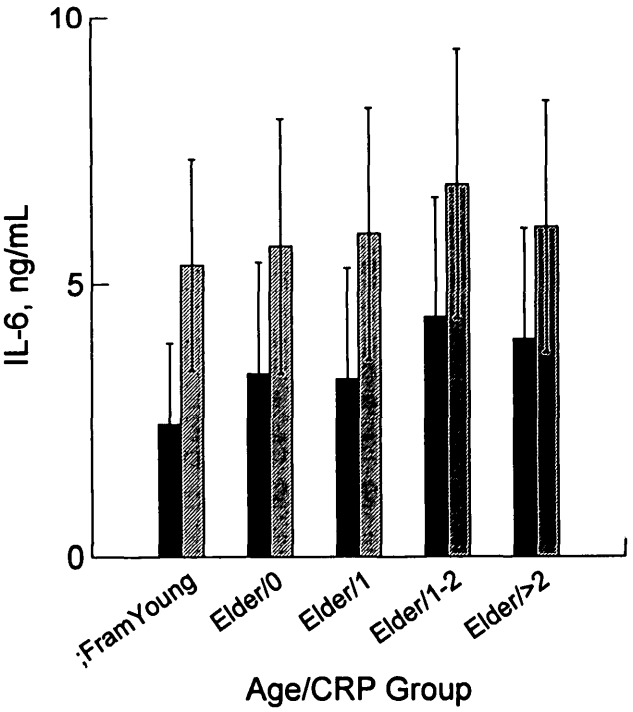


Figure 1. Production of IL-6 by PBMC from young and elderly subjects at four levels of serum CRP under unstimulated conditions (RPMI 1640 medium and autologous heat-treated serum only, filled bars) and after ex vivo stimulation with PHA (hatched bars). ANOVA for trend $p < .0001$ for unstimulated; p = NS for PHA. Young versus Elderly/0 CRP group, $p < .20$; Elderly/0 versus Elderly/1–2, $p < .06$; Elderly/0 versus Elderly/>2, $p < .008$. Error bars indicate 1 *SD*.

Table 1. Demographic and Clinical Aspects of the Study Populations

	Framingham Young Control	Framingham Elderly				
		All	CRP = 0 μg/dL	CRP = 1 μg/dL	CRP = 2 μg/dL	CRP > 2 μg/dL
Number	21	854	575	69	44	166
Age, y (range)	39 (22–54)	78.6 (72–94)	78.6 (72–94)	78.1 (72–92)	78.0 (72–88)	78.6 (72–94)
Sex (% female)	81	63	64	69	61	56
Hemoglobin (g/dL)	13.5 ± 1.6	13.6 ± 1.4	13.8 ± 1.2	13.7 ± 1.3	13.4 ± 1.6	13.5 ± 1.4
Albumin (g/L)	44.0 ± 4.2	42.0 ± 3.8	42.2 ± 2.7	42.2 ± 4.5	41.4 ± 3.0	41.4 ± 3.8
C-reactive protein (CRP), median (range) (μg/dL)	0 (0–1)	.00	.00	1.0	2.0	5.5 (3–63)
Aspartate aminotransferase (AST) (U/L)	22.1 ± 5.8	23.0 ± 9.0	23.1 ± 9.0	23.5 ± 6.5	20.6 ± 6.2	22.7 ± 8.9
Creatinine (g/dL)	0.9 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.4

IL-1Ra. — Production of IL-1Ra was higher in the elderly group than in the young group both under unstimulated conditions [9.9 ± 1.9 ng/mL (n = 701) vs 1.0 ± 4.3 ng/mL, p < .0001], and after ex vivo stimulation with 1 ng/mL of LPS [14.5 ± 1.9 ng/mL (n = 710) vs 0.8 ± 4.4 ng/mL, p < .0001]. As shown in Figure 2, IL-1Ra was elevated in all four elderly Framingham subgroups, regardless of CRP level, when compared to the young control subjects (p < .0001). Stimulation with LPS did not increase IL-1Ra production in the young subjects, but did significantly increase it in all four elderly Framingham subgroups (median increase, 4 ng/mL, p < .0001). In contrast to IL-6, there was no correlation between IL-1Ra production and production of either IL-1β or TNF-α in either the young or elderly subjects. There was a very weak correlation between IL-1Ra and IL-6 production by unstimulated PBMC (r = .07, p < .06).

IL-1β and TNF-α production. — There were no differences in production of the agonist cytokines IL-1β or TNF-α between the young and elderly subjects either from unstimulated cells or after ex vivo stimulation with 1 ng/mL of LPS, 100 ng/mL of LPS, or heat-killed *S. epidermidis* (n = 814, p = .11–.66). After the elderly were categorized by CRP level, there was a weak trend toward increasing production of IL-1β under unstimulated conditions with increasing CRP (p = .006, Figure 3). No such trend was seen after cells were stimulated with LPS at 1 ng/mL or 100 ng/mL or with heat-killed *S. epidermidis*. TNF-α production also did not differ between young and elderly subjects under

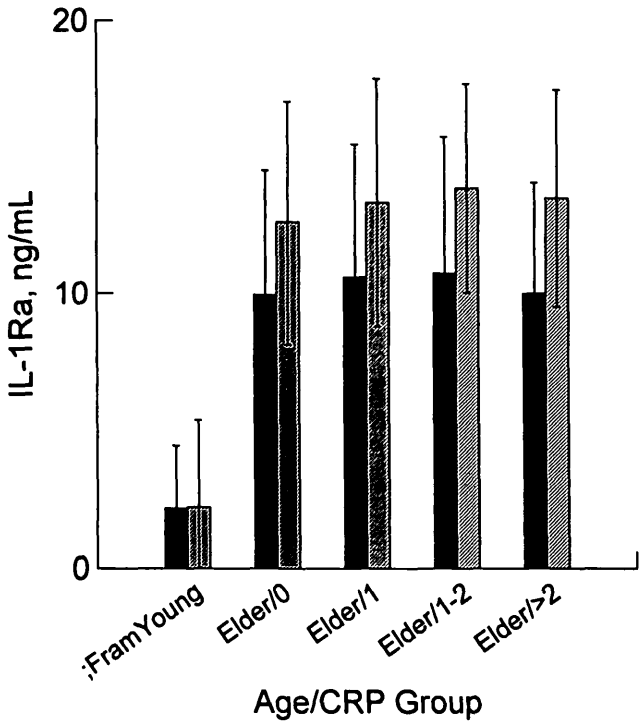
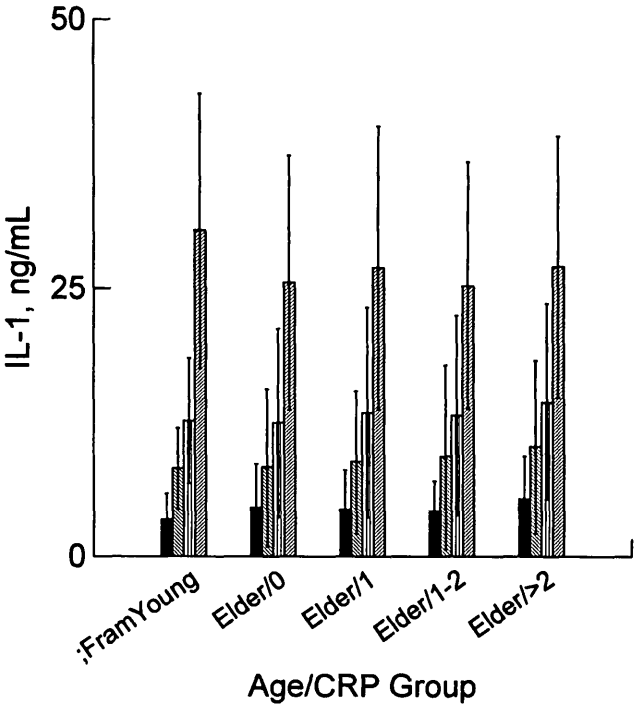


Figure 2. Production of IL-1Ra by PBMC from young and elderly subjects at four levels of serum CRP under unstimulated conditions (RPMI 1640 medium and autologous heat-treated serum only, filled bars) and after ex vivo stimulation with 1 ng/mL LPS (hatched bars). ANOVA for trend p < .0001 for both conditions.



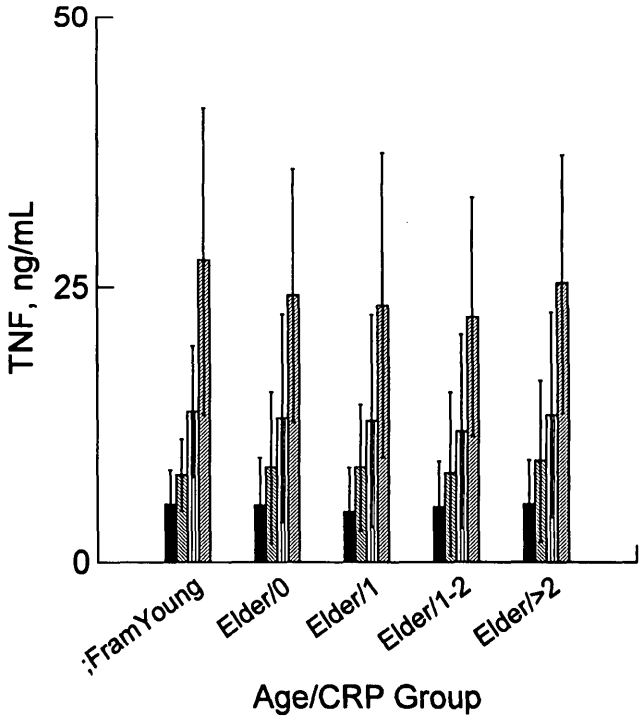


Figure 3. Production of IL-1β (top) and TNF-α (bottom) by PBMC from young and elderly subjects at four levels of serum CRP, under unstimulated conditions (open bars), or stimulation with 1 ng/mL LPS (left hatched bars), 100 ng/mL LPS (filled bars), or heat-killed *S. epidermidis* (right hatched bars). IL-1β, ANOVA for trend, p < .06 under unstimulated conditions only; p = NS under all other conditions. TNF-α, p = NS under all conditions.

any stimulation conditions, and there was no trend in TNF-α production with CRP level under any stimulation conditions (Figure 3). The dose-response of IL-1β and TNF-α production with escalating stimulation was well preserved in the elderly subjects, regardless of their CRP status.

Precursor IL-1β production. — There was no difference in the amount of pro-IL-1β produced in response to 1 ng/ml of LPS by elderly subjects, regardless of CRP status, compared to the young group [11.0 ± 1.0 ng/mL (n = 766) vs 8.3 ± 1.3 ng/mL, $p < .48$].

Discussion

The present study is, to our knowledge, the largest to date examining cellular production of inflammatory cytokines in an ambulatory elderly population. We found, as have others, that IL-6 production is increased in the elderly compared to young subjects (6,23). Compared to our young healthy controls, we found that IL-6 was elevated even in our elderly subjects without detectable CRP. The difference in IL-6 production between young and elderly subjects was greater in the elderly subjects with higher CRP levels. This suggests that IL-6 elevation in the elderly is related to both age and inflammation (as indicated by elevated CRP) and may not be purely a phenomenon of advancing age. Cells from elderly subjects showed a similar increment in IL-6 in response to PHA stimulation, although the proportional increase was somewhat higher in the young group (Figure 2). This observation suggests that the capacity to respond to an exogenous stimulus is not seriously impaired with age.

In addition, this study shows for the first time that production of IL-1Ra by PBMC from elderly subjects is increased with age. However, unlike IL-6, we saw no effect of CRP status on IL-1Ra production, as IL-1Ra was equally elevated in the elderly subjects regardless of CRP level. The finding of increased IL-1Ra production in the elderly regardless of CRP level (Figure 2) suggests that this counter-regulatory cytokine may be responding to an age-related, but not necessarily an inflammation-related, stimulus. With respect to the measurement of IL-1Ra, it should be noted that all cells were cultured in the presence of 1% autologous heat-treated serum to allow each culture to carry the individual's own microenvironment to the greatest degree possible. It is known that IgG in serum will induce the production of IL-1Ra but not IL-1β. Thus, these cultures do not represent truly unstimulated conditions for IL-1Ra and should not be interpreted as indicative of IL-1Ra production under basal conditions. Nonetheless, because the major outcome is a comparison of young versus old cells treated under the same conditions, we believe that IgG stimulation is acceptable. When cells were cultured in the presence of 1 ng/mL of LPS, a further increase in IL-1Ra production was seen only in elderly subjects, indicating that cells from elderly adults may be primed to make more IL-1Ra in response to stimulation than cells from young adults. Because the action of IL-1Ra inhibits the effects of IL-1β, we hypothesize that increased production of IL-1Ra in the elderly may be a novel mechanism of immunosuppression in the elderly. It is intriguing to speculate, for example, that loss of estrogen and androgen with age (41–43) leads to an increased production of IL-6, which stimulates inflammation and leads to a counter-regulatory response by IL-1Ra. Further studies are needed to understand the stimuli that lead to increased IL-1Ra in this population.

In contrast to the results with IL-6 and IL-1Ra, we found no age effect on production of IL-1β or TNF-α, and a very weak trend toward an increase in IL-1β production with increasing CRP. In this, our findings agree with those of some (1–4) but not all (5–7,20,22) previous studies. The increase in IL-1Ra production without a concommitant increase in agonist cytokine production would tend to tilt the agonist–antagonist balance in favor of the latter, which would be consistent with an immunosuppressive effect of aging.

An important limitation of the present study is that our elderly population included people taking medications and with a variety of chronic illnesses. Thus, these results cannot be said to represent the effects of aging per se in the absence of the effects of degenerative illnesses. However, our population is an ambulatory one, and all members of the cohort had to be able to come to the clinic for evaluation. As shown in Table 1, our elderly subjects had normal hepatic and renal function, suggesting that they were not severely ill. For example, only 4 subjects out of 862 had a serum albumin below 35 g/L. By classifying subjects according to their serum CRP levels, we attempted to discriminate between elderly subjects with laboratory evidence of a systemic inflammatory process from those without it.

A second limitation is the size of our control group. This group was chosen because of the importance of obtaining cells from control subjects under the same conditions of blood drawing and transport as the elderly group. Because of funding constraints, we were limited to subjects under age 55 available on site at Framingham not taking medications and with normal CRP values. Because a small control group should bias the study in favor of finding no difference, this limitation should not have an adverse effect on our conclusions regarding IL-6 and IL-1Ra, although it limits our power regarding age-related differences in IL-1β and TNF-α production.

The effect of age on mononuclear cell cytokine production has been unclear. Our results suggest that the effect of age on cytokine production is complex and that there is an important interaction between age and inflammation. Further, these data suggest that the effect of age is specific to each particular cytokine. Nevertheless, it appears that changes in production of several cytokines — including IL-2, IL-6, and IL-1Ra — occur with aging, and that increased IL-1Ra could play a role in the decline in inflammatory response that is seen in the elderly. The role of increased IL-6 production in aging also remains to be fully clarified. Whether this cytokine production profile represents a healthy adaptive response to aging or an age-related failure of immune regulation remains to be determined.

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References

1. Mooradian AD, Reed RL, Scuderi P. Serum levels of tumor necrosis factor alpha, interleukin-1 alpha and beta in healthy elderly subjects. *Age.* 1991;14:61–64.
2. Rudd AG, Banerjee DK Interleukin-1 production by human monocytes in ageing and disease. *Age Ageing.* 1989;18:43–6.
3. Bradley SF, Vibhagool A, Kunkel SL, Kauffman CA. Monokine secretion in aging and protein malnutrition. *J Leukocyte Biol.* 1989;45: 510–4.
4. Bradley SF, Vibhagool A, Fabrick S, Terpenning MS, Kauffman CA. Monokine production by malnourished nursing home patients. *Gerontology.* 1990;36:165–70.
5. Fagiolo U, Cossarizza A, Scala E, Fanales-Belasio E, Ortolani C, Cozzi E, Monti D, Franceschi C, Paganelli R. Increased cytokine production in mononuclear cells of healthy elderly people. *Eur J Immunol.* 1993;23:2375–8.
6. Effros RB, Svoboda K, Walford RL. Influence of age and caloric restriction on macrophage IL-6 and TNF production. Lymphokine *Cytokine Res.* 1991;10:347–51.
7. Namizu T, Chang M-P, Makinodan T. Influence of age on the production and regulation of interleukin-1 in mice. *Immunology.* 1985;55: 447–55.
8. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumor necrosis factor. *Lancet.* 1989;i:1122–5.
9. Beutler B, Cerami A. Cachectin (tumor necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response. *Endocr Rev.* 1988;9:57–63.
10. Kunkel SL, Remick DG. *Cytokines in health and disease.* New York: Marcel Dekker, Inc., 1992.
11. Dinarello CA, Wolff SM. The role of interleukin-1 in disease. *N Engl J Med.* 1993;328:106–13.
12. Reichlin S. Neuroendocrine-immune interactions. *N Engl J Med.* 1993;329:1246–53.
13. Zimmerman GA, Martin SB, Tracey KJ. The neuroimmunology of tumour necrosis factor-alpha. *Clin Immunother.* 1994;1:67–78.
14. Bedell SE, Bush BT. Erythrocyte sedimentation rate: from folklore to facts. *Am J Med.* 1985;78:1001–9.
15. Christou W, Tellado-Rodriguez J, Chartrand L, Giannas B, Kapadia B, Meakins J, Rode H, Gordon J. Estimating mortality risk in preoperative patients using immunologic, nutritional, and acute-phase response variables. *Ann Surg.* 1989;210:69–77.
16. Bottiger LE, Svedberg CA. Normal erythrocyte sedimentation rate and age. *Br Med J.* 1967;2:85–7.
17. Miller A, Green M, Robinson D. Simple rule for calculating normal erythrocyte sedimentation rate. *Br Med J.* 1983;286:266.
18. Rall LC, Roubenoff R, Harris TB. Albumin as a marker of nutritional and health status. In: Rosenberg IH, ed. *Nutritional assessment of elderly populations.* New York: Raven Press, 1995:1–18.
19. Salive ME, Cornoni-Huntley J, Phillips CL, Guralnik JM, Cohen HJ, Ostfeld AM, Wallace RB. Serum albumin in older persons: relationship with age and health status. *J Clin Epidemiol.* 1992;45:213–21.
20. Liao Z, Caucino JA, Schnipper SM, Stanley ER, Small CB, Rosenstreich DL. Increased urinary cytokine levels in the elderly. *Aging Immunol Infect Dis.* 1993;4:139–53.
21. Ernst DN, Weigle WO, Noonan DJ, McQuitty DN, Hobbs MV. The age-associated increase in IFN-gamma synthesis by mouse CD8+ T cells correlates with shifts in the frequencies of cell subsets defined by membrane CD44, CD45RB, 3G11, and MEL-14 expression. *J Immunol.* 1993;151:575–87.
22. Liao Z, Tu JH, Small CB, Schnipper SM, Rosenstrich DL. Increased urine interleukin-1 levels in aging. *Gerontology.* 1993;39:19–24.
23. Daynes RA, Araneo BA, Ershler WB, Maloney C, Li G-Z, Ryu S-Y. Altered regulation of IL-6 production with normal aging. *J Immunol.* 1993;150:5219–30.
24. Roubenoff R, Roubenoff RA, Cannon JG, Kehayias JJ, Zhuang H, Dawson-Hughes B, Dinarello CA, Rosenberg IH. Rheumatoid cachexia: cytokine-driven hypermetabolism and loss of lean body mass in chronic inflammation. *J Clin Invest.* 1994;93:2379–86.
25. Roubenoff R, Rall LC. Humoral mediation of changing body composition during aging and chronic inflammation. *Nutr Rev.* 1993;51: 1–11.
26. Roubenoff R, Kehayias JJ. The meaning and measurement of lean body mass. *Nutr Rev.* 1991;46:163–75.
27. Tzankoff SP, Norris AH. Longitudinal changes in basal metabolism in man. *J Appl Physiol.* 1978;45:536–9.
28. Frontera WR, Hughes VA, Lutz KJ, Evans WJ. A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. *J Appl Physiol.* 1991;71:644–50.
29. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, Evans WJ. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med.* 1994;330:1769–75.
30. Tsujinaka T, Fujita J, Ebisui C, Yano M, Kominami E, Suzuki K, Tanaka K, Katsume A, Ohsugi Y, Shiozaki H, Monden M. Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. *J Clin Invest.* 1996; 97:244–9.
31. Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Constantini F. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO J.* 1994;13:1189–96.
32. Kania DM, Binkley N, Checovich M, Havighurst T, Schilling M, Ershler WB. Elevated plasma levels of interleukin-6 in postmenopausal women do not correlate with bone density. *J Am Geriatr Soc.* 1995;43:236–9.
33. McKane WR, Khosla S, Peterson JM, Egan K, Riggs BL. Circulating levels of cytokines that modulate bone resorption: effects of age and menopause in women. *J Bone Min Res.* 1994;9:1313–8.
34. Dinarello CA, Cannon JG, Mancilla J, Bishai I, Lees J, Coceani F. Interleukin-6 as an endogenous pyrogen: induction of prostaglandin E2 in brain but not in peripheral blood mononuclear cells. *Brain Res.* 1991;562:191–203.
35. Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood.* 1994;83:113–8.
36. Hauptmann B, Van Damme J, Dayer JM. Modulation of IL-1 inflammatory and immunomodulatory properties by IL-6. *Eur Cytokine Netw.* 1991;2:39.
37. Aderka D, Le JM, Vilcek J. IL-6 inhibits lipopolysaccahride-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. *J Immunol.* 1989;143:3517–22.
38. Fattori E, Cappelletti M, Costa P, Sellitto C, Cantoni L, Carelli M, Faggioni R, Fantuzzi G, Ghezzi P, Poli V. Defective inflammatory response in interleukin-6 deficient mice. *J Exp Med.* 1994;180:1243– 50.
39. van de Loo FAJ, Arntz OJ, van den Berg WB. Role of IL-6 in IL-1 induced cartilage insult in mice. *Arthritus Rheum.* 1995;38(Suppl 9):S160.
40. Steel DM, Whitehead AS. The major acute phase proteins: C-reactive protein, serum amyloid P component, and serum amyloid A protein. *Immunol Today.* 1994;15:81–8.
41. Pottratz ST, Bellido T, Mocharla H, Crabb D, Manologas SC. 17-Beta-estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. *J Clin Invest.* 1994;93:944–50.
42. Lapp CA, Thomas ME, Lewis JB. Modulation by progesterone of interleukin-6 production by gingival fibroblasts. *J Periodontol.* 1995; 66:27–84.
43. Granowitz EV, Porat R, Mier JW, Pribble JP, Stiles DM, Bloedow DC, Catalano MA, Wolff SM, Dinarello CA. Pharmacokinetics, safety, and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. *Cytokine.* 1992;4:353–60.
44. Arend WP. Interleukin-1 receptor antagonist. *Adv Immunol.* 1993;54: 167–93.
45. Endres S, Cannon JG, Ghorbani R, Dempsey RA, Sisson SD, Lonnemann G, van der Meer JWM, Wolff SM, Dinarello CA. In vitro production of IL-1β, IL-1α, TNF, and IL-2 in healthy subjects: distribu-

tion, effect of cyclooxygenase inhibition and evidence of independent gene regulation. *Eur J Immunol.* 1989;19:2327–33.
46. Schindler R, Clark BD, Dinarello CA. Dissociation between interleukin-1β mRNA and protein synthesis in human peripheral blood mononuclear cells. *J Biol Chem.* 1990;265:10232–7.
47. Dinarello CA, Lonnemann G, Maxwell R, Shaldon S. Ultrafiltration to reject human interleukin-l-inducing substances derived from bacterial cultures. *J Clin Microbiol.* 1987;25:1233–8.
48. van der Meer JWM, Endres S, Lonnemann G, Cannon JG, Ikejima T, Okusawa S, Gelfand JA, Dinarello CA. Concentrations of immunoreactive human tumor necrosis factor-α produced by human mononuclear cells in vitro. *J Leukocyte Biol.* 1988; 43:216–23.
49. Cannon JG, van der Meer JWM, Kwiatkowski D, Endres S, Lonnemann G, Burke JF, Dinarello CA. Interleukin-1β in human plasma: optimization of blood collection, plasma extraction, and radioimmunoassay methods. *Lymphokine Res.* 1988;7:457–67.
50. Poutsiaka DD, Clark BD, Vannier E, Dinarello CA. Production of interleukin-1 receptor antagonist and interleukin-1beta by peripheral blood mononuclear cells is differentially regulated. *Blood.* 1991;78: 1275–81.

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