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Plasma Concentration of Interleukin-6 and the Risk of Future Myocardial Infarction Among Apparently Healthy Men

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Background—Interleukin-6 (IL-6) plays a central role in inflammation and tissue injury. However, epidemiological data evaluating the role of IL-6 in atherogenesis are sparse.

Methods and Results—In a prospective study involving 14 916 apparently healthy men, we measured baseline plasma concentration of IL-6 in 202 participants who subsequently developed myocardial infarction (MI) and in 202 study participants matched for age and smoking status who did not report vascular disease during a 6-year follow-up. Median concentrations of IL-6 at baseline were higher among men who subsequently had an MI than among those who did not (1.81 versus 1.46 pg/mL; $P=0.002$). The risk of future MI increased with increasing quartiles of baseline IL-6 concentration (P for trend <0.001) such that men in the highest quartile at entry had a relative risk 2.3 times higher than those in the lowest quartile (95% CI 1.3 to 4.3, $P=0.005$); for each quartile increase in IL-6, there was a 38% increase in risk ($P=0.001$).This relationship remained significant after adjustment for other cardiovascular risk factors, was stable over long periods of follow-up, and was present in all low-risk subgroups, including nonsmokers. Although the strongest correlate of IL-6 in these data was C-reactive protein ($r=0.43$, $P<0.001$), the relationship of IL-6 with subsequent risk remained after control for this factor ($P<0.001$).

Conclusions—In apparently healthy men, elevated levels of IL-6 are associated with increased risk of future MI. These data thus support a role for cytokine-mediated inflammation in the early stages of atherogenesis. (***Circulation***. 2000;101:1767-1772.)

Key Words: myocardial infarction ■ risk factors ■ inflammation ■ epidemiology ■ cytokines

Interleukin (IL)-6 is a pleiotropic cytokine with a broad range of humoral and cellular immune effects relating to inflammation, host defense, and tissue injury.[1,2] Produced in response to several factors, including infection, IL-1, interferon-γ, and tumor necrosis factor,[3–5] IL-6 is a central mediator of the acute-phase response and a primary determinant of hepatic production of C-reactive protein.[6,7]

Although elevated levels of IL-6 have been reported in some chronic inflammatory conditions,[2] epidemiological data evaluating the potential role of IL-6 in early atherogenesis are sparse. However, experimental studies indicate that vascular endothelial and smooth muscle cells from normal and aneurysmal arteries produce IL-6,[8–10] that IL-6 gene transcripts are expressed in human atherosclerotic lesions,[11,12] and that IL-6 may have procoagulant effects.[13–15] Furthermore, prospective studies of apparently healthy[16,17] as well as high-risk[18–20] individuals indicate that elevated levels of C-reactive protein, a potential surrogate for IL-6 activity,[21] are associ-

ated with first coronary and cerebrovascular events. Finally, elevated levels of IL-6 and other acute-phase proteins have been reported among patients with acute coronary syndromes,[22–25] even among those without overt plaque rupture or acute tissue trauma.[26]

On the basis of these data and the hypothesis that atherosclerosis fundamentally represents a chronic inflammatory disorder,[27,28] we sought to determine whether plasma levels of IL-6 might be elevated among apparently healthy individuals at risk for future myocardial infarction. We further sought to determine whether any relationship between IL-6 and subsequent vascular risk was modified by other cardiovascular risk factors, including markers of chronic inflammation.

Methods

We performed a prospective, nested, case-control study of IL-6 as a potential marker for future myocardial infarction among participants in the Physicians' Health Study, a randomized, double-blind, placebo-controlled trial of aspirin (325 mg PO every other day) and β-carotene (50 mg PO every other day) in the primary prevention of

TABLE 1.   Baseline Characteristics of Study Participants

	Controls (n=202)	Cases (n=202)	P
Age, y	59.1±8.8	59.2±8.8	...
Smoking status, %			
Never	44.3	44.3	
Past	40.3	40.3	
Current	15.4	15.4	
Body mass index, kg/m^2	24.9±3.0	25.7±3.2	0.02
History of hyperlipidemia, %	8.8	15.8	0.04
Total cholesterol, mg/dL	212.6±34.6	227.0±40.5	0.001
HDL cholesterol, mg/dL	51.0±13.9	45.9±13.7	0.001
Triglycerides, mg/dL	145.4±88.2	200.8±153.2	0.001
History of hypertension, %	18.9	26.4	0.07
Blood pressure, mm Hg			
Systolic	127.5±12.0	130.4±12.9	0.03
Diastolic	79.4±6.8	80.7±8.5	0.1
Family history of CAD, %	13.9	18.2	0.2
Diabetes, %	2.5	4.5	0.3
Alcohol use, %			0.3
Daily	27.7	21.3	
Weekly	41.1	50.5	
Monthly	12.4	11.9	
Rarely/never	18.8	16.3	
Exercise >1/week, %	72.2	65.7	0.2

CAD indicates coronary artery disease.

cardiovascular disease and cancer conducted among 22 071 US male physicians 40 to 84 years of age with no prior history of myocardial infarction, stroke, transient ischemic attack, or cancer.[29] Before randomization, between August 1982 and December 1984, potential participants were asked to provide baseline blood samples, which were collected in EDTA and processed for long-term storage at −80°C. Of randomized study participants, 14 916 (68%) provided baseline plasma samples. At the time of blood collection, all study participants were taking active oral aspirin.

For cases of incident myocardial infarction reported during study follow-up, hospital records, death certificates, and autopsy reports were reviewed by a committee of physicians using standardized criteria to confirm or refute reported events. Reported myocardial infarction was confirmed if symptoms met World Health Organization criteria and the event was associated with increased concentrations of diagnostic cardiac enzymes or characteristic ECG changes. Deaths due to coronary disease were confirmed on the basis of autopsy reports, circumstances of death, symptom patterns, and a history of coronary disease. Silent myocardial infarctions were not included, because they could not be dated accurately.

Study participants who provided an adequate baseline plasma sample for analysis and who had a confirmed myocardial infarction during follow-up (cases) were each matched with 1 control subject. Controls were study participants who also provided adequate baseline plasma samples and who remained free of reported vascular disease during follow-up. Controls were randomly selected from study participants who met the matching criteria of age (±1 year), length of study follow-up (6-month intervals), and smoking status (past smoker, current smoker, or never smoked). With these criteria, 202 cases and 202 matched controls were evaluated in this analysis.

Stored plasma obtained at baseline from each case and control subject was thawed and assayed for IL-6 by use of a commercially available ELISA (R&D Systems). In pilot data based on these frozen plasma samples, coefficients of variation ranged between 5% and
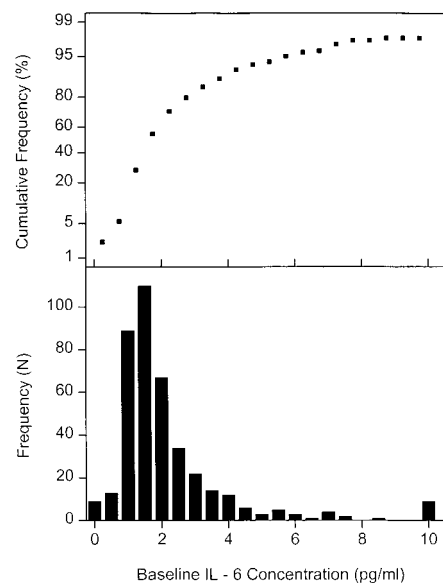

Figure 1. Frequency distribution and cumulative frequency of baseline Il-6 levels among apparently healthy men participating in the Physicians' Health Study.

11%, and repeat determinations on the same plasma sample were highly correlated ($r=0.93$, $P<0.001$). Blood specimens were analyzed in blinded pairs, with the position of the case specimen varied at random within pairs to reduce the possibility of systematic bias and minimize interassay variability. Methods used to evaluate baseline lipid parameters, homocysteine, fibrinogen, C-reactive protein, and tissue-type plasminogen activator have been described elsewhere.[16,30–32]

Because IL-6 levels were skewed, median concentrations were computed, and the significance of any differences in median values between cases and controls was assessed by Wilcoxon rank-sum test. We used logistic regression analysis, conditioned on the matching variables of age and smoking, to determine relative risks of future myocardial infarction after dividing the study sample into quartiles of IL-6 based on the distribution of the control values. Adjusted estimates of risk were obtained with multivariate models that additionally controlled for body-mass index; history of diabetes, hypertension, or hyperlipidemia; parental history of premature atherosclerosis; and randomized treatment assignment. Stratified analyses were performed in low-risk subgroups and by duration of follow-up. All probability values are 2-tailed, and all CIs were computed at the 95% level.

Results

Baseline characteristics of the study participants are shown in Table 1. Owing to matching, the distribution of age and

TABLE 2.   Baseline Plasma Concentrations of IL-6 According to Case and Control Status

	Median IL-6, pg/mL		
	Controls	Cases	P
All participants	1.46	1.81	0.002
Nonsmokers	1.39	1.70	0.002
No hypertension	1.40	1.77	0.003
No hyperlipidemia	1.41	1.80	0.009
No diabetes	1.42	1.78	0.001
No obesity	1.38	1.68	0.006
No family history of CAD	1.48	1.82	0.007

CAD indicates coronary artery disease.

TABLE 3. Crude and Adjusted Relative Risks of Future Myocardial Infarction Among Apparently Healthy Men According to Baseline Level of IL-6

| | Quartile of IL-6 (range, pg/mL) | | | | |
	1 (<1.04)	2 (1.04–1.46)	3 (1.47–2.28)	4 (>2.28)	*P* for Trend
Crude analysis					
Total cohort					
RR	1.0	1.4	2.8	2.3	<0.001
95% CI	...	0.7–2.6	1.6–5.1	1.3–4.3	
P	...	0.3	0.001	0.005	
Nonsmokers					
RR	1.0	1.4	2.5	2.6	0.001
95% CI	...	0.7–2.7	1.4–4.7	1.3–4.8	
P	...	0.3	0.003	0.01	
Total and HDL cholesterol adjusted					
Total cohort					
RR	1.0	1.5	2.9	2.3	0.003
95% CI	...	0.7–2.9	1.6–5.4	1.2–4.3	
P	...	0.3	0.001	0.01	
Nonsmokers					
RR	1.0	1.6	2.6	2.5	0.003
95% CI	...	0.8–3.1	1.4–5.1	1.3–5.1	
P	...	0.2	0.004	0.01	
Fully adjusted					
Total cohort					
RR	1.0	1.9	3.5	2.3	0.01
95% CI	...	0.9–3.9	1.8–6.9	1.1–4.6	
P	...	0.08	0.001	0.03	
Nonsmokers					
RR	1.0	2.1	3.1	2.7	0.009
95% CI	...	1.0–4.5	1.6–6.7	1.1–5.7	
P	...	0.06	0.002	0.02	

RR indicates relative risk.

Models for total cohort matched on age and smoking status. Fully adjusted models additionally controlled for total and HDL cholesterol, body mass index, diastolic blood pressure, diabetes, family history of premature coronary artery disease, alcohol use, and exercise frequency.

smoking was identical for cases and controls. As expected in a prospective study of myocardial infarction, cases had higher baseline levels of total cholesterol and triglycerides, lower levels of HDL cholesterol, and a greater prevalence of hypertension and obesity than controls.

The overall frequency distribution of baseline IL-6 levels is presented in Figure 1. As shown, IL-6 levels ranged between 0.015 and 10.01 pg/mL in a pattern virtually identical to that expected from fresh blood samples obtained in otherwise healthy populations (0.01 to 11.5 pg/mL; data on file, R&D Systems, Minneapolis, Minn).

Median plasma concentrations of IL-6 at baseline were significantly higher among men who went on to experience a first myocardial infarction than among men who remained free of reported cardiovascular disease during follow-up (1.81 versus 1.46 pg/mL; *P*=0.002). As shown in Table 2, these differences were present in the total cohort as well as in the low-risk subgroups of nonsmokers and those with no history of hypertension, hyperlipidemia, diabetes, or obesity or a family history of premature myocardial infarction (all *P*≤0.001).

As shown in Table 3, the relative risk of first myocardial infarction increased with increasing quartiles of baseline IL-6 concentration (*P* for trend <0.001) such that men in the highest quartile at baseline had a relative risk 2.3 times higher than those in the lowest quartile (95% CI 1.3 to 4.3, *P*=0.005); overall, each quartile increase in baseline plasma concentration of IL-6 was associated with a statistically significant 38% increase in risk of future myocardial infarction (95% CI 15% to 66%, *P*=0.001).The relationship between baseline IL-6 level and risk of future myocardial infarction was not altered in analyses that adjusted for baseline differences in total cholesterol, HDL cholesterol, body mass index, blood pressure, diabetes, a family history of
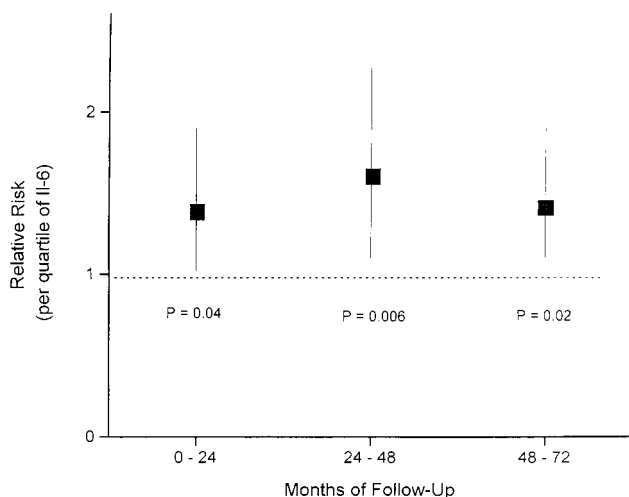
Figure 2. Relative risk of future myocardial infarction associated with each increasing quartile of baseline IL-6 concentration according to months of follow-up.

Figure 3. Median baseline IL-6 levels according to number of traditional risk factors present (hypertension, hyperlipidemia, smoking, diabetes, age >60 years, family history, and body mass index >27.3 kg/m²).

premature coronary artery disease, alcohol use, and exercise frequency (Table 3).

Smokers had significantly higher median concentrations of IL-6 than did nonsmokers (2.23 versus 1.58 pg/mL; $P<0.001$). However, because we matched case and control subjects on smoking status, confounding by this factor was minimized. Moreover, in analyses limited to nonsmokers, the relationship between baseline level of IL-6 and subsequent risk remained highly significant such that the relative risks of future myocardial infarction from lowest to highest quartiles of IL-6 among nonsmokers were 1.0, 1.4, 2.5, and 2.6 (P for trend 0.001). As in the total cohort, these effects were minimally altered in analyses that adjusted for lipid and nonlipid risk factors (Table 3).

To assess whether the effect of baseline IL-6 levels on risk of future myocardial infarction varied over time, we stratified our analyses by months of follow-up. Statistically significant increases in risk were found to be associated with elevations of IL-6 in the first 2 years of follow-up ($P=0.04$), in years 2 to 4 ($P=0.006$), and in years 4 to 6 ($P=0.02$). As shown in Figure 2, the relative risk associated with each increasing quartile of baseline IL-6 level was stable over long periods of time, with no significant time trends noted.

Log-normalized concentration of IL-6 was not correlated with total cholesterol ($r=-0.01$, $P=0.9$) or triglycerides ($r=0.03$, $P=0.5$) and was modestly correlated with HDL cholesterol ($r=0.15$, $P=0.003$). Modest correlations were also found between IL-6 and several nontraditional markers of vascular risk, including antigen level for tissue-type plasminogen activator ($r=0.21$, $P=0.004$), total plasma homocysteine ($r=0.15$, $P=0.003$), fibrinogen ($r=0.24$, $P=0.002$), and soluble intercellular adhesion molecule-1 ($r=0.19$, $P=0.001$). The strongest correlate of IL-6 in these data was log-normalized plasma concentration of C-reactive protein ($r=0.43$, $P=0.001$). However, the relationship between IL-6 and myocardial infarction remained statistically significant after controlling for this inflammatory marker. Specifically, in analyses that adjusted for baseline plasma concentration of C-reactive protein, a 44% increase in relative
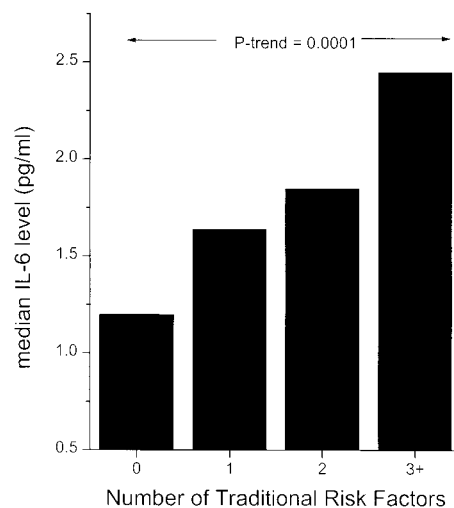
risk of future myocardial infarction was associated with each increasing quartile of IL-6 (95% CI 12% to 86%, $P=0.005$).

Nonlipid cardiovascular risk factors that correlated with IL-6 levels included age ($r=0.15$, $P=0.002$), systolic ($r=0.27$, $P=0.001$) and diastolic ($r=0.21$, $P=0.001$) blood pressure, and body mass index ($r=0.26$, $P=0.001$). As shown in Figure 3, median levels of IL-6 increased with increasing number of traditional risk factors (hypertension, hyperlipidemia, smoking, diabetes, age >60 years, family history, and body mass index >27.3 kg/m²) (P for trend 0.001). However, as shown in Tables 2 and 3, adjustment for these factors had a minimal effect on the relationship between IL-6 levels and future risk of myocardial infarction. Alcohol consumption also appeared to affect IL-6 such that daily drinkers had significantly higher plasma levels than those who consumed alcohol on a less frequent basis (2.7 versus 2.9 pg/mL; $P=0.001$). By contrast, daily exercisers tended to have lower levels of IL-6 than those who exercised less frequently, although this association was not statistically significant (2.1 versus 2.3 pg/mL; $P=0.2$). As shown in our multivariate analyses, additional control for these factors had no effect on our overall results.

We repeated these analyses for events that occurred before the termination of the randomized aspirin component of the Physicians' Health Study. In this subgroup, as in the cohort as a whole, each increasing quartile of IL-6 was associated with an increased risk of subsequent myocardial infarction (P for trend 0.001) such that those with the highest levels at baseline had a relative risk 4.1 times higher than those with the lowest levels (95% CI 1.7 to 9.5, $P=0.001$). The highest risk was observed among those with elevated levels of IL-6 who were randomly assigned to placebo (relative risk 7.3, $P=0.001$). The reduction in risk of first myocardial infarction attributable to aspirin was similar for those with and without elevated levels of IL-6.

Discussion

These data indicate that baseline levels of the inflammatory cytokine IL-6 are significantly elevated among apparently

healthy men at risk for future myocardial infarction. The relationship between IL-6 level and risk was not altered in analyses that adjusted for baseline differences in total cholesterol, HDL cholesterol, body mass index, blood pressure, diabetes, a family history of premature coronary artery disease, alcohol use, or exercise frequency; it was stable over the 6-year follow-up period and was present even among all low-risk subgroups evaluated, including nonsmokers.

Prior data evaluating the role of IL-6 among healthy individuals at risk for future coronary events are sparse. In the setting of acute ischemia, however, it has recently been shown that IL-6 levels increase with the acute-phase response and that these elevations may be a marker for plaque instability.[24–26] However, because blood samples in the present study were collected at baseline, we can exclude the possibility that acute ischemia was a cause of IL-6 elevation in these data. Thus, if an enhanced inflammatory response is present among individuals with a propensity for acute plaque rupture,[33] then our data indicate that such effects are present and can be clinically detected many years in advance of first myocardial infarction.

Elevated levels of IL-6 have previously been observed in several autoimmune disorders, including arthritis, Castleman syndrome, psoriasis, mesangial proliferative glomerulonephritis, and inflammatory bowel disease.[2] In this regard, the current data provide support for the hypothesis that atherosclerosis represents, at least in part, a fundamentally inflammatory condition.[27]

The strongest correlates of IL-6 in these data were C-reactive protein and fibrinogen, a finding that would be expected because IL-6 is a primary stimulant for hepatic production of acute-phase proteins.[6,7] In our data, the effects of IL-6 on subsequent risk remained statistically significant after we controlled for these latter factors. Thus, the current data also help to explain why several acute-phase proteins, such as C-reactive protein, serum amyloid A, albumin, and fibrinogen, have been associated with increased vascular risk.[34]

Limitations of these data should be considered. We relied on a single baseline blood sample and thus cannot take into account any variation of IL-6 that may occur over time. Furthermore, because our baseline blood samples were not obtained at a uniform time of day, our data may be limited by any diurnal variation in IL-6 that might exist, a potential issue because both glucocorticoids and catecholamines increase IL-6 levels, and the plasma half-life of IL-6 is <6 hours. It is important to recognize, however, that both of these potential limitations would tend to increase random misclassification in our data, an effect that, if anything, would lead to an underestimation of true effects. Finally, because our samples were stored at −80°C, we cannot exclude the possibility of protein degradation. Such an effect is unlikely, however, because the distribution of IL-6 in our study was quite similar to that observed in studies that used fresh blood samples. Furthermore, even if such an effect were present, it would not affect the validity of our results, because both case and control samples were handled identically and in a blinded fashion throughout the study. In addition, because all our study participants were taking oral aspirin at the time of blood

sampling, any effect of this agent on IL-6 levels could not have affected our main results.[35]

Stimuli underlying IL-6 production in apparently healthy men at risk for future myocardial infarction are uncertain. It is possible, for example, that preclinical atherosclerosis is itself an inflammatory stimulus and that IL-6 is a marker rather than a cause of disease. On the other hand, because monocyte-derived macrophages are abundant in atherosclerotic plaque and IL-6 gene transcripts are expressed in human atheroma, it is also possible that increased IL-6 production from endothelium and vascular smooth muscle has direct effects on plaque proliferation and stability.[27,28] IL-6 levels also increase with infection,[1,2,36] and it has been hypothesized that infection might aggravate atherogenesis.[37] In this cohort, however, baseline IgG titers directed against *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, and cytomegalovirus were not associated with increased vascular risk or with increased levels of inflammatory markers.[38,39]

Together, these prospective epidemiological data support a fundamental role for cytokine-mediated inflammation in the early stages of atherogenesis, data that corroborate and extend the recent finding that IL-6 levels are associated with increased mortality in the elderly.[40] As such, we believe these data support the possibility that anti-inflammatory therapies might provide a new approach to cardiovascular disease treatment and prevention.

Acknowledgments

References

1. Van Snick J. Interleukin-6: an overview. *Annu Rev Immunol*. 1990;8:253–278.
2. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med*. 1998;128:127–137.
3. Van Deventer SJH, Buller HR, Ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic and complement pathways. *Blood*. 1990;76:2520–2526.
4. Ng SB, Tan YN, Guy GR. Differential induction of the interleukin-6 gene by tumor necrosis factor and interleukin-1. *J Biol Chem*. 1994;269:19021–19027.
5. Sanceau J, Kaisho T, Hirano T, Wietzerbin J. Triggering of the human interleukin-6 gene by interferon-γ and tumor necrosis factor-α in monocyte cells involves cooperation between interferon regulatory factor-1, NFkB, and SP1 transcription factors. *J Biol Chem*. 1995;270:27920–27931.
6. Baumann H, Gauldie J. Regulation of hepatic acute phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. *Mol Biol Med*. 1990;7:147–159.
7. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J*. 1990;265:621–636.
8. Loppnow H, Libby P. Adult human vascular endothelial cells express the IL6 gene differentially in response to LPS or IL1. *Cell Immunol*. 1989;122:493–503.
9. Loppnow H, Libby P. Comparative analysis of cytokine induction in human vascular endothelial and smooth muscle cells. *Lymphokine Res*. 1989;8:293–299.
10. Szekanecz Z, Shah MR, Pearce WH, Koch AE. Human atherosclerotic abdominal aortic aneurysms produce interleukin (IL)-6 and interferon-gamma but not IL-2 and IL-4: the possible role for IL-6 and interferon-gamma in vascular inflammation. *Agents Actions*. 1994;42:159–162.

11. Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, Kano S, Shimada K. Interleukin-6 gene transcripts are expressed in human atherosclerotic lesions. *Cytokine*. 1994;6:87–91.

12. Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interluekin-8 protein and gene expression in human arterial atherosclerotic wall. *Atherosclerosis*. 1996;127:263–271.

13. Mestries JC, Kruithof EKO, Gascon MP, Herodin F, Agay D, Ythier A. In vivo modulation of coagulation and fibrinolysis by recombinant glycosylated human interleukin-6 in baboons. *Eur Cytokine Netw*. 1994;5: 275–281.

14. Van der Poll T, Levi M, Hack CE, Ten Cate U, van Deventer SJH, Erenberg AJM, de Groot ER, Jansen J, Gallati H, Buller HR, Ten Cate JW, Aarden LA. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. *J Exp Med*. 1994;179:1253–1259.

15. Stouthard JML, Levi M, Hack CE, Beenhof CHN, Romijn HA, Sauerwein HP, van der Poll T. Interleukin-6 stimulates coagulation, not fibrinolysis, in humans. *Thromb Haemost*. 1996;76:738–742.

16. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979.

17. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation*. 1998;98:731–733.

18. Haverkate F, Thompson SG, Pyke SDM, Gallimore JR, Pepys MB, for the European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet*. 1997;349:462–466.

19. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol*. 1997;17:1121–1127.

20. Kuller LH, Tracy RP, Shaten J, Meilahn EN, for the MRFIT Research Group. Relationship of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *Am J Epidemiol*. 1996;144: 537–547.

21. Bataille R, Klein B. C-reactive protein levels as a direct indicator of interleukin-6 levels in humans in vivo. *Arthritis Rheum*. 1992;35: 982–984.

22. Miyao Y, Yasue H, Ogawa H, Misumi I, Masuda T, Sakamoto T, Morita E. Elevated plasma interleukin-6 levels in patients with acute myocardial infarction. *Am Heart J*. 1993;126:1299–1304.

23. Guillen I, Blanes M, Gomez-Lechon MJ, Castell JV. Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6. *Am J Physiol*. 1995;269:R229–R235.

24. Biasucci LM, Bitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C, Rebuzzi AG, Ciliberto G, Maseri A. Elevated levels of interleukin-6 in unstable angina. *Circulation*. 1996;94:874–877.

25. Biasucci LM, Liuzzo G, Fantuzzi G, Caligiuri G, Rebuzzi AG, Ginnetti F, Dinarello CA, Maseri A. Increasing levels of interleukin (IL)-1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation*. 1999;99:2079–2084.

26. Liuzzo G, Buffon A, Biasucci LM, Gallimore JR, Caligiuri G, Vitelli A, Altamura S, Ciliberto G, Rebuzzi AG, Crea F, Papys MB, Maseri A. Enhanced inflammatory response to coronary angioplasty in patients with severe unstable angina. *Circulation*. 1998;98:2370–2376.

27. Ross R. Mechanisms of disease: atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.

28. Libby P. Molecular bases of the acute coronary syndromes. *Circulation*. 1995;91:2844–2850.

29. Steering Committee of the Physicians' Health Study Research Group. Final report of the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med*. 1989;321:129–135.

30. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med*. 1991;325:373–381.

31. Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ. Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risks of future venous thromboembolism. *Circulation*. 1997;95: 1777–1782.

32. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. *Lancet*. 1993;341:1165–1168.

33. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes: implications for plaque rupture. *Circulation*. 1994;90:775–778.

34. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA*. 1998;279: 1477–1482.

35. Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouxas P, Nihoyannopoulos P. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation*. 1999;100:793–798.

36. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J Clin Invest*. 1999;103:571–577.

37. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation*. 1997;96:4095–4103.

38. Ridker PM, Hennekens CH, Stampfer MJ, Wang F. Prospective study of herpes simplex virus, cytomegalovirus, and the risk of future myocardial infarction and stroke. *Circulation*. 1998;98:2796–2799.

39. Ridker PM, Kundsin R, Stampfer MJ, Poulin S, Hennekens CH. A prospective study of *Chlamydia pneumoniae* IgG seropositivity and risks of future myocardial infarction. *Circulation*. 1999;99:1161–1164.

40. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH, Heimovitz H, Cohen HJ, Wallace R. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999;106:506–512.