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Increased serum concentrations of interleukin-1β in patients with coronary artery disease

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Abstract

***Objective*—To assess serum interleukin-1β(IL-1β) concentrations in patients with ischaemic heart disease, to characterise subgroups of patients with raised IL-1β concentrations, and to examine whether serum IL-1β concentrations correlate with non-specific indices of inflammation.**

***Design*—Survey study of patients with ischaemic heart disease.**

***Setting*—Cardiac catheterisation laboratory of a tertiary medical centre.**

***Patients*—Consecutive patients with angina pectoris and patients recovering from uncomplicated acute myocardial infarction and undergoing elective coronary angiography.**

***Results*—Mean(SD) serum IL-1β concentrations were higher ($P < 0.001$) in patients with angina and < 50% coronary artery stenosis (n = 11; 18·8(19·9) pg/ml), patients with angina $\geqslant$ 50% stenosis (n = 23; 10·2(11·4) pg/ml), and patients 8(0·8) days post-infarction (n = 13; 4·4(5·8) pg/ml) than in 15 healthy, age-matched controls (0·3(0·5) pg/ml). Serum IL-1β concentrations did not correlate with total blood leucocyte counts ($r = -0.07$, P = NS), blood lymphocyte counts ($r = -0.24$, P = NS), and blood monocyte counts ($r = -0.29$, P = NS), or with fibrinogen ($r = -0.16$, P = NS) and C-reactive protein concentrations (9(10·5) mg/dl *v* 14·1(19) mg/dl for patients with undetectable and detectable concentrations, respectively, P = NS).**

***Conclusion*—Serum IL-1β concentrations are raised in patients with ischaemic heart disease, in particular in those with minimal coronary artery disease and angina. The precise role of IL-1β in coronary artery disease remains to be determined.**

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Keywords: interleukin-1β; inflammation; atherosclerosis

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Interleukin-1 (IL-1) is a 17 kDa proinflammatory glycoprotein.[1–4] Interleukin 1α and 1β (IL-1α and IL-1β, respectively) are isoforms of IL-1, with IL-1β being the predominant circulating isoform in humans.[4] Monocytes, macrophages, and macrophage-derived cells are the main sources of IL-1, although other cell types, such as endothelial cells, also produce IL-1.[4] Once released by the stimulated cell, IL-1 can exert either local effects on the surrounding milieu or widespread effects on various organs via plasma transport.

IL-1 may play a major part in the evolution of atherosclerotic coronary artery disease,[4–8] through several mechanisms. Being a pro-inflammatory cytokine,[1–4] IL-1 may mediate the inflammatory response occurring in the vascular wall during atherogenesis. In fact, IL-1β has been shown to induce post-cardiac transplantation coronary arteriopathy by augmenting infiltration of inflammatory cells into the vascular wall[9–12] and to increase endothelial cell adhesiveness to leucocytes,[13 14] by inducing adhesion molecule presentation on the cell surface of vascular endothelium.[15] In addition, IL-1 may enhance atherogenesis by promoting vascular smooth muscle cell proliferation,[15 16] increasing endothelial cell procoagulant activity,[17] and affecting lipid metabolism.[18] Moreover, IL-1 may have direct effects on the heart, including suppression of catecholamine inotropy.[19 20] The aims of the present study were (*a*) to assess whether serum concentrations of IL-1β are raised in patients with ischaemic heart disease, (*b*) to characterise subgroups of patients with ischaemic heart disease according to angiographic and clinical criteria and raised IL-1β concentrations, and (*c*) to examine whether serum IL-1β concentrations correlate with commonly used non-specific indices of inflammation.

Patients and methods

PATIENTS

The study protocol was approved by our institutional review board. All patients gave informed consent after the purpose of the study was explained to them.

We studied 34 consecutive patients undergoing elective coronary angiography because they had typical anginal pain and 13 consecutive patients recovering from uncomplicated acute myocardial infarction (8·1(0·8) days post-infarction), 11 of whom had been treated with streptokinase. Patients did not necessarily have non-invasive evaluation for ischaemia before coronary angiography. Patients were referred for coronary angiography at the discretion of the attending physician. In all patients experiencing chest pain, the cause was presumed to be cardiac after other causes were ruled out. Heparin treatment was stopped at least six hours before blood tests and coronary angiography. All other drugs were adminis-

Demographic, clinical, angiographic and laboratory characteristics of patients with angina without significant coronary artery stenosis (1), patients with angina and significant coronary artery stenosis (2), and patients after myocardial infarction (3)

	1	2	3	P value
Patients	11	23	13	
Age (y) (mean (SD))	54·6 (13·7)	66·1 (9·8)	63·1 (12·1)	0·03
Sex (male)	8 (73%)	17 (74%)	9 (69%)	NS
Previous myocardial infarction	0 (0%)	16 (70%)	3 (23%)	0·02*
Previous coronary angioplasty	0 (0%)	5 (22%)	1 (8%)	NS*
Diabetes mellitus	3 (27%)	7 (30%)	2 (15%)	NS
Hypertension	6 (55%)	10 (43%)	8 (62%)	NS
Hypercholesterol aemia	4 (36%)	14 (61%)	6 (46%)	NS
Coronary artery disease:				NS*
1 VESSEL	0 (0%)	6 (26%)	5 (38%)	
2 VESSEL	0 (0%)	6 (26%)	2 (15%)	
3 VESSEL	0 (0%)	11 (48%)	6 (46%)	
Blood leucocytes (mean (SD)):				
Total (× 109/1)	7·5 (1)	7·3 (1·8)	7·9 (2·3)	NS
Lymphocytes (× 109/1)	2 (0·6)	2·4 (0·7)	2·1 (0·8)	NS
Monocytes (× 109/1)	0·4 (0·1)	0·4 (0·1)	0·5 (0·2)	NS
Fibrinogen (g/l)	3·9 (0·8)	3·8 (0·9)	5·3 (1·3)	0·0006
C-Reactive protein (> 0·5 mg/dl)	2 (18%)	3 (13%)	3 (23%)	NS
Drug therapy:				
Aspirin	7 (64%)	20 (87%)	13 (100%)	< 0·05
Nitrates	4 (36%)	15 (65%)	3 (23%)	< 0·05
β-Blockers	1 (9%)	4 (17%)	4 (31%)	NS
Calcium-channel blockers	6 (55%)	15 (65%)	3 (23%)	0·05
Diuretics	2 (18%)	6 (26%)	1 (8%)	NS
ACE inhibitors	2 (18%)	6 (26%)	5 (38%)	NS

*P value for comparison between subgroups 2 and 3. P value for coronary artery disease represents differences in distribution of one, two, and three vessel disease in both groups. ACE, angiotensin converting enzyme.

tered as usual. Patients with unstable angina underwent coronary angiography after their symptoms had abated with pharmacological therapy (asymptomatic for 2–3 days). Patients with severe heart failure (NYHA class III-IV), neoplastic, immunological, infectious, or inflammatory disease were excluded.

ANGIOGRAPHIC DATA

The degree of coronary artery stenosis in coronary angiography (Judkin's technique) was determined in at least two projections by two independent investigators. Stenosis was regarded as significant if ⩾ 50% of the luminal diameter was occluded. The study group was divided into three subgroups: (1) stable (n = 6) and unstable angina (n = 5) without significant coronary artery stenosis, (2) stable (n = 9) and unstable angina (n = 14) with significant coronary artery stenosis, and (3) post-infarction (n = 13).

IL-1β ASSAY

Immediately before coronary angiography, venous blood was drawn and centrifuged for five minutes at 2000 rpm, and the serum was separated and stored at −20°C. Serum IL-1β concentrations were measured using a highly sensitive immunoassay (Quantikine HS, R&D systems, Minneapolis, MN, USA). The detection limit of the assay is 0·059 pg/ml. Fifteen healthy, age-matched volunteers (with no history of ischaemic heart disease or other systemic diseases) served as a control group.

OTHER INDICES OF INFLAMMATION

Venous blood samples were also obtained before coronary angiography for other commonly used non-specific indices of inflammation. Blood leucocyte and differential counts were determined using an automated haematology analyser (Cell-Dyn 1600, Sequoia-Turner Corporation, Mountainview, CA, USA). Fibrinogen concentrations were determined using an automated coagulation assay

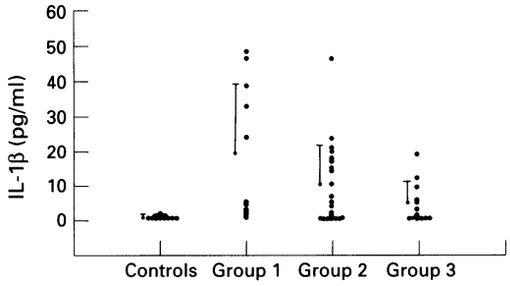

Figure 1 Serum IL-1β concentrations in normal healthy controls, patients with angina without significant coronary artery stenosis (1), patients with angina with significant coronary artery stenosis (2), and patients after myocardial infarction (3).

(Automated Coagulation Laboratory 1000, Instrumentation Laboratory, USA) with normal values of 2–4 g/l. C-reactive protein was assayed by rate nephelometry using a latex-agglutination method (Immunostics, NJ, USA). The threshold for detection using this method in our laboratory is > 0·5 mg/dl, and values under 1·2 mg/dl are regarded as normal. We did not use sensitive assays to determine concentrations of C-reactive protein below the threshold of detection.

STATISTICAL ANALYSIS

Mean (SD) was calculated for continuous variables, and absolute frequencies were measured for discrete variables. For continuous variables, differences in IL-1β concentrations between groups were examined for statistical significance by the Mann-Whitney rank-sum test or Kruskal-Wallis statistic, and differences in other variables (that is, age, leucocytes, etc) were compared by one-way analysis of variance (ANOVA). The χ^2 test was applied for discrete variables. When there were small numbers of patients in each category, Fisher's exact test was performed.The Spearman rank test was performed for correlations. All tests were 2-tailed, and P values ⩽ 0·05 were regarded as statistically significant.

Results

The table shows the demographic and clinical characteristics of the three subgroups. Patients with angina and minimal coronary artery disease were younger (P = 0·03), but there was no difference between the three subgroups in the distribution of gender or risk factors. Patients in subgroup 2 were significantly more likely to have a history of previous myocardial infarction (P = 0·02) than patients in subgroup 3 but there was no significant difference in their history of previous angioplasty. The number of vessels involved was similar in subgroups 2 and 3. Fibrinogen concentrations were significantly higher in post-infarction patients (P = 0·0006). There was no difference in total blood leucocyte counts among the three groups or in blood monocyte and lymphocyte counts. C-reactive protein concentrations were detected in the same proportion of patients in each group. Drug treatment at the time of coronary angiography was slightly different in the three subgroups.

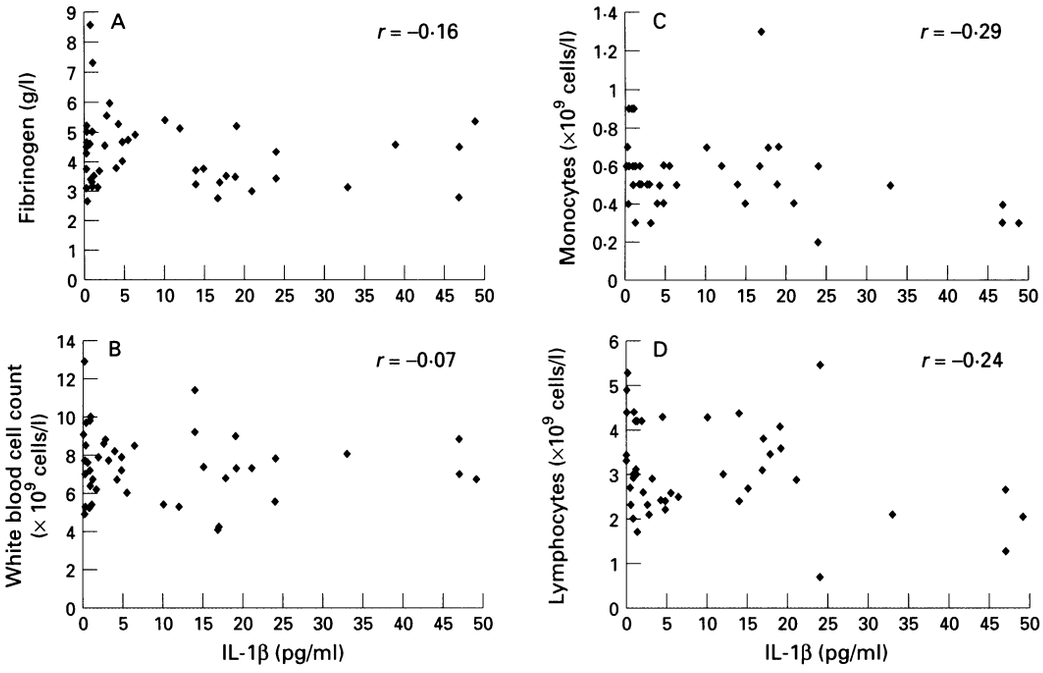

Figure 2 Correlation between serum IL-1β concentrations and (A) fibrinogen concentrations, and (B) total blood leucocyte counts, (C) lymphocyte counts, and (D) monocyte counts. There was no significant correlation between serum IL-1β concentrations and these non-specific indices of inflammation.

Aspirin treatment (100–250 mg per day) was more prevalent in patients in subgroup 3, whereas nitrate and calcium-channel blocker treatment was more common in patients in subgroup 2.

Figure 1shows the distribution of serum IL-1β concentrations in the subgroups and in healthy controls. Serum IL-1β concentrations were significantly lower ($P < 0·001$) in controls (0·3 (0·5) pg/ml) than in patients in groups 1, 2, and 3 (18·8 (19·9) pg/ml, 10·2 (11·4) pg/ml, and 4·4 (5·8) pg/ml, respectively). In groups 1 and 2, there was no difference in IL-1β concentrations between patients with stable or unstable angina (group 1 15·4 (20·5) pg/ml in patients with stable angina *v* 22·9 (20·7) pg/ml in patients with unstable angina, P = NS; group 2 14·2 (13·9) pg/ml in patients with stable *v* 7·7 (9·2) pg/ml in patients with unstable angina, P = NS). There was no correlation between serum IL-1β concentrations and fibrinogen concentrations ($r = -0·16$, P = NS) or blood leucocyte counts ($r = -0·07$, P = NS), including monocytes ($r = -0·29$, P = NS) and lymphocytes ($r = -0·24$, P = NS) (fig 2). Similarly, serum IL-1β concentrations were not significantly different in patients with and without detectable C-reactive protein concentrations (9 (10·5) mg/dl *v* 14·1 (19) mg/dl for patients with undetectable and detectable concentrations, respectively, P = NS).

Discussion

MAJOR FINDING

We found that serum IL-1β concentrations were raised in patients with coronary artery disease, especially in those with angina and minimal coronary artery stenosis. Serum concentrations of IL-1β were also slightly raised in patients recovering from acute myocardial infarction. There was no correlation between serum IL-1β concentrations and other non-specific indices of inflammation.

PREVIOUS STUDIES

A recent report by Matsumori *et al*[21] found that circulating IL-1β concentrations were undetectable in healthy controls and in patients recovering from acute myocardial infarction and patients with angina pectoris. Similarly, Latini *et al*[22] reported undetectable concentrations of IL-1β in patients in the early phase of acute myocardial infarction. It is difficult to explain the disparity between our study and the other two studies, because neither of the other two studies[21 22] provide angiographic data, and Matsumori *et al*[21] do not provide clinical information. However, we used a highly sensitive assay for IL-1β (sensitivity of 0·059 pg/ml), whereas the assays used by Matsumori *et al*[21] and Latini *et al*[22] were of much lower sensitivity (20 pg/ml and 5 pg/ml, respectively).

IL-1β AND ACUTE MYOCARDIAL ISCHAEMIA/INFARCTION

IL-1β is regarded as an important mediator of the inflammatory process taking place in the myocardium in the course of early myocardial ischaemia and reperfusion.[23] Moreover, increased concentrations of IL-1 receptor antagonist have been detected in the early phase of acute myocardial infarction.[22] Therefore, our finding of slightly raised concentrations of circulating IL-1β in patients post-infarction accords with its presumed proinflammatory role in response to ischaemia and reperfusion. Raised IL-1β concentrations in our patients with angina might also be the result of myocardial ischaemia.

IL-1β AND CORONARY ARTERY DISEASE

Inflammation and active atherogenesis

Given the considerable evidence linking an inflammatory response in the vascular wall with the evolution of atherosclerosis[24] and coronary artery disease[25–27] on the one hand, and the broad proinflammatory activities of IL-1β on the other, it is reasonable to attribute the raised serum IL-1β concentrations in our patients

with angina to an active inflammatory response in the vascular wall during early atherogenesis. One could argue that the lack of correlation between serum IL-1β and other non-specific indices of inflammation (blood leucocyte count and differential count and fibrinogen and C-reactive protein concentrations) indicates that IL-1β concentrations do not accurately reflect inflammatory activity. The lack of correlation between IL-1β and other indices of inflammation may be partially explained by differences in circulating half-life (IL-1β having a short half-life). Moreover, the studies which showed increased concentrations of C-reactive protein in various myocardial ischaemic states[28-30] did not establish that there was an active inflammatory process in the atheroma. In fact, the authors of a recent study that showed that C-reactive protein concentrations are of prognostic significance in unstable angina[28] acknowledge that the protein most probably reflects an "unknown process" rather than inflammation in the atheroma.[31] In addition, because fibrinogen is also influenced by thrombogenesis and anti-thrombotic treatment, it does not accurately reflect active inflammation. Thus the selective increase in IL-1β concentrations and not in other non-specific acute phase reactants supports a specific role for IL-1β in the pathophysiology of coronary artery disease.

Endothelial dysfunction

In our study the highest concentrations of IL-1β were found in patients with angina and minimal coronary artery disease. These patients were younger than patients with overt coronary artery disease but had similar risk factors. Thus at least some of them could be regarded as being in the early stages of atherosclerosis, which are characterised by altered vasomotor regulation of the coronary vasculature in the absence of pronounced morphological changes.[32-34] These patients may have been experiencing angina because of endothelial dysfunction.

Impaired production and/or secretion of vasodilators such as endothelium-derived relaxing factor (nitric oxide), are thought to be involved in atherosclerotic endothelial dysfunction.[34] It is of interest that IL-1 is involved in the regulation of nitric oxide metabolism by inducing production of nitric oxide synthase,[35-37] the enzyme responsible for nitric oxide formation. Moreover, IL-1, like endothelium-derived relaxing factor, may induce some of its actions by binding to guanylate cyclase and increasing intracellular cyclic guanine monophosphate concentrations.[15] Similarly, IL-1β was recently reported to induce production of another vasodilator of endothelial origin, C-type natriuretic peptide,[38] which also exerts its actions by binding to guanylate cyclase. It could be that IL-1β retains vasomotor control of atherosclerotic coronary arteries by tilting the vasomotor scale in favour of vasodilators.

CLINICAL IMPLICATIONS

If IL-1β has a crucial role in the inflammatory response in atherogenesis, selective inhibition of IL-1β by receptor antagonists[4 39] might reduce the vascular immunoinflammatory reaction, and thus inhibit atherogenesis and subsequent morbidity.[4 40] However, this strategy might prove to be two-edged, because IL-1β might be instrumental in maintaining vasomotor tone, as suggested above. Moreover, it has been reported that IL-1β may be cardioprotective in myocardial ischaemia.[41 42] Hopefully, as we gain more insight into the role of IL-1β in ischaemic heart disease, and the significance of raised serum IL-1β concentrations in patients with ischaemic heart disease, serum IL-1β concentrations will be used for diagnostic purposes in these patients.

LIMITATIONS

The major limitation of this study is the lack of histopathological evidence of inflammation in patients with raised IL-1β concentrations. Also, because we did not measure local IL-1β concentrations, we can only assume that increased systemic concentrations reflect a similar local increase. In addition we measured serum IL-1β concentrations once only. Especially in patients post-infarction, concentrations may be different in earlier/later stages of myocardial infarction.[22] Nor do we know how concomitant drug treatment influences IL-1β concentrations. Thus differences between subgroups may also be the result of differences in pharmacotherapy. For example, the higher prevalence of aspirin treatment in post-infarction patients might have reduced the rise in IL-1β concentrations, although the dose of aspirin used is much lower than that required for a potent anti-inflammatory effect.

We used a non-sensitive assay for C-reactive protein. A recent study in which a sensitive assay was used showed that although C-reactive concentrations were higher in patients with unstable angina and a poor prognosis,[27] C-reactive protein concentrations in many patients with angina (stable and unstable) were in 0·3 mg/dl or lower. This shortcoming should be borne in mind when the correlation between IL-1β and C-reactive protein concentrations we report is considered. Endothelial dysfunction (impaired coronary flow reserve and/or response to acetylcholine) as a possible cause for angina was not assessed in patients belonging to group1.

Last, it is well known that the pathophysiology of myocardial ischaemia[22] and atherosclerosis[23] involve a vast network of synergistic and inhibitory cytokines. For example, changes in IL-1 receptor antagonists may also rise in these processes, thus blunting the effects of IL-1. In the present study we examined changes in only one cytokine.

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