Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease

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Abstract

***Objective*—To determine whether serum concentrations of the cytokines tumour necrosis factor α (TNFα) and interleukin 6 (IL-6), which regulate C reactive protein, are associated with cardiovascular risk factors and prevalent coronary heart disease.**

***Design*—A population based cross sectional study.**

***Subjects and methods*—198 men aged 50 to 69 years were part of a random population sample drawn from south London. Serum cytokine and C reactive protein concentrations were determined by enzyme linked immunosorbent assay. The presence of coronary heart disease was determined by Rose angina questionnaire and Minnesota coded electrocardiogram.**

***Results*—Serum TNFα concentrations were positively related to body mass index and *Helicobacter pylori* infection, but inversely related to alcohol consumption. IL-6 concentrations were positively associated with smoking, symptoms of chronic bronchitis, age, and father having a manual occupation. TNFα was associated with increased IL-6 and triglycerides, and reduced high density lipoprotein cholesterol. IL-6 was associated with raised fibrinogen, sialic acid, and triglycerides. ECG abnormalities were independently associated with increases in IL-6 and TNFα, each by approximately 50% ($P < 0.05$ for TNFα, $P < 0.1$ for IL-6). The corresponding increases in men with an abnormal ECG or symptomatic coronary heart disease were 28% for TNFα and 36% for IL-6 ($P = 0.14$ for TNFα and $P < 0.05$ for IL-6).**

***Conclusions*—This study confirms that many of the phenomena with which C reactive protein is associated, are also associated with serum levels of cytokine, which may be the mechanism.**

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Keywords: C reactive protein; interleukin 6; TNFα; cardiovascular risk; coronary heart disease

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Cardiovascular risk factors as established in prospective studies could be considered to fall into two broad groups: endogenous and exogenous (lifestyle) risk factors. Endogenous risk factors in turn fall into four broad groups: lipids, glucose and hyperinsulinaemia; clotting factors; haematological factors (viscosity, white blood cell count); and hypertension. These risk factors have been found to cluster in the same individual in a various epidemiological studies.[1–3] Currently established lifestyle risk factors include social class, smoking, obesity, alcohol consumption, and diet. These lifestyle risk factors have in turn been shown to have effects on many of the endogenous risk factors.[1 4] This suggests that a common underlying mechanism may explain much of their influence on the development of cardiovascular disease.

Inflammation may be this mechanism. Most cardiovascular risk factors are changed in an adverse direction by acute inflammation: fibrinogen and the white blood cell count rise, glucose rises, HDL falls, and triglycerides rise.[5–7] We have shown recently that low levels of systemic inflammation, as measured by serum C reactive protein in normal subjects, are related to many of these endogenous risk factors and that these levels of inflammatory activity are influenced in turn by many of the exogenous (lifestyle) cardiovascular risk factors.[8] C reactive protein production by the liver is regulated by cytokines, principally interleukin 6 (IL-6), and tumour necrosis factor α (TNFα), which is the main trigger for the production of IL-6 by a variety of cells.[9] The effect of these cytokines is modulated by cortisol and growth factors such as insulin.[10]

In vitro and animal challenge experiments suggest that IL-6 and TNFα play important roles in the regulation of the synthesis of other acute phase proteins which are established risk factors for atherosclerosis, such as fibrinogen and factor VIII.[10] These cytokines also have profound effects on lipid metabolism in animal challenge experiments.[11] TNFα has also been implicated in insulin resistance, which produces changes in lipids and glucose associated with cardiovascular risk.[12] Until recently it was assumed that many of the actions of cytokines were local and that they were not detectable in the serum. This was because immunoassays were insufficiently sensitive to detect their concentrations except in the acutely unwell. Much of the earlier work on serum concentrations of cytokines was performed with bioassays, but there are problems of lack of specificity with such assays.[13] There are now various reports of the determination of serum cytokines in normal subjects using modern high sensitivity enzyme linked immunosorbent assays (ELISAs) based on high affinity monoclonal antibodies.[14]

We aimed to test the hypothesis that many exogenous (lifestyle) cardiovascular risk factors are associated with alterations in circulating concentrations of the inflammatory cytokines IL-6 and TNFα, and that the concentrations of these cytokines are in turn associated with serum levels of many endogenous risk factors. We also investigated whether the serum values of these cytokines are higher in subjects with coronary heart disease.

Methods

An age stratified random sample of males with Caucasian names, aged 50 to 69 years, were recruited from general practices in the Wandsworth, Merton and Sutton District Health Authority, South London. Six hundred and twelve subjects were invited to St George's Hospital for examination, of whom 413 (68%) attended and 388 were white Caucasian subjects. Information was obtained on history and symptoms of coronary heart disease,[15] lifestyle, and socioeconomic circumstances, as described previously.[16] Subjects who answered yes to the question "have you ever had a heart attack?" or who had a history of myocardial infarction recorded in their general practitioner's records were considered to have had a myocardial infarct. Cardiovascular risk factor analysis was performed on 300 of these subjects and serology for *Helicobacter pylori* and *Chlamydia pneumoniae* was also performed on all of them, as described previously.[16] Electrocardiograms (ECGs) were Minnesota coded[17] and the Whitehall criteria for ischaemic heart disease were adopted.[18] The first consecutive 198 subjects with sufficient plasma and full cardiovascular risk factor profiles were included in the present study of IL-6 and TNFα. The sample size was limited to 198 by resource constraints.

C reactive protein was measured by in-house ELISA as previously described.[8] Serum IL-6 and TNFα concentrations were determined using high sensitivity assays marketed by R&D systems (Oxfordshire, UK). These assays have been used by others to detect serum cytokine concentrations in normal subjects.[14] These assays had a lower limit of detection of 0·18 pg/ml for TNFα and 0·1 pg/ml for IL-6.

STATISTICAL ANALYSIS

Serum IL-6, TNFα, and C reactive protein distributions were positively skewed. Log transformation resulted in normalisation of the distributions, and statistical analyses were performed on the $\log^{10}$ of these variables. The relation of $\log^{10}$ IL-6 and $\log^{10}$ TNFα to age (continuous variable), smoking (current/ex-/never), chronic infection, alcohol consumption (current: more than one drink per week; non-drinker: one or fewer drinks per week), and body mass index (BMI) (continuous variable) was analysed using multiple regression in Statview. For categorical variables with more than two categories including missing values, dummy variables were created to produce binary variables. These determinants were controlled for each other, for own social class (manual/non-manual), and father's social class (manual/non-manual/unknown). In eight subjects, the father's occupation was unknown. In nine subjects the alcohol consumption was unknown and was coded as missing.

Regression models were also analysed with each of the endogenous risk factors in turn as the outcome. These included as explanatory variables: age as a continuous variable; smoking habit (never, former, current); pack-years of smoking; current daily cigarette consumption; years since last smoked; own social class (Registrar General's classification: I, II, III non-manual, III manual, IV, V, unclassified) and father's social class, BMI, alcohol consumption, $\log^{10}$ TNFα, and $\log^{10}$ IL-6. In these models the outcome variables triglycerides and glucose were log transformed, as they had a positively skewed distribution.

The relation of cytokines to the risk of cardiovascular disease was performed using multiple regression in Statview with the log cytokine as the outcome variable, and coronary disease as one of the dependent variables.

Results

The mean (SD) age of the subjects included in this study was 59·1 (5·42) years compared with 59·0 (5·46) years for the 300 subjects with full risk factor profiles, 59·1 (5·41) years for the 413 subjects who attended for the study, and 59·1 (5·39) years for the 612 subjects invited to

Table 1 Relation of TNFα and IL-6 to exogenous cardiovascular risk factors

	TNFα (pg/ml)						*IL-6 (pg/ml)*					
	Unadjusted			*Adjusted¶*			*Unadjusted*			*Adjusted*		
	Geometric mean			*Geometric mean¶*			*Geometric mean*			*Geometric mean¶*		
Exposure (No exposed):	*No*	*Yes*	*t*	*No*	*Yes*	*t*	*No*	*Yes*	*t*	*No*	*Yes*	*t¶*
Father manual (147)	0·85	0·89	0·36	0·93	0·87	0·45	1·51	2·27	3·03**	1·60	2·14	2·01*
Manual (102)	0·79	0·97	1·53	0·80	0·96	1·25	1·81	2·19	1·63	1·96	2·05	0·33
Current smoker (54)	0·91	0·84	0·79	0·90	0·84	0·34	1·82	2·58	2·67**	1·79	2·70	2·40*
Phlegm (47)	0·87	0·91	0·30	0·88	0·88	0·04	1·78	2·88	3·54**	1·85	2·58	2·37*
H pylori (105)	0·72	1·04	2·80**	0·76	1·00	2·02*	1·83	2·15	1·32	1·95	2·06	0·52
Current drinker (135)	1·14	0·81	2·43*	1·13	0·80	2·37*	1·71	2·12	1·67	1·67	2·15	1·97*
BM (per kg/m²) § 27·1 (3·92)	1·04 (1·01 to 1·07)		2·33*	1·04 (1·01 to 1·07)		2·40*	1·02 (0·99 to 1·05)		1·57	1·03 (1·00 to 1·06)		1·73
Age (per year) § 59 (5·42)	1·02 (1·00 to 1·05)		1·89	1·02 (1·00 to 1·05)		1·74	1·02 (1·00 to 1·04)		1·68	1·02 (1·00 to 1·05)		2·02*

¶The variables in the table are mutually adjusted for each other and for past smoking (ex-smoker).
§Continuously distributed variables. The coefficients shown are for the relative change in cytokine for a unit change, after full adjustment. The mean and SD are shown for these variables.
*P < 0·05; **P < 0·01.

Table 2 Relation of TNFα and IL-6 to endogenous cardiovascular risk factors

Risk factor	*Mean (SD)§*	*Difference in risk factor across interquartile range of TNFα and IL-6*	
		TNFα¶	*IL-6¶*
Fibrinogen (g/l)	2·75 (0·59)	0·16 (0·07, 0·26)*	0·16 (0·07, 0·26)***
Total cholesterol (mmol/l)	6·00 (1·01)	0·045 (−0·13, 0·22)	0·045 (−0·13, 0·22)
Log^{10} triglyceride (mmol/1)	1·44 (1·06–2·03)	0·027 (0, 0·06)*	0·037 (0·004, 0·068)*
LDL (mmol/l)	1·47 (0·43)	−0·02 (−0·09, 0·04)	0·018 (−0·090, 0·055)
HDL (mmol/l)	1·13 (0·31)	−0·05 (−0·09, −0·004)***	−0·05 (−0·09, −0·004)*
Log^{10} glucose (mmol/l)	5·2 (4·9-5·6)	0·004 (−0·02, 0·008)	0·004 (−0·009, 0·02)
Systolic BP (mm Hg)	5·2 (4·9-5·6)	−1·26 (−4·64, 2·12)	−0·26 (−0·64, 2·12)
Log^{10} C reactive protein (mg/l)	1·73 (0·76–3·96)	1·25 (1·02, 1·49)**	1·60 (1·33, 1·92)***
Sialic acid (g/l)	0·71 (0·12)	0·05 (0·033, 0·049)***	0·049 (0·033, 0·049)***
Log^{10} IL-6 (pg/ml)	1·72 (1·21–2·93)	1·32 (1·14, 1·52)***	

¶Adjusted for age, body mass index (BMI), father's social class in six categories, current occupation in six categories, current and ex-smoking, current daily cigarette consumption, pack years, years since last smoked, and current alcohol consumption.
§The mean (SD), and for log^{10} transformed variables median (interquartile range).
HDL, high density lipoprotein; LDL, low density lipoprotein.
*P < 0·05; **P < 0·01; ***P < 0·001.

attend originally. The respective proportion of subjects who had a manual occupation were 51%, 52%, and 52%, with occupations of those who were invited but failed to attend unknown.

The intra-assay and interassay variation for IL-6 was 5% and 12%, respectively, and for TNFα, 6% and 19%. Serum IL-6 concentrations were measurable in all subjects, with a mean concentration of 3·49 pg/ml, median 1·73 pg/ml (interquartile range 1·21 to 2·91 pg/ml, range 0·48 to 67·1 pg/ml). Serum TNFα was below the limit of detection in 17 cases. For the purposes of statistical analysis these samples were assigned a value of 0·09 pg/ml (half the lower limit of detection). The mean TNFα concentration was 1·21 pg/ml, median 1·082 pg/ml (interquartile range 0·60 to 1·50 pg/ml, range 0·09 to 6·51 pg/ml).

ASSOCIATIONS WITH EXOGENOUS CARDIOVASCULAR RISK FACTORS

Table 1 shows the relation between exogenous (lifestyle) risk factors for coronary heart disease and TNFα and IL-6, both before and after adjustment for each other. *H pylori* seropositivity and BMI were positively related to serum TNFα concentrations both before and after adjustment. Alcohol consumption, on the other hand, was negatively associated with serum TNFα concentrations. Age, chronic bronchitis, father's social class, alcohol consumption, father's occupation manual, and smoking were positively related to serum IL-6 concentrations.

ASSOCIATIONS WITH ENDOGENOUS CARDIOVASCULAR RISK FACTORS

The relations between TNFα and IL-6 and endogenous cardiovascular risk factors are shown in table 2. The coefficients shown represent the change in risk factor on for a change in TNFα and IL-6 from the 25th to the 75th centile of the distribution of values for this population. There were strong positive associations between TNFα and C reactive protein, fibrinogen, and sialic acid, and a strong negative relation with high density lipoprotein (HDL) cholesterol. There was a weaker association with serum triglycerides, and none with blood glucose or low density lipoprotein (LDL) cholesterol. IL-6 was strongly positively related to sialic acid, fibrinogen, and C reactive protein. There was a weaker relation with triglycerides, and a negative relation with HDL cholesterol; as with TNFα, no relation with blood glucose or LDL cholesterol was found.

ASSOCIATION WITH CORONARY HEART DISEASE

Table 3 shows the association of serum TNFα and IL-6 concentrations with coronary heart disease. There were 50 subjects with evidence of coronary disease; 26 had an abnormal ECG (13 had Q wave infarcts, four had T wave inversion, six had ST depression, and three had left bundle branch block), and a further 24 had a normal ECG but a history of myocardial infarction or symptoms of angina on the Rose angina questionnaire. Relations were stronger with abnormal ECGs than with symptomatic heart disease, particularly with TNFα. The magnitude of the associations was little diminished by controlling for exogenous risk factors, except that of IL-6 with symptomatic coronary disease, but the relation of TNFα with all (prevalent) coronary heart disease and of IL-6 with abnormal ECG became of borderline statistical significance (0·1 > P > 0·05).

Table 3 Relation of TNFα and IL-6 to electrocardiographic (ECG) abnormalities and prevalent heart disease

	TNFα (pg/ml) geometric means			*IL-6 (pg/ml) geometric means*		
	CHD	*normals*	*TNFα ratio of means*	*CHD*	*normals*	*IL-6 ratio of means*
Unadjusted						
Abnormal ECG	1·25	0·83	1·51 (1·02 to 2·20)*	2·96	1·88	1·57 (1·12 to 2·21)**
Prevalent CHD (n = 50)§	1·10	0·89	1·25 (0·93 to 1·69)	2·72	1·80	1·51 (1·16 to 1·97)**
Adjusted¶						
Abnormal ECG (n = 26)	1·28	0·83	1·53 (1·00 to 2·37)*	2·77	1·91	1·45 (0·98 to 2·12)
Prevalent CHD (n = 50)§	1·09	0·85	1·28 (0·93 to 1·77)	2·62	1·93	1·36 (1·02 to 1·81)*

¶Adjusted for age, body mass index (BMI), father's social class in six categories, current occupation in six categories, current and ex-smoking, current daily cigarette consumption, pack years, years since last smoked, and current alcohol consumption.
§Prevalent refers to abnormal ECG plus subjects with symptomatic coronary disease defined by Rose angina questionnaire alone.
CHD, coronary heart disease.
*P ⩽ 0·05; **P < 0·01.

Discussion

This study is the first to examine in detail factors associated with serum IL-6 and TNFα concentrations within the conventional reference range and to explore their relation to risk factors for cardiovascular disease. This is also the first suggestion of a relation between IL-6 and TNFα and ECG abnormalities indicative of past or present ischaemic heart disease and symptoms of angina or myocardial infarction.

We were able to detect serum concentrations of these cytokines in virtually all the subjects tested, and found that a variety of phenomena were associated with changes in serum cytokines. Gastric inflammation produced by *H pylori* infection, and bronchial inflammation underlying symptoms of chronic bronchitis were both associated with raised serum concentrations of TNFα or IL-6. The reason why inflammation in one area should be associated with raised serum concentrations of one cytokine and not the other is unclear. Smoking was associated with raised IL-6, and some of this relation was accounted for by chronic bronchitis. It is also possible that vascular endothelium could synthesise cytokines in response to the products of cigarette smoke. The observation that alcohol consumption is associated with reduced TNFα is consistent with studies which showed that alcohol suppresses TNFα production by macrophages.[19] The positive association of IL-6 with alcohol consumption was unexpected, or a direct effect of alcohol on IL-6 synthesis. The association of BMI with raised serum TNFα is consistent with recent work showing that the adipocytes of obese subjects synthesise increased amounts of TNFα mRNA and that this returns to normal on losing weight.[17]

The associations of TNFα and IL-6 with fibrinogen and sialic acid are consistent with in vitro experiments on isolated hepatocytes.[9] Both IL-6 and TNFα have been shown to influence lipid metabolism in animals through two mechanisms.[11 21] First, both stimulate fatty acid synthesis by the liver, and second, TNFα stimulates lipolysis by adipocytes. These changes could explain the association of both with serum triglycerides. The reason for the strong association of TNFα with a reduced serum level of HDL is uncertain. It has been speculated that the reduced HDL seen in inflammation results from increased serum concentrations of serum amyloid A protein replacing apoA1 as an apolipoprotein in HDL particles, and that this leads to increased catabolism.[22] The association of alcohol with raised HDL cholesterol concentrations could be explained by its association with reduced serum TNFα. A recent report of the association of alcohol consumption with increased insulin sensitivity[23] may be explained by the effects of alcohol on TNFα production. TNFα expression by adipose tissue has been linked to insulin resistance.[12]

Serum cytokine concentrations may be associated with ECG abnormalities and symptoms of angina through their effects on endogenous risk factors, and hence provide mechanisms whereby exogenous risk factors can result in increased risk of cardiovascular disease. However, it is increasingly appreciated that a key pathological process in atherosclerosis is inflammation.[24] Hence it is possible that the raised serum concentrations of cytokines are a consequence of inflammation in the arterial wall, and that levels of certain endogenous risk factors are merely epiphenomena of this process, while others are causes of atherosclerosis and thereby of raised levels of circulating cytokines.

On the other hand direct and indirect effects of TNFα and IL-6 shown in vitro and in vivo could have important effects on the development of the atherosclerotic lesion. Both have intense proinflammatory, growth promoting, and procoagulant effects.[25] TNFα and IL-6 generated away from the arterial wall could produce these effects, as could locally produced cytokines. Exogenous risk factors for cardiovascular disease such as cigarette smoke and alcohol could, if distributed throughout the body, affect the inflammatory process and consequent production of IL-6 and TNFα in the arterial wall directly. They could also have effects on cytokine production at distant sites, in the lungs for example in smokers. Other risk factors, such as obesity and *H pylori* infection, are unlikely to have effects directly at the site of the atherosclerotic lesion, but could influence the atheroma process through distant production of cytokines, or through stimulating circulating white blood cells to produce them.

In conclusion, we have confirmed that many of the phenomena with which C reactive protein is associated are also associated with serum levels of cytokines, which is as would be expected from in vitro studies. Our study provides confirmation that similar mechanisms may be operating in vivo. The associations of serum concentrations of TNFα and IL-6 with cardiovascular risk factors observed in this study require confirmation in larger cross sectional and prospective studies, but if confirmed they suggest new and exciting markers of, or mechanisms for, the pathogenesis of atherosclerosis.

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