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Circulating levels of MCP-1 and eotaxin are not associated with presence of atherosclerosis or previous myocardial infarction

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Abstract

The chemokines are a family of signalling proteins that participate in regulation of the immune system and have been implicated in the pathogenesis of vascular diseases. Deleting the gene encoding the chemokine MCP-1 in mouse models of atherosclerosis reduces lipid lesion formation and circulating chemokines are upregulated in man immediately following myocardial infarction (MI) or coronary angioplasty. We have therefore investigated whether circulating levels of two chemokines (MCP-1 and eotaxin) differ between subjects with and without atherosclerosis. We have used three different methods of measuring the presence and extent of atherosclerosis in human subjects: duplex ultrasonography of the carotid arteries and clinical diagnosis of coronary heart disease on individuals from the general population and coronary angiography on patients with suspected heart disease. There was no difference in the levels of circulating MCP-1 or eotaxin, measured by ELISA, between subjects with and without atherosclerosis. Furthermore, any increase in circulating MCP-1 following acute MI must be short-lived, since chemokine levels were not different in subjects who had had an MI previously compared to those who had not. We conclude that although there may be a transient increase in circulating chemokine levels following coronary angioplasty, there is no difference in the levels of circulating MCP-1 or eotaxin in subjects with and without atherosclerosis.

Keywords: Angiography; Chemokine; Coronary artery disease; Cytokine; Immunology

1. Introduction

The chemokines are a large family of signalling molecules that have a role in the maintenance of the immune system [1]. Most chemokines act as chemoattractants for leukocytes, although the specificity of particular chemokines for different leukocyte subsets varies. The chemokine family is comprised of several sub-families that are distinguished by the presence or absence of intervening amino acids between two conserved cysteine residues in their primary sequence. The two major sub-families of chemokines are designated CC and CXC chemokines, by the presence or absence of one intervening residue. These two sub-families vary in their

Abbreviations: 1VD, 2VD, 3VD, subjects with 1, 2 or 3 of their coronary arteries heavily diseased (see Section 2); ANOVA, analysis of variance; CHD, coronary heart disease; ELISA, enzyme-linked immunosorbent assay; GLM, general linear model; HDL, cholesterol in the high-density lipoprotein fraction; LDL, cholesterol in the low-density lipoprotein fraction; MaGiCAD, the Metabonomics and Genomics in Coronary Artery Disease Study; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; n.s., not statistically significant; NCA, subjects with normal coronary arteries; PAI-1, plasminogen activator inhibitor-1; PTCA, percutaneous transluminal coronary angioplasty

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specificity of action—most CC chemokines are chemoattractants for monocytes and T-lymphocytes whereas most CXC chemokines are chemoattractants for neutrophils [1].

MCP-1, an extensively studied CC chemokine, is a potent chemoattractant for monocytes, whereas eotaxin, another CC chemokine, is a potent eosinophil chemoattractant [2]. Both MCP-1 and eotaxin are present in the circulation, although circulating levels of eotaxin are typically somewhat lower than circulating levels of MCP-1 [3]. Due to their leukocyte chemoattractant properties, both MCP-1 and eotaxin are thought to be involved in the regulation of the immune system. Consistent with this, patients with diseases that involve misregulation of the immune system typically have higher levels of circulating chemokines (e.g. MCP-1 in HIV infection [4] and eotaxin in asthma [5]).

Several lines of evidence suggest that chemokines play an important role in leukocyte recruitment during atherogenesis. Recent studies have demonstrated that knocking out the *JE* gene encoding MCP-1 in three different mouse models of atherosclerosis reduces both macrophage accumulation into the vessel wall and atherosclerosis lesion development [6–8]. Deletion of the MCP-1 receptor gene (*ccr*2) has a similar effect [9]. Furthermore, MCP-1 is present in human atherosclerotic plaques [10–12].

Eotaxin may also be involved in the pathogenesis of atherosclerosis, since levels of both eotaxin and its receptor (CCR3) are upregulated in human atherosclerotic plaques [13].

MCP-1 has been shown to be elevated 24 h following myocardial infarction (MI) [14], in patients with restenosis following percutaneous transluminal coronary angioplasty [15,16] and in patients with congestive heart failure [17], but it is not yet known if the levels of circulating MCP-1 are associated with the presence or severity of atherosclerotic disease.

We used three different methods to measure the presence and extent of atherosclerosis or coronary heart disease [18], and assessed the levels of circulating chemokines in these subjects. We show that levels of circulating MCP-1 and eotaxin are not associated with the presence of atherosclerosis or with coronary heart disease in any of the subject groups studied. Furthermore there is no difference in circulating MCP-1 or eotaxin between subjects who have had a MI and those who have not.

2. Materials and methods

The investigation conforms with the principles outlined in the declaration of Helsinki

2.1. Subjects examined for carotid atherosclerosis and coronary heart disease

Recently, the MRC Environmental Epidemiology Unit has carried out several studies in cohorts of people born in the Jessop Hospital for Women, Sheffield, UK. These people were traced using the National Health Service Central Register and those still living in the city were invited to take part in research into the processes by which environment in early life influences adult disease. We took the opportunity to examine the relation between serum concentrations of chemokines and carotid atherosclerosis and coronary heart disease in 446 men and women aged 66–75 years. The study sample has been described previously [19], and is termed the 'Sheffield cohort' herein for clarity.

Subjects underwent a colour duplex ultrasonographic examination of the common, internal and external carotid arteries and carotid bifurcation. Intima-media thickness of the far wall was measured three times in the common carotid artery and twice in the internal carotid artery on each side. In the statistical analysis, intima-media thickness was defined as the average of all measurements made. The maximum degree of stenosis was estimated as a percentage of lumen diameter loss on each side, and each subject placed into one of the following four categories: no atherosclerotic plaques, up to 30% stenosis, 31–50% stenosis and more than 50% stenosis.

Separately, but in the same cohort of subjects, coronary heart disease (CHD) was defined as the presence of one or more of the following: angina according to the Rose/WHO cardiovascular questionnaire, electrocardiograph codes 1-1, 1-2 (Q and QS codes), or a history of coronary artery bypass grafting or coronary angioplasty. Of the 446 subjects, 111 had coronary heart disease by this definition. Characteristics of the Sheffield study cohort when analysed in this way are shown in Table 1.

Table 1
Characteristics of subjects with and without coronary heart disease (the Sheffield cohort)

	No CHD	CHD	*p*
n	335	111	
Age	70.2	70.0	*n.s.*
Sex (% male)	57	63	*n.s.*
Smokers (%)			
Current	22.7	19.1	*n.s.*
Current + past	68.4	71.8	*n.s.*
Systolic blood pressure (mm Hg)	144	144	*n.s.*
Diastolic blood pressure (mm Hg)	80	79	*n.s.*
Body mass index (kg/m^2)	26.8	27.8	0.04
HDL (mM)	1.3	1.2	< 0.01
LDL (mM)	4.1	4.1	*n.s.*
Total triglyceride (mM)	1.6	1.8	0.02
Total cholesterol (mM)	6.1	6.0	*n.s.*
Creatinine (mg/ml)	95	99	0.05

Values are means, unless otherwise stated. Subjects were classified as given in Section 2. Statistical significance was examined using ANOVA or the χ^2 test. HDL and LDL = cholesterol in the high and low-density lipoprotein fractions respectively, n.s. = not statistically significant

2.2. Subjects examined for coronary atherosclerosis by angiography

Two groups of subjects were examined for the potential relationship between chemokine levels and coronary atherosclerosis.

The first group of subjects with coronary atherosclerosis (termed the 'Papworth NCA/3VD cohort') was selected as follows. Subjects presented to their general practitioner with chest pain and were subsequently found to have a positive exercise electrocardiogram. Individuals with diabetes or left ventricular hypertrophy were excluded from the study. Following a coronary angiogram, the number of major coronary arteries (left anterior descending, circumflex or right coronary artery) that had >50% stenosis was noted. Consecutive patients arriving at Papworth Hospital (Cambridgeshire, UK) that were categorised as having either normal coronary arteries (NCA; neither artery significantly stenosed) or three vessel disease (3VD) were recruited, and this group therefore represented subjects at the extremes of the disease severity. Characteristics of this group are given in Table 2.

The second group of subjects examined for coronary atherosclerosis by angiography are the first 440 patients recruited into the Metabonomics and Genomics in Coronary Artery Disease Study (MaGiCAD). MaGiCAD recruitment is ongoing and currently comprises some 1000 patients who have been referred for a diagnostic coronary angiogram. The only patients excluded from the study were those returning for a check-up following a heart transplant. A blood sample was taken from the patient's arterial sheath (through which the catheter was to be inserted) prior to administration of the contrast medium. Serum was prepared from this arterial blood sample in the same way as when the blood sample was taken from the cubital vein. In a subset of 15 patients we confirmed that there was no difference between the levels of MCP-1 measured in the venous sample drawn from the cubital vein compared to the arterial blood sample taken from the femoral artery (<1% difference in mean circulating venous levels versus arterial levels for both MCP-1 and eotaxin). The result of the angiogram was used to categorise each patients into one of five categories using a formula based largely on that described by Rinqvist et al. [20], Appendix I, 1. The only modification made was that those patients who did not have 1, 2 or 3-vessel disease (1, 2 and 3VD) were further subdivided into NCA (no angiographically-defined disease noted at all) and Mild categories. Patients in the Mild category had noteable atherosclerotic lesions, but none of the lesions put the patient into the 1VD group due to size and/or location of the lesions.

Table 2
Characteristics of the Papworth NCA/3VD cohort: subjects with severe atherosclerosis (3VD) and subjects with normal coronary arteries (NCA)

	NCA	3VD	*p*
n	31	41	
Age	57.1	63.0	<0.01
Sex (% male)	26	95	<0.01
Previous MI (%)	3	56	<0.01
Smokers (%)			
Current	8	7	n.s.
Current + past	38	78	<0.01
Systolic blood pressure (mm Hg)	146	135	n.s.
Diastolic blood pressure (mm Hg)	81.0	73.7	n.s.
Body mass index (kg/m^2)	26.5	27.8	n.s.
HDL (mM)	1.1	0.8	<0.01
LDL (mM)	3.9	4.7	0.03
Total triglyceride (mM)	1.30	1.88	<0.05
Total cholesterol (mM)	5.7	6.4	<0.05
Creatinine (mg/ml)	100.7	101.8	n.s.

Values shown are arithmetic or geometric means (BMI and triglycerides geometric means; remaining variables arithmetic means), unless otherwise stated. Due to the large age and sex difference between the groups, values shown are marginal means, adjusted for age and sex. Subjects were selected as described in Section 2. Statistical significance of the differences between the two groups were examined using the Student's *t*-test (for age), the χ^2 test (sex and smoking) or a GLM univariate model, adjusting for age and sex (remaining variables).

During monitoring of the data it was noted that there was a significant effect of patients having taken a diazepam and/or clopidogrel pre-med prior to their angiogram on the levels of circulating MCP-1 measured. Those patients that had received a pre-med had levels of circulating MCP-1 12% lower than those who did not ($p = 0.001$). This effect is particularly important as the patients with more severe coronary artery disease are more likely to have received a pre-med. All patients who had received either a diazepam and/or a clopidogrel pre-med were therefore excluded from all further analysis. Characteristics of the remaining 251 patients are given in Table 3. Of these, 74 (32%) had previously had an MI (18 patients with unknown MI status excluded).

2.3. Preparation of blood samples

For the preparation of serum from subjects undergoing coronary angiography, blood was removed either from the cubital vein (Papworth NCA/3VD cohort detailed in Table 2) using a 19-gauge needle and transferred into a polypropylene tube or from the femoral artery (MaGiCAD subjects detailed in Table 3). The blood was then allowed to clot for between 2 and 3 h and then the cells spun out at 4000 × *g*. The supernatant (serum) was collected, aliquoted and stored at −80 °C until use.

Preparation of samples from the Sheffield cohort (detailed in Table 1) has been described previously [19].

2.4. Assay of MCP-1 and eotaxin concentrations in serum and plasma

Measurements of MCP-1 and eotaxin concentration in serum were performed using the respective Quantikine ELISA kits (R&D Systems, Abingdon, Oxon.) in accordance with the manufacturers instructions. Inter-assay precision for the MCP-1 kit is given by R&D Systems as approximately 6%, and for the eotaxin kit approximately 10%. According to the manufacturer, typical values for MCP-1 in normal human

Table 3
Characteristics of male subjects with varying degree of coronary atherosclerosis (the MaGiCAD study)

	NCA	Mild	1VD	2VD	3VD	*p*
n	56	34	53	45	63	
Age	58.5	63.5	64.1	63.9	66.3	<0.0001
Sex (% male)	48	65	77	82	90	<0.0001
Previous myocardial infarction (%)	2	12	50	45	44	<0.0001
Smokers (%)						
Current	19.6	20.6	20.8	18.2	9.5	n.s.
Current + past	62.5	64.7	81.1	77.3	84.1	0.033
Systolic blood pressure (mm Hg)	132	145	134	135	135	n.s.
Diastolic blood pressure (mm Hg)	78	81	75	78	73	0.04
Body mass index (kg/m^2)	28.0	27.9	27.8	28.5	27.3	n.s.
HDL (mM)	1.21	1.19	0.92	0.97	0.93	<0.0001
LDL (mM)	2.87	2.37	2.21	2.18	2.41	0.006
Total triglyceride (mM)	1.51	2.00	1.76	2.00	1.51	n.s.
Total cholesterol (mM)	4.85	4.73	4.55	4.45	4.32	0.002
Creatinine (mg/ml)	88.7	91.1	100.8	96.7	101.4	<0.0001

Values shown are means with a *p* for trend calculated using linear regression, unless otherwise stated. Subjects were selected as described in Section 2.

serum are 200–722 pg/ml (mean 370 pg/ml). Typical values for eotaxin in normal human serum are given as 50–642 pg/ml (mean 163 pg/ml).

2.5. Statistical analysis

The normality of the data distributions was checked using the Kolgomorov–Smirnoff test. Typically, circulating levels of MCP-1 and eotaxin were not normally distributed, and therefore both were logarithmically transformed prior to all analyses.

For analysis of correlations between variables, the Pearson's *r*-test was used.

For comparisons between two groups of data, either a *t*-test or Mann–Whitney *U* (non-parametric)-test were used.

Linear regression was used to determine statistical likelihood of a linear trend across subject groups.

A univariate general linear model was used to analyse the effect of subject group on the levels of circulating MCP-1 and circulating eotaxin, with correction for other covariates, such as age and sex as described in the text.

Due to the large age and sex differences between the groups described in Table 2, a univariate general linear model was used to analyse differences in other variables between the two subject groups, adjusting for age and sex.

For all statistical tests $p < 0.05$ was taken to indicate statistical significance. Furthermore, prior to any analyses, the MaGiCAD data was trimmed and winsorized to ±3 standard deviations.

3. Results

3.1. MCP-1 and eotaxin in carotid atherosclerosis and coronary heart disease

In a group of subjects collected in Sheffield, UK, carotid artery atherosclerosis was determined as described in Section 2. Circulating levels of the two chemokines MCP-1 and eotaxin were then measured in serum samples from the same patients. There was no statistically significant relation between carotid intima-media thickness and serum levels of either chemokine. The age- and sex-adjusted correlation coefficient between intima-media thickness and serum MCP-1 was 0.021 ($p = 0.658$), while that between intima-media thickness and serum eotaxin was 0.024 ($p = 0.659$). Concentrations of MCP-1 and eotaxin tended to be higher in participants with a greater degree of carotid stenosis after adjustment for age and sex, but these relations were not statistically significant after further adjustment for serum cholesterol, smoking status and blood pressure, *p* for trend = 0.209 and 0.142, respectively (see Fig. 1).

In the same subjects, the presence of atherosclerosis in the coronary arteries was estimated by determining the presence of coronary heart disease (CHD), as described in Section 2. Although the presence of CHD is not a direct measure of atherosclerosis, it is indicative of increased atherosclerosis in the coronary blood vessels. We compared circulating levels of MCP-1 and eotaxin in people with and without CHD, and found no significant difference in either chemokine. After adjustment for age and sex, geometric mean levels of MCP-1 were 289 pg/ml in subjects with CHD versus 306 pg/ml in subjects without CHD ($p = 0.171$). Age- and sex-adjusted geometric mean levels of eotaxin were 141 pg/ml in both subject groups ($p = 0.927$).

3.2. MCP-1 and eotaxin in coronary atherosclerosis

A better way of determining the extent of coronary atherosclerosis is to use a coronary angiogram to determine the degree of narrowing of the lumen of the coronary arteries. Therefore, we determined the levels of MCP-1 and eotaxin in the serum from 72 subjects, 31 of whom had normal coronary arteries (NCAs) and 41 of whom had extensive atherosclerosis (3VDs) as determined by coronary angiography (the Papworth NCA/3VD cohort). There was no significant difference between the two subject groups for either MCP-1 or eotaxin,

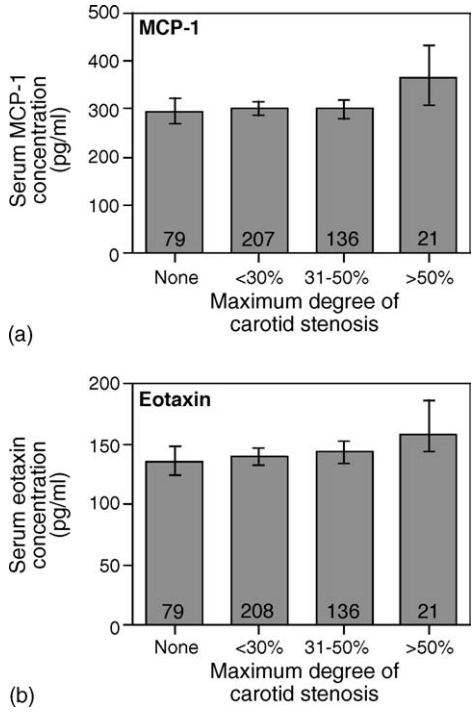

Fig. 1. Serum levels of MCP-1 (a) and eotaxin (b) in subjects with varying degrees of carotid stenosis (the Sheffield cohort). Values shown are geometric means, adjusted for age, sex, serum cholesterol, smoking status and blood pressure, with 95% confidence intervals. The number of subjects in each group is shown at the base of each bar. Linear regression was used to test for a statistical difference.

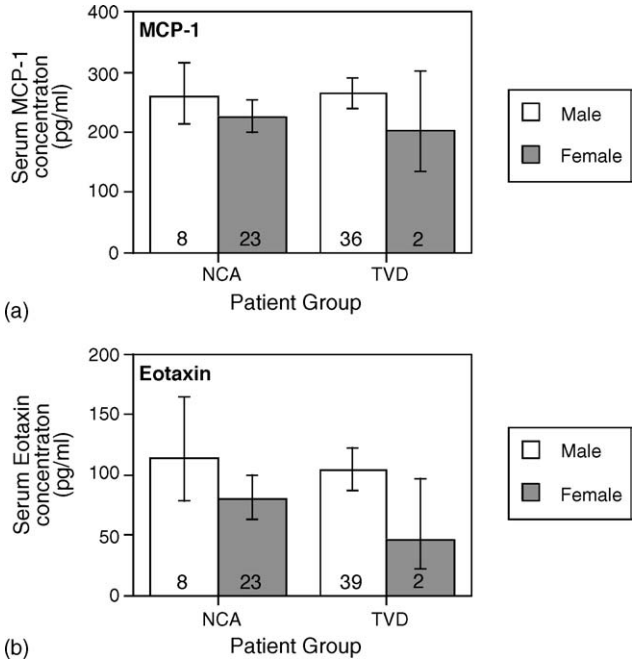

Fig. 2. Serum levels of MCP-1 (a) and eotaxin (b) in subjects with severe atherosclerosis (3VD) and subjects with normal coronary arteries (NCA; the Papworth NCA/3VD cohort). Values shown are geometric means, adjusted for age and sex, with 95% confidence intervals. The number of subjects in each group is shown at the base of each bar. A GLM univariate analysis was used to test for a statistical difference.

after adjustment for age and sex ($p = 0.917$ and 0.315, respectively, Fig. 2). Serum levels of MCP-1 were 19% higher in males than in females, although this difference is of borderline significance ($p = 0.085$) while eotaxin levels were 60% higher in men ($p = 0.013$). Levels of circulating MCP-1 and eotaxin correlated in these subjects ($r = 0.414$, $p = 0.0004$).

For a more comprehensive analysis of any differences in the levels of MCP-1 between patients with varying degrees of atherosclerosis and patients without overt atherosclerosis, we measured circulating MCP-1 in the first 440 patients recruited into the Metabonomics and Genomics in Coronary Artery Disease Study (MaGiCAD). Circulating levels of MCP-1 were measured in all 440 patients using serum prepared from blood obtained from the arterial sheath through which the catheter was to be inserted. However, during data monitoring it was found that the levels of MCP-1 were significantly elevated in those patients who had received a pre-med prior to their angiogram, and these patients were therefore excluded from all further analysis (see Section 2). In the remaining 251 patients there was a small, marginally statistically significant trend to increasing levels of circulating MCP with increasing severity of disease ($r = 0.119$, $p = 0.059$). Following adjustment for age and sex, this small association became weaker ($r = 0.101$, $p = 0.114$), while further adjustment for serum cholesterol, smoking status and blood pressure reduced the association even more ($r = 0.072$, $p = 0.265$; Fig. 3).

3.3. Circulating chemokines in subjects who have had a myocardial infarction

Approximately one-third of the subjects for whom coronary atherosclerosis was measured using angiography had previously had a MI. We analysed the levels of circulating MCP-1 and eotaxin in subjects from the Papworth NCA/3VD cohort and the MaGiCAD study subjects to see if there was a difference between those who had previously had a MI and those who had not. There was no difference

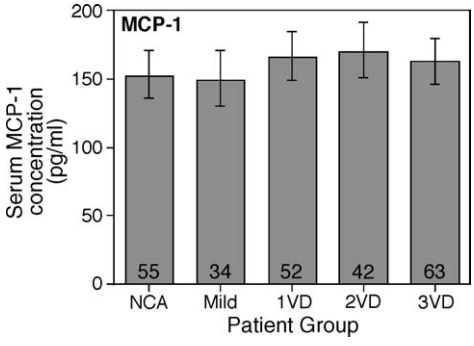

Fig. 3. Serum levels of MCP-1 in subjects with varying degrees of coronary atherosclerosis (the MaGiCAD study). Values shown are geometric means, adjusted for age, sex, serum cholesterol, smoking status and blood pressure, with 95% confidence intervals. The number of subjects in each group is shown at the base of each bar. Linear regression was used to test for a statistical difference.

in either circulating MCP-1 or eotaxin for those subjects who had had a previous MI compared to those that had not in the Papworth NCA/3VD cohort. After adjustment for age and sex, geometric mean levels of MCP-1 were 246 and 252 pg/ml in the no MI and MI groups respectively ($p = 0.750$). Likewise, age and sex-adjusted geometric means of serum eotaxin concentrations were 97 and 87 pg/ml in the no MI and MI groups, respectively ($p = 0.457$).

In the subjects from the MaGiCAD cohort, circulating MCP-1 was 11% higher in patients that had had a MI compared to those that had not (geometric means 171 pg/ml versus 154 pg/ml), and this was of borderline statistical significance ($t = -1.95$, $p = 0.053$). Adjustment for age and sex had little effect ($p = 0.068$), whereas further adjustment for serum cholesterol, smoking status and blood pressure reduced the effect somewhat (geometric means 169 pg/ml versus 155 pg/ml; $p = 0.122$).

4. Discussion

This study has shown no robust significant difference in the levels of MCP-1 or eotaxin between subjects with and without atherosclerosis, irrespective of the way in which extent of atherosclerosis is estimated. We have also demonstrated that there is no long-term elevation in the levels of circulating MCP-1 or eotaxin following myocardial infarction.

The use of more than one method to estimate the extent of atherosclerosis is important [18]. Atherosclerosis is a very diverse disease, and it is still unclear whether the presence of atherosclerotic plaques in one region of the vasculature will have the same underlying biological basis as in the other. In order to increase our power to detect any difference in circulating chemokine levels between subjects with and without atherosclerosis, we have used several different methods to estimate the presence and extent of atherosclerosis. Firstly, we have used colour duplex ultrasonography to measure both the maximum stenosis in the carotid arteries and the intima-media thickness. Secondly, we used the Rose/WHO cardiovascular questionnaire and the medical records of subjects to define the presence or absence of coronary heart disease. Thirdly, we have used coronary angiography to measure the extent of coronary stenosis, and used this data to classify people into categories depending on their number of diseased coronary arteries. These three methods will each have in-built bias in the way in which they determine the presence and extent of atherosclerosis, thus by using each of them we reduce the possibility that the conclusions that we draw have only limited applicability and increase the probability of determining any possible link between circulating MCP-1 and eotaxin and atherosclerosis.

Our report is consistent with one previous report that, although lacking power, suggested that there was little difference in circulating MCP-1 concentrations between subjects with overt atherosclerosis and age-matched controls [21]. However, there have been several reports demonstrating that levels of circulating chemokines are different in other vascular conditions. Economou et al. [22] measured circulating levels of MCP-1 and eotaxin in patients undergoing coronary angiography or PTCA. In their hands (also using the R&D Systems Quantikine ELISA), levels of MCP-1 and eotaxin were higher in patients than healthy controls, and were elevated following the PTCA procedure. Moreover, Hokimoto et al. showed that those patients who had restenosis after PTCA had significantly higher levels of MCP-1 in their plasma at 48 h and 3 months after angioplasty than patients who did not have restenosis after their angioplasty [15]. One explanation for increased levels of circulating chemokine levels following angioplasty is the presence of heparin. Heparin is administered routinely prior to angioplasty as an anti-coagulant. Addition of heparin to blood cells ex vivo significantly increases the concentration of MCP-1 and eotaxin detected by ELISA in the liquid fraction (data not shown). Consistent with this, Morita et al. [3] show that eotaxin levels are higher in plasma prepared with heparin as the anticoagulant than with EDTA as the anticoagulant.

Circulating MCP-1 has also been shown to be upregulated 24 h following myocardial infarction [14]. However, in the present study, we show that this increase is not long-lived, since subjects who have had an MI at some point in the past do not have elevated circulating MCP-1 or eotaxin levels compared with subjects who have not had a previous MI. A previous study has also shown that circulating levels of MCP-1, RANTES and MIP-lα were elevated in patients with congestive heart failure, with MCP-1 especially upregulated in patients with coronary artery disease [17]. In summary, therefore, it is not clear why increased levels of circulating MCP-1 have been found in some subjects with some vascular diseases and not others, but it is likely to depend on the method of preparation of the blood fraction, the assay used to measure MCP-1 and the precise nature of the disease being examined.

We have found no consistent difference in the levels of circulating MCP-1 and eotaxin between men and women, nor have we found a correlation with age. Inadera et al. showed that there was a statistically significant positive correlation between serum MCP-1 levels and age [21], but they examined subjects with a much greater age range (20–72) than the present study. They also found that there was a tendency for MCP-1 levels to be higher in males than females, although for most age ranges this difference was not statistically significant.

We found a consistent correlation between circulating MCP-1 and circulating eotaxin concentrations. Since MCP-1 and eotaxin have similar roles in vitro, there may be a similar mechanism regulating their circulating levels in vivo.

Several previous reports have investigated the effect on levels of circulating MCP-1 of treatment with statins [23,24] or thiazolidinediones [25,26]. However, no consistent pattern has yet emerged. In the MaGiCAD cohort of patients, levels of circulating MCP-1 were not different in patients taking statins compared to those who were not, nor were levels of

MCP-1 different in patients taking any one particular statin or in patients taking higher doses of statins ($p > 0.05$ in all cases). Insufficient patients were taking thiazolidinediones for a statistical meaningful conclusion to be drawn.

In the MaGiCAD study patients who had a pre-med had lower levels of serum MCP-1 and this was not related to the extent of their coronary atherosclerosis by angiography. Of the 181 patients who had a pre-med, 60% had 10 mg diazepam, 7% had 300 mg clopidogrel and 33% had both. It is not clear which of the two drugs may be having the effect on serum MCP-1 concentrations. However, nearly 20% of the 440 patients were taking clopidogrel regularly at a dose of 75 mg OD, and this had no effect on serum MCP-1 concentrations (data not shown).

In summary, we have used three methods to estimate the prevalence of atherosclerosis, and correlated this with circulating levels of MCP-1 and eotaxin. We find no robust statistically significant difference in the levels of these circulating chemokines between subjects with and without atherosclerosis, or who have had or not had a previous MI. By analysing circulating MCP-1 levels in nearly 1000 subjects and eotaxin levels in over 500 subjects the present study has sufficient power to detect all but the most marginal of differences. We conclude that circulating MCP-1 and eotaxin levels are not different between subjects with and without atherosclerosis.

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