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Associations of Four Circulating Chemokines with Multiple Atherosclerosis Phenotypes in a Large Population-Based Sample: Results from the Dallas Heart Study

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Specific chemokines contribute to vascular inflammation and may be useful biomarkers to detect atherosclerosis. The chemokines CXCL1 and CCL11 have previously been studied in animal or human models of atherosclerosis, while CXCL2 and CCL23 have not. Among 2,454 subjects enrolled in the Dallas Heart Study, a multi-ethnic population-based sample, we measured plasma CCL11, CCL23, CXCL1, and CXCL2, and associated levels with coronary artery calcium (CAC) by computed tomography, and aortic wall thickness, plaque burden, and compliance by magnetic resonance imaging. Elevated chemokine levels were defined as greater than or equal to the median for CCL11 and CCL23 and greater than or equal to the upper detection limit for CXCL1 and CXCL2. Elevated CCL23 ($P < 0.01$) and CXCL1 ($P = 0.01$), but not CCL11 and CXCL2, associated with CAC in univariable analyses. After adjustment for traditional risk factors, elevated CCL23 remained associated with CAC (OR 1.3, 95% CI 1.0–1.7; $P = 0.02$), while the association with CXCL1 was modestly attenuated (OR 1.4, 95% CI 1.0–2.1; $P = 0.06$). CCL23 also associated with aortic wall thickness, plaque, and compliance in univariable analyses ($P < 0.05$ for each), but these associations were attenuated after multivariable adjustment. The novel chemotactic protein, CCL23, which has not been previously studied in atherosclerosis, is independently associated with coronary atherosclerosis, suggesting that this chemokine merits further study in animal and human models.

Introduction

Chemokines, the secreted proteins that recruit specific cell types to inflammatory sites, have emerged as major contributors to vascular inflammation. Considerable efforts have focused on characterizing the role of individual chemokines in the processes leading to atherosclerotic plaque development and progression. Study of circulating chemokines, therefore, may provide greater understanding of the underlying pathophysiology of atherosclerosis, as well as identifying novel biomarkers and potential targets for drug discovery.

Several chemokines that play an active role within the atherosclerotic plaque, including monocyte chemoattractant protein (MCP)-1 (CCL2), CCL4, CCL5, CXCL10, CXCL1, and CXCL16, have been identified (Deo and others 2004; Charo and Ransohoff 2006; Ardigo and others 2007; Zernecke and others 2008; de Oliveira and others 2009). It is possible that other less well-studied chemokines may also have important associations with atherosclerosis. Studies of circulating levels of CCL11 (eotaxin), a chemoattractant for eosinophils, have yielded conflicting results with regard to associations with atherosclerotic phenotypes (Haley and others 2000; Mosedale and others 2005; Sheikine and others 2006). Although CCL23 has demonstrated chemotactic activity on monocytes as well as activated T lymphocytes, with minimal activity on neutrophils, it has not been well-studied in relation to atherosclerosis (Forssmann and others 1997). CXCL1, also known as GRO-α (growth related oncogene α), is a chemoattractant for neutrophils, T lymphocytes, and monocytes, and induces free radical production, leading to endothelial cell damage (Bechara and others 2007). Expression is up-regulated under shear stress conditions in murine models of atherosclerosis; moreover, it has been shown to be present in human atherosclerotic plaques (Bechara and

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others 2007). CXCL2, also known as GRO-β, is a chemokine secreted by activated monocytes. It attracts neutrophils (Jabs and others 2007) and has similar activity as CXCL1; however, much less is known about the potential role of CXCL2 in atherosclerosis.

To further evaluate the role of novel circulating chemokines in atherosclerosis, we performed a large, cross-sectional evaluation of the associations between multiple atherosclerosis phenotypes and plasma levels of CCL11, CCL23, CXCL1, and CXCL2 in the Dallas Heart Study, a large, multi-ethnic population-based study.

Materials and Methods

Study population

The Dallas Heart Study (DHS) is a multi-ethnic, population-based, probability-based sample of 6,101 Dallas County residents (Victor and others 2004). The current study was performed in 3,177 DHS subjects, aged 30–65, who underwent measurement of plasma levels of CCL23, CXCL1, CXCL2, and CCL11. A subset of the study population underwent electron beam-computed tomography (EBCT) to measure coronary artery calcium (CAC; n = 2,435), and magnetic resonance imaging to measure aortic wall thickness (n = 2,238), aortic plaque burden (n = 2,223), and aortic compliance (n = 2,355).

Definition of variables

Hypercholesterolemia was defined as a calculated, fasting low-density lipoprotein (LDL) cholesterol ≥160 mg/dL; total cholesterol ≥240 mg/dL; or use of a statin medication. Hypertriglyceridemia was defined as a fasting triglyceride concentration ≥200 mg/dL. Low high-density lipoprotein (HDL) cholesterol was defined as HDL-C <40 mg/dL in men, and <50 mg/dL in women. Hypertension was defined as an average (based on 5 measurements) systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg at the first study visit, or use of any anti-hypertensive medication. Diabetes was defined as a fasting glucose level ≥126 mg/dL; or a random, non fasting glucose >200 mg/dL; or use of a hypoglycemic medication. Impaired fasting glucose was defined as >100 mg/dL. Glomerular filtration rate (GFR; mL/min per 1.73 m^2 body surface area) was estimated with the modified Modification of Diet in Renal Disease (MDRD) Study formula: GFR = $186 \times (\text{serum creatinine}^{-1.154}) \times (\text{age}^{-0.203}) \times (0.742 \text{ if female}) \times (1.21 \text{ if black})$. Chronic kidney disease was defined as an estimated GFR ≤60 mL/min per 1.73 m^2 BSA (Wallace and others 2006). Height, weight, hip, and waist circumference were measured at the same time as imaging was performed and body mass index and waist-to-hip ratio were calculated based on these measurements. Dual energy X-ray absorptiometry was used to measure bone density, total lean body mass, and total fat mass. Left ventricular hypertrophy was determined by cardiac MRI, and was defined as left ventricular mass indexed to body surface area >89 g/m^2 in women and >112 g/m^2 in men (Drazner and others 2005). Family history of myocardial infarction was defined as a mother, sister, or daughter who had a myocardial infarction at <55 years of age, or a father, brother, or son who had myocardial infarction at <50 years old (Victor and others 2004).

Measurement of chemokines and other biomarkers

Venous blood samples were collected in EDTA collection tubes and maintained at 4°C for <4 h, followed by centrifugation at 4°C. Aliquots of plasma were frozen at −80°C and shipped to Biosite, Inc. (an Inverness Medical Company, Waltham, MA), where they were thawed for measurement of the biomarkers. Sandwich immunoassays for CCL23, CXCL1, and CXCL2 were performed on Biosite's proprietary device platform, whereas an ELISA assay for CCL11 was performed in 384-well microtiter plates.

CXCL1 and CXCL2 are highly homologous. Antibodies to CXCL1 were generated at Biosite by immunization with CXCL1; using this antibody library, cross-reacting antibodies for CXCL2 were identified. Further screening was performed using antibody libraries created by immunization with C-terminal peptides for CXCL1 and CXCL2. The final antibody pairs for the CXCL1 and CXCL2 assays included one nonspecific and one specific antibody for each chemokine. The specific antibody pairs for CXCL1 and CXCL2 were tested and showed no cross-reactivity with each other or with CXCL3, CXCL4, CXCL5, CXCL7, or IL-8.

For CCL11, the minimal detection limit (MDL) was 10 pg/mL and average coefficient of variation (CV) across the assay range was 14%; for CCL23, the MDL was 0.13 ng/mL and the average CV was 13%; for CXCL1 the MDL was 0.07 ng/mL and the average CV was 20%; for CXCL2, the MDL was 0.13 ng/mL and the CV was 23%.

Adiponectin was measured using a sandwich ELISA assay (Biosite, Inc.) with a minimum detection limit of 0.24 μg/mL; samples were diluted 2,000-fold for measurement. The intra- and inter-assay CVs ranged from 4.6% to 9.0% at concentrations of 7.9 and 158 μg/mL, respectively. Serum fructosamine was measured to estimate glycemic control using a standard Roche/Hitachi fructosamine assay (Roche Diagnostics, Indianapolis, IN) given that whole blood samples were not available for glycosolated hemoglobin measurement. sICAM-1 and sVCAM-1 were measured using a competitive ELISA on a Luminex platform (Biosite, Inc.). The MDL for sICAM-1 was 100 ng/mL and for sVCAM-1 was 200 ng/mL. High-sensitivity C-reactive protein (CRP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), MCP-1, caspase-3, leptin, peptidoglycan recognition protein-1 (PGLYRP-1), lymphotoxin β receptor (LTβR), soluble endothelial cell adhesion molecule (sESAM), soluble receptor for advanced glycation end products (sRAGE), troponin T, cystatin C, lipoprotein-associated phospholipase A2 (LP-PLA2), and oxidized low-density lipoprotein (oxLDL) were measured as previously reported (Deo and others 2004; Abdullah and others 2005; Khera and others 2006; Wallace and others 2006; Brilakis and others 2008; Matulevicius and others 2008; Rohatgi and others 2009; Tsimikas and others 2009). Insulin levels were measured, and the homeostasis model of insulin resistance (HOMA-IR) was calculated as previously reported (Grundy and others 2008).

Atherosclerosis assessment

Coronary artery calcium (CAC) was determined using the average of 2 EBCT measurements. Subjects with an average CAC score > 10 Agatston units were defined as CAC-positive (Jain and others 2004). MRI was performed using a 1.5 Tesla whole-body MRI system (Intera, Philips Medical

Systems, Best, The Netherlands). Six transverse slices of the infrarenal abdominal aorta were taken. Image analysis was performed using the Magnetic Resonance Analytical Software Systems (MASS) cardiac analysis software package (Version 4.2 beta, Medis Medical Imaging Systems, Inc.). Using a free hand drawing tool, adventitia and luminal borders were drawn for the slices obtained, and atherosclerotic plaque was contoured based on hyperintense signal that protruded from the vessel wall. Using previously described methods, aortic wall thickness (AWT) and aortic plaque burden (APB) were calculated (Matulevicius and others 2008).

Statistical methods

Univariable associations between plasma levels of the chemokines and age, other biomarkers, body indices, and lipids were assessed by Spearman rank correlation coefficients. The relationships between atherosclerotic risk factors and these chemokines were assessed by the chi-squared test for contingency tables or Fisher's exact test where appropriate, with CCL11 and CCL23 dichotomized at the median value and CXCL1 and CXCL2 categorized as detectable and undetectable. Logistic regression was performed to assess associations between the chemokines and CAC score >10 in unadjusted and adjusted models including age, sex, race, hypertension, diabetes, current smoking, hypercholesterolemia, hypertriglyceridemia, low HDL, and BMI. Odds ratios were computed for CCL23 and CCL11 levels above their respective median values (versus less than or equal to the median values). CCL23 and CCL11 were also analyzed as continuous variables, calculating the odds ratios for a one log-unit change in log-transformed values. Because most individuals had undetectable levels of CXCL1 and CXCL2, odds ratios were calculated for those with detectable (versus undetectable) levels of these chemokines. Multivariable linear regression was performed to evaluate associations between the chemokines and aortic wall thickness (AWT), aortic plaque burden (APB), and aortic compliance with identical variables as in the logistic regression models. To ensure adequate linearity assumptions, CCL23 and CCL11 were log-transformed. Furthermore, all chemokines were dichotomized as described above, with the adjusted β-coefficient representing the incremental change in the average outcome measure for an elevated chemokine versus not elevated, holding other variables fixed.

All statistical analyses were performed using SAS Version 9.1.3 (Cary, NC). All statistical tests reported are 2-tailed, with $\alpha = 0.05$.

Results

The study cohort included 3,177 individuals, with a median age of 44 years [IQR 37–52], 37% women, and 30% Caucasian, 51% African American, and 17% Hispanic. The distributions of chemokines are shown in Figure 1. Most individuals had undetectable levels of CXCL1 and CXCL2 and both CCL11 and CCL23 displayed right-skewed distributions.

Associations of cytokines with atherosclerosis risk factors and biomarkers

CCL23 associated with older age (Table 1), white race, hypertension, hypercholesterolemia, and chronic kidney disease (Table 2). Moderate correlations were observed with a number of inflammatory markers, including sESAM, PGLYRP-1, LTβR, NT-proBNP, and cystatin C (Table 1). A weak association was observed between CCL23 and MCP-1 (Table 1). CXCL1 associated with African-American race, hypertension, diabetes, and chronic kidney disease (Table 2). CXCL2 also associated with African-American race and chronic kidney disease (Table 2), but not hypertension or diabetes. CXCL2 further correlated with levels of sESAM and caspase-3 (Table 1). Moderate correlations were observed between CXCL2 and both sICAM-1 and sVCAM-1 (Table 1). CCL11 was associated with non-African-American race (Table 2) and sRAGE (Table 1).

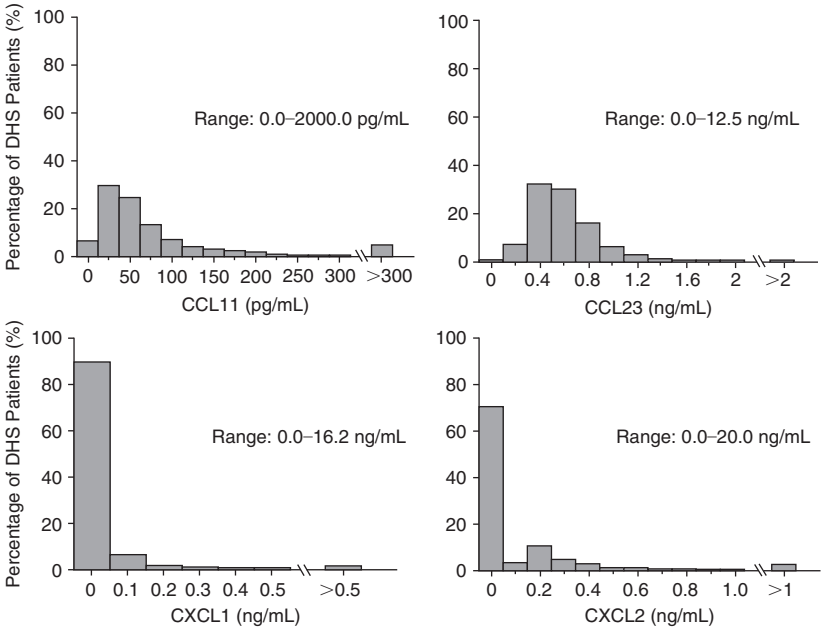

FIG. 1. Distribution of chemokines in Dallas Heart Study (DHS) subjects.

Table 1. Spearman Rank Correlations

	CCL23	*P*	*CXCL1*	*P*	*CXCL2*	*P*	*CCL11*	*P*
Age	0.117	<0.0001	0.02	0.32	0.025	0.16	0.010	0.58
Biomarkers								
CCL23	n/a	n/a	0.107	<0.0001	0.135	<0.0001	0.015	0.41
CXCL1	0.107	<0.0001	n/a	n/a	0.318	<0.0001	−0.043	0.02
CXCL2	0.135	<0.0001	0.318	<0.0001	n/a	n/a	−0.005	0.78
CCL11	0.015	0.41	−0.043	0.02	−0.005	0.78	1.000	0.000
sESAM	0.424	<0.0001	0.180	<0.0001	0.250	<0.0001	0.077	<0.0001
PGLYRP-1	0.416	<0.0001	0.101	<0.0001	0.138	<0.0001	0.010	0.57
LTβR	0.352	<0.0001	0.157	<0.0001	0.142	<0.0001	0.043	0.02
hsCRP	0.225	<0.0001	0.075	<0.0001	0.045	0.01	−0.069	0.000
MCP-1	0.117	<0.0001	0.076	<0.0001	0.118	<0.0001	0.104	<0.0001
Caspase-3	0.192	<0.0001	0.217	<0.0001	0.329	<0.0001	0.027	0.13
sRAGE	0.021	0.24	−0.066	0.000	−0.049	0.01	0.429	<0.0001
NT-proBNP	0.188	<0.0001	0.072	<0.0001	0.050	0.01	0.027	0.14
Troponin I	0.050	0.01	0.053	0.003	0.015	0.35	0.008	0.66
Cystatin C	0.285	<0.0001	0.026	0.16	0.047	0.01	0.019	0.30
sICAM-1	0.052	0.003	0.081	<0.0001	0.105	<0.0001	0.062	0.001
sVCAM-1	0.047	0.01	0.108	<0.0001	0.199	<0.0001	0.078	<0.0001
LP-PLA2	0.17	<0.0001	−0.005	0.768	0.01	0.567	0.08	<0.0001
ox LDL	−0.03	0.079	0.09	<0.0001	0.006	0.000	<0.08	<0.0001
Body Indices								
Waist/hip ratio	0.028	0.15	−0.038	0.05	−0.042	0.03	0.060	0.002
BMI	0.016	0.40	0.017	0.37	−0.018	0.35	−0.100	<0.0001
Systolic BP	0.046	0.02	0.049	0.01	0.021	0.27	−0.009	0.63
Diastolic BP	0.016	0.41	0.043	0.02	0.031	0.11	−0.037	0.05
Lipid Panel								
Total cholesterol	0.096	<0.0001	−0.022	0.21	0.026	0.14	0.030	0.09
LDL	0.102	<0.0001	−0.018	0.32	0.006	0.74	0.015	0.41
Triglycerides	0.108	<0.0001	−0.024	0.17	−0.007	0.68	0.086	<0.0001
HDL	−0.095	<0.0001	0.020	0.27	0.048	0.01	−0.068	0.000
Other Markers								
Adiponectin	0.036	0.04	−0.003	0.89	0.048	0.01	0.087	<0.0001
Leptin	0.012	0.54	0.035	0.06	0.013	0.51	−0.129	<0.0001
Fructosamine	0.008	0.66	0.071	<0.0001	0.071	<0.0001	0.054	0.002
HOMA	−0.032	0.09	0.034	0.08	−0.011	0.58	−0.054	0.01
GFR-MDRD	−0.206	<0.0001	0.030	0.10	−0.017	0.33	−0.026	0.14

Abbreviations: hsCRP, High-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; BNP, brain natriuretic peptide; MCP-1, monocyte chemoattractant protein-1; PGLYRP-1, peptidoglycan recognition protein-1; LTBR, lymphotoxin β-receptor; sRAGE, soluble receptor for advanced glycation end products; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble intercellular adhesion molecule 1; LP-PLA2, lipoprotein-associated phospholipase A2; ox LDL, oxidized low-density lipoprotein; DEXA, dual energy X-ray absorptiometry; BMI, body mass index; GFR, glomerular filtration rate calculated by Modification of Diet in Renal Disease formula; HOMA, homeostasis model assessment of insulin resistance.

The strongest correlations among chemokines occurred between CXCL1 and CXCL2 ($\rho = 0.32$; $P < 0.0001$), followed by weaker correlations between CCL23 and CXCL1 and CXCL2 ($\rho = 0.11$ and 0.14, respectively; $P < 0.0001$). CCL11 did not correlate with any other chemokine.

Univariable associations of cytokines with measures of subclinical atherosclerosis

Prevalent CAC (Agatston score > 10) was more common among subjects with CCL23 above the median compared to those with levels below the median (25% versus 18%, $P < 0.05$; Fig. 2). Similarly, subjects with detectable levels of CXCL1 ($n = 236$; ~10%) had a higher prevalence of CAC (28% versus 21%, $P < 0.05$; Fig. 2). There was no significant difference in CAC prevalence based on detectable levels of CXCL2 or levels of CCL11 above the median (Fig. 2).

Increasing aortic wall thickness (AWT) and aortic plaque burden (APB) were associated with higher levels of CCL23 (AWT: $P < 0.01$; APB: $P < 0.03$), but not with any of the other chemokines (Table 3). Aortic compliance was inversely associated with CCL23, CXCL1, and CXCL2 ($P < 0.05$ for each phenotype; Table 3). No associations were seen between CCL11 and any of the atherosclerosis phenotypes.

Multivariable associations with subclinical atherosclerosis

Levels of CCL23 above the median value remained associated with CAC in multivariable analyses adjusting for

Table 2. Associations of Chemokines and Atherosclerosis Risk Factors

	CCL23			*CXCL1*			*CXCL2*			*CCL11*		
Variable	*<0.56*† *(n = 1,587)*	*>0.56*† *(n = 1,590)*	*P value**	*= 0 (n = 2,850)*	*>0 (n = 327)*	*P value**	*= 0 (n = 2,234)*	*>0 (n = 943)*	*P value**	*<50.05*† *(n = 1,588)*	*>50.05*† *(n = 1,589)*	*P value**
Men	704 (44%)	698 (44%)	0.80	1,283 (45%)	119 (36%)	0.003	1,026 (46%)	376 (40%)	0.002	642 (40%)	760 (48%)	<0.0001
Women	883 (56%)	892 (56%)		1,567 (55%)	208 (64%)		1,208 (54%)	567 (60%)		946 (60%)	829 (52%)	
African-American	896 (56%)	727 (46%)	<0.0001	1,379 (48%)	244 (75%)	<0.0001	1,096 (49%)	527 (56%)	0.0004	959 (60%)	664 (42%)	<0.0001
Caucasian	392 (25%)	554 (35%)	<0.0001	910 (32%)	36 (11%)	<0.0001	661 (30%)	285 (30%)	0.72	388 (24%)	558 (35%)	<0.0001
Hispanic	265 (17%)	276 (17%)	0.62	498 (18%)	43 (13%)	0.05	424 (19%)	117 (12%)	<0.0001	217 (14%)	324 (20%)	<0.0001
Other race	34 (2%)	33 (2%)	0.90	63 (2%)	4 (1%)	0.31	53 (2%)	14 (2%)	0.14	24 (2%)	43 (3%)	0.03
Hypertension	498 (32%)	571 (37%)	0.01	927 (33%)	142 (45%)	<0.0001	724 (33%)	345 (37%)	0.02	561 (36%)	508 (33%)	0.05
Diabetes	189 (12%)	187 (12%)	0.90	325 (11%)	51 (16%)	0.03	254 (11%)	122 (13%)	0.21	185 (12%)	191 (12%)	0.742
Metabolic syndrome	497 (31%)	574 (36%)	0.004	952 (33%)	119 (36%)	0.28	755 (34%)	316 (34%)	0.88	536 (34%)	535 (34%)	0.96
Impaired fasting glucose	80 (5%)	98 (6%)	0.17	160 (6%)	18 (6%)	0.95	127 (6%)	51 (5%)	0.76	93 (6%)	85 (5%)	0.54
Current smokers	479 (30%)	438 (28%)	0.09	813 (29%)	104 (32%)	0.21	668 (30%)	249 (26%)	0.05	469 (30%)	448 (28%)	0.41
Hypercholesterolemia	164 (10%)	248 (16%)	<0.0001	365 (13%)	47 (14%)	0.43	279 (12%)	133 (14%)	0.22	198 (12%)	214 (13%)	0.40
Low HDL	600 (38%)	723 (45%)	<0.0001	1,185 (42%)	138 (42%)	0.83	936 (42%)	387 (41%)	0.65	633 (40%)	690 (43%)	0.04
Family history of MI	174 (11%)	167 (11%)	0.68	295 (10%)	46 (14%)	0.04	231 (10%)	110 (12%)	0.27	178 (11%)	163 (10%)	0.39
Statin use	85 (6%)	112 (7%)	0.05	173 (6%)	24 (8%)	0.33	126 (6%)	71 (8%)	0.04	93 (6%)	104 (7%)	0.39
ACE inhibitor	132 (8%)	174 (11%)	0.01	262 (9%)	44 (13%)	0.01	210 (9%)	96 (10%)	0.50	168 (11%)	138 (9%)	0.07
Chronic kidney disease	106 (7%)	183 (12%)	<0.0001	239 (8%)	50 (15%)	<0.0001	173 (8%)	116 (12%)	<0.0001	157 (10%)	132 (8%)	0.12

Values are number of people with the condition (% = column percents); **P* values are from chi-squared test; †splits are at median of each biomarker.
Abbreviations: ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein cholesterol; MI, myocardial infarction.

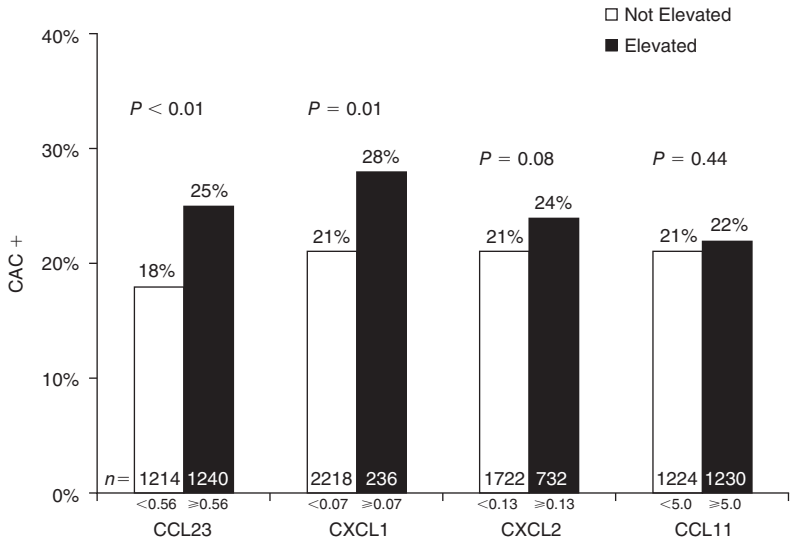

FIG. 2. Coronary artery calcium (CAC) and chemokines.

Table 3. Associations Between Chemokines and Atherosclerosis

	Unadjusted		*Adjusted*	
	OR [95% CI]	*P value*	*OR [95% CI]*	*P value*
CAC (n = 2,435)				
CCL23 > median*	1.5 [1.2, 1.8]	0.0001	1.3 [1.0, 1.7]	0.02
Log CCL23 continuous†	1.3 [1.2, 1.4]	<0.0001	1.2 [1.0, 1.3]	0.02
Detectable CXCL1	1.5 [1.1, 2.0]	0.01	1.4 [1.0, 2.1]	0.06
Detectable CXCL2	1.2 [1.0, 1.5]	0.08	1.3 [1.0, 1.6]	0.06
CCL11 > median*	1.1 [0.9, 1.3]	0.56	1.0 [0.8, 1.3]	0.97
Log CCL11 continuous†	1.0 [0.9, 1.1]	0.86	1.0 [0.8, 1.1]	0.48
	Unadjusted		*Adjusted*	
	Beta	*P value*	*Beta*	*P value*
AWT (n = 2,238)				
CCL23 > median*	0.034	0.01	−0.001	0.96
Log CCL23 continuous†	0.092	<0.0001	0.021	0.25
Detectable CXCL1	0.019	0.41	0.012	0.55
Detectable CXCL2	−0.002	0.89	0.005	0.71
CCL11 > median*	0.017	0.21	0.000	0.98
Log CCL11 continuous†	0.026	0.23	0.003	0.88
APB (n = 2,223)				
CCL23 > median*	0.189	0.03	0.029	0.69
Log CCL23 continuous†	0.133	0.002	0.011	0.76
Detectable CXCL1	0.125	0.38	0.078	0.52
Detectable CXCL2	−0.07	0.45	−0.076	0.34
CCL11 > median*	0.162	0.06	0.043	0.56
Log CCL11 continuous†	0.006	0.88	−0.038	0.30
Aortic compliance (n = 2,355)				
CCL23 > median*	−1.054	0.03	−0.098	0.81
Log CCL23 continuous†	−0.063	0.002	−0.001	0.94
Detectable CXCL1	−2.245	0.01	−1.136	0.09
Detectable CXCL2	−1.101	0.04	−0.532	0.22
CCL11 > median*	0.093	0.85	0.141	0.73
Log CCL11 continuous†	−0.005	0.80	−0.005	0.79

*Non-continuous versions are above/below median.
†Betas, OR for continuous versions are for 1 standard deviation increase.

traditional cardiovascular risk factors (OR 1.3; 95% CI 1.0–1.7; $P = 0.02$; Table 3). In contrast, the associations of CCL23 with AWT, APB, and aortic compliance were attenuated after multivariable adjustment and no longer significant (Table 3). Findings were similar when CCL23 was modeled as a log-transformed continuous variable (Table 3).

The unadjusted association between detectable CXCL1 and CAC was modestly attenuated and of borderline statistical significance after adjustment for traditional risk factors (OR 1.4; 95% CI 1.0–2.1; $P = 0.06$; Table 3). In multivariable analyses, CXCL2 and CCL11 were not associated with any atherosclerosis phenotype (Table 3).

Discussion

Inflammation within the atherosclerotic plaque is mediated by specific chemokines that recruit target inflammatory cells into the developing atheroma. In this study, we measured levels of 4 novel chemokines in a large, population-based sample, and correlated these levels with traditional atherosclerosis risk factors and with markers of subclinical coronary and peripheral atherosclerosis. Of the 4 chemokines measured, the most robust evidence for an association with atherosclerosis emerged with CCL23; this cytokine associated with a number of traditional risk factors as well as with CAC independent of these risk factors. We also found associations of CCL23 with atherosclerosis in the abdominal aorta in univariable analyses, but these were attenuated and no longer statistically significant after adjustment for traditional risk factors. To our knowledge, this is the first study evaluating plasma levels of CCL23 as a cardiac biomarker. In addition, we found that levels of CXCL1 associated with CAC in unadjusted analysis, but the association was of only borderline statistical significance after adjusting for traditional risk factors. We observed no association of CXCL2 and CCL11 with any of the atherosclerotic phenotypes.

Only limited data are available from studies measuring circulating CCL23 in humans. In patients with rheumatoid arthritis, levels of CCL23 have been shown to correlate with C-reactive protein, erythrocyte sedimentation rate, and rheumatoid factor (Inmaculada Rioja and others 2008). This cytokine has not been well-studied in animal models of atherosclerosis, as no mouse homolog for this cytokine has yet been identified. Moreover, no reports have been published assessing its association with coronary risk factors or atherosclerosis in humans. Our findings of associations between plasma levels of CCL23 and CAC should prompt further exploration to identify a mouse homolog for this cytokine that would permit more in depth exploration of the role of this cytokine in atherosclerotic plaque development and progression. In humans, identification of genetic variants in the CCL23 gene that cause variation in circulating plasma levels would facilitate Mendelian Randomization experiments that could help to evaluate a potential causal role in atherosclerosis (Shah and de Lemos 2009).

In contrast to CCL23, CXCL1 has been more extensively studied in atherosclerosis. One study reported higher circulating levels of CXCL1 among patients with unstable angina compared to patients with stable angina (Breland and others 2008). Moreover, CXCL1 has been identified within human atherosclerotic plaques (Bechara and others 2007; Breland and others 2008). *In vitro* studies suggest that CXCL1 promotes uptake of LDL by macrophages and secretion of matrix metalloproteinases from vascular smooth muscle cells (Breland and others 2008). We observed a univariable association of CXCL1 with CAC that was attenuated and of borderline significance after multivariable adjustment for traditional risk factors. This finding suggests that if CXCL1 is involved in atherosclerosis development and progression, it may lie within the etiologic pathways linking risk factors with disease progression.

CXCL2 has similar activity as CXCL1, but much less is known about its role in atherosclerosis. In our analysis, we did not demonstrate an association between CXCL2 and CAC. The discordant associations between CXCL1 and CXCL2 with CAC and relatively modest correlations with each other suggest distinct roles of these 2 closely related GRO cytokines in atherosclerosis.

Interestingly, we found no association between CCL11 and any atherosclerosis phenotype. Prior studies have reported inconsistent associations of this eosinophil chemotactic protein with coronary heart disease. CCL11 has been shown to be up-regulated within the arterial wall of patients with atherosclerosis compared to normal controls (Haley and others 2000; Economou and others 2001). Levels of CCL11 have been associated with clinically significant coronary artery disease (based on a history of MI) (Ardigo and others 2007) and with coronary angiographic evidence of atherosclerosis (Emanuele and others 2006). In contrast, other investigators have not found an association between levels of CCL11 and atherosclerosis (Mosedale and others 2005; Sheikine and others 2006). Our findings within the largest and most ethnically diverse study population reported for CCL11 provide definitive null data with regard to the association between this cytokine and subclinical atherosclerosis. These null data should deter future epidemiologic research with this cytokine in the atherosclerotic process.

Potential role of CCL23 in atherosclerosis

CCL23 is a potent chemoattractant for monocytes, while having less activity for eosinophils and neutrophils (Forssmann and others 1997). Several alternative splice variants of this chemokine have been identified that may have a role in human disease. CCL23 was first isolated from aortic endothelium (Patel and others 1997), and we speculate that individuals with higher circulating levels of CCL23 may also have increased secretion of this protein from endothelial cells within the atherosclerotic plaque, although this has not yet been established. This chemokine pathway may operate in parallel or in synergy with other chemokine pathways recruiting monocytes into the vascular wall, including MCP-1 (CCL2), CCL4, and CCL5 (Charo and Ransohoff 2006; Mestas and Ley 2008; Zernecke and others 2008). Our findings of a correlation between CCL23 and MCP-1 support this hypothesis. We also observed a robust association of CCL23 with sESAM, a junctional adhesion molecule, but only weak associations with sVCAM-1 and sICAM-1. CCL23 acts upon activated lymphocytes (Forssmann and others 1997) as well, a finding that is reinforced by its strong correlation with LTβR, a receptor that binds activated lymphocytes and ensures efficient trafficking to sites of inflammation (Luther and others 2000; Drayton and others 2003; Browning and others 2005). We observed modest positive correlations of CCL23 with LDL-C, triglycerides, and oxidized phospholipids and an inverse correlation with HDL-C; the role of

oxidized lipids in stimulating production of CCL23 merits further exploration in cell culture experiments. In aggregate these findings suggest that CCL23 may work in concert with specific vascular inflammatory mechanisms and support further study of CCL23 in atherosclerosis.

Study limitations

Several important limitations merit comment. First, our study was an exploratory, cross-sectional study, and thus cannot provide clear insight into causation. Although our findings should be considered only hypothesis generating, they do provide an avenue for further exploration. A second important limitation is that the majority of subjects had undetectable levels of CXCL1 and CXCL2, which limited assessment of linear associations between levels of these cytokines and atherosclerosis. Because of this limitation, we could not evaluate differences below the detection limit that may potentially be of clinically importance. For CXCL1, in particular, additional study in higher risk populations and/or with more sensitive assays appears warranted, given the borderline-independent associations between this cytokine and atherosclerosis observed in the present study.

Author Disclosure Statement

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