

CXCL16 Is a Marker of Inflammation, Atherosclerosis, and Acute Coronary Syndromes in Humans

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Objectives	This study was designed to determine the association of CXCL16 with inflammation, atherosclerosis, and acute coronary syndromes.
Background	Vascular inflammation coincides with uptake of modified lipoproteins in the pathogenesis of atherosclerosis. CXCL16 is a protein that shares scavenger receptor function, promoting uptake of modified lipids, with the activities of an inflammatory chemokine. However, the role of CXCL16 in atherosclerosis remains uncertain.
Methods	The effect of inflammatory stimuli on CXCL16 gene and protein expression was studied in macrophages, mice, and humans, and the association of sol-CXCL16 with risk factors, atherosclerosis, and acute coronary syndromes was determined in humans.
Results	Endotoxin induction of CXCL16 in human macrophages was attenuated by aspirin, nuclear factor (NF)-kappa-B inhibition and peroxisome proliferator-activated receptor (PPAR)-gamma agonists. Experimental human endotoxemia (n = 6) led to an 8-fold increase in whole-blood CXCL16 messenger ribonucleic acid (p < 0.001) and a 1.7-fold increase in soluble (sol)-CXCL16 (p < 0.001), a cleaved active chemokine. Rosiglitazone-blocked endotoxin induced sol-CXCL16 in mice (p = 0.001), and pioglitazone (n = 28), compared to placebo (n = 28), lowered plasma sol-CXCL16 in metabolic syndrome subjects (p < 0.05). In a nested case-control study of acute and chronic coronary artery disease (n = 699), sol-CXCL16 levels correlated with inflammatory and metabolic risk factors and were associated with chronic coronary artery disease (odds ratio [OR] [95% confidence interval], above vs. below median; 1.60 [1.01 to 2.58]; p = 0.04) and acute coronary syndromes (OR 2.52 [1.32 to 4.82], p = 0.005) following adjustment for established risk factors, medications, and C-reactive protein levels.
Conclusions	Our findings suggest that CXCL16 may play a pro-inflammatory role in human atherosclerosis, particularly in acute coronary syndrome. (J Am Coll Cardiol 2007;49:442-9) © 2007 by the American College of Cardiology Foundation

Vascular inflammation and uptake of modified lipoprotein converge in atherosclerosis (1). Accumulation of lipids in the vascular wall serves as an attractant for invading immunologic cells. Modified lipoproteins are recognized by pattern recognition receptors, termed scavenger receptors, ex-

pressed by macrophages (2). In the setting of limited clearance, scavenger receptors promote unrestricted uptake of modified lipids, leading to foam cell formation and early atherosclerotic lesions (2). Vascular accumulation of immune cells and progression of atherosclerosis is accompa-

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Squibb, GlaxoSmithKline, KOS Pharmaceuticals, Merck & Co., Merck/Schering-Plough, Pfizer, Schering-Plough, and Takeda; Dr. Lazar is a recipient of an unrestricted Freedom to Discover Award from Bristol-Myers Squibb, has research funding from GlaxoSmithKline, and is a consultant for Abbot Laboratories. Dr. Wilensky's potential conflicts of interest are as follows: Scientific Advisory Board member for Topspin Medical, Medeikon, and Boston Scientific Corp.; stock options in Topspin Medical and Medeikon; equity interest in Johnson & Johnson Company; research grants from Boston Scientific Corporation, GlaxoSmithKline Inc., and Medeikon; and Speaker's Bureau for Pfizer Corp. Dr. Szapary is now an employee of Wyeth Pharmaceuticals.

Manuscript received July 10, 2006; revised manuscript received August 24, 2006, accepted September 11, 2006.

nied by an inflammatory state that reflects risk of cardiovascular disease (CVD) (3).

Recently, a protein called CXCL16 has been identified, which combines scavenger receptor functions with the properties of an inflammatory chemokine (4). CXCL16, a transmembrane protein, is composed of an extracellular chemokine domain fused to a trans-membrane mucin stalk (4). The chemokine domain acts as an attractant for cells expressing the CXCR6 receptor (4,5), but also as a scavenger receptor facilitating uptake of oxidized low-density lipoprotein (ox-LDL), phosphatidylserine, and bacteria (6). The extracellular part of CXCL16 undergoes cleavage by the metalloproteinase ADAM 10 creating a soluble chemokine (sol-CXCL16) (4,7), which activates CXCR6-expressing T-cells (4,5,7). CXCL16 has been implicated in variety of inflammatory diseases such as hepatitis (8) and encephalomyelitis (9), but its role in atherosclerosis is debated (10,11).

CXCL16 is expressed in macrophages (5) and aortic smooth muscle cells (12,13), and expression is enriched in atherosclerotic plaques (6,14). Pro-inflammatory stimuli increase CXCL16 expression (4,7,13), enhancing uptake of ox-LDL and facilitating foam cell formation (14). Furthermore, sol-CXCL16 stimulates vascular cell proliferation, and a preliminary study found that a polymorphism of the CXCL16 gene was associated with the severity of coronary artery disease (CAD) (15). Despite this evidence, a recent rodent study suggests an atheroprotective effect of CXCL16 (11), and plasma sol-CXCL16 levels were reported to be decreased in patients with coronary atherosclerosis (10), calling into question CXCL16's role in human atherosclerosis. We describe the inflammatory regulation of CXCL16 in cells, mice, and humans and its association with CVD risk factors, stable CAD, and acute coronary syndromes (ACS) in humans.

Methods

Macrophage studies. Human and mouse macrophages were isolated and differentiated as described (16). Experiments, after overnight equilibration with serum-free medium (Gibco/Invitrogen, Carlsbad, California), included treatment as indicated with lipopolysaccharide (LPS) (Sigma, St. Louis, Missouri), aspirin (Sigma), SN50, and control peptide (Biomol, Plymouth Meeting, Pennsylvania). Human macrophages were treated in the presence of 5 ng/ml granulocyte macrophage colony-stimulating factor. Adenoviruses overexpression of activated I-kappa-B kinase (gift, Steven Shoelson) or control vector was performed as described (16).

Ribonucleic acid (RNA) and protein quantification. Ribonucleic acid quantification was performed by Taqman real-time polymerase chain reaction (16) using primers and probes (Perkin Elmer/Applied Biosystems) for CXCL16, tumor necrosis factor (TNF)-alpha, 18s-RNA, or beta-actin. CXCL16 protein concentrations in cell media, mouse serum, and human plasma were assessed with a commercial enzyme-linked immunosorbent assay (ELISA) (R&D Systems) and normalized

to cell protein or per ml plasma (in vivo studies). Plasma C-reactive protein (CRP) (high-sensitivity turbidimetric immunoassay; Wako Ltd., Osaka, Japan), resistin, TNF-alpha, and soluble intercellular adhesion molecule (sol-ICAM1) (ELISAs, Linc, St. Louis, Missouri) were determined by commercial assays. Intra- and interassay coefficients of variance, derived from pooled human plasma, were 4.7% and 6.7% for sol-CXCL16, 8.0% and 8.3% for CRP, 4.6% and 4.3% for resistin, 8.66% and 20.4% for TNF-alpha, and 8.3% and 14.8% for sol-ICAM-1, respectively.

Rodent studies. At 8 weeks of age, LDL receptor null (LDL-R^{-/-}) mice on C57BL/6 background (Jackson Laboratories, Bar Harbor, Maine) were fed rosiglitazone (30 mg/kg/day in 0.25% w:v methylcellulose) or vehicle for 7 days and injected intraperitoneally with LPS (200 ng/g), and blood was drawn for analysis of sol-CXCL16. Animal care procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Human endotoxemia study. Healthy volunteers (3 men, 3 women) ages 18 to 40 years were studied as described previously (16). Serial blood samples were collected for 24 h before and after the intravenous administration of human research grade endotoxin 3 ng/kg (NIH Clinical Center, CCRE; lots 1 and 2; NIHCC PDS #67801). For this and other human studies, the Penn Institutional Review Board approved each study and written informed consent was provided by participants.

Clinical trial of pioglitazone. As described (17), 56 participants were recruited to a placebo-controlled trial of pioglitazone (30 mg for 6 weeks and then 45 mg for 6 weeks) effects on atherogenic biomarkers in non-diabetic, metabolic syndrome individuals.

Association study of plasma CXCL16 with coronary atherosclerosis. As described (18), 3,850 consecutive patients undergoing coronary angiography at Penn were recruited to an Institutional Review Board-approved protocol of risk factors for CVD. A 3-arm nested case-control sample was randomly selected: 1) control patients with no angiographic CAD (n = 235), 2) chronic CAD cases (≥ 2 vessels $\geq 70\%$ stenosis; n = 268), and 3) ACS cases (n = 196), defined as CAD (any vessel $\geq 20\%$ stenosis) in the setting of myocardial infarction or unstable angina (chest pain or dyspnea with elevated cardiac enzymes or dynamic electrocardiogram changes) within 48 h of angiography. Exclusions included ACS, cardiomyopathy, previous CAD,

Abbreviations and Acronyms

ACS	= acute coronary syndromes
CAD	= coronary artery disease
CRP	= C-reactive protein
CVD	= cardiovascular disease
ELISA	= enzyme-linked immunosorbent assay
HDL	= high-density lipoprotein
IFN	= interferon
LPS	= lipopolysaccharide
ox-LDL	= oxidized low-density lipoprotein
RNA	= ribonucleic acid
TG	= triglycerides
TNF	= tumor necrosis factor

coronary angioplasty or coronary stent (for control patients), cardiac transplant, or evaluation for vascular surgery or organ transplantation.

The metabolic syndrome was defined using a modified (body mass index >29 kg/m² in men and >25 in women (19) version of the updated National Cholesterol Education Program definition (20). Fasting blood samples, drawn into ethylenediaminetetraacetic acid tubes before (<1 h) angiography, were kept at 4°C until same-day processing for plasma, which was stored at -80°C. For this study, plasma sol-CXCL16 levels were measured in plasma aliquots that had undergone 1 or 2 freeze-thaw cycles. Total and high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and glucose levels were measured enzymatically on a Cobas Fara II (Roche Diagnostic Systems Inc., New Jersey). We calculated LDL-cholesterol using the Friedewald formula except when TG levels were >400 mg/dl.

Statistical analysis. Data are reported as mean ± SEM or median and interquartile range (25th, 75th percentile) and as proportions for categorical variables. For experiments with multiple treatments, analysis of variance (ANOVA) was used to test for differences in means; when significant differences were found, post hoc Scheffe corrected *t* tests were used for comparisons. The effects of rosiglitazone (in mice), pioglitazone (in humans), and human endotoxemia on CXCL16 were tested by repeated-measures ANOVA.

The primary hypothesis tested in the case-control study was that sol-CXCL16 was associated with both chronic CAD and ACS beyond established CVD risk factors and CRP compared with control patients. We present Spearman correlations with other continuous variables and a multivariable linear regression model (log-transformed sol-CXCL16 as outcome) examining which factors were independently associated with sol-CXCL16. Unadjusted associations with case-control status were examined using ANOVA. Logistic regression models were fit to test for association of quartiles (because of non-linear associations) of sol-CXCL16 and CRP levels with chronic CAD and ACS. Models were adjusted for: 1) age, gender, and race; 2) established risk factors (total cholesterol, HDL-C, triglycerides, smoking, history of hypertension and diabetes, family history of CAD, body mass index), and medications (beta-blocker, lipid therapy, aspirin, heparin within the previous 24 h, angiotensin-converting enzyme inhibitors), as well as age, gender, and race; and 3) CRP levels, established risk factors, age, gender, and race. Gender and race interactions were assessed by likelihood-ratio test. Statistical analyses were performed using Stata 9.0 software (Stata Corp., College Station, Texas).

Results

Induction of CXCL16 by LPS in vivo and attenuation by anti-inflammatory drugs that target nuclear factor (NF)-kappa-B. Levels of whole-blood CXCL16 messenger ribonucleic acid (mRNA) (Fig. 1A) and circulating sol-

CXCL16 (Fig. 1B) increased following an initial early cytokine induction (TNF-alpha) during experimental endotoxemia. Thus, this extends to humans evidence in vitro that cytokines induce CXCL16 expression in human macrophages.

In vitro, aspirin blocked LPS induction of CXCL16 mRNA (Fig. 2A) and protein (Fig. 2B) in human macrophages at concentrations (5 mM) that inhibit I-kappa-B kinase (21). Given this, we blocked this pathway using SN50, a cell-permeable peptide that prevents the translocation of NF-kappa-B to the nucleus (22). SN50 significantly reduced the induction of sol-CXCL16 by LPS (Fig. 2C). In contrast, constitutive activation of NF-kappa-B, by overexpression of activated I-kappa-B kinase, induced secretion of sol-CXCL16 to an extent similar to LPS (Fig. 2D).

CXCL16 is down-regulated by peroxisome proliferator-activated receptor (PPAR)-gamma activation in vitro and in vivo. Rosiglitazone, a thiazolidinedione (TZD) PPAR-gamma agonist with anti-inflammatory and anti-

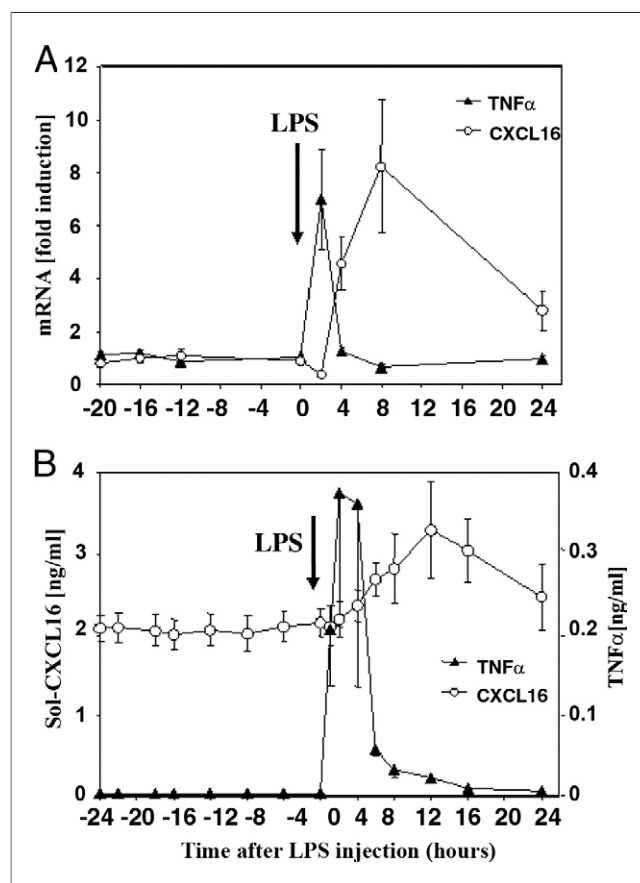


Figure 1 Human Endotoxemia Induces CXCL16 In Vivo

(A) Whole-blood CXCL16 and tumor necrosis factor (TNF)-alpha messenger ribonucleic acid (mRNA) and (B) plasma protein levels were measured serially for 20 h before and 24 h after intravenous lipopolysaccharide (LPS) (3 ng/kg) in 6 healthy volunteers. Repeated-measures ANOVA revealed significant effects on CXCL16 mRNA (F = 6.89, p < 0.001) and sol-CXCL16 (F = 6.15, p < 0.001) levels.

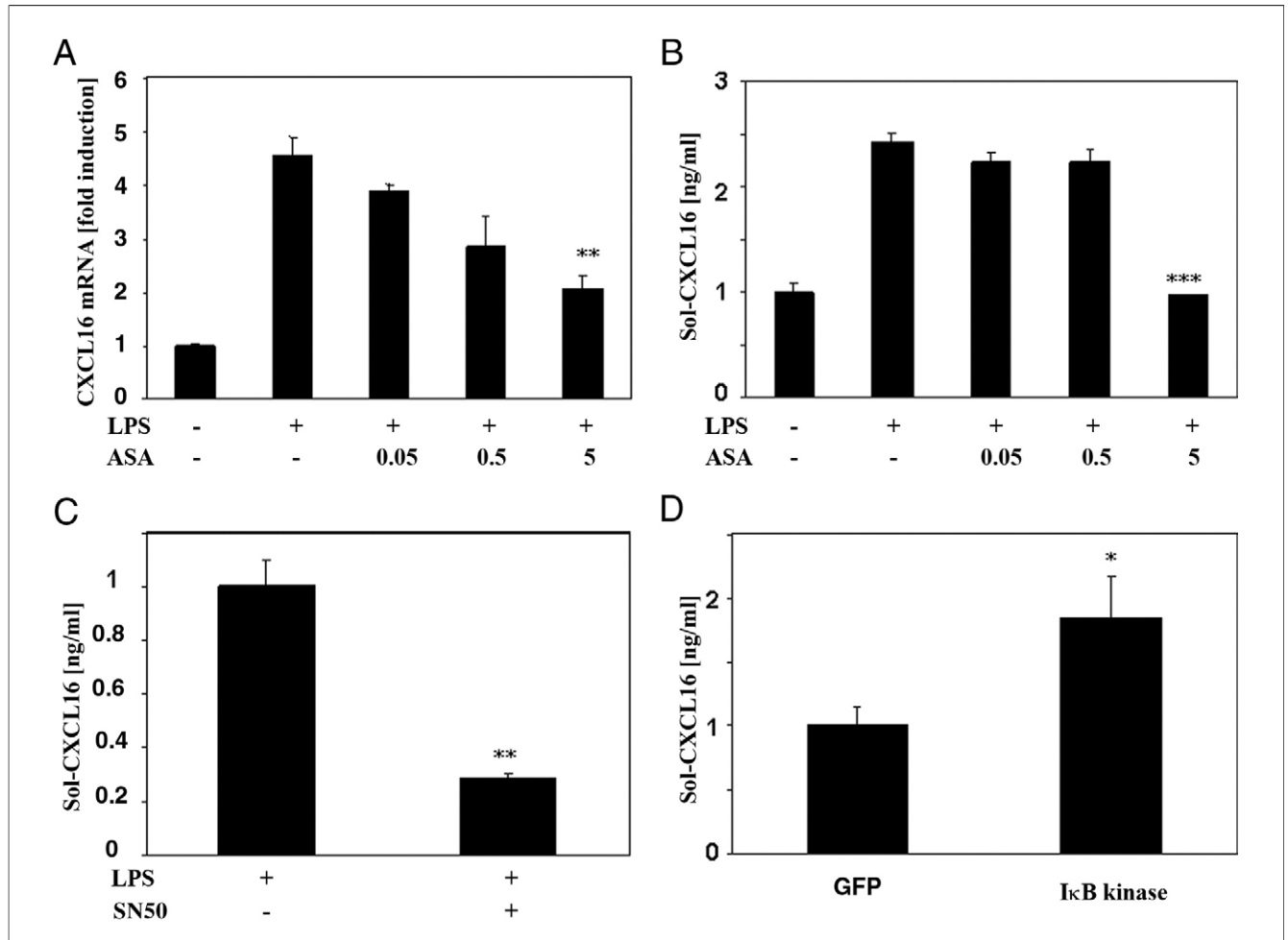


Figure 2 Inhibition of CXCL16 Induction by Anti-Inflammatory Drugs

Down-regulation of (A) CXCL16 mRNA ($F = 19.4$, $p = 0.001$) and (B) CXCL16 protein secretion ($F = 71.9$, $p < 0.001$) by aspirin (ASA) in human macrophages. Cells were pre-treated with aspirin for 2 h and then for 24 h with LPS ($1 \mu\text{g/ml}$). $**p < 0.01$, $***p < 0.001$ by t test compared to LPS-induced CXCL16 levels. (C) Down-regulation of CXCL16 mRNA in human macrophages by the nuclear factor-kappa-B inhibitor SN50 (t test $p = 0.002$). Cells were pretreated with SN50 or control peptide at $100 \mu\text{g/ml}$ for 2 h, then for 24 h with LPS ($1 \mu\text{g/ml}$). (D) Induction of CXCL16 in human macrophages by adenovirus over-expression (24 h) of activated I-kappa-B kinase compared to control virus ($*t$ test $p = 0.03$). Results of representative experiments with triplicate samples are expressed as mean \pm (SEM). GFP = green fluorescent protein; other abbreviations as in Figure 1.

atherosclerotic effects in mice (23) and humans (24), is known to attenuate inflammation, in part, by inhibition of NF-kappa-B (25). Pretreatment with rosiglitazone reduced LPS-induction of CXCL16 protein in macrophages (Fig. 3A). Rosiglitazone (30 mg/kg orally for 7 days) reduced by 50% LPS-induced sol-CXCL16 levels in LDL-R^{-/-} mice (Fig. 3B). Further, sol-CXCL-16 levels (ng/ml) were modestly but significantly reduced by 12-weeks of pioglitazone (from 2.29 [range 1.68 to 2.60] to 2.13 [range 1.66 to 2.60]; $n = 28$, $p = 0.03$) but not placebo (from 2.22 [range 1.74 to 2.70] to 2.22 [range 1.71 to 2.64]; $n = 28$, $p = 0.82$; between-group $p = 0.05$) in metabolic syndrome subjects (17).

Association of sol-CXCL16 levels with CVD risk factors and atherosclerosis in humans. Before undertaking association studies of CXCL16, we established the stability and reproducibility of sol-CXCL16 in human plasma. Plasma

levels did not vary in 6 healthy subjects at multiple time points over the 24 h before (median pairwise $r^2 = 0.89$). CXCL16 levels were remarkably stable in paired samples stored at -80°C over a 3-month period in the 28 subjects in the placebo arm of the pioglitazone trial ($r^2 = 0.89$, median [interquartile range] coefficient of variance = 1.25% [range 6.3% to 9.4%]). Sol-CXCL16 levels (median [interquartile range] ng/ml) were 2.50 (range 2.24 to 3.07) after 1 freeze-thaw and 2.51 (range 2.21 to 2.84) after a second freeze-thaw in paired analysis of 35 plasma samples.

The characteristics of the PENN-CATH case-control study sample are summarized in Table 1. Briefly, 85% of the sample was Caucasian, 55% was male, and the average age was 62 years. As expected, several established risk factors, including family history, cigarette smoking, diabetes, metabolic syndrome, prior myocardial infarction,

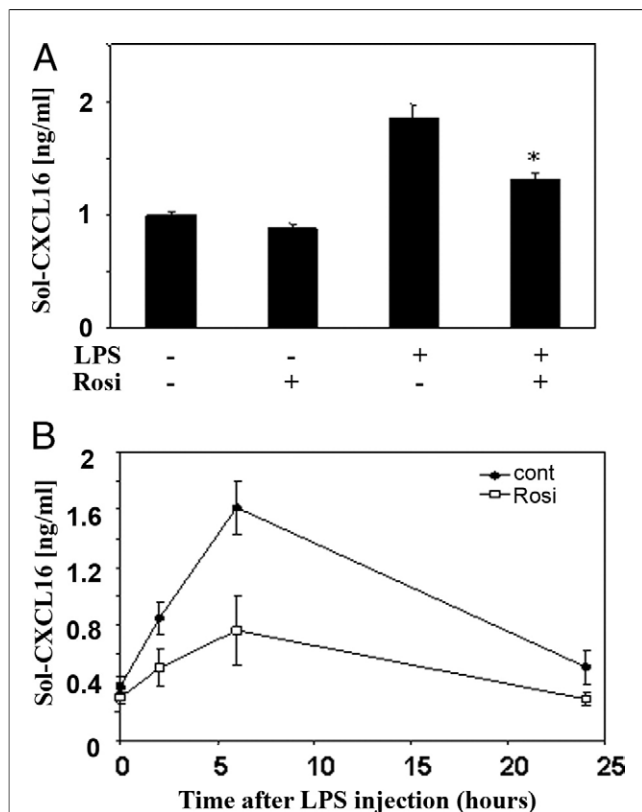


Figure 3 Inhibition of Sol-CXCL16 by Rosiglitazone In Vitro and In Vivo

(A) Down-regulation of sol-CXCL16 protein by rosiglitazone (Rosi) in mouse macrophages (* $F = 38.4$, $p = 0.005$; $p < 0.01$ rosiglitazone/LPS versus LPS alone). Cells were pretreated with rosiglitazone ($1 \mu\text{M} \times 24 \text{ h}$) and then with LPS ($1 \mu\text{g}/\text{ml} \times 24 \text{ h}$). (B) LPS-dependent induction of plasma sol-CXCL16 is blocked by rosiglitazone in mice ($F = 8.83$, $p = 0.01$). LDLR $^{-/-}$ mice (8 per group) were treated for 7 days with rosiglitazone (30 mg/kg) or vehicle and injected intraperitoneally with LPS (200 ng/g). Abbreviations as in Figure 1.

and HDL-C and TG levels, as well as use of cardiac medications, were higher in cases compared with control patients; smoking and a history of myocardial infarction were more prevalent in ACS than chronic CAD cases. Plasma levels of both CRP and sol-CXCL16 were higher in women than men within each case-control category.

Association of sol-CXCL16 levels with CVD risk factors.

We found significant associations of plasma sol-CXCL16 levels with inflammatory and metabolic factors (Table 2). In particular, sol-CXCL16 was correlated with levels of CRP, sol-ICAM1, and resistin, an inflammatory adipokine (16), as well as higher TG and lower HDL-C. Levels (median [interquartile range], ng/ml) were modestly but significantly greater in patients with the metabolic syndrome compared to those without (2.65 [range 2.27 to 3.20] vs. 2.45 [range 2.08 to 2.93], $p < 0.001$). Notably, within any case-control group, levels of sol-CXCL16 did not differ significantly between statin users and non-statin users (e.g., in ACS cases, 2.80 [range 2.40 to 3.31] vs. 2.77 [range 2.39 to 3.22], $p = 0.75$). In multivariable linear regression,

female gender ($p < 0.001$), increasing age ($p = 0.001$), African-American race ($p < 0.005$), cigarette smoking ($p < 0.005$), increasing TG ($p < 0.01$), and lower HDL-C ($p = 0.05$), as well as levels of resistin ($p < 0.001$) and CRP ($p = 0.05$), were independently associated with sol-CXCL16, even after adjusting for medications and case-control status.

Association of sol-CXCL16 with coronary atherosclerosis.

The association of sol-CXCL16 levels with CAD case-control status was similar across gender and race (all interactions $p > 0.4$); therefore, results are combined for men and women. Compared with control patients, sol-CXCL16 levels (Table 1) were higher ($F = 21.54$; $p < 0.001$) in patients with chronic CAD ($p < 0.01$ vs. control patients) and ACS ($p < 0.001$ vs. control patients; $p = 0.001$ vs. chronic CAD). In logistic regression, quartiles of sol-CXCL16 levels were positively and incrementally associated with ACS compared with control patients following adjustment for age, gender, race, medications, and all traditional CVD risk factors, and even with further adjustment for plasma CRP levels (Table 3), levels above the median were strongly associated with ACS in fully adjusted models that included medications and CRP data (odds ratio 2.52 [range 1.32 to 4.82]; $p = 0.005$). The association of sol-CXCL16 quartiles with chronic CAD was attenuated after adjusting for CVD risk factors (Table 3); however, levels above the median were statistically significant predictors of chronic CAD in fully adjusted models (odds ratio 1.61 [range 1.01 to 2.58]; $p = 0.04$).

Discussion

CXCL16 is remarkable among scavenger receptors because it combines scavenger receptor and inflammatory chemokine functions (4). Preliminary studies in humans have provided conflicting evidence for a role in atherosclerosis (10,15). We found that atherogenic inflammatory signals induce CXCL16 in human macrophages in vitro and in vivo and therapies that attenuate NF-kappa-B signaling inhibit CXCL16 induction. In humans, sol-CXCL16 levels were modestly but positively associated with metabolic dyslipidemia and inflammatory risk factors. Consistent with this pro-inflammatory profile, sol-CXCL16 levels were independently associated with coronary atherosclerosis, particularly ACS.

Human endotoxemia is an established model of innate immunity activation that may provide mechanistic insight into the role of specific inflammatory pathways in human disease. Lipopolysaccharide binds CD14, which delivers it to its signaling receptor, toll-like receptor 4, leading to translocation of NF-kappa-B to the nucleus driving gene transcription (26). Here we demonstrate that CXCL16 is induced during human endotoxemia and that activation of NF-kappa-B is necessary and sufficient for CXCL16 induction in human macrophages. We found that aspirin, at doses

Table 1 Characteristics of the Case-Control Study Sample

	Control Cases (n = 235)	Chronic Cases (n = 268)	Acute Cases (n = 196)
Age (yrs)	61 ± 11	63 ± 11	62 ± 10
Men (%)	46.4%	52.6%	69.9%*
Race (%)			
Caucasian	78.7%	88.1%†	88.8%*
African American	18.7%	11.6%	7.6%
Other	2.6%	0.3%	3.6%
History (%)			
Family history of CAD	33.6%	45.2%*	45.4%*
Hypertension	57.5%	72.0%	64.8%
Current smokers	24.7%	37.7%*	71.0%*†
Diabetes	16.6%	32.1%*	31.1%*
Prior myocardial infarction	6.0%	32.1%*	71.0%†
Body mass index (kg/m ²)	29.9 ± 7.0	29.9 ± 5.8	29.1 ± 6.0
Metabolic syndrome	43.8%	62.6%*	53.9%*
Laboratory profile			
Total cholesterol (mg/dl)	177 ± 36	177 ± 46	175 ± 36
LDL cholesterol (mg/dl)	107 ± 31	105 ± 38	104 ± 33
HDL cholesterol (mg/dl)			
Men	42 ± 12	39 ± 19*	41 ± 10
Women	52 ± 15	46 ± 12.0*	45 ± 13*
Triglycerides (mg/dl)	94 (67-133)	117 (79-171)†	124 (93-169)*
C-reactive protein (mg/dl)			
Men	1.74 (0.77-3.76)	2.23 (1.00-4.06)	4.14 (2.34-6.91)*
Women	2.94 (1.06-4.46)	2.88 (1.42-4.88)	5.18 (3.13-7.78)*
CXCL16 (ng/ml)			
Men	2.19 (1.95-2.54)	2.5 (2.07-2.9)*	2.70 (2.32-3.01)*
Women	2.45 (2.11-2.95)	2.69 (2.28-3.21)*	3.20 (2.74-3.68)*
Medical treatment (%)			
Statin	25.2	52.6*	45.3*
Aspirin	50.0	76.5*	86.7*
Beta-blocker	35.8	64.5*	62.7*
ACE-I or ARB	29.1	40.6‡	35.6‡
Insulin or oral hypoglycemic	12.6	25.6‡	20.0‡
Intravenous heparin	10.0	16.4	63.0†

Data presented as percentages for categorical data and as mean ± SD or median (interquartile range) for continuous variables. *p < 0.001 versus controls; †p < 0.001 for ACS cases versus CAD cases; ‡p < 0.01 versus control patient.

ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CAD = coronary artery disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

that attenuate NF-kappa-B signaling (21), potentially inhibited CXCL16 induction, whereas atheroprotective TZDs (23,24), which antagonize NF-kappa-B via activation of PPAR-gamma, also down-regulate CXCL16 in mice and humans. These data provide evidence that CXCL16 is activated by inflammatory signals that promote atherosclerotic CVD.

A role for CXCL16 in atherosclerosis was first suggested by findings of its accumulation in atherosclerotic plaques (6,14), its implication in foam cell formation as a scavenger receptor (14), and a genetic study that associated a CXCL16 polymorphism with CAD (15). Sol-CXCL16 has several inflammatory functions and serves as a T-cell activator (4,5,8,27), one of the first immune cells found in developing atherosclerotic lesions (1). T-cells co-localize with CXCL16-expressing endothelial cells in plaques (27), and CXCR6, the only receptor for

CXCL16, is highly expressed in immune and vascular cells (4,5,28,29). Indeed, interferon (IFN)-gamma is a strong inducer of CXCL16 expression (14) whereas CXCL16 is a stimulator of T-cell IFN-gamma production (27), suggesting that CXCL16 may regulate atherogenic effects of T-cells and IFN-gamma (1).

Surprisingly, Aslanian and Charo (11) reported recently that CXCL16 gene deficiency in LDL-R^{-/-} mice was associated with accelerated atherosclerosis, despite a reduced capacity of macrophages to accumulate oxidized LDL. This study should be considered in the context of conflicting reports for the role in mouse atherosclerosis of better characterized scavenger receptors SR-A and CD36. Despite most mouse studies supporting atherogenic effects of SR-A and CD36 (Suzuki, Sakguchi, Febbraio), recent work in apoE-deficient mice suggest no effect or even atheroprotective roles (30). Overall, there remains great controversy

Table 2 Spearman Correlations of Sol-CXCL16 Levels With Cardiovascular Risk Factors

Variable	Men (n = 387)	Women (n = 312)	All (n = 699)
Age	0.09	0.16*	0.15†
Systolic BP	-0.06	-0.04	-0.04
Plasma glucose	0.03	0.05	0.06
Body mass index	0.02	0.09	0.06
LDL cholesterol	-0.05	-0.07	-0.04
HDL cholesterol	-0.07	-0.21†	-0.09‡
Triglycerides	0.08	0.21†	0.13†
CRP	0.26†	0.27†	0.28†
Soluble ICAM-1	0.15*	0.18*	0.16†
Resistin	0.15*	0.31†	0.23†

*p < 0.01; †p < 0.001; ‡p < 0.05.

BP = blood pressure; CRP = high-sensitivity C-reactive protein; HDL = high-density lipoprotein; ICAM = intercellular adhesion molecule; LDL = low-density lipoprotein.

regarding the role of several scavenger receptors in mouse atherosclerosis.

Mouse models of atherosclerosis may provide only limited insight into the inflammatory pathophysiology of complex atherosclerotic outcomes in humans. Despite this, the possibility that CXCL16 might be atheroprotective in humans needs to be considered. Although we found that plasma levels of sol-CXCL16 were positively related to coronary atherosclerosis, these data conflict with a recent report by Sheikine et al. (10) suggesting that patients with CAD had reduced levels of sol-CXCL16. In their study, however, only a small number of patients (n = 17) were examined at the time of presentation with ACS. Both studies used the same ELISA, and sol-CXCL16 levels were similar in both control populations. Our study had a much larger sample of ACS cases, in which we observed the strongest association with sol-CXCL16. We suggest that findings of negative regulation by anti-inflammatory therapies, correlation with metabolic and inflammatory CVD risk factors, and strong positive association with ACS in an

appropriately sized sample provide indirect support for a pro-inflammatory role for CXCL16 in human CVD. Overall, conflicting findings underscore the need for additional clinical studies and experiments using diverse animal models.

The concept that several circulating inflammatory markers may provide prognostic value is consistent with the complex pathophysiology of atherosclerosis and its diverse manifestations (1). In this context, we found that sol-CXCL16 levels were strong independent predictors of ACS as well as independent of and superior to CRP in association with chronic CAD. Plasma levels were remarkably stable under basal conditions, suggesting that sol-CXCL16 may have favorable biomarker characteristics for epidemiologic and clinical application. Overall, our findings require replication in prospective, population-based studies of diverse CVD end points that also compare the diagnostic and prognostic value of sol-CXCL16 to additional biomarkers.

In conclusion, we found that inflammatory signals that promote atherosclerosis also induce CXCL16 in vitro and in vivo. Consistent with this pro-inflammatory induction, sol-CXCL16 levels were positively associated with coronary atherosclerosis. Despite conflicting reports, our studies provide support for CXCL16 as a pro-inflammatory factor in human atherosclerosis, especially acute coronary syndromes.

Acknowledgments

The authors thank Steven E. Shoelson and Dongsheng Cai for providing the adenovirus-expressing activated I-kappa-B kinase, the Immunology Core at the PENN Center for AIDS Research for peripheral blood monocytes, and the PENN General Clinical Research Center (NIH M01-RR00040) and its nursing staff for outstanding patient care.

Table 3 Multivariable Association of Plasma Sol-CXCL16 and CRP Quartiles* With CAD

	Model 1		Model 2		Model 3	
	Chronic CAD Cases	ACS Cases	Chronic CAD Cases	ACS Cases	Chronic CAD Cases	ACS Cases
CRP						
Q2	1.54 (0.97-2.47)	3.11 (1.55-6.82)	1.54 (0.86-2.77)	2.85 (1.07-7.56)	1.44 (0.79-2.60)	2.49 (0.89-7.01)
Q3	0.99 (0.61-1.61)	5.13 (2.60-10.09)	0.62 (0.33-1.14)	2.64 (1.05-6.64)	0.56 (0.29-1.06)	2.22 (0.83-5.95)
Q4	1.85 (1.07-3.22)	16.44 (8.15-33.17)	1.58 (0.77-3.22)	15.03 (5.35-42.20)	1.38 (0.66-2.85)	13.16 (4.51-38.4)
CXCL16						
Q2	1.26 (0.78-2.04)	3.62 (1.90-6.92)	1.42 (0.81-2.50)	2.57 (1.11-5.94)	1.30 (0.72-2.31)	2.92 (1.14-7.47)
Q3	2.71 (1.59-4.60)	9.76 (4.91-19.38)	2.01 (1.08-3.73)	4.27 (1.72-10.59)	1.96 (1.04-3.70)	3.48 (1.27-9.52)
Q4	2.75 (1.60-4.73)	14.10 (6.90-28.80)	1.81 (0.93-3.51)	7.33 (2.84-18.94)	1.71 (0.86-3.40)	8.0 (2.77-23.0)

Results are presented as odds ratio (95% confidence interval) of CAD status comparing incremental quartiles (Q) of (a) CRP or (b) sol-CXCL16 data to their lowest quartile. Model 1 adjusted for age, gender, and race; Model 2 adjusted for age, gender, race, and risk factors (total cholesterol, HDL cholesterol, triglycerides, smoking status, history of hypertension and diabetes mellitus, family history of CAD, body mass index, and medications [beta-blocker, lipid lowering therapy, aspirin, heparin, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers]); Model 3 adjusted for CRP data (when modeling sol-CXCL16) or sol-CXCL16 data (when modeling CRP) as well as age, gender, race, and risk factors (as per Model 2). Quartile cut points were 0.09 to <2.16, 2.16 to <2.54, 2.54 to <3.02, and 3.02 to 8.29 ng/ml for sol-CXCL16 and 0.01 to <1.35, 1.35 to <3.01, 3.01 to <4.87, and 4.87 to 9.5 mg/dl for CRP. There were no significant differences by gender in the association of sol-CXCL16 levels with ACS or chronic CAD cases.

ACS = acute coronary syndrome; CAD = coronary artery disease, other abbreviations as in Table 2.

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