Increased CD36 Expression Signals Monocyte Activation Among Patients With Type 2 Diabetes

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OBJECTIVE — To explore the hypothesis that CD36, a scavenger receptor and fatty acid translocase, is upregulated in peripheral blood mononuclear cells (PBMCs) among patients with type 2 diabetes and is a biomarker of PBMC activation and inflammation.

RESEARCH DESIGN AND METHODS — We used a cross-sectional observational design to study a multi-racial/ethnic population sample consisting of Caucasians, Hispanics, and Native Americans with type 2 diabetes ($n = 33$) and nondiabetic control subjects ($n = 27$). PBMC CD36 mRNA/protein and plasma high sensitivity (hs) C-reactive protein (hsCRP), hs–interleukin-6 (hsIL-6), and adiponectin were measured.

RESULTS — Unadjusted PBMC CD36 mRNA and protein were 1.56- and 1.63-fold higher, respectively, among type 2 diabetic subjects versus control subjects. PBMC CD36 protein was directly associated with CD36 mRNA, plasma hsCRP, and hsIL-6 and inversely associated with plasma adiponectin in both groups.

CONCLUSIONS — Increased CD36 expression is a biomarker of PBMC activation and inflammation and may become a useful tool in cardiovascular disease risk stratification.

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The prevalence of type 2 diabetes is increasing in epidemic proportions among minority populations. Cardiovascular disease (CVD) is a major cause of morbidity and mortality among diabetic patients. New methods for risk stratification are needed to reduce the burden of CVD among patients with diabetes.

CD36, a 88-kDa transmembrane glycoprotein and a cell surface scavenger receptor for oxidized low-density lipoproteins (oxLDL), plays a critical role in the pathogenesis of atherosclerosis and CVD (1,2). Ligation of oxLDL by CD36 in macrophages induces activation of nuclear factor κ–light-chain enhancer of activated B-cells (NFκB) and production of proinflammatory cytokines, e.g., tumor necrosis factor α/β (TNF-α/β), interleukin (IL)-1β, IL-6, and interferon β/γ. Production of proinflammatory cytokines is reduced in CD36-deficient macrophages (2). The size of atherosclerotic lesions is reduced by inactivation of CD36 and increased by reintroduction of CD36 in apolipoprotein E/CD36-deficient mice. CD36 expression is increased in the presence of high glucose concentrations. Therefore, assessment of CD36 levels may become a valuable tool in CVD risk stratification among patients with type 2 diabetes.

The majority of previous clinical studies of CD36 expression have been conducted in a single racial/ethnic group. In contrast, the present study explored the hypothesis that there is coordinated upregulation of peripheral blood mononuclear cell (PBMC) CD36 mRNA and protein, signaling PBMC activation and increased production of proinflammatory cytokines among non-Hispanic whites, Hispanics, and American Indians with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Clinical protocol

All participants were studied in the General Clinical Research Center at the University of New Mexico. We recruited participants with ($n = 33$) and without ($n = 27$) type 2 diabetes. Patients with type 2 diabetes had two or more fasting plasma glucose values >126 mg/dl or received hypoglycemic medications. Nondiabetic participants had a fasting plasma glucose level <100 mg/dl and A1C <6.1% and were not receiving hypoglycemic medications. Patients with end-stage renal disease, with liver disease, or who were pregnant, malnourished, or receiving insulin were excluded. We obtained a venous blood sample after an overnight fast for a chemistry profile, lipid panel, cytokines, high sensitivity (hs) C-reactive protein (CRP), adiponectin, A1C, nonesterified fatty acids, and PBMC isolation.

Isolation of PBMCs, extraction of RNA and protein, and quantification of CD36 γ mRNA and protein

PBMCs were isolated and stored at −80°C. PBMC RNA and protein were isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA). mRNA was reverse-transcribed to cDNA using random hexamer primers. Quantitative PCR was performed using SYBR Green RT-PCR reagents (Applied Biosystems, Foster City, CA) in the MiniOpticon System (Bio-Rad,

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Table 1—*Unadjusted and adjusted mean CD36 mRNA/protein and Pearson product moment correlations (r)*

	CD36 mRNA (log r.u.)		CD36 protein (log r.u.)	
	Type 2 diabetes	Nondiabetic control subjects	Type 2 diabetes	Nondiabetic control subjects
n	33	27	33	27
Unadjusted mean	0.23 ± 0.02†	0.16 ± 0.02	0.28 ± 0.02§	0.18 ± 0.02
Model 1	0.21 ± 0.02	0.17 ± 0.02	0.27 ± 0.01*	0.21 ± 0.02
Model 2	0.20 ± 0.02	0.18 ± 0.02	0.26 ± 0.01†	0.20 ± 0.01
CD36 mRNA (log r.u.)	NA	NA	$r = 0.64$‡	$r = 0.63$‡
PPAR-γ mRNA (log r.u.)	$r = 0.73$‡	$r = 0.72$‡	$r = 0.33$#	$r = 0.46$*
hsCRP (log mg/dl)	$r = 0.55$†	$r = 0.21$	$r = 0.45$†	$r = 0.43$*
hsIL-6 (log pg/ml)	$r = 0.40$*	$r = 0.49$†	$r = 0.64$‡	$r = 0.49$†
TNF-α (log pg/ml)	$r = -0.20$	$r = 0.09$	$r = -0.28$	$r = -0.02$
Adiponectin (log μg/ml)	$r = -0.61$‡	$r = -0.67$‡	$r = -0.60$‡	$r = -0.56$†
BMI (kg/m^2)	$r = 0.20$	$r = 0.03$	$r = 0.09$	$r = 0.17$
HOMA-IR (log r.u.)	$r = 0.18$	$r = 0.03$	$r = 0.12$	$r = -0.07$
QUICKI (log r.u.)	$r = -0.20$	$r = 0.05$	$r = -0.16$	$r = 0.19$

Data are least squares means ± SEM unless otherwise indicated. The *P* value is from a *t* test of equality of the means. Model 1 adjusts for hypertension (HTN), hsIL-6, PPAR, adiponectin, and QUICKI. Model 2 adjusts for hsIL-6, adiponectin, and QUICKI. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$, §$P < 0.0001$, #$P = 0.08$. HOMA-IR, homeostasis model assessment–insulin resistance; QUICKI, quantitative insulin sensitivity check index; r.u., relative units.

Richmond, CA). CD36 protein was measured using Western blot (rabbit anti-CD36 antibody, sc9154; Santa Cruz Technology, Santa Cruz, CA; mouse anti-actin antibody, A4700, Sigma, St. Louis, MO; goat anti-rabbit IgG-HRP, #172-1019, and goat anti-mouse IgG-HRP, #170-6516, Bio-Rad).

Statistical analysis

Pearson correlations of CD36 mRNA and protein levels with anthropometric and laboratory measurements were determined in diabetic and nondiabetic participants. Means of CD36 mRNA and protein across groups stratified by race/ethnicity, sex, diabetes, and hypertension status were compared using ANOVA. The relationship of diabetes status to CD36 expression was assessed in multiple linear regression analyses that included markers of inflammation, obesity, and insulin resistance as covariates. Statistical analyses were performed in SAS version 9.2.

RESULTS

Study participants

There were no significant differences in the distributions of age, sex, and race/ethnicity, respectively, between diabetic and nondiabetic participants. Male and female non-Hispanic whites, Hispanics, and American Indians were represented in each group. BMI, blood pressure, triglycerides, HDL cholesterol, nonesterified fatty acids, fasting plasma glucose, A1C, and insulin levels were higher among diabetic versus nondiabetic participants. No interactions were identified between race/ethnicity, diabetes status, and CD36 mRNA ($P = 0.22$) or protein levels ($P = 0.18$).

PBMC CD36 expression and circulating levels of biomarkers and mediators of inflammation

Unadjusted CD36 mRNA and protein levels were higher among diabetic versus nondiabetic participants (Table 1). PBMC CD36 protein, but not CD36 mRNA, levels remained higher among patients with type 2 diabetes versus control subjects, after adjusting for markers of inflammation, blood pressure, and insulin sensitivity. Plasma hsCRP, hsIL-6, and TNF-α were higher among diabetic versus nondiabetic participants.

CD36 mRNA and protein levels were tightly correlated among diabetic and nondiabetic participants, respectively. PBMC CD36 and peroxisome proliferator–activated receptor (PPAR)-γ mRNA levels were directly correlated in both groups. PBMC CD36 protein and PPAR-γ mRNA levels were directly correlated among nondiabetic participants. PBMC CD36 mRNA and protein levels were directly correlated with plasma hsCRP and hsIL-6 and inversely correlated with plasma adiponectin in each group (Table 1). CD36 mRNA was inversely associated with blood pressure among diabetic participants ($P = 0.02$). No associations were observed between CD36 expression and plasma HDL, LDL, or total cholesterol or nonesterified fatty acids.

CONCLUSIONS

— Our results are in concert with previous reports demonstrating that unadjusted PBMC CD36 mRNA and protein levels were upregulated among patients with type 2 diabetes (3,4). After adjustment for potential confounders, only CD36 protein levels remained higher among diabetic participants, suggesting that hyperglycemia has a greater impact on translation versus transcription. PBMC CD36 expression among diabetic participants was strongly correlated with hsCRP and hsIL-6, which are strong independent predictors of CVD (5–7). Therefore, increased PBMC CD36 expression is a sign of monocyte activation and a biomarker for a proinflammatory state. PBMC CD36 mRNA and protein levels were inversely correlated with plasma adiponectin levels, which, in turn, were inversely correlated with insulin resistance, suggesting a pathogenic link between insulin resistance and atherosclerosis. Therefore, CD36 may be important in the pathogenesis of CVD among patients with type 2 diabetes (4,8–11). The higher age of the diabetic patients may have contributed to observed higher levels of plasma hsCRP, hsIL-6, and TNF-α (12). Furthermore, it is reasonable to speculate that CD36 may also be protective in aging, since it may delay

age-related corneal neovascularization (13).

A strength of the present study is the inclusion of non-Hispanic whites, Hispanics, and American Indians. Limitations include the cross-sectional design and the limited number of African Americans.

In summary, in a multiracial/ethnic group of patients with type 2 diabetes, *1*) unadjusted PBMC CD36 mRNA and protein levels were upregulated and *2*) CD36 expression was a biomarker of monocyte activation and a proinflammatory state. Additional studies are needed to assess the use of CD36 expression in CVD risk stratification.

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References

1. Han J, Hajjar DP, Febbraio M, Nicholson AC. Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36. J Biol Chem 1997;272:21654–1659
2. Janabi M, Yamashita S, Hirano K, Sakai N, Hiraoka H, Matsumoto K, Zhang Z, Nozaki S, Matsuzawa Y. Oxidized LDL-induced NF-kappa B activation and subsequent expression of proinflammatory genes are defective in monocyte-derived macrophages from CD36-deficient patients. Arterioscler Thromb Vasc Biol 2000;20:1953–1960
3. Cipolletta C, Ryan KE, Hanna EV, Trimble ER. Activation of peripheral blood CD14+ monocytes occurs in diabetes. Diabetes 2005;54:2779–2786
4. Zhang HM, Zhang XL, Zhou X, Li D, Gu JG, Wu JJ. [Mechanism linking atherosclerosis and type 2 diabetes: increased expression of scavenger receptor CD36 in monocytes.]. Chin Med J (Engl) 2005;118:1717–1722
5. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996;144:537–547
6. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767–1772
7. Yu Z, Liu W, Liu D, Fan L. The regulatory role of Hyper-IL-6 in the differentiation of myeloid and erythroid progenitors derived from human cord blood. Cell Immunol 2006;241:32–37
8. Griffin E, Re A, Hamel N, Fu C, Bush H, McCaffrey T, Asch AS. A link between diabetes and atherosclerosis: glucose regulates expression of CD36 at the level of translation. Nat Med 2001;7:840–846
9. Handberg A, Levin K, Højlund K, Beck-Nielsen H. Identification of the oxidized low-density lipoprotein scavenger receptor CD36 in plasma: a novel marker of insulin resistance. Circulation 2006;114:1169–1176
10. Handberg A, Lopez-Bermejo A, Bassols J, Vendrell J, Ricart W, Fernandez-Real JM. Circulating soluble CD36 is associated with glucose metabolism and interleukin-6 in glucose-intolerant men. Diab Vasc Dis Res 2009;6:15–20
11. Sampson MJ, Davies IR, Braschi S, Ivory K, Hughes DA. Increased expression of a scavenger receptor (CD36) in monocytes from subjects with Type 2 diabetes. Atherosclerosis 2003;167:129–134
12. Leonarduzzi G, Gamba P, Gargiulo S, Sottero B, Kadl A, Biasi F, Chiarpotto E, Leitinger N, Vendemiale G, Serviddio G, Poli G. Oxidation as a crucial reaction for cholesterol to induce tissue degeneration: CD36 overexpression in human promonocytic cells treated with a biologically relevant oxysterol mixture. Aging Cell 2008;7:375–382
13. Mwaikambo BR, Sennlaub F, Ong H, Chemtob S, Hardy P. Genetic ablation of CD36 induces age-related corneal neovascularization. Cornea 2008;27:1037–1041