Increased CD36 Expression Signals Monocyte Activation Among Patients with Type 2 Diabetes Mellitus

Abbreviated Title: CD36 and Monocyte Activation in Diabetes

Yijuan Sun, MD, MS1, Marina Scavini, MD2, Robert A Orlando, PhD3, Glen H Murata1, MD, Karen S Servilla, MD1, Antonios H Tzamaloukas, MD1, Ronald Schrader, PhD4, Edward J. Bedrick, PhD2, Mark R Burge, MD2, Nada A Abumrad, PhD5, Philip G Zager, MD2

1Department of Medicine, Veteran Affairs Medical Center, 2Departments of Medicine and Biochemistry & Molecular Biology, 4Clinical Translational Sciences Center, University of New Mexico Health Sciences Center, Albuquerque, NM, and 5Department of Medicine, Center for Human Nutrition, Washington University, St Louis, MO

Author for Correspondence:
Philip G. Zager, MD
Email: pzag@unm.edu

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Objective: Explore the hypothesis that CD36, a scavenger receptor and fatty acid translocase, is up-regulated in peripheral blood mononuclear cells (PBMCs) among patients with type 2 diabetes and is a biomarker of PBMC activation and inflammation.

Design and Setting: 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 non-diabetic controls (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 diabetes versus controls. 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.

The prevalence of type 2 diabetes mellitus 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 88kDa 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 kappa-light-chain-enhancer of activated B cells (NFκB) and production of pro-inflammatory cytokines e.g., tumor necrosis factor α/β (TNFα/β), interleukin-1β (IL-1β), IL-6, and interferon β/γ. Production of pro-inflammatory 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 up-regulation of PBMC CD36 mRNA and protein, signaling PBMC activation and increased production of pro-inflammatory cytokines among Non-Hispanic Whites (NHW), 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 ≥ 2 FPG values >126mg/dL or received hypoglycemic medications. Non-diabetic participants had a fasting plasma glucose (FPG) <100mg/dL and HbA1c <6.1% and were not receiving hypoglycemic medications. Patients with end-stage renal disease, liver disease or who were pregnant, malnourished, receiving insulin were excluded. We obtained a venous blood sample after an overnight fast for a chemistry profile, lipid panel, cytokines, hsCRP, adiponectin, HbA1c, non-esterified fatty acids (NEFA) and PBMC isolation.

**Isolation of PBMCs, extraction of RNA and protein, quantification of CD36 γmRNA and protein.** PBMC were isolated and stored at -80°C. PBMC RNA and protein were isolated using TRIZOL reagent (Invitrogen Inc.). Messenger RNA was reverse transcribed to cDNA using random hexamer primers. Quantitative PCR was performed using SYBR Green RT-PCR reagents (Applied Biosystems) in the MiniOpticon System (Bio-Rad). CD36 protein was measured using Western Blot (rabbit anti-CD36 antibody, sc9154, Santa Cruz, CA; mouse anti-actin antibody, A4700, SIGMA; goat anti-rabbit IgG-HRP, #172-1019 and goat anti-mouse IgG-HRP, #170-6516, Bio-Rad).

**Statistical analyses:** Pearson correlations of CD36 mRNA and protein levels with anthropometric and laboratory measurements were determined in diabetic and non-diabetic participants. Means of CD36 mRNA and protein across groups stratified by race/ethnicity, gender, 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 Ver. 9.2.

**RESULTS**

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**Study participants.** There were no significant differences in the distributions of age, gender and race/ethnicity, respectively, between diabetic and non-diabetic participants. Male and female NHW, Hispanics and American Indians were represented in each group. Body mass index, blood pressure, triglycerides, HDL-cholesterol, NEFA, FPG, HbA1c and insulin levels were higher among diabetic versus non-diabetic participants. No interactions were identified between race/ethnicity, diabetes status and CD 36 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 non-diabetic participants (Table 1). PBMC CD 36 protein, but not CD36 mRNA, levels remained higher among patients with type 2 diabetes versus controls, after adjusting for markers of inflammation, blood pressure and insulin sensitivity. Plasma hsCRP, hsIL-6 and TNF-α were higher among diabetic versus non-diabetic participants.

CD36 mRNA and protein levels were tightly correlated among diabetic and non-diabetic participants, respectively. PBMC CD36 and PPARγ mRNA levels were directly correlated in both groups. PBMC CD 36 protein and PPARγ mRNA levels were directly correlated among non-diabetic 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- total cholesterol or NEFA.

**DISCUSSION**

Our results are in concert with previous reports demonstrating that unadjusted PBMC CD36 mRNA and protein levels were up-
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regulated 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 pro-inflammatory 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 NHW, 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 up-regulated; and (2) CD36 expression was a biomarker of monocyte activation and a pro-inflammatory state. Additional studies are needed to assess the use of CD36 expression in CVD risk stratification.

Author Contributions: Sun, Yijuan: researched data, wrote manuscript; Scavini, Marina: researched data, edited manuscript; Orlando, Robert: contributed to discussion, reviewed / edited manuscript; Murata, Glen: statistical analysis; Servilla, Karen: contributed to discussion, reviewed / edited manuscript; Tzamaloukas, Antonios: reviewed / edited manuscript; Schrader, Ronald: statistical analysis; Bedrick, Edward: statistical analysis, reviewed / edited manuscript; Abumrad, Nada: contributed to discussion, reviewed / edited manuscript; Zager, Philip: wrote manuscript, contributed to discussion, reviewed / edited manuscript.

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REFERENCES


Table 1. Unadjusted and adjusted mean CD36 mRNA/Protein and Pearson Product Moment Correlations (r)

<table>
<thead>
<tr>
<th></th>
<th>Type 2 Diabetes (n=33)</th>
<th>Non-diabetic Controls (n=27)</th>
<th>Type 2 Diabetes (n=33)</th>
<th>Non-diabetic Controls (n=27)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CD36 mRNA (log r.u.)</td>
<td>CD36 mRNA (log r.u.)</td>
<td>CD36 protein (log r.u.)</td>
<td>CD36 protein (log r.u.)</td>
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<tr>
<td>Unadjusted Mean</td>
<td>0.23 ± 0.02**</td>
<td>0.16 ± 0.02</td>
<td>0.28 ± 0.02****</td>
<td>0.18 ± 0.02</td>
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<tr>
<td>Model 1</td>
<td>0.21± 0.02</td>
<td>0.17± 0.02</td>
<td>0.27± 0.01*</td>
<td>0.21± 0.02</td>
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<td>Model 2</td>
<td>0.20± 0.02</td>
<td>0.18± 0.02</td>
<td>0.26± 0.01**</td>
<td>0.20± 0.01</td>
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<td>CD36 mRNA (log r.u.)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
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<td>PPARγ mRNA (log r.u.)</td>
<td>r = 0.73 ***</td>
<td>r = 0.72 ***</td>
<td>r = 0.33 (p=.08)</td>
<td>r = 0.46 *</td>
</tr>
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<td>hsCRP (log mg/dl)</td>
<td>r = 0.55 **</td>
<td>r = 0.21</td>
<td>r = 0.45 **</td>
<td>r = 0.43 *</td>
</tr>
<tr>
<td>hsIL-6 (log pg/ml)</td>
<td>r = 0.40 *</td>
<td>r = 0.49**</td>
<td>r = 0.64 ***</td>
<td>r = 0.49 **</td>
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<td>TNF α (log pg/mL)</td>
<td>r = -0.20</td>
<td>r = 0.09</td>
<td>r = -0.28</td>
<td>r = -0.02</td>
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<td>Adiponectin (log µg/ml)</td>
<td>r = -0.61 ***</td>
<td>r = -0.67 ***</td>
<td>r = -0.60 ***</td>
<td>r = -0.56 **</td>
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<td>BMI (kg/m²)</td>
<td>r = 0.20</td>
<td>r = 0.03</td>
<td>r = 0.09</td>
<td>r = 0.17</td>
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<td>HOMA-IR (log r.u.)</td>
<td>r = 0.18</td>
<td>r = 0.03</td>
<td>r = 0.12</td>
<td>r = -0.07</td>
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<td>QUICKI (log r.u.)</td>
<td>r = -0.20</td>
<td>r = 0.05</td>
<td>r = -0.16</td>
<td>r = 0.19</td>
</tr>
</tbody>
</table>

r.u., relative units. Means are expressed as least squares means ± SE. The p-value is from a t-test of equality of the means. Model 1 adjusts for HTN, hsIL6, PPAR, Adiponectin, and QUICKI. Model 2 adjusts for hsIL6, Adiponectin, and QUICKI. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001. PPARγ, peroxisome proliferator-activated receptor gamma; hsCRP, high-sensitivity C-reactive protein; hsIL-6, high-sensitivity interleukin-6; TNF α, tumor necrosis factor-alpha; HOMA-IR, homeostatic model assessment-insulin resistance; QUICKI, quantitative insulin-sensitivity check index.