Circulating soluble intercellular adhesion molecule-1 (sICAM-1) and other novel markers have been correlated with a variety of cardiovascular outcomes, including the likelihood of future clinical cardiovascular events (1–9).

We assessed circulating markers to explore relationships between subclinical atherosclerosis, bone density and metabolism, and fat distribution and metabolism in relation to type 2 diabetes mellitus. Serum sICAM-1, soluble vascular cell adhesion molecule-1 (sVCAM-1), s-E-selectin, monocyte chemoattractant protein-1, and interleukin-6 were evaluated as markers of inflammation; adiponectin, leptin, and soluble leptin receptor were markers of adiposity; and collagen type I C-terminal propeptide, bone-specific alkaline phosphatase, osteocalcin, and N-telopeptide cross-link of type I collagen were evaluated as markers of bone metabolism in families with siblings concordant for type 2 diabetes mellitus (10, 11) as well as unaffected family members. This study population included 42 men and 38 women, ranging in age from 39 to 81 years. Sixty-nine participants (86%) had type 2 diabetes mellitus, and 19 (24%) were African American.

Serum was obtained from a morning fasted blood sample and stored at −70°C before analysis. Commercially available assays from R&D Systems were used for measuring serum sE-selectin, sVCAM-1, monocyte chemoattractant protein-1, and sICAM-1 (assay BBE1B). Adiponectin was measured by RIA and leptin by ELISA with reagents and protocols from LINCO Research, Inc. Soluble leptin receptor was measured by an ELISA from ALPCO Diagnostics. Collagen type I C-terminal propeptide, bone-specific alkaline phosphatase, and osteocalcin were measured by ELISAs with reagents and protocols from Quidel, and N-telopeptide cross-link of type I collagen was measured by an ELISA from Wampole Laboratories. Intraassay CVs were <7%.

One of the African-American individuals, the focus of this report, had undetectable concentrations of sICAM-1, as measured by a common monoclonal antibody-based sandwich ELISA (BBE1B), but at the same time had the highest concentrations of sE-selectin, sVCAM-1, and soluble leptin receptor of the 80 individuals investigated (Table 1). Lack of serum immunoreactivity could result from complete absence of ICAM-1 expression, impaired release of ICAM-1 from the plasma membrane into the bloodstream, enhanced degradation or clearance of ICAM-1, or a missing or altered epitope in the ICAM-1 protein. To investigate these possibilities, we performed DNA sequence analysis of the coding and promoter regions of the ICAM-1 gene in this individual and family members. All coding regions of the ICAM-1 gene and 1.4

Table 1. Biomarker values for the K29M ICAM-1 homozygous individual and the entire study group.

<table>
<thead>
<tr>
<th>Serum marker</th>
<th>Group Mean</th>
<th>Range</th>
<th>SD</th>
<th>K29M individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1, μg/L</td>
<td>266</td>
<td>0–626</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>sICAM-1, μg/L</td>
<td>217</td>
<td>133–434</td>
<td>55</td>
<td>434</td>
</tr>
<tr>
<td>sVCAM-1, μg/L</td>
<td>598</td>
<td>222–1786</td>
<td>241</td>
<td>1786</td>
</tr>
<tr>
<td>sE-selectin, μg/L</td>
<td>71.7</td>
<td>17–384</td>
<td>51</td>
<td>384</td>
</tr>
<tr>
<td>IL-6, ng/L</td>
<td>4.30</td>
<td>1.15–11.7</td>
<td>2.33</td>
<td>7.41</td>
</tr>
<tr>
<td>MCP-1, ng/L</td>
<td>341</td>
<td>143–654</td>
<td>108</td>
<td>345</td>
</tr>
<tr>
<td>sLeptin receptor kU/L</td>
<td>24.0</td>
<td>9.0–80.8</td>
<td>11</td>
<td>80.8</td>
</tr>
<tr>
<td>Leptin, μg/L</td>
<td>16.8</td>
<td>0.8–89.5</td>
<td>17</td>
<td>8.3</td>
</tr>
<tr>
<td>ADPN, mg/L</td>
<td>12.2</td>
<td>3.1–46.5</td>
<td>9.06</td>
<td>18.1</td>
</tr>
<tr>
<td>CICP, μg/L</td>
<td>101</td>
<td>41–311</td>
<td>40</td>
<td>168</td>
</tr>
<tr>
<td>BALP, U/L</td>
<td>22.6</td>
<td>14.2–63.5</td>
<td>8</td>
<td>36.9</td>
</tr>
<tr>
<td>OC, μg/L</td>
<td>8.9</td>
<td>5.3–29.0</td>
<td>3</td>
<td>5.7</td>
</tr>
<tr>
<td>Nx, mol/L</td>
<td>10.8</td>
<td>3.7–26.1</td>
<td>4</td>
<td>8.7</td>
</tr>
</tbody>
</table>

*K29M ICAM-1 was not detected by assay BBE1B but was by assay BMS-201INST.

Note the extreme values for sICAM-1, sVCAM-1, s-E-selectin, and soluble leptin receptor in the K29M ICAM-1 homozygous individual.

* sICAM-1 was measured by assay BMS-201INST.

+ sICAM-1 was measured by assay BBE1B.

+ IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; ADPN, adiponectin; CICP, collagen type I C-terminal propeptide; BALP, bone-specific alkaline phosphatase; OC, osteocalcin; Nx, N-telopeptide cross-link of type I collagen.
kb of the promoter were amplified from genomic DNA by PCR (primer sequences available on request), and cleaned PCR products were sequenced with Big Dye Terminators on an ABI 3730 (Applied Biosystems). The individual with undetectable serum sICAM-1 was found to be homozygous for an A>T substitution at nucleotide 100 of exon 2, which encodes a lysine-to-methionine substitution at position 29 in the mature ICAM-1 protein (residue 56 of the precursor peptide, which contains a 27-amino acid leader sequence). Originally identified in Kilifi, Kenya, by Fernandez-Reyes et al. (12), this K29M ICAM-1 variant (also designated ICAM-1Kilifi) has been the subject of some investigation (12–18) and is represented in dbSNP by rs5491. The frequency of this allele in Kenya was 33.2%. In our study, based in Forsyth County, NC, this allele was present in 37 African-American probands at a frequency of 20% compared with a frequency of 0.4% in a group of 255 Caucasian probands.

Because monoclonal antibody specificity was a primary suspect in the failure to detect the K29M variant, we used an alternative commercial human sICAM-1 ELISA (BMS201INST; Bender MedSystems) to reevaluate the 80 study samples. With this alternative assay, sICAM-1 was detected in the individual homozygous for K29M ICAM-1 at the highest concentration among the sample population, consistent with the findings for the other adhesion molecules for this individual. Comparison of the sICAM-1 values obtained from the two assays in the remaining 18 African Americans (13 AA genotype and 5 AT heterozygotes) showed that sICAM-1 in AT heterozygotes was recognized to a greater extent by BMS201INST than by BBE1B (Fig. 1).

The results demonstrate that commercially available sICAM-1 assays vary markedly in their ability to recognize a common ICAM-1 variant (20% allele frequency) in African-American populations. The serum concentration of K29M sICAM-1, undetectable with one assay, was extremely high with the other, an effect presumably related to limited ability of a monoclonal antibody in the BBE1B assay to recognize the K29M region of the ICAM-1 protein. The binding characteristics of several monoclonal antibodies for this variant have been investigated (12), and one antibody in particular (BBA4; R&D Systems) was found to have markedly reduced ability to interact with the K29M variant. The antibodies used in the BBE1B ELISA and their recognition domains are proprietary information and not public knowledge. Regardless of the specifics of the antibody used, based on these studies it appears that the BBE1B ELISA has little or no ability to recognize the K29M ICAM-1. These findings have important implications for investigations into the relationships between serum concentrations of sICAM-1 and cardiovascular and other disease outcomes, particularly in African-American populations.

K29M is located in domain 1 near the NH₂ terminus of the ICAM-1 protein, a region critical for binding of the malarial organism Plasmodium falciparum, the human rhinovirus LFA-1, and fibrinogen. In vitro studies have demonstrated that K29M ICAM-1 has an altered ability to
bind to \textit{P. falciparum} (15, 16), reduced affinity for LFA-1, and no apparent affinity for fibrinogen (16). Important insights into the function of ICAM-1 may be obtained from future investigations of relationships in additional individuals homozygous and heterozygous for K29M ICAM-1.

In summary, previous studies exploring sICAM-1 as a marker for cardiovascular and other diseases may need to be reevaluated in light of the demonstration that commercial sICAM-1 ELISAs vary markedly in their ability to recognize this ICAM-1 variant, which is common (20–35\% allele frequency) in African-American populations.

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References


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Detection of Fetal DNA and RNA in Placenta-Derived Syncytiotrophoblast Microparticles Generated in Vitro

Fetal DNA and RNA can be readily detected in maternal plasma samples (1–4). Most of this material appears to be of placental origin (5), and it appears to be in a predominantly cell-free form (2), whereas circulatory mRNA is membrane-encapsulated (6).

Pregnancy is associated with the release of microparticles by the syncytiotrophoblast membrane into the maternal circulation (7). These particles, frequently termed STBM, are released by turnover of the syncytiotrophoblast monolayer covering the entire villous tree (8–11). This process of normal physiologic syncytiotrophoblast turnover involves the release of apoptotic material into the maternal circulation by the extrusion of syncytial knots and the associated release of STBM (8–11). The amount of material that is released by apoptotic shedding of syncytial knots (and STBM) is several grams per day (9), and the circulating concentrations are increased significantly in preeclampsia (11).

STBM particles have been suggested to evoke the mild maternal inflammatory response accompanying normal pregnancies (12), and increased release has been proposed to play a role in the etiology of preeclampsia by triggering maternal endothelial cell damage (13, 14).

As these particles are difficult to detect and prepare from maternal blood samples, use is frequently made of in vitro-prepared particles to study their physiologic activity (13). In this context, we have recently extensively examined three different modes of STBM preparation: mechanical dissection of fresh placental villous tissues; in vitro