CD11c/CD18 Expression Is Upregulated on Blood Monocytes During Hypertriglyceridemia and Enhances Adhesion to Vascular Cell Adhesion Molecule-1


Objective—Atherosclerosis is associated with monocyte adhesion to the arterial wall that involves integrin activation and emigration across inflamed endothelium. Involvement of β3-integrin CD11c/CD18 in atherogenesis was recently shown in dyslipidemic mice, which motivates our study of its inflammatory function during hypertriglyceridemia in humans.

Methods and Results—Flow cytometry of blood from healthy subjects fed a standardized high-fat meal revealed that at 3.5 hours postprandial, monocyte CD11c surface expression was elevated, and the extent of upregulation correlated with blood triglycerides. Monocytes from postprandial blood exhibited an increased light scatter profile, which correlated with elevated CD11c expression and uptake of lipid particles. Purified monocytes internalized triglyceride-rich lipoproteins isolated from postprandial blood through low-density lipoprotein–receptor–related protein-1, and this also elicited CD11c upregulation. Laboratory-on-a-chip analysis of whole blood showed that monocyte arrest on a vascular cell adhesion molecule-1 (VCAM-1) substrate under shear flow was elevated at 3.5 hours and correlated with blood triglyceride and CD11c expression. At 7 hours postprandial, blood triglycerides decreased and monocyte CD11c expression and arrest on VCAM-1 returned to fasting levels.

Conclusion—During hypertriglyceridemia, monocytes internalize lipids, upregulate CD11c, and increase adhesion to VCAM-1. These data suggest that analysis of monocyte inflammation may provide an additional framework for evaluating individual susceptibility to cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2011;31:00-00.)

Key Words: adhesion molecules ■ atherosclerosis ■ leukocytes

Neointimal thickening due to monocyte recruitment and lipid deposition in arteries begins during childhood, but complications due to atherosclerosis do not manifest until later in life. Prevention requires early identification of individuals at risk for cardiovascular disease. Conventional risk factors, including smoking, diabetes, hypertension, or dyslipidemia, can identify those at increased risk; however, many individuals with cardiovascular events have only 1 or none of these factors. Thus, there is a need for personalized tests of susceptibility to detect disease and guide therapy.

In animal models, hypercholesterolemia induces vascular cell adhesion molecule-1 (VCAM-1) expression on aortic endothelium, and its induction precedes accumulation of macrophages and T lymphocytes at sites of lesion formation. The β3-integrin very late antigen-4 (VLA-4) is the primary monocyte receptor that binds to VCAM-1 and supports rolling, which on activation mediates cell arrest. VLA-4/VCAM-1 interactions are important for the progression of atherosclerosis, as hypercholesterolemic mice that express VCAM-1 lacking one of the VLA-4 binding domains exhibit reduced lesion area. Recent evidence suggests the β3-integrin CD11c/CD18 also recognizes VCAM-1 as an adhesive ligand during monocyte recruitment in shear flow. Antibody blocking experiments on inflamed human aortic endothelium revealed that CD11c and VLA-4 cooperate to support arrest and transendothelial migration. Furthermore, our recent study showed that monocytes isolated from mice deficient in CD11c exhibited lower arrest efficiency and an inability to adhesion strengthen on recombinant VCAM-1 under shear flow.

Monocyte CD11b/CD18 is elevated during postprandial lipemia and following exposure to triglyceride-rich lipoproteins (TGRL); however, the effect on CD11c/CD18 expression is unknown. It is also unclear how native TGRL modulates monocyte upregulation of these proteins. Low-density lipoprotein (LDL)-receptor related protein-1 (LRP-1)
mediates TGRL clearance from the blood in the liver and TGRL uptake into macrophages.\textsuperscript{13,14} We demonstrate that blood monocytes express LRP-1 and it is involved in binding TGRL, which leads to upregulation of monocyte CD11c in vitro. Thus, we hypothesized that during periods of hypertri-
glyceridemia, CD11c may be upregulated on circulating monocytes and contribute to enhancing arrest on VCAM-1.

We report that in healthy subjects, monocyte uptake of circulating lipoprotein and upregulation of cell surface CD11c correlates with blood triglyceride following a high-fat meal. Applying a laboratory-on-a-chip assay of monocyte adhesion to VCAM-1 under shear flow revealed that arrest correlated
with postprandial triglycerides, was dependent on CD11c, and returned to fasting levels as the triglyceride peak sub-
sided. As atherosclerosis is a chronic disease exacerbated by and returned to fasting levels as the triglyceride peak sub-
sided, we hypothesized that cytokines levels may
elevated, and returned to fasting levels as the triglyceride peak sub-
sided, we hypothesized that cytokines levels may
increase and be associated with the observed activation.

\textbf{Methods}

An expanded Methods section is available online at http://atvb.ahajournals.org.

\textbf{Results}

\textbf{Blood Triglycerides and Monocyte Inflammation Are Elevated Postprandial}

Blood triglyceride concentration increased an average of 85% from fasting levels 3.5 hours postprandial, a period that coincides with the peak in triglycerides after ingestion of a high-fat meal.\textsuperscript{12} Glucose and apoB100 remained unchanged at this time point, but there were significant decreases in total cholesterol, LDL, and high-density lipoprotein (HDL) cholesterol (Supplemental Table II).

Surface receptors were detected by flow cytometry of antibody-labeled whole blood samples to define a baseline for monocyte inflammation and avoid activation that occurs during isolation.\textsuperscript{19} Following the peak in blood triglycerides at 3.5 hours, monocytes exhibited a significant increase in cell surface expression of CD14, CD11b, and CD11c and a decrease in CD62L (Figure 1). In contrast, VLA-4 expression was not increased (data not shown). Granulocytes did not exhibit a significant increase in any measured surface antigens (Supplemental Figure II).

Monocyte markers of inflammation were increased post-
prandially, and we hypothesized that cytokines levels may also be increased and associated with the observed activation. Tumor necrosis factor-\(\alpha\), interferon-\(\gamma\), interleukin-1\(\beta\), interleukin-6, and interleukin-8 were all significantly in-
creased after the meal, whereas interleukin-10, a potent antiinflammatory cytokine,\textsuperscript{20} remained unchanged. It is note-
worthy that the relative increase in cytokines did not correlate
with the change in monocyte surface CD11c or triglyceride level in blood. Endotoxin was not a factor in the inflammatory response because levels detected in serum were low (4 IU/mL) and remained unchanged by the meal (Table 1).

Increase in Monocyte CD11c Correlates With Blood Lipid Levels

We have reported that CD11c expression is increased on monocytes in dyslipidemic mice, and we were interested in whether CD11c correlated with blood lipid levels in our human cohort. Relative increases in CD11c correlated the strongest with the absolute value of a subject’s fasting and postprandial triglycerides (Table 2) but not the percentage increase in triglycerides (Supplemental Figure IIIA). Percentage postprandial triglycerides (Table 2) but not the percentage increase in HDL cholesterol, total cholesterol, and fasting LDL but not HDL (Table 2).

Inflammatory Response Following Triglyceride Increase Subsidies by 7 Hours

The extent of monocyte CD11c increase correlated with postprandial triglycerides at 3.5 hours, and we hypothesized that its expression might decrease as triglycerides reverted back to fasting levels. To address this hypothesis, we recalled 6 subjects selected for high postprandial triglycerides (410±116 mg/dL, mean±SD) and robust monocyte CD11c increase. Venous blood was obtained after a 10-hour overnight fast, and then the meal was administered. Participants returned 3.5 and 7 hours following ingestion for postprandial blood draws. Clinical characteristics and serum biochemistry at 0, 3.5, and 7 hours postprandial are displayed in Supplemental Tables III and IV. For these subjects, triglyceride concentration at 7 hours showed a trend to decrease but was not significantly different from 3.5 hours (Figure 2A). However, the elevation in CD11c and CD11b expression on monocytes was significantly decreased at 7 hours compared with the level at 3.5 hours (Figure 2B).

Table 2. Correlations Between the Postprandial Increase in Monocyte CD11c and Blood Lipids

<table>
<thead>
<tr>
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<th>Pearson r</th>
<th>95% CI</th>
<th>P Value</th>
<th>r²</th>
</tr>
</thead>
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<tr>
<td>Fasting</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Triglyceride</td>
<td>0.65</td>
<td>0.42 to 0.81</td>
<td>&lt;0.0001</td>
<td>0.43</td>
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<td>0.22 to 0.72</td>
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<td>Total cholesterol:HDL ratio</td>
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<td>0.004</td>
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<tr>
<td>Non-HDL cholesterol</td>
<td>0.46</td>
<td>0.16 to 0.68</td>
<td>0.004</td>
<td>0.21</td>
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<tr>
<td>Total cholesterol</td>
<td>0.45</td>
<td>0.16 to 0.68</td>
<td>0.004</td>
<td>0.21</td>
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<tr>
<td>LDL cholesterol</td>
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<td>0.12 to 0.66</td>
<td>0.008</td>
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<td>HDL cholesterol</td>
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<td>0.05</td>
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<td>Postprandial</td>
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<tr>
<td>Triglyceride</td>
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<tr>
<td>Total cholesterol:HDL ratio</td>
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<td>0.14 to 0.67</td>
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<td>0.20</td>
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<td>Total cholesterol</td>
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<td>0.14 to 0.67</td>
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<td>HDL cholesterol</td>
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<td>LDL cholesterol</td>
<td>0.23</td>
<td>−0.12 to 0.53</td>
<td>0.197</td>
<td>0.05</td>
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Correlations between the postprandial increase in monocyte CD11c expression from fasting levels (as measured by flow cytometry) and blood lipids. Data are from 40 subjects (24 female). Data are partitioned on fasting and postprandial values and listed in descending order based on strength of correlation.
Monocytes Take Up Lipid Particles in Hypertriglyceridemic Blood

We have previously reported that monocytes in the blood of hypercholesterolemic mice fed a high-fat diet internalized lipids, and this correlated with elevated light scatter and cell surface CD11c as detected by flow cytometry and confirmed by histology. Here, we report that monocyte light scatter significantly increased in the blood of subjects at 3.5 hours postprandial (Figure 3A and 3B). Monocytes with elevated light scatter profiles expressed significantly more CD11c than cells with low light scatter (Figure 3C). By 7 hours, these trends reversed, as indicated by a decrease in the scatter distribution and CD11c expression of monocytes in blood. Inspection of peripheral blood films confirmed a 1-fold increase in the percentage of vacuolated monocytes at the 3.5-hour time point (Supplemental Figure IVA and IVB). To establish whether these vacuoles consisted of neutral lipids, monocytes from whole blood were sorted on the basis of CD14 expression and scatter profile. Monocytes from 3.5 hours postprandial blood exhibited punctate Oil Red O–positive droplets, which were not detected in fasting blood (Figure 3D and 3E and Supplemental Figure IVC and IVD).

Monocytes Internalize TGRL and Upregulate CD11c In Vitro

We hypothesized that the lipid droplets observed in monocytes were due to uptake of TGRL in the blood. In support of this, we found that monocytes expressed LRP-1 (Supplemental Figure VA), an LDL-family receptor that mediates macrophage uptake of TGRL. To determine its potential role, TGRL was isolated from postprandial blood, labeled with Alexa Fluor 488, and incubated with MNCs from fasting healthy subjects. Internalization was visualized by confocal immunofluorescence microscopy and quantified by flow cytometry. The LRP-1 antagonist RAP inhibited TGRL uptake by 30%, whereas TGRL endocytosis was abrogated at 10°C (Figure 4A and 4B). We also found that LRP-1 antibody binding was inhibited in the presence of TGRL, suggesting that binding of lipoprotein particles resulted in receptor internalization (Supplemental Figure VB).

Additional experiments were performed to examine the capacity of isolated TGRL to induce CD11c upregulation on monocytes. A 30-minute incubation with TGRL induced a 40% increase in CD11c expression, a level similar to that observed ex vivo, and this increase was inhibited by RAP.
Monocyte Adhesion to VCAM-1 Under Shear Stress Correlates With Postprandial Triglycerides and Depends on CD11c

To assess the consequences of elevated postprandial triglycerides on monocyte function, we measured monocyte adhesion to recombinant VCAM-1 in diluted whole blood under defined shear stress at fasting, 3.5 hours postprandial, and 7 hours postprandial using a laboratory-on-a-chip microfluidic flow channel (Supplemental Figure VIA to VIC). Monocytes in diluted and sheared whole blood recruited avidly to the VCAM-1 substrate (Supplemental Figure IVD) and were rarely observed rolling, but they quickly transitioned to firm arrest, in agreement with previous work. A direct correlation was observed between a subject’s increase in monocyte arrest from fasting and his or her peak in postprandial triglycerides at 3.5 hours (Figure 5A).

Previously, we have reported that cooperative binding of CD11c and VLA-4 to VCAM-1 contributes to the conversion of monocyte rolling to arrest. To investigate the contribution of CD11c to the enhanced monocyte adhesion, we measured monocyte arrest in the presence of CD11c blocking antibody 496K, VLA-4 blocking antibody HP2.1, or IgG control for 6 subjects selected for high postprandial triglycerides and CD11c expression (Figure 5B). Monocyte arrest was significantly elevated at 3.5 hours but decreased to fasting levels by 7 hours. Blood monocyte counts in our subjects ranged from $2 \times 10^5$ to $10 \times 10^7$ per mL and remained constant at 3.5 and 7 hours after the meal, consistent with previous studies. This confirms that increased monocyte arrest was due to postprandial changes in monocyte function, not simply changes in blood concentration. Inhibition of CD11c with antibody significantly decreased monocyte adhesion at 3.5 hours postprandial but not at fasting or 7 hours postprandial. Consistent with previous observations, blocking VLA-4 function with HP2.1 significantly blocked adhesion at all time points, demonstrating that monocyte arrest was dependent on binding to VCAM-1. Moreover, the contribution of CD11c to adhesion correlated with the time point of its peak in expression.

Discussion

We quantified the acute inflammatory response of circulating monocytes following an increase in blood triglycerides, which was characterized by elevated expression of CD11c, an integrin receptor recently shown to regulate adhesion to VCAM-1 on inflamed endothelium. This was accompanied by an increase in CD11b expression and a reduction in CD62L, suggesting monocyte activation. Monocyte activation was found to subside by 7 hours postprandial, a time when blood triglyceride was declining. Monocytes in post-
postprandial blood exhibited cytoplasmic lipid droplets, suggesting that lipid uptake in the circulation induces cell surface expression of CD11c. Increase in monocyte arrest on VCAM-1 also correlated with postprandial triglycerides, and this was dependent on integrin cooperativity between VLA-4 and CD11c. These data are consistent with a recent report showing that apoE<sup>−/−</sup> mice fed a high-fat diet have increased CD11c expression on blood monocytes, which take up lipid and accumulate in atherosclerotic lesions. In that study, genetic deletion of CD11c in crossedb apoE<sup>−/−</sup>/CD11c<sup>−/−</sup> mice significantly diminished the efficiency of monocyte arrest on VCAM-1 in shear flow and reduced atherosclerosis. These data in human and mouse models implicate CD11c as a functional integrin that participates in recruitment on VCAM-1 during periods of elevated blood lipids, such as the postprandial phase, a period when incidents linked to coronary disease are prevalent.

Our data set assessed a cohort of healthy subjects selected without regard to age or gender. The most striking change in serum biochemistry following the meal was an 85% increase in triglycerides. Epidemiological evidence links elevated nonfasting triglyceride with increased risk of atherosclerosis and associated cardiovascular events. A causal relationship between triglyceride and monocyte CD11c levels was recently demonstrated in humans with metabolic syndrome and obese mice. In both cases, high triglyceride levels correlated with elevated expression of CD11c on blood monocytes that decreased following diet-induced weight loss. Here, we observed in an acute study design that postprandial hypertriglyceridemia transiently increased monocyte CD11c and primed them for adhesion to VCAM-1. Our studies provide a plausible link between the epidemiological data in humans and mouse models that reveal increased monocyte recruitment to nascent arterial lesions under conditions in which VCAM-1 is elevated. For example, in dyslipidemic mice, atherosclerotic lesion size is increased in proportion to the number of accumulated monocytes. The importance of CD11c in this pathogenesis was highlighted by our recent study showing that apoE<sup>−/−</sup>/CD11c<sup>−/−</sup> mice have smaller aortic lesions because of decreased macrophage content.

A novel finding of this study was the close correlation between postprandial triglyceride concentration, CD11c expression, and monocyte adhesion under shear flow. Increase in CD11c and monocyte adhesion did not correlate with the relative increase in triglycerides following the meal but rather with the absolute concentration in the blood. Stable monocyte arrest was dependent on VLA-4 and CD11c to recognize distinct epitopes on VCAM-1. Furthermore, VLA-4 was dependent on high-affinity CD11c that supported increased levels of monocyte arrest because the blocking antibody 496K acts allosterically on CD11c by stabilizing a low affinity conformation. These data are consistent with our previous observation of cooperativity between CD11c and VLA-4 in binding VCAM-1 on TGRL-primed and inflamed human aortic endothelium. A potential mechanism by which upregulation of CD11c expression potentiates VLA-4 function may involve cross-talk in signaling such as that previously demonstrated between β<sub>1</sub> and β<sub>2</sub>. Thus, the clinical relevance of elevated CD11c on monocytes in the postprandial circulation is their enhanced potential to home to lesions where endothelial VCAM-1 expression is elevated.

Our study and others demonstrate that monocytes internalize lipids during postprandial lipemia and after exposure to TGRL. How blood monocytes internalize unmodified lipoprotein particles is unclear. Recent studies using cell lines and mice indicate that macrophages uptake native lipoproteins through LRP-1, apoB48 receptor, CD36, and pinocytosis. We show that freshly isolated monocytes internalized TGRL through LRP-1 (and possibly other LDL-family receptors), resulting in upregulation of CD11c. Interestingly, several in vivo studies with LDL receptor<sup>−/−</sup> or apoE<sup>−/−</sup> mice indicated that macrophage-specific LRP-1 deficiency was associated with increased atherosclerotic lesions. Although this suggests an atheroprotective role for LRP-1, our data and those of others demonstrate an essential role of LRP-1 in macrophage uptake of remnant lipoproteins and highlight the complexity of LRP-1 in atherogenesis. In this regard, we are actively studying how binding or internalization of lipoproteins upregulates CD11c expression. A plausible pathway may involve signaling following ligand binding to LRP-1, which induces activation of PLC and a rise in intracellular IP<sub>3</sub> and Ca<sup>2+</sup> levels that in turn activate protein kinase C, Because protein kinase C is important for cell degranulation through its modulation of proteins involved in late exocytosis, a mechanism contributing to monocyte activation could be that LRP-1 binding TGRL triggers activation of protein kinase C and release of CD11c stored in secretory vesicles.

In summary, we show an increase in monocyte inflammatory response in a population of subjects with high circulating triglycerides. Monocytes were found to internalize lipids and upregulate cell surface CD11c as early as 3.5 hours after a high-fat meal, a period coincident with peak blood triglycerides. The inflammatory response was transient, subsiding by 7 hours. Using a dynamic blood film in a laboratory-on-a-chip format revealed that monocyte arrest on VCAM-1 was dependent on CD11c and correlated with a subject’s postprandial triglycerides. Thus this assay may prove useful in identifying high-risk individuals early in disease progression. Given that the initial presentation of coronary disease for many patients is sudden death or myocardial infarction, these data warrant longitudinal studies to determine whether monocyte CD11c expression correlates with the development of more severe atherosclerotic disease.

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**Disclosures**

None.
References


34. Gower et al. CD11c Is Upregulated on Postprandial Monocytes
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