Increased Expression of Fc-gamma receptors on Monocytes in Patients with Nascent Metabolic Syndrome

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Abstract

The Metabolic Syndrome (MetS) confers an increased propensity to diabetes and cardiovascular disease (CVD). C-reactive protein (CRP) levels are increased in MetS and predict cardiovascular events. Fc gamma receptors (FcγRs) are immune receptors on macrophages and include FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). CRP has been shown to be processed by CD32 and CD64 in human monocytes. While MetS is characterized by increased CRP and monocyte pro-inflammatory activity, there is sparse data on FcγR expression on monocytes in MetS compared to controls and this was the aim of the study. Following informed consent, MetS (n = 50) and healthy control subjects (n = 40) were studied. CR32 and CD64 expression on monocytes was examined both by flow cytometry and western blotting. Immunoprecipitation studies were performed to examine Syk tyrosine kinase activity, as a measure of downstream signals. Thus, we examined Syk kinase activity by immunoprecipitation with Syk antibody (Anti-Syk) and western blotting for phosphotyrosine (pY antibody).

Introduction

• The Metabolic Syndrome (MetS) affects 1 in 3 US adults and confers an increased propensity to both diabetes and cardiovascular disease (CVD).
• C-reactive protein (CRP) has been shown to be a cardiovascular marker and high levels of CRP have been shown to predict cardiovascular events.
• CRP levels are increased in patients with MetS and predict cardiovascular events in MetS.
• In addition to being a cardiovascular risk marker, CRP appears to be pro-atherogenic.
• Fcgamma receptors (FcγRs) are cell-surface immune receptors that mediate phagocytosis, release of inflammatory mediators and stimulation of the immune response in macrophages.
• The main FcγRs on monocytes include FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16).
• The proatherogenic effects of CRP appear to be mediated by the Fc gamma receptors (FcγR), CD32 and CD64.

Aim

While MetS is a pro-inflammatory state characterized by increased monocyte pro-inflammatory activity (11-14), there is sparse data on FcγR expression on monocytes in MetS without the confounders of diabetes or CVD compared to controls and this was the aim of the study since these patients have elevated CRP levels.

Materials & Methods

• All subjects were recruited from Sacramento County, California, through fliers and advertisements in the newspaper.
• The subjects (aged 21-69 years) with MetS (n = 50) and healthy control subjects (n = 40) were studied.
• MetS was defined using the modified criteria of the NCEP ATP III as described previously.
• Nascent MetS - no diabetes or cardiovascular disease; must have at least three risk features of MetS and/or not on hypertension or antihypertensive medications.
• Control subjects needed to have ≤2 features of MetS and not be on blood pressure medications. Other exclusion criteria for control subjects were fasting plasma glucose (>100 mg/dL) and triglycerides (TGs) >200 mg/dL.
• Informed consent was obtained from participants in the study, which was approved by the institutional review board at the University of California Davis.
• Mononuclear cells were isolated from fasting heparinized blood by Ficoll Hypaque centrifugation followed by magnetic separation using the depletion technique.
• Monocytes from control and Nascent MetS subjects were incubated with human CD32 and CD64 antibodies (InviroGen) or isotype controls, and surface expression of CD32 and CD64 was analyzed using BD FACSArray (Franklin Lakes, NJ) after gating for CD14.
• Using antibodies to CD32 and CD64 and beta actin (loading control) (Invivogen, San Diego, CA), western blotting was performed on monocyte lysates.
• CRP signals through activation of CD32 and CD64 via ITAM and ITIM receptors (17), the former leading to activation signals and the latter resulting in inhibitory signaling pathways. These ITAM tyrosine residues are phosphorylated by cross-linking of the receptors with specific antibodies and are associated with Syk tyrosine kinase that activates downstream signals. Thus, we examined Syk kinase activity by immunoprecipitating with Syk (Anti-Syk) and western blotting for phosphotyrosine (pY antibody).

Results

Table 1: Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=40)</th>
<th>MetS (n=50)</th>
<th>P value</th>
<th>(C vs. MetS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48±12</td>
<td>51±10</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>92±15</td>
<td>107±12*</td>
<td>&lt;0.0001</td>
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<td>BMI (kg/m²)</td>
<td>30.5±6.7</td>
<td>34.7±6.6*</td>
<td>0.005</td>
<td></td>
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<tr>
<td>BP-diastolic (mmHg)</td>
<td>73±8</td>
<td>82±9</td>
<td>&lt;0.0001</td>
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<tr>
<td>BP-systolic (mmHg)</td>
<td>89±7</td>
<td>99±11*</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>188±32</td>
<td>201±28</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Total Triglycerides (mg/dL)</td>
<td>80 (59,96)</td>
<td>151</td>
<td>&lt;0.0001</td>
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<td>HDL Cholesterol (mg/dL)</td>
<td>54±15</td>
<td>41±11*</td>
<td>&lt;0.0001</td>
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<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>118±26</td>
<td>130±21</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.3 (0.5,2.8)</td>
<td>3.7 (1.7,5.6)*</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1 (0.9,2.8)</td>
<td>2.8 (1.9,5.8)*</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
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Fig 1: Monocyte CD32 and CD64 Surface Expression

Fig 2: Monocyte CD32 and CD64 Western Blotting

Fig 3: Syk Kinase and Tyrosine Phosphorylation

Summary and Conclusion

• Surface expression of CD32 and CD64, were significantly increased in MetS compared to controls even following adjustment for BMI and waist circumference.
• Expression of these FcγRs increased significantly with number of features of MetS (p for trend < 0.001) and correlated with levels of hsCRP (p<0.01).
• CRP following engagement of CD32 and CD64 signals via Syk kinase. Syk tyrosine phosphorylation was increased in monocytes of patients with MetS compared to controls.

• Conclusions: We provide novel evidence of increased FcγR expression and activity in monocytes of patients with MetS that correlate with the number of features of MetS and with hsCRP.

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* p<0.05 compared to Controls