Interleukin-10 Associates With Adiponectin Predominantly in Subjects With Metabolic Syndrome

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Background  
Inflammation and the metabolic syndrome (MetS) are important risk factors in cardiovascular disease. There is accumulating evidence that decreased adiponectin levels are associated with MetS. Recently, it was shown that adiponectin induces the expression of a potent anti-inflammatory cytokine, interleukin (IL)-10, in vitro. The aim of this study is to investigate the association of IL-10 levels with other pro-inflammatory and anti-inflammatory factors including adiponectin levels in vivo.

Methods and Results  
MetS components were assessed in 117 drug-naïve middle-aged men. Serum levels of high-sensitive C-reactive protein (hs-CRP), IL-6, adiponectin, IL-10 and tumor necrosis factor-α (TNF-α) were measured in these subjects. A significant decrease in adiponectin (5.15±1.79 μg/ml vs 6.87±3.55 μg/ml, p<0.02) and an increase in IL-6 (1.50±1.50 pg/ml vs 1.06±0.78 pg/ml, p<0.05) levels were associated with MetS. The serum IL-10 level exhibited a significant positive correlation with IL-6, hs-CRP, and TNF-α levels, but not with adiponectin in healthy individuals. However, IL-10 exhibited a significant correlation with adiponectin, especially in the subjects with MetS.

Conclusions  
Serum IL-10 levels correlated with inflammatory proteins, but not with adiponectin. However, IL-10 positively associated with adiponectin especially in the subjects with MetS. IL-10 might be involved in the inflammatory network of MetS in relation to adiponectin.  

Key Words: Adiponectin; Cytokine; IL-10; Inflammation; Metabolic syndrome

The metabolic syndrome (MetS) is an epidemiological target for preventing cardiovascular disease (CVD) and is now considered a pathological condition common to atherosclerosis and MetS. Various studies have shown that inflammation also associates with CVD, and that inflammatory markers are predictive of cardiovascular events.

Adiponectin, an anti-inflammatory protein, has been demonstrated to be insulin-sensitizing and an anti-atherogenic factor including coronary spasms and is considered a key component of MetS. The serum adiponectin level is known to decrease with an increase in number of MetS components. Interleukin (IL)-10 has multifaceted anti-inflammatory properties including deactivation of macrophages and T cells and exhibits a protective effect against atherogenesis. Recently, it was shown that adiponectin induces expression of IL-10 in human macrophages and that anti-atherogenic effects of adiponectin are partially mediated by the induction of IL-10. In addition, IL-10 production by lipopolysaccharide-stimulated blood cells is inhibited in MetS and Type 2 diabetes. However, there has been no report showing the association between IL-10 and other inflammatory markers in subjects with or without MetS.

In the present study, we examined serum levels of pro-inflammatory and anti-inflammatory proteins, including adiponectin and IL-10 in drug-naïve, middle-aged Japanese men. For the first time, we will determine the unique associations between IL-10 and other inflammatory markers in subjects with or without MetS.

Methods  

Study Population  
The subjects were individuals who underwent a health examination in the Osaka University Health Care Center in 2004. Individuals with a history of acute illness within 2 weeks or who had taken any medicine were excluded in order to avoid the effects on serum levels of inflammatory proteins. A total of 117 drug-naïve Japanese men, aged 40–59 years of age, were evaluated in terms of MetS components. Informed consent was obtained from all subjects prior to participation in the study, and the study was approved by the Ethics Committee of Osaka University.

Risk Factor Assessment  
Information on medical history and use of medicines was obtained by questionnaires, and was reconfirmed by trained nurses. Waist circumference at the umbilical level was measured in the late exhalation phase when subjects were in a standing position. Abdominal obesity was diagnosed if a waist circumference was ≥85 cm by using the guidelines for abdominal obesity in Japanese individuals. In this study, according to the definition by the International Diabetes Federation (IDF), MetS was defined as the existence of abdominal obesity and at least 2 of 4 MetS components were defined as follows: (1) hypertriglyceridemia: a serum level of triglycerides ≥150 mg/dl; (2) low high-den-
sity lipoprotein (HDL)-cholesterolemia: a serum level of HDL-cholesterol (C) <40 mg/dl; (3) hypertension: a systolic blood pressure ≥130 mmHg, or a diastolic blood pressure ≥85 mmHg; and (4) high fasting glucose: plasma level of glucose ≥100 mg/dl. In addition to the IDF criteria, the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) criteria and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria were applied to assess the significance of abdominal obesity. Abdominal obesity is not an essential component in these criterions and the cut-off point of high fasting glucose in the NCEP-ATP III criteria is 110 mg/dl.

**Laboratory Measurements**

Serum was collected from subjects after an overnight fast and kept at ≤-20°C until assay. The serum high-sensitive C-reactive protein (hs-CRP) level was measured by an immunoenzyme assay (Dase Behring, Marburg, Germany). The serum IL-6 concentration was measured in duplicate by a sandwich enzyme-linked immunoenzyme assay (Dase Behring, Marburg, Germany). C-reactive protein (hs-CRP) level was measured by using an adiponectin ELISA kit (R&D Systems Inc, MN, USA) and a Quantikine human TNF-α, tumor necrosis factor-α (TNF-α) kit (Otsuka Pharmaceutical Co, Tokushima, Japan), as described previously.18,19 Both IL-10 and tumor necrosis factor-α (TNF-α) were measured by using a human IL-10 ultra-sensitive immunoassay kit (Biosource International Inc, CA, USA) and a Quantikine human TNF-α high sensitive immunoassay kit (R&D Systems Inc, MN, USA), respectively.

**Statistical Analysis**

All values are presented as mean±SD. A Student’s t-test was used to assess the difference between the 2 groups. Pearson’s correlation coefficients were calculated among skewed variables after logarithmic transformation of variables. Values of p<0.05 were considered to be statistically significant.

**Results**

Profiles of the study subjects are shown in Table 1. The mean age is 48.6±5.4 years old and the mean body mass index (BMI) is 24.1±3.1. The frequency of abdominal obesity defined by waist circumference was 51.3%. Twenty-nine men were diagnosed as having a MetS defined by IDF criteria (24.8%; Table 2). Subjects with a diagnosed MetS showed significant worsening of metabolic risk factors including obesity (BMI and waist), insulin resistance (fasting plasma glucose, hemoglobin (Hb) A1c, immunoreactive insulin, and homeostasis model assessment of insulin resistance and dyslipidemia (triglycerides and HDL-C) as compared to subjects without a diagnosis for MetS (Table 2). By using AHA/NHLBI or NCEP-ATPIII criteria, the number of subjects with MetS was 30 (25.6%) or 22 (18.8%), respectively (data not shown). Profiles of the subjects with MetS defined by AHA/NHLBI or NCEP-ATPIII criteria were almost the same as those defined by IDF criteria. Subjects with MetS defined by NCEP-ATPIII criteria showed a slightly higher BMI and a higher fasting plasma glucose level as compared with those defined by IDF criteria, probably because of the high cut-off point of fasting plasma glucose level (data not shown). Subjects with MetS showed a significantly decreased se-

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<th>n</th>
<th>BMI (kg/m²)</th>
<th>Waist (cm)</th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>Hemoglobin A1c (%)</th>
<th>IRI (µU/ml)</th>
<th>HOMA-IR</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Log Adiponectin (µg/ml)</th>
<th>Log hs-CRP (mg/L)</th>
<th>Log TNF-α (pg/ml)</th>
<th>Log IL-10 (pg/ml)</th>
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<tr>
<td>MetS (–)</td>
<td>88 (75.2%)</td>
<td>23.3±2.7</td>
<td>82.8±6.9</td>
<td>91±8</td>
<td>3.5±1.6</td>
<td>0.80±0.38</td>
<td>103±59</td>
<td>58±13</td>
<td>1.8±1±4</td>
<td>-0.06±0.52</td>
<td>-0.39±0.50</td>
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<tr>
<td>MetS (+)</td>
<td>29 (24.8%)</td>
<td>26.8±2.7</td>
<td>92.3±5.4</td>
<td>10±22</td>
<td>10.8±16.7</td>
<td>2.57±3.4</td>
<td>182±102</td>
<td>49±10</td>
<td>1.59±0.33</td>
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<td>0.0051</td>
<td>0.049</td>
<td>0.21</td>
<td>0.36</td>
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Data are mean±SD. MetS, metabolic syndrome; MetS (–), subjects without MetS; MetS (+), subjects with MetS; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; IL, interleukin; hs-CRP, high-sensitive C-reactive protein; TNF-α, tumor necrosis factor-α. Other abbreviations see in Table 1.
Adiponectin level (5.15±1.79 μg/ml vs 6.87±3.55 μg/ml, p<0.015) and a significantly increased IL-6 level (1.50±1.50 pg/ml vs 1.06±0.78 pg/ml, p<0.05) when compared to those without it. As shown in Table 2, log-transformed adiponectin and IL-6 levels also demonstrated significant changes. Log-transformed hs-CRP, TNF-α, and IL-10 levels were increased in the subjects with MetS, but no significant differences were obtained by the presence of MetS. Multiple logistic regression analysis with MetS as a dependent variable demonstrated that log adiponectin and log hs-CRP were significant determinants (B=–2.63 and 1.13, p=0.021 and 0.026, respectively). These data support the hypothesis that adiponectin is a key molecule of MetS.

As shown in Fig 1, the serum IL-10 level exhibited significant positive correlations with inflammatory markers including hs-CRP [r=0.29 (95% confidence interval (CI); 0.12–0.45)] (Fig 1a), IL-6 [r=0.25 (95% CI; 0.07–0.41)] (Fig 1b), and TNF-α [r=0.30 (95% CI; 0.05–0.52)] (Fig 1c). However, the serum levels of IL-10 did not correlate with adiponectin levels [r=0.06 (95% CI; –0.13–0.24)].

The correlation between IL-10 and adiponectin was re-evaluated in the subjects with or without MetS (Fig 2). Interestingly, the IL-10 levels significantly correlated with adiponectin levels especially in the subjects with MetS [r=0.46 (95% CI; 0.11–0.70)] (Fig 2b). Using a Spearman rank correlation test also proved that there was a significant correlation between IL-10 and adiponectin levels in the subjects with MetS (r=0.22, p=0.07, and 0.19, (95% CI; –0.16–0.54, –0.42–0.31, and –0.32–0.62), respectively).

**Discussion**

MetS involves multiple risk factor clustering, and intra-abdominal fat is known to be a major determinant of NCEP-ATP III criteria for MetS. In 2005, the IDF proposed new criteria for MetS, with abdominal obesity being considered as an essential component. Inflammation is an important underlying etiology of MetS through its various effects mediated by adipose tissue-derived factors (eg,
leptin, adiponectin, TNF-$\alpha$, IL-6, IL-10, etc)$^{11}$ We demonstrated a significant decrease in adiponectin and an increase in IL-6 levels by the presence of MetS. Although hs-CRP level was increased in MetS, it was not significant in our study population. The stable and ultra-sensitive measurement of IL-6 by CLEIA might detect a minimum increase of IL-6 that preceded hs-CRP elevation in apparently healthy subjects. 

Many studies have demonstrated associations between inflammatory proteins and MetS, abdominal obesity, or insulin resistance. Multivariate analyses have revealed the importance of abdominal obesity and insulin resistance in low-grade inflammation.$^{22}$ Accordingly, inflammatory status might be changed by the definitions of MetS as to whether abdominal obesity is considered essential or not. Therefore, we used the IDF definition in order to access low-grade inflammation in MetS.

Adiponectin is an anti-inflammatory protein.$^{2}$ The serum adiponectin level was associated with MetS more clearly and independently than the other inflammatory factors did. These data support the hypothesis that adiponectin is a key component of MetS$^{3}$ or an important marker for MetS$^{4}$ Recently, it was shown that the anti-inflammatory effects of adiponectin could be partially mediated by the induction of IL-10, a potent anti-inflammatory cytokine.$^{13}$ However, so far, there is no report evaluating the circulating levels of IL-10 that preceded hs-CRP elevation in apparently healthy subjects. 

For the first time, this present study demonstrated the physiological levels of IL-10 as well as significant associations between levels of IL-10 and CRP, IL-6, and TNF-$\alpha$ in healthy middle-aged men. Recently, a positive correlation between serum IL-10 and hs-CRP levels was reported in 312 Korean individuals including 269 women.$^{23}$ Serum levels of IL-10 are likely to correlate positively with serum levels of inflammatory factors despite its anti-inflammatory properties. As shown in the present study, an elevation of serum IL-10 might be a compensatory reaction for pro-inflammatory activation in healthy subjects.

In addition, a clear correlation was found between adiponectin and IL-10 levels predominantly in the subjects with MetS. The correlation between adiponectin and IL-10 levels was recently demonstrated in android obese women$^{24}$ Because there were only 14 abdominal obese subjects who exhibited no MetS components, a significant correlation between adiponectin and IL-10 levels was not observed in the present study. Moreover, in the subjects with MetS, the significant association between IL-10 and IL-6, CRP, and TNF-$\alpha$ levels was abolished. Interestingly, the presence of MetS clearly altered the association between IL-10 and adiponectin levels. In our study subjects, in which there was a relatively low number of subjects with MetS, different definitions of MetS did not show clear differences within each component of MetS. However, the significant positive correlation between IL-10 and adiponectin in the subjects with MetS defined by IDF criteria was weakened in the subjects with MetS that were defined by AHA/NHLBI criteria or NCEP-ATP III criteria, in which abdominal obesity is not an essential component. Thus, abdominal obesity might exert influence on inflammatory networks in MetS, most likely irrespective of the MetS components.

The mechanism of the link between abdominal obesity and serum IL-10 and adiponectin levels are speculated as follows. Immunohistochemical analysis of adipose tissue demonstrated the presence of macrophages. It has been reported that macrophages are a source of many adipose tissue-derived proteins$^{25}$ The finding of increased numbers of macrophages infiltrating the visceral fat tissue in obese individuals suggests that adipose tissue itself is a source and site of inflammation.$^{3,26}$ Circulating IL-10 is mainly derived from T-cells and macrophages in bone marrow etc, but increased visceral fat can be an alternative source for circulating IL-10 in abdominally obese subjects. Abdominally obese subjects might also have large numbers of macrophages infiltrating into their visceral fat. Although the level of secretion of adiponectin from fat cells is low in MetS, adiponectin might locally activate macrophages and thereby induce IL-10 secretion. Thus, a significant positive correlation between IL-10 and adiponectin levels was observed in the abdominally obese subjects with MetS. In contrast, adiponectin does not induce IL-10 locally in lean adipose tissue where low amounts of macrophages are accumulated, and therefore there is no correlation between adiponectin and IL-10 levels. That is to say, the induction of IL-10 might thus be a compensatory reaction to the inflammation accompanied by clustering of MetS components in subjects who suffer from abdominal obesity.

As inflammation is greatly influenced by the presence of diabetes, we performed the same analysis excluding 2 subjects who showed a high HbA1c level (26.0%). There was still a significant correlation between IL-10 and adiponectin levels ($r=0.51, p=0.006$ (95% CI, 0.16–0.740)), however, a significant increase of IL-6 levels in MetS was abolished. Diabetes might contribute a pro-inflammatory reaction that is represented by IL-6. Inflammatory status is also influenced by gender. Although we evaluated the correlation between IL-10 and adiponectin levels in women ($r=0.16, p=0.20$ (95% CI, −0.19–0.09), n=64), we could not further evaluate inflammation in the presence of MetS. The prevalence of MetS in women is very low in our institution (1%), partly because of the elimination of the subjects who had been taking medications and those who were from the relatively younger population. Further studies can elucidate the significance of diabetes and gender difference in the relationship between inflammation and MetS.

In conclusion, the serum adiponectin level is associated with MetS more clearly than the other inflammatory factors. As a potent anti-inflammatory cytokine, IL-10 positively associates with adiponectin predominantly in the subjects with MetS defined by the IDF criteria. These data suggest that IL-10 might be involved in the inflammatory network of MetS in relation to adiponectin.

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