Autoantibodies against oxidized low-density lipoprotein (LDL) and carotid atherosclerosis in patients with rheumatoid arthritis

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Abstract

Objectives

To examine the relationship between autoantibodies against oxidized low-density lipoprotein (oxLDL-Abs) and the progression of carotid atherosclerosis in patients with rheumatoid arthritis (RA).

Methods

Fifty RA patients without evidence of risk factors for atherosclerosis (RA group) and 30 healthy volunteers (normal group) were investigated. The mean intima-media thickness of the common carotid artery (mean CCA-IMT) was measured by high-resolution B-mode ultrasonography. The titer of IgG oxLDL-Abs was measured by enzyme-linked immunosorbent assay. The relationships among mean CCA-IMT, IgG oxLDL-Ab titer and patient factors such as body mass index, systolic blood pressure, diastolic blood pressure, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum lipid levels were examined.

Results

Mean CCA-IMT, CRP, ESR and titer of IgG oxLDL-Abs were significantly higher in the RA group than in the normal group. Although mean CCA-IMT showed a positive correlation only with age in multivariate analysis, IgG oxLDL-Ab titers in the RA group were positively associated with mean CCA-IMT and independently with age and sex by multiple regression analysis.

Conclusions

IgG oxLDL-Abs appear to be associated with the degree of carotid atherosclerosis in patients with RA, and are independent of traditional risk factors for atherosclerotic diseases. These results suggest a possible link between autoimmune mechanisms and accelerated atherosclerosis in RA.

Key words

Rheumatoid arthritis, atherosclerosis, autoantibodies against oxidized low-density lipoprotein (LDL).
Introduction
Coronary vascular disease and stroke are well recognized as major causes of mortality in patients with autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), (1, 2) and increased arterial intima-media thickness (IMT) has also been demonstrated in these patients (3, 4). Although traditional atherosclerosis risk factors play essential roles, several studies have also suggested the importance of systemic inflammation rather than traditional risk factors in the progression of atherosclerosis in autoimmune disorders (3-5). The potential mechanisms involved in the accelerated atherosclerosis associated with autoimmune disorders may involve various cellular and humoral inflammatory mediators, immune complex-mediated endothelial cell damage, prothrombotic states, and autoantibodies (6).

Autoantibodies against oxidized low-density lipoprotein (oxLDL-Abs) have been reported to be one of the autoimmune agents associated with atherosclerosis (6), and the serum titer of oxLDL-Abs is reportedly increased in patients with atherosclerotic and autoimmune disorders (6-10). In the present study, we investigated the relationship between oxLDL-Abs and atherosclerosis in patients with RA.

Patients and methods
Subjects
Between October 2002 and March 2003, 50 RA patients who had been referred to Niigata Prefectural Senami Hospital for surgery or rehabilitation were entered in the study (RA group). All patients fulfilled the diagnostic criteria of the American College of Rheumatology for RA and had no history of any atherosclerotic disorders, hypertension, diabetes mellitus, hyperlipidemia, or other complications that might affect the measurements performed in the study. Thirty healthy volunteers (normal group) were selected for comparison. Informed consent was obtained from all 80 subjects. The background data for the RA and normal groups are summarized in Table I and II. In this study, body mass index was significantly lower in the RA group than in the normal group.

Carotid ultrasonography
Ultrasonography of the common carotid artery (CCA) was performed using a high resolution real-time scanner with a 7.5-MHz transducer (SSD-2200; Aloka Co., Ltd., Tokyo, Japan), and all examinations were performed by a single observer who was unaware of the clinical and biochemical data for the subjects. Measurement of the mean CCA-IMT was performed as described previously (11). All subjects were examined in the supine position and the bilateral CCA and carotid bifurcations were carefully scanned in the longitudinal and transverse views. The IMT was measured at 0.5, 1 and 2 cm below the bifurcation on each side in plaque-free arterial segments, and the average value was taken as the mean CCA-IMT.

Anti-oxidized LDL antibodies and other measurements
The titer of IgG oxLDL-Abs was measured by enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (OLAB; Biomedica, Wien, Austria), as described previously (12). Serum samples stored at -20°C were diluted 1:50 and tested in 96-well microtiter wells precoated with copper-oxidized LDL. Double wavelength reading at 450 and 620 nm was performed and the difference in absorbance was calculated with a microplate reader. The titer was calculated from a

| Table I. Clinical characteristics of patients with rheumatoid arthritis (RA group) and healthy controls (normal group). |
|-----------------|-----------------|-----------------|
|                 | RA group (n = 50) | Normal group (n = 30) | p     |
| Age, mean ± SD  | 60.5 ±12.2       | 60.4 ±12.9       | NS    |
| Sex, male/female| 4/46             | 2/28             | NS    |
| Body mass index, mean ± SD | 21.1 ± 3.7       | 23.8 ± 3.5       | 0.002 |
| Smokers/non-smokers | 4/46           | 3/27             | NS    |

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OxLDL-Ab and CCA-IMT in RA/ Y. Wada et al.

Table II. Clinical characteristics of patients with rheumatoid arthritis (RA group).

<table>
<thead>
<tr>
<th>Disease duration, mean ± SD</th>
<th>12.4 ± 11.4 yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMARDs, yes/no</td>
<td>44/6</td>
</tr>
<tr>
<td>Glucocorticoid, yes/no</td>
<td>33/17</td>
</tr>
<tr>
<td>Functional class</td>
<td>RA stage</td>
</tr>
<tr>
<td>I</td>
<td>I</td>
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<td>II</td>
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<td>III</td>
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<td>IV</td>
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</tr>
</tbody>
</table>

Functional class: RA functional status determined by American College of Rheumatology criteria; RA stage: determined by Steinbrocker criteria; DMARDs: disease-modifying antirheumatic drugs.

Table III. Laboratory findings and intima-media thickness of the common carotid artery (CCA-IMT) in RA group and normal group.

<table>
<thead>
<tr>
<th></th>
<th>RA group (n = 50)</th>
<th>Normal group (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/hour)</td>
<td>56.0 ± 27.1</td>
<td>17.5 ± 12.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>2.65 ± 2.71</td>
<td>0.17 ± 0.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>218.0 ± 336.9</td>
<td>1.00 ± 3.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.83 ± 0.65</td>
<td>5.01 ± 0.72</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.92 ± 0.34</td>
<td>1.19 ± 0.48</td>
<td>0.011</td>
</tr>
<tr>
<td>HDLC (mmol/l)</td>
<td>1.56 ± 0.41</td>
<td>1.50 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>LDLC (mmol/l)</td>
<td>2.91 ± 0.59</td>
<td>2.99 ± 0.64</td>
<td>NS</td>
</tr>
<tr>
<td>sBP (mmHg)</td>
<td>121.3 ± 11.5</td>
<td>117.6 ± 10.8</td>
<td>NS</td>
</tr>
<tr>
<td>dBP (mmHg)</td>
<td>74.7 ± 7.0</td>
<td>73.6 ± 7.4</td>
<td>NS</td>
</tr>
<tr>
<td>OxLDL-Ab (EU/ml)</td>
<td>305.1 ± 299.9</td>
<td>198.6 ± 151.8</td>
<td>0.039</td>
</tr>
<tr>
<td>aCL (U/ml)</td>
<td>1.49 ± 5.083</td>
<td>0.52 ± 2.573</td>
<td>0.19</td>
</tr>
<tr>
<td>CCA-IMT (mm)</td>
<td>0.61 ± 0.10</td>
<td>0.53 ± 0.08</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; TC: total cholesterol; TG: triglycerides; HDLC: high density lipoprotein cholesterol; LDLC: low density lipoprotein cholesterol; sBP: systolic blood pressure; dBP: diastolic blood pressure; oxLDL-Ab: antibodies against oxidized-LDL; aCL: anticardiolipin antibodies; CCA-IMT: mean intima-media thickness of common carotid artery.

Table II. Clinical characteristics of patients with rheumatoid arthritis (RA group).

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</tr>
</tbody>
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Functional class: RA functional status determined by American College of Rheumatology criteria; RA stage: determined by Steinbrocker criteria; DMARDs: disease-modifying antirheumatic drugs.

Results

Table III shows the laboratory findings and the mean CCA-IMT in the RA group and the normal group, as expected, ESR was significantly higher in the RA group than in the normal group. Although there were no significant differences in serum cholesterol levels between the two groups, the titer of oxLDL-Ab and the mean CCA-IMT were significantly higher in the patients with RA. Figure 1 shows the distributions of the titers of oxLDL-Ab in the RA and normal groups. OxLDL-Ab were elevated over the cut-off value in 9 patients (18%) in the RA group. Table IV shows the correlation between mean CCA-IMT and other variables in the RA and normal groups. Although the mean CCA-IMT was correlated only with age in patients with RA, it was positively correlated with age, systolic blood pressure, CRP, and ESR in the normal group. We also examined the correlations between CCA-IMT and the clinical features of the RA patients listed in Table II; however, no significant relationships were apparent (data not shown).

To examine the factors that correlated independently with mean CCA-IMT in patients with RA, stepwise multiple regression analysis was performed for each group (Table V). Although age and titer of oxLDL-Ab were selected as independent variables that showed a significant positive correlation with CCA-IMT in the RA group, only age...
was selected in the normal group. The next analysis was performed on all study subjects (n = 80) using RA status and the interaction between RA status and oxLDL-Abs as additional independent variables. Table VI shows that age, RA status and interaction between RA status and oxLDL-Abs were selected as independent variables in multiple regression analysis in all subjects.

Discussion
oxLDL-Abs are known as one of the autoimmune agents associated with atherosclerosis (6), and the serum titer of oxLDL-Abs is reported to be elevated in many pathological conditions, including cardiovascular diseases and autoimmune disorders (6-10). However, the mechanism by which these autoantibodies act against the development of atherosclerosis remains controversial. Salonen et al. reported a positive correlation between progression of atherosclerosis and serum levels of oxLDL-Abs (7), and Hulthe et al. also demonstrated a positive correlation between CCA-IMT and oxLDL-Abs in healthy subjects (13). Increased levels of oxLDL-Abs are also found in cardiovascular diseases, hypertension, and peripheral arterial disease (8,14,15). These studies suggest that elevated levels of oxLDL-Abs may predict the development of atherosclerosis and cardiovascular disorders. On the other hand, several studies have suggested an inverse correlation between CCA-IMT and oxLDL-Abs in healthy subjects (6, 12). Our study also indicated a similar inverse trend, without statistical significance, in the normal group. In addition, immunization with oxLDL results in a reduction of atherosclerotic lesions with increased levels of oxLDL-Abs in experimental animal models (16). OxLDL-Abs are known to be detectable in healthy subjects as well as in patients with atherosclerotic diseases, and humoral immunity to oxidized LDLs is thought to reduce the incidence of atherosclerosis by neutralizing them (17).

These apparently conflicting results may be due, at least in part, to methodological differences in the detection of oxLDL-Abs. There are several antigenic forms of LDL used in immunoassay, and the nature of their antigenic epitopes remains uncertain (6, 18). OxLDL-Abs also belong to different sub-classes, such as IgA, IgG, and IgM (6), and Karvonen et al. reported that only IgM oxLDL-Abs had an inverse association with carotid atherosclerosis.

Table IV. Correlation coefficient between mean CCA-IMT in RA group and normal group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA group (n = 50)</th>
<th>Normal group (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male = 1/female = 0)</td>
<td>-0.005</td>
<td>0.04</td>
</tr>
<tr>
<td>Age</td>
<td>0.57***</td>
<td>0.78***</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>ESR (1 hour)</td>
<td>-0.24</td>
<td>0.49**</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.09</td>
<td>0.43*</td>
</tr>
<tr>
<td>RF</td>
<td>-0.211</td>
<td>0.23</td>
</tr>
<tr>
<td>TC</td>
<td>0.05</td>
<td>0.35</td>
</tr>
<tr>
<td>TG</td>
<td>0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>HDLC</td>
<td>0.13</td>
<td>0.27</td>
</tr>
<tr>
<td>LDLc</td>
<td>-0.02</td>
<td>-0.16</td>
</tr>
<tr>
<td>sBP</td>
<td>0.24</td>
<td>0.43*</td>
</tr>
<tr>
<td>dBP</td>
<td>0.03</td>
<td>-0.09</td>
</tr>
<tr>
<td>OxLDL-Ab</td>
<td>0.15</td>
<td>-0.25</td>
</tr>
<tr>
<td>aCL</td>
<td>0.23</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001.
ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; TC: total cholesterol; TG: triglycerides; HDLC: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; sBP: systolic blood pressure; dBP: diastolic blood pressure; oxLDL-Ab: antibodies against oxidized-LDL; aCL: anticardiolipin antibodies; CCA-IMT: mean intima-media thickness of common carotid artery.

Table V. Results of stepwise multiple regression analysis of factors affecting CCA-IMT in RA group and normal group.

<table>
<thead>
<tr>
<th>Selected variables</th>
<th>Standardized Beta</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA group (n = 50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.57</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OxLDL-Ab</td>
<td>0.24</td>
<td>0.044</td>
</tr>
<tr>
<td>Normal group (n = 30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.78</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Selected from Sex, Age, BMI, ESR, CRP, RF, TC, TG, HDLC, LDLc, sBP, dBP, and OxLDL-Ab.
CCA-IMT: mean intima-media thickness of common carotid artery; BMI: body mass index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; TC: total cholesterol; TG: triglycerides; HDLC: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; sBP: systolic blood pressure; dBP: diastolic blood pressure; oxLDL-Ab: antibodies against oxidized-LDL.

Table VI. Results of stepwise multiple regression analysis of factors affecting CCA-IMT in all subjects (n = 80).

<table>
<thead>
<tr>
<th>Selected variables</th>
<th>Standardized Beta</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.61</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Interaction RAstatus and OxLDL-Ab</td>
<td>0.23</td>
<td>0.019</td>
</tr>
<tr>
<td>RAstatus (RA= 1/normal = 0)</td>
<td>0.20</td>
<td>0.035</td>
</tr>
</tbody>
</table>

*Selected from Sex, Age, BMI, ESR, CRP, RF, TC, TG, HDLC, LDLc, sBP, dBP, and OxLDL-Ab.
CCA-IMT: mean intima-media thickness of common carotid artery; BMI: body mass index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; TC: total cholesterol; TG: triglycerides; HDLC: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; sBP: systolic blood pressure; dBP: diastolic blood pressure; oxLDL-Ab: antibodies against oxidized-LDL.
These studies suggest possible functional differences according to epitope or isotype, and it will be important to determine these differences in order to clarify the relationship between ox-LDL-Abs and the development of atherosclerosis. Apart from these problems, immunological status in patients with autoimmune disorders seems quite different from that in healthy subjects. Elevated levels of oxLDL-Abs have been found in patients with several autoimmune disorders, and oxLDL-Ab titers in SLE and RA patients are reportedly related to disease severity (9, 10). Although in atherosclerosis and markers associated with RA (3,4). Kumeda et al demonstrated that the duration and clinical severity of RA patients, cross-reactivity between aCL and oxLDL-Abs has been reported in several autoimmune disorders (6). These findings suggest that levels of oxLDL-Abs reflect abnormal immunological activity in autoimmune disorders, such as high production of autoantibodies or immunoglobulins. Although it remains unknown whether oxLDL-Abs have a “promotive” or “protective” role in the development of atherosclerosis, these observations indicate that the process of atherosclerosis might involve some kind of immunological abnormality or autoimmune mechanism. Recently, several studies have examined the relationship between carotid atherosclerosis and markers associated with systemic inflammation in patients with RA (3,4). Kumeda et al demonstrated that the duration and clinical severity of RA were independently associated with increased CCA-IMT (3). Rincon et al. showed that increased CCA-IMT and the presence of carotid plaque were associated with markers of systemic inflammation such as CRP and ESR both in RA patients and in healthy subjects (4). These studies have suggested a close relationship between systemic inflammation and the progression of atherosclerosis in patients with RA. Although we have suggested that the positive correlation between CCA-IMT and oxLDL-Abs is affected by RA status, in this study CCA-IMT was not correlated with serum inflammatory markers or clinical features in RA patients. As RA disease activity may be modified by changes in lifestyle or medication, it may be difficult to evaluate disease status by examination at a single time-point. An appropriate marker of long-term disease activity is needed for future studies. In summary, this study has demonstrated a positive correlation between carotid atherosclerosis and oxLDL-Abs in patients with RA, independent of age, sex, or other traditional risk factors. As our findings suggest a possible link between autoimmune mechanisms and acceleration of atherosclerosis in RA, further studies will be needed to investigate the functional role of these autoantibodies in the human body in various immunological states.

References

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