Autoantibodies to OxLDL Are Decreased in Individuals With Borderline Hypertension

Ruihua Wu, Ulf de Faire, Carola Lemne, Joseph L. Witztum, Johan Frostegård

Abstract—Elevated antibody levels to oxidized low-density lipoprotein (aOxLDL) have been shown to correlate with the degree of atherosclerosis in some studies. On the other hand, immunization of experimental animals with OxLDL, leading to enhanced aOxLDL levels, inhibits the development of the disease. The role of antibodies to OxLDL during different stages of disease development is thus not clear. The objective of this study was to determine the level of aOxLDL in early cardiovascular disease, such as borderline hypertension (BHT). Seventy-three men with BHT were matched with 75 age-matched normotensive (NT) men (diastolic blood pressures, 85 to 94 and <80 mm Hg, respectively). Antibody levels to epitopes of OxLDL were determined by use of conventional and chemiluminescence ELISA techniques. Presence of carotid atherosclerosis was determined by B-mode ultrasonography; atherosclerotic plaques were detected in 29 individuals. BHT men had significantly lower aOxLDL levels of IgG class (P=0.001) and IgM class (P=0.001) than NT controls, as determined using chemiluminescence ELISA. Similar results were obtained using conventional ELISA, with which aOxLDL of IgG (P=0.0002) and IgM (P=0.026) classes and antibody levels to malondialdehyde–LDL were significantly lower in BHT individuals. There was no difference in antibody levels between individuals with or without carotid atherosclerosis. It is not clear whether the decreased aOxLDL levels in BHT are due to a decreased immune reaction to OxLDL or to an increased consumption of aOxLDL due to binding to early atherosclerotic lesions. The possible implications of these findings are discussed. (Hypertension. 1999;33:53-59.)

Key Words: hypertension, borderline , atherosclerosis , low-density lipoproteins, oxidized , antibodies

Hypertension is a major risk factor for atherosclerosis, and, recently, borderline hypertension (BHT) also has been shown to be a risk factor for atherosclerosis.1,2 In hypertension, alterations of immune function including decreased T-cell responses, abnormalities in complement function, and enhanced immunoglobulin levels have been reported.3,4 However, available data are comparatively scarce, and little is known about the role of the immune system in early stages of hypertension such as BHT. We recently demonstrated that antibody levels to immunogenic heat shock proteins are enhanced in BHT, which may reflect the enhanced mechanical stress to the endothelium at lesion-prone sites in the vascular tree, which precedes development of atherosclerosis at these sites.5

Atherosclerosis is a chronic inflammation in the vascular wall, where activated T cells, monocytes, and macrophages are present in significant amounts.5–8 Oxidized low-density lipoprotein (OxLDL) is taken up by specific scavenger receptors in macrophages, which develop into foam cells,9 and antibodies to OxLDL (aOxLDL) are present both in the atherosclerotic lesions and in plasma.10 In some studies but not all, antibodies to OxLDL have been demonstrated to be related to the degree of atherosclerosis.11–15 On the other hand, immunization of experimental animals with OxLDL such that high aOxLDL titers were generated decreased atherosclerotic lesions.16–18 This suggests that antibody levels may differ in their significance depending on titers and on the relative stage of atherosclerosis.

To investigate the role of immune reactions to OxLDL in the early development of cardiovascular disease, we studied the aOxLDL levels in a group of 73 middle-aged men with BHT compared with age-matched normotensive (NT) control subjects. Surprisingly, the serum aOxLDL levels were decreased in patients with BHT. The possible implications of this observation are discussed.

Methods

Study Group

Patients were recruited from a population screening program as previously described.19 BHT was defined as diastolic blood pressure (DBP) of 85 to 94 mm Hg, and the screening identified 81 men who remained within the range for BHT during repeated measurements over a 3-year period. From the same population, 80 age-matched
control subjects were recruited, whose blood pressures were measured on 2 occasions a few weeks apart and were <80 mm Hg on both occasions.

The study was approved by the local Ethics Committee of Karolinska Hospital and conducted in accordance with the Declaration of Helsinki. All subjects gave informed consent before entering the study. Of the 81 men with BHT and the 80 NT control subjects who agreed to participate, 73 in the BHT group and 75 in the NT group completed all procedures of the present study. None of the subjects had any other illnesses or were regularly using any drugs known to influence blood pressure or metabolic or inflammatory variables.

**Study Protocol**

All subjects were investigated according to the same schedule. BHT and NT control subjects were investigated simultaneously when possible and no more than 4 weeks apart. Blood samples for analyses of metabolic and inflammatory variables were taken between 8:00 and 9:30 AM, after 8 to 12 hours of fasting. All samples were drawn after 15 minutes of rest in the supine position.

**Blood Pressure Measurements**

An identical procedure was followed on each occasion during the entire recruitment period. All blood pressure measurements were performed with a mercury sphygmomanometer. The cuff was adjusted according to the circumference of the arm and placed at the level of the heart. Blood pressure was recorded as the mean of 2 measurements taken after 5 minutes of rest in the supine position. Systolic and diastolic blood pressure measurements were defined according to Korotkoff I and V. The same specially trained nurse performed the measurements on all occasions.

Twenty-four-hour ambulatory blood pressure was measured using the ambulatory Del Mar Avionics P-IV (P-IV, model 1990; Del Mar Avionics, Irvine, Calif) measuring BP every 15 minutes for the complete 24-hour period. Patients completed a diary during the period noting body posture, going to bed, waking up, and so forth. Data were transferred to a computer unit at the end of the period. Artifacts were defined as any of the following: SBP <50 mm Hg, SBP >250 mm Hg, DBP >SBP, DBP <30 mm Hg, DBP >150 mm Hg. No other editing was performed.

**Carotid Ultrasound**

The right and left carotid arteries were examined with a duplex scanner (Acuson model 128XP/5; Acuson, Mountain View, Calif) using a 7.0-MHz linear array transducer as previously described.2 The subjects were investigated in the supine position and intima-media thickness was determined in the far wall as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-ventitia echo. Plaque was defined as a localized intima-media thickening with a thickness >1 mm and a 100% increase in thickness compared with normal adjacent wall segments. Plaque occurrence was scored as present or absent. Plaque was screened for in the common, internal, and external carotid arteries on both sides, as described previously.2

**Body Stature**

All patients were weighed wearing only underwear, using the same scale (Delta 707, SECA, Germany). Length was measured with a special ruler, which was fixed to the wall. Waist circumference was measured at the level of the umbilicus, and the hips were measured at the level of the greatest circumference. Body mass index (BMI) was subsequently calculated as weight in kilograms divided by height in meters squared.

**Analysis of Plasma Lipoprotein and Insulin Levels**

Lipid and lipoprotein levels were determined by a combination of preparative ultracentrifugation followed by lipid analyses in the lipoprotein fractions as previously described.1 Venous blood samples were taken for determination of plasma insulin concentration (Radio-Immuno Assay, Kabi Pharmacia, Sweden).

**Analysis of Total Serum Immunoglobulin Levels**

Serum immunoglobulins IgG, IgM, and IgA were determined by immunoturbidimetry. Specific anti-IgG, anti-IgM, and anti-IgA reagents and calibrators were obtained from Dako (Copenhagen, Denmark). The turbidimetric reaction was quantified in a Hitachi 911 analyzer by measuring light transmission at 340 nm wavelength.

**Lipids and Reagents**

LDL was isolated from plasma of healthy donors by sequential preparative ultracentrifugation and oxidized by use of copper ions as described for ELISA determination of antibody levels in Stockholm21 and for chemiluminescence determinations in San Diego.22 Malondialdehyde (MDA)–LDL was prepared as previously described.22

**Determination of Antibodies Against Lipoproteins and Cardiolipin by ELISA**

IgG and IgM antibodies against OxLDL were determined by ELISA essentially as described.23 OxLDL was diluted to 2 μg/mL in coating buffer (carbonate-bicarbonate buffer 50 mmol/L, pH 9.7), and 100 μL/well was used to coat ELISA plates (Costar 2581). The plates were kept at 4°C overnight, washed 4 times with phosphate-buffered saline (PBS) containing 0.05% Tween-20, and then blocked with 20% adult bovine serum albumin in PBS (20% ABS-PBS) for 2 hours at room temperature. They were then incubated with 100 μL serum and diluted 1:30 in 20% ABS-PBS at 4°C overnight.

Antibodies against cardiolipin (aCL) were analyzed essentially as described.21 Briefly, Titer-Tek 96-well polyvinylchloride microplates (Flow Laboratories, Costa Mesa, Calif) were coated with 50 μL/well of 50 μg/mL cardiolipin dissolved in ethanol and allowed to dry overnight at 37°C. Blocking was accomplished with 20% ABS-PBS for 2 hours. Fifty microliters of serum samples, diluted 1:30 in 20% ABS-PBS, was added to each well.

After 3 washings with PBS, the plates were incubated with 50 μL/mL of alkaline phosphatase–conjugated goat anti-human IgG (Sigma A-3150; Sigma Chemical, St Louis, Mo) diluted 1:9000, or IgM (Sigma A-3275) diluted 1:7000 with PBS at 37°C for 2 hours. After 4 washings, 100 μL of substrate (phosphatase substrate tablets, Sigma 104) 5 mg in 5 mL diethanolamine buffer, pH 9.8, was added. The plates were incubated at room temperature for 30 minutes and read in an ELISA Multiskan Plus spectrophotometer at 405 nm. Each determination was done in triplicate. All samples were measured in a single assay, and the coefficient of variation was 10% to 15%.

**Chemiluminescence Immunoassay for Antibody Binding to OxLDL**

The chemiluminescent assay was performed as previously described, with modifications.12The assays were performed using 96-well white round-bottomed MicroFluor plates (Dynatech Laboratories Inc, Chantilly, Va). Plates were coated overnight at 4°C with 50 μL of Cu-oxidized LDL or MDA-LDL (5 μg/mL) in Tris-buffered saline (TBS with 0.27 mmol/L EDTA and 20 μmol/L butylated hydroxytoluene). The plates were washed 4 times with washing TBS buffer. The serum samples were diluted 1:250 in TBS containing 2% bovine serum albumin (BSA) and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature. After washing, the amount of antibody bound was measured with alkaline phosphatase–labeled goat anti-human IgG (Sigma A-3187) diluted 1:45 000 in TBS containing 2% BSA and incubated in wells for 1 hour at room temperature.
Determination of OxLDL-Epitopes and LDL-Containing Immune Complexes

The EO6-epitope concentration on apolipoprotein (apo) B-100–containing particles was measured by a chemiluminescent modification of a previously described assay.26 This sandwich assay uses an anti-human apo B-100 antibody, MB47, to capture LDL and a biotin-labeled anti-OxLDL antibody, EO6,26 to measure the amount of the EO6-epitope present on the LDL. The EO6-epitope appears to be an oxidized phospholipid.26

The procedure was as follows: 96-well white microtiter plates (Microlit2, Dynex Technologies Inc, Chantilly, Va) were incubated with 50 μL of 10 μg/mL MB47 in 50 mmol/L Tris-HCL (pH 7.4) containing 150 mmol/L NaCl, 0.27 mmol/L EDTA, and 0.02% NaN3 (TBS) overnight at 4°C. After washing the plates 3 times with washing buffer (TBS) containing 0.001% apoprotinin with an automated plate washer (Micro Plate Washer model 1550, Bio-Rad Laboratories, Hercules, Calif) a 50-fold dilution of serum in TBS containing 2% BSA (BSA/TBS) was added to the MB47-coated plates (50 μL/well) and incubated for 2 hours at room temperature.

At this dilution of serum, the amount of LDL-apoB100 saturates the capacity of MB47 binding, and thus in each well an equal number of apo B–containing particles are bound. This has been verified by demonstrating that biotinylated MB24, another apoB-specific antibody that binds to an apo B epitope distinctly different from that of MB47,27 binds equally to each well.

After incubation, the plates were washed as above and 10 μg/mL biotin-labeled EO6 in BSA/TBS was added to the plates (50 μL/well) and incubated overnight at 4°C. After washing the plates as above, 10,000-fold diluted avidin-conjugated alkaline phosphatase (NeutrAvidin, alkaline phosphatase–conjugated; Pierce, Rockford, Ill) in BSA/TBS containing 1 mmol/L MgCl2 and 1 mmol/L ZnCl2 was added to the plates (50 μL/well) and incubated for 1 hour at room temperature. The plates were then washed 4 times with washing buffer, and 50% Lumi-Phos 530 in distilled water was added to the plates (30 μL/well) and incubated for 1.5 hours at room temperature in the dark. The chemiluminescence was read on a MLX microtiter plate luminometer (Dynex Technologies Inc., Chantilly, Va). Data are expressed in relative light units (RLU), measured over 100 ms. All samples were measured in a single assay, and the intra-assay coefficients of variation of low and high standards were 6% to 8%. Data are expressed as absolute amounts of EO6 bound per well (in RLU).

To detect LDL-immune complexes in sera, 96-well white round-bottomed MicroFluor plates (Dynatech Laboratories, Inc) were coated with 10 μg/mL of MB47 overnight at 4°C. The plates were washed 4 times with TBS. Sera were then added at 1:50 dilution in TBS containing 2% BSA for 2 hours at room temperature. The level of IgG or IgM bound to the LDL was then determined by the addition of alkaline phosphatase–labeled goat anti-human IgG and IgM as indicated above. As noted above, the amount of LDL bound per well was demonstrated to be equal, and, thus the amount of IgG or IgM bound (expressed as RLU) is normalized to equivalent numbers of LDL particles.

Statistical Methods

For skewed variables, nonparametric tests were used for comparisons between the groups (Mann-Whitney U test), whereas Student’s t test was used for normally distributed variables. Spearman rank correlation coefficients were calculated to estimate interrelations between antibody levels, metabolic variables, and blood pressure levels. The significance level was put at P<0.05. Values in the text are given as mean±SE as indicated.

Results

Characteristics of Cases and Controls

Basic characteristics of the 2 study groups are presented in Table 1. The mean±SE blood pressure level in the NT group was 125±11/75±5 / mm Hg as compared with 141±10/89±6 / mm Hg in the BHT group. The BHT group also had a significantly higher BMI and waist/hip ratio.

The BHT men had a significantly altered metabolic profile with fasting hyperinsulinemia and dyslipoproteinemia (Table 1), as previously presented.28 In the BHT group, 26% of the subjects had detectable carotid plaques on 1 or both sides, whereas the corresponding figure for the NT group was 16% (19 versus 10 subjects, NS).

Antibody Levels

aOxLDL levels were determined using 2 different methods as developed in 2 different laboratories, ELISA at Karolinska Institute and chemiluminescence at the University of California, San Diego. There was a strong correlation between the methods used (Figure), both between IgG (r=0.76, P<0.0001) and IgM (r=0.71, P<0.0001).

In the material as a whole, the aOxLDL level of IgG and IgM types were significantly lower in the BHT group

Table 1. Basic Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotension (n=75)</th>
<th>Borderline Hypertension (n=73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.0±0.7</td>
<td>50.0±0.7</td>
<td></td>
</tr>
<tr>
<td>BP systolic, mm Hg</td>
<td>125±1.3</td>
<td>141±1.2</td>
<td></td>
</tr>
<tr>
<td>BP diastolic, mm Hg</td>
<td>75±0.5</td>
<td>89±0.23</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.6±0.34</td>
<td>25.9±0.34</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.90±0.006</td>
<td>0.92±0.006</td>
<td>0.022</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>37</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.5±0.1</td>
<td>5.5±0.1</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.27±0.03</td>
<td>1.16±0.03</td>
<td>0.016</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td>1.34±0.1</td>
<td>1.57±0.1</td>
<td>0.015</td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td>14.2±0.5</td>
<td>17.4±0.7</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

HDL indicates high density lipoprotein. Values are mean±SE.

Group differences were determined by Student’s t test or Mann-Whitney U test (skewed variables).

89±2 / mm Hg in the BHT group. The BHT group also had a significantly higher BMI and waist/hip ratio.

The BHT men had a significantly altered metabolic profile with fasting hyperinsulinemia and dyslipoproteinemia (Table 1), as previously presented.28 In the BHT group, 26% of the subjects had detectable carotid plaques on 1 or both sides, whereas the corresponding figure for the NT group was 16% (19 versus 10 subjects, NS).

Correlation between IgG antibody levels to OxLDL as determined by ELISA and chemiluminescence (r=0.76, P<0.0001).

Similar data were found for IgM.
compared with the NT group using both ELISA and chemiluminescence techniques (Table 2). Antibody levels to malondialdehyde–LDL (aMDA-LDL) of IgG and IgM were also significantly lower in the BHT group (Table 2). There was no difference in aCL between the groups tested (Table 2).

DBP was negatively associated with aOxLDL titers using chemiluminescence, of IgG class (R = 0.33, P = 0.0001) and IgM class (R = 0.31, P = 0.0002) and of IgG class using ELISA (R = 0.21, P = 0.01). SBP was negatively associated with aOxLDL titers using chemiluminescence, of IgG class (R = 0.18, P = 0.032) and IgM class (R = 0.20, P = 0.019).

Twenty-four–hour DBP measurements were also negatively associated with aOxLDL titers using chemiluminescence (R = 0.316, P = 0.0001). Twenty-four–hour SBP readings were also negatively associated with aOxLDL titers using chemiluminescence, of IgM class (R = 0.28, P = 0.008). There was no difference in antibody levels tested between smokers and nonsmokers (data not shown).

To exclude the possibility that differences in antibody levels simply reflected differences in total antibody levels, IgG and IgM levels were determined. There was no difference between the BHT group and control subjects (IgG 9.71 ± 1.9 versus 9.76 ± 2.3 mg/mL and IgM 2.25 ± 0.8 versus 2.1 ± 0.9 mg/mL, respectively).

**Immune-Complexes and Oxidation-Related Epitopes**

There was no difference between BHT individuals and control subjects in the presence of immune complexes in sera with LDL (Table 2). Neither was there any difference in the presence of oxidation-specific epitopes on LDL, defined by the monoclonal antibody EO6 (Table 2).

There was no significant difference between individuals with plaque (n = 29) compared with those without (n = 117) in any of the antibody levels tested. Neither was there any difference in LDL immune complexes or in the EO6-epitope in subjects with carotid plaques as compared with those without (Tables 2 and 3).

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**Correlations to Metabolic Variables**

In the population as a whole, and the 2 groups separately, there were no significant correlations between aOxLDL or aMDA-LDL on one hand and lipoprotein levels, BMI, waist-to-hip ratio, or intimal-medial thickness on the other (data not shown). There was no correlation between these antibody titers and other markers for the metabolic syndrome, including fasting insulin and insulin resistance (data not shown). Age was not associated with aOxLDL or aMDA-LDL levels (data not shown).

**Discussion**

The main finding in this report is that antibody levels to epitopes of OxLDL such as those generated by copper ion–mediated oxidation or MDA formation are decreased in BHT. aOxLDL levels were negatively associated with the

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**TABLE 2. aCL, aOxLDL, and aMDA-LDL Levels, Immune Complexes to LDL, and Oxidation Epitopes in BHT and NT Groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ig Class</th>
<th>NT (n = 75)</th>
<th>BHT (n = 73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>aOxLDL by ELISA</td>
<td>IgM</td>
<td>0.172 ± 0.008</td>
<td>0.145 ± 0.007</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>0.136 ± 0.01</td>
<td>0.098 ± 0.009</td>
<td>0.0002</td>
</tr>
<tr>
<td>aOxLDL by chemiluminescence</td>
<td>IgM</td>
<td>43.836 ± 5.39</td>
<td>26.852 ± 2.264</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>20.096 ± 1.732</td>
<td>11.257 ± 1.044</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>aMDA-LDL by ELISA</td>
<td>IgM</td>
<td>0.177 ± 0.006</td>
<td>0.151 ± 0.005</td>
<td>0.0031</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>0.397 ± 0.014</td>
<td>0.355 ± 0.01</td>
<td>0.044</td>
</tr>
<tr>
<td>aCL by ELISA</td>
<td>IgM</td>
<td>0.143 ± 0.01</td>
<td>0.138 ± 0.013</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>0.30 ± 0.022</td>
<td>0.33 ± 0.017</td>
<td>-</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>IgM</td>
<td>12.259 ± 810</td>
<td>10.080 ± 640</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>14.490 ± 598</td>
<td>14.247 ± 655</td>
<td>-</td>
</tr>
<tr>
<td>EO6-epitope</td>
<td></td>
<td>17.514 ± 2.369</td>
<td>14.322 ± 1.385</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE. Group differences were determined by Mann-Whitney U test. Values for ELISA assays are OD and values for chemiluminescence assays are relative light units in 100 ms. EO6-epitope indicates oxidation epitopes present on LDL as determined by binding of EO6 to LDL captured by MB47.

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**TABLE 3. aCL, aOxLDL, and aMDA-LDL Levels, Immune Complexes to LDL, and aOxLDL Epitope in Individuals With or Without Carotid Plaques**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ig Class</th>
<th>Without Plaque (n = 113)</th>
<th>With Plaque (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aOxLDL by ELISA</td>
<td>IgM</td>
<td>0.157 ± 0.007</td>
<td>0.165 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>0.119 ± 0.007</td>
<td>0.107 ± 0.015</td>
</tr>
<tr>
<td>aOxLDL by chemiluminescence</td>
<td>IgM</td>
<td>35.778 ± 3.306</td>
<td>34.149 ± 3.306</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>15.496 ± 997</td>
<td>16.719 ± 3.729</td>
</tr>
<tr>
<td>aMDA-LDL by ELISA</td>
<td>IgM</td>
<td>0.163 ± 0.005</td>
<td>0.161 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>0.373 ± 0.009</td>
<td>0.369 ± 0.026</td>
</tr>
<tr>
<td>aCL by ELISA</td>
<td>IgG</td>
<td>0.32 ± 0.036</td>
<td>0.31 ± 0.016</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>0.162 ± 0.02</td>
<td>0.145 ± 0.01</td>
</tr>
<tr>
<td>LDL immune complexes</td>
<td>IgM</td>
<td>11.188 ± 611</td>
<td>11.169 ± 949</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>14.728 ± 493</td>
<td>12.901 ± 965</td>
</tr>
<tr>
<td>EO6-epitope</td>
<td></td>
<td>14.823 ± 1.297</td>
<td>20.489 ± 4.573</td>
</tr>
</tbody>
</table>

EO6-epitope indicates oxidation epitopes present on LDL as determined by binding of EO6 to LDL captured by MB47. Values are mean ± SE. There was no significant difference between groups.

Group differences were determined by Mann-Whitney U test.
baseline blood pressure measurement: diastolic, systolic, and 24-hour determinations. The underlying cause of these findings is not clear but may include immunological, metabolic, and hormonal factors, because BHT patients have been shown to often have changes compatible with metabolic syndrome.19

Several factors argue against the possibility that this unexpected finding was due either to an artifact or to generally decreased immune reactivity. There was a close correlation between measured aOxLDL levels when 2 different methods, ELISA and chemiluminescence, were used in 2 different laboratories. This indicates a high reproducibility of determinations of aOxLDL levels. Furthermore, similar data were obtained using 2 different models for generation of epitopes of OxLDL and were observed both for IgG and IgM. In contrast, we have recently reported that antibody levels to heat shock proteins5 and also to endothelial cells28 were enhanced in subjects with BHT, suggesting that the findings demonstrated here are not likely to reflect a general decreased humoral response in BHT. In addition, total antibody concentrations showed no difference between control subjects and BHT patients, indicating that the results do not simply reflect changes in total Ig levels.

In principle, decreased antibody levels may reflect either an increased consumption of antibodies against OxLDL in BHT individuals as compared with normal control subjects, or a decreased production of antibodies, or both. Immune complexes against apoB—containing particles in sera were not higher in BHT individuals than in control subjects. Although the exact nature of the LDL immune complexes was not defined, this finding would argue against the possibility that decreased aOxLDL levels were low because of increased binding to oxidized LDL in plasma and because of subsequent immune complex formation and enhanced removal. However, it is still possible that the antibodies could be trapped in early atherosclerotic lesions in the artery wall or other tissues. Individuals with carotid plaque did not differ significantly in their antibody levels as compared with those without carotid plaque, arguing against this possibility, although this may be an insensitive indication of antibody tissue binding.

There was no significant difference between NT and BHT individuals in the presence of an epitope of OxLDL in serum, as determined by the binding of the monoclonal antibody EO6, which detects an oxidized phospholipid epitope on OxLDL. Although the EO6 epitope may represent only 1 epitope among the many that may form when LDL undergoes oxidation, it suggests that the decreased immune reactivity to OxLDL in BHT individuals may not simply be due to a decreased exposure to the antigen.

During recent years the role of the immune system in atherosclerosis has attracted increasing attention. Activated T cells and monocytes are present in the lesions. Activated T cells and monocytes present in the lesions.9–8 OxLDL has been identified as a possible factor inducing the inflammatory component of atherosclerosis, because OxLDL activates monocytes29,30 and lymphocytes to secrete proinflammatory cytokines.31–33 Other effects include enhancement of endothelial adhesiveness34,35 and smooth muscle cell proliferation.36 These proatherogenic effects may be related to platelet activating factor–like lipids formed in LDL during oxidation.36–38 OxLDL and components thereof, at higher concentrations than those inducing cell stimulatory responses, also have toxic and inhibitory effects on cells, and OxLDL may thus induce injury and inflammation in the vascular wall also by this mechanism.39–41

Available data in experimental animal models on the role that specific immune reactions play in atherosclerosis indicate that T and B cells are not a prerequisite for the development of atherosclerotic lesions in the presence of severe hypercholesterolemia but that they may play an important role in moderate degrees of hypercholesterolemia.42,43 A number of experimental studies indicate an important modulating role in the initiation and development of atherosclerosis. Cell-mediated immune reactions have been reported to be related both to an increase and a decrease in the development of atherosclerosis when different animal models were used. In a recent report, as much as a 42% decrease in atherosclerotic lesions was detected in apo E knockout mice in the presence of moderate hypercholesterolemia with a combined immunodeficiency, as compared with apo E knockout control mice.44 In another study in which apo E knockout mice were crossed into interferon-γ receptor knockout mice, atherosclerosis was greatly diminished.45 Likewise, interferon-γ mediated graft arteriosclerosis in transplanted mouse hearts.46 Furthermore, in T-cell ablated, immune-deficient atherosclerosis prone C57BL/6J mice, atherosclerosis was inhibited.47 Other studies indicate that cell-mediated immune reactions in rabbit models48 and in atherosclerosis-prone C57BL/6J mice suppress the development of atherosclerosis.49 These apparently conflicting data may be related to the different animal models used, but it is also possible that the role of the cell-mediated immune system in atherogenesis may depend on the disease stage and on the presence of other risk factors.

Likewise, the role of humoral immune reactions in atherogenesis is not clear. In general, it may be hypothesized that the physiological function of antibodies to OxLDL and related compounds is to participate in the removal of these obnoxious agents from the artery wall. This is clearly the role of antibody reactions in general, eg, in infectious diseases. In this context, low aOxLDL levels may predispose to progression of atherosclerosis. In line with this are recent reports indicating that immunization of experimental animals with OxLDL, leading to dramatically enhanced aOxLDL levels, inhibits atherosclerosis progression.16–18 On the other hand, several studies have demonstrated an association between the degree of atherosclerosis and antibody levels to OxLDL,11,12 and it is possible that at a later stage of disease development enhanced antibody levels may simply reflect the chronic inflammation in the artery wall. Indeed in later stages of vascular disease, as may occur in essential hypertension (as opposed to BHT), increased aOxLDL levels have been reported.49 Thus, during the earliest stages of atherosclerosis, the humoral response may be limited and the antibodies generated may be bound by antigens in tissue or plasma. Later, as lesions expand, a more robust humoral response may occur, and plasma titers rise as the generation of antibodies exceeds binding to tissues.
Antibodies to OxLDL in Borderline Hypertension

Taken together, the data herein indicate that in BHT, characterized by early cardiovascular changes, the serum titers to OxLDL were decreased. The recent observation that immunization of experimental animals with OxLDL decreased the progression of atherosclerosis suggests that under some circumstances the immune reaction to OxLDL may be protective. Possibly, the increased titers seen in later stages, such as overt hypertension or frank atherosclerosis, represent an attempt to respond to the enhanced content of oxidized lipoproteins. Whether this response is sufficient to be relevant is not known. Similarly, whether an enhanced immune response to OxLDL, as could be achieved by immunization, is beneficial or not, also remains to be shown.

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References


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