Oxidized Low-Density Lipoprotein/β₂-Glycoprotein I Complexes and Autoantibodies to oxLig-1/β₂-Glycoprotein I in Patients With Systemic Lupus Erythematosus and Antiphospholipid Syndrome

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Abstract

Oxidized low-density lipoprotein (oxLDL) interacts with β₂-glycoprotein I (β₂-GPI) via oxLDL-derived specific ligands (oxLig-I) forming complexes. The prevalence and significance of oxLDL/β₂-GPI complexes and antibodies to oxLig-1/β₂-GPI were evaluated in patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS). The oxLDL/β₂-GPI complex was 69% positive (above mean + 3 SD of control subjects) in 97 consecutive patients with SLE, 62% in 40 patients with SLE with secondary APS, and 60% in 50 control patients with SLE without APS. IgG anti–oxLig-1/β₂-GPI antibody was positive in 31 (32%) of 97 consecutive patients with SLE, in 26 (65%) of 40 patients with SLE with secondary APS, and in 6 (19%) of 32 control patients with SLE. Anti–oxLig-1/β₂-GPI antibodies were 93.7% specific with a positive predictive value of 90.0% for APS, better than anticardiolipin antibodies (80.0% specific, 71.4% predictive value). These results confirm that oxLDL/β₂-GPI complexes are common in SLE and suggest a possible immunogenic role in APS. In contrast, IgG anti–oxLig-1/β₂-GPI antibodies not only are associated with but also are clinically useful risk factors for APS.

Vascular thromboembolic events, pregnancy morbidity (miscarriages and fetal loss), and thrombocytopenia in association with the presence of elevated serum levels of antiphospholipid antibodies are common clinical features of the antiphospholipid syndrome (APS). APS is classified as primary if there is no coexisting autoimmune disease or secondary when present in the context of an autoimmune disorder. There is considerable evidence to suggest a pathogenic role of antiphospholipid antibodies in the development of these clinical features.1-3 Antiphospholipid antibodies are a heterogeneous group of autoantibodies characterized by their reactivity to anionic phospholipids, phospholipid/protein complexes, and certain proteins presented on suitable surfaces in the absence of phospholipids, ie, activated cell membranes and oxygenated polystyrene.4-6

Several plasma proteins that participate in coagulation and interact with anionic phospholipids have been reported to function as antiphospholipid cofactors, eg, β₂-glycoprotein I (β₂-GPI), prothrombin, protein C, protein S, and annexin V. β₂-GPI is the most extensively studied of the cofactors and has been shown to be a relevant antigenic target for antiphospholipid antibodies.7,8 β₂-GPI is a 50-kd, single-chain polypeptide composed of 326 amino acid residues, arranged in 5 homologous repeats known as complement control protein domains. β₂-GPI’s fifth domain contains a patch of positively charged amino acids that likely represents the binding region for phospholipids.9-11

Oxidized low-density lipoprotein (oxLDL) has an important pathogenic role in early events leading to atherosclerosis,12,13 and oxLDL has been shown to be a proinflammatory chemotactic agent for macrophages and T lymphocytes, cells with a central role in atherogenesis.14
During the 1980s, oxLDL was localized in atherosclerotic lesions of rabbit and man, and it can be immunogenic, as antibodies to oxLDL have been demonstrated in patients with autoimmune disorders such as systemic lupus erythematosus (SLE) and APS. More recently, the premature (or accelerated) development of atherosclerosis has been recognized in patients with autoimmune diseases. The traditional risk factors for atherosclerosis failed to account for these changes, and alternative mechanisms have been proposed such as increased levels of autoantibodies to Lp(a), oxLDL, and phospholipids, as well as certain biochemical and genetic abnormalities. An immune component in the pathogenesis of atherosclerosis is becoming apparent, and antiphospholipid antibodies might be possible participants.

\[ \beta_2 \text{-GPI also has been localized in human atherosclerotic lesions by immunohistochemical staining, which suggests a role for } \beta_2 \text{-GPI (and antiphospholipid antibodies) in atherosclerosis. Hasunuma et al reported that Cu}^{2+}\text{-oxidized LDL, unlike native LDL, binds to } \beta_2 \text{-GPI. In vitro macrophage uptake of oxLDL was partly but significantly decreased when } \beta_2 \text{-GPI was added compared with oxLDL alone. This uptake was inhibited by polyinosinic acid, a scavenger receptor blocker. The addition of an antiphospholipid antibody, either } \beta_2 \text{-GPI-dependent anticardiolipin (aCL) or anti-} \beta_2 \text{-GPI, resulted in a significant increase of complex uptake by macrophages. Because the increased uptake was not affected by polyinosinic acid, it was suggested that macrophage Fc} \gamma \text{ receptors were involved. This mechanism might be relevant to the development of atherosclerosis in patients with APS. Kobayashi et al recently demonstrated a covalent interaction between oxLDL and } \beta_2 \text{-GPI that resulted in a “stable” (or nondissociable) oxLDL/} \beta_2 \text{-GPI complex. The ligand on the oxLDL molecule (oxLig-1, 7-ketocholesterol-9-carboxynonanoate) responsible for the interaction with } \beta_2 \text{-GPI has been identified and isolated. Increased in vitro macrophage uptake also has been reported when oxLDL/} \beta_2 \text{-GPI antibody complexes were used. In addition, high serum levels of oxLDL/} \beta_2 \text{-GPI complexes and autoantibodies to these complexes have been associated with venous and arterial thrombosis in patients with APS.}

To study the second population, we used serum samples from 97 consecutive patients with SLE and 120 with RA. The patients with SLE attended the Rheumatology Clinic, Western General Hospital, Guadalajara, Mexico, from January to October 2001, and the patients with RA from April to August 2002. The samples were stored at \(-20^\circ C\) until tested. The diagnoses of SLE and RA were established according to American College of Rheumatology Classification Criteria. Control samples consisted of 20 serum samples from patients with syphilis and 34 from healthy blood bank donors. Six (30%) of the syphilis samples were positive for IgG aCL antibodies and none for anti-\( \beta_2 \text{-GPI} \) antibodies.

Of the patients with SLE, 93 (96%) were women, and of the patients with RA, 102 (85.0%) were women. The mean age for patients with SLE was 31 years (range, 18-82 years) and for patients with RA was 49 years (range, 19-80 years). Informed consent was obtained from all patients and institutional review board approval from Western General Hospital.

For 6 patients with SLE, at least 4 serum samples were obtained at different intervals during a follow-up period of 12 months. The hospital records were reviewed, and clinical and serologic measures of disease activity corresponding to the time of the samples were recorded. Scoring for the SLE-DAI was as follows: 0, inactive; 1 to 5, minimal; 6 to 10, moderate; more than 10, high. SLE-DAI scores were generated by the attending rheumatologist from 24 weighted clinical and serologic descriptors of disease activity. Anti–double-stranded DNA antibodies, C3 and C4 serum levels, and erythrocyte sedimentation rate were also recorded.

To study the second population, we used serum samples from 90 selected patients with SLE classified into 2 subgroups: 50 without APS and no clinical history of antiphospholipid antibodies and 40 with secondary APS. The clinical diagnosis was established according to the Sapporo criteria for the classification of APS. Of the patients with SLE, 82 were females and 8 were males. The mean age was 38.7 years (range, 17-74 years). A separate...
group of 60 serum samples from healthy blood donors were used as control samples.

Monoclonal Antibodies

The following monoclonal antibodies were used to develop the enzyme-linked immunosorbent assay (ELISA) tests for measuring oxLDL/β₂-GPI complexes and anti–oxLig-1/β₂-GPI antibodies: WB-CAL-1 monoclonal antibody reactive to β₂-GPI (IgG2a, κ) derived from a NZW x BXSB F1 mouse, a model of spontaneous APS,33 and EY2C9 monoclonal anti-β₂-GPI antibody (IgM) established from peripheral blood lymphocytes of patients with APS.34 Both monoclonal antibodies bind only to β₂-GPI/negatively charged phospholipid (or oxLDL) complexes and not with monomeric (free) β₂-GPI in solution. 1D2 is an IgG murine monoclonal antibody specific to human apolipoprotein B-100. 1D2 equally reacts with native and oxLDL.

Purification of Human β₂-GPI

Human β₂-GPI was purified from fresh normal plasma as previously described35 with slight modifications. Briefly, human plasma was precipitated with 70% perchloric acid, extensively dialyzed against tris(hydroxymethyl)aminomethane–sodium chloride buffer, pH 7.4 (Hepes buffer), and concentrated before loading into a heparin column (Amersham Biosciences, Piscataway, NJ). Pooled β₂-GPI fractions were dialyzed against sodium acetate–sodium chloride buffer (pH 4.8) and concentrated. This preparation was then loaded into a carboxymethylcellulose column (Sigma-Aldrich, St Louis, MO), and β₂-GPI fractions were pooled, dialyzed against sodium acetate–sodium chloride buffer, concentrated at approximately 1 mg/mL, and stored at −70°C until used. The β₂-GPI preparation contained more than 95% of protein, and a 50-kd, single, diffuse band was demonstrated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. In addition, the immunoreactivity of the purified β₂-GPI was checked by using an anti-β₂-GPI ELISA procedure before use.

LDL Purification and Oxidation

LDL was isolated by ultracentrifugation of fresh normal human plasma in EDTA–potassium bromide solutions as described.36 LDL (d = 1.019-1.063 g/mL) was adjusted to a concentration of 100 µg/mL based on the protein concentration. The LDL fraction was oxidized with a 5-µmol/L concentration of copper sulfate in a 10-mmol/L concentration of sodium chloride, pH 7.4 (Hepes buffer), at 37°C for 12 hours. Oxidation was terminated by the addition of EDTA (at a final concentration of 1 mmol/L), and oxLDL was dialyzed extensively against Hepes buffer containing EDTA. The degree of oxidation was measured using the thiobarbituric acid reactive substance procedure.37

ELISA Procedure for oxLDL/β₂-GPI Complexes

Monoclonal antibody against β₂-GPI (WB-CAL-1) was coated onto Immulon 2HB microplates (Dynex Technologies, Chantilly, VA) by incubating 50 µL per well of 5 µg/mL of WB-CAL-1 in phosphate-buffered saline (PBS), pH 7.4, overnight at 2°C to 4°C. WB-CAL-1 is an IgG murine monoclonal antibody against human β₂-GPI used in this assay to capture oxLDL/β₂-GPI complexes via its reactivity with β₂-GPI.

The plate was blocked with PBS–1% nonfat dry milk for 1 hour. Then, 100 µL of serum samples diluted 1:25 in PBS–nonfat dry milk containing a 10-mmol/L concentration of magnesium chloride were added to the appropriate microwells and incubated for 2 hours at room temperature. Magnesium chloride dissociates intermediate oxLDL/β₂-GPI complexes (ie, electrostatically bound), permitting the specific detection of nondissociable and covalently bound complexes present in serum samples.26 The microwells were washed 4 times with PBS–0.05% polysorbate 20 between each step. Biotinylated 1D2 (antihuman apolipoprotein B-100) antibody diluted in PBS–1% nonfat dry milk was added to the microwells and incubated for 1 hour at room temperature, followed by horseradish peroxidase–streptavidin. Color was developed with tetramethylbenzidine–hydrogen peroxide, and the reaction was stopped with 0.36N sulfuric acid. Optical density was read at a wavelength of 450 nm (650 nm reference).

The intra-assay precision (coefficient of variation percentage) ranged from 7.2% to 12.3% for weakly and 4.5% to 8.9% for moderately and strongly reactive samples. The serum oxLDL/β₂-GPI complex concentration (expressed in units per milliliter) was calculated against a reference curve built with 2-fold serial dilutions of oxLDL/β₂-GPI complex solution. The complexes were prepared in advance by incubating equal amounts of Cu²⁺-oxLDL and purified human β₂-GPI, pH 7.4, for 12 hours at 37°C. The unit value was derived arbitrarily from the protein concentration of the oxLDL/β₂-GPI complex used in the reference curve. A normal cutoff value for the assay was established by testing serum samples from healthy blood donors (mean ± 3 SD). Figure II shows that only oxidized (not native) LDL reacted with exogenous human β₂-GPI forming oxLDL/β₂-GPI complexes.

ELISA for IgG Anti–oxLig-1/β₂-GPI Antibodies

The ELISA procedure described by Kobayashi et al27 was used with slight modifications. We coated 50 µL of 100 µg/mL of oxLig-1 (7-ketocholesteryl-9-carboxynonanoate) in ethanol onto Immulon 2HB microplates by evaporation. The synthesis and characterization of oxLig-1 has been reported.28 The plate was blocked with 1% bovine serum albumin (BSA) for 1 hour at room temperature and washed. Then 50 µL of 30 µg/mL of β₂-GPI in PBS–3%
BSA was added to oxLig-1–coated microwells to permit complex formation. These oxLig-1/β₂-GPI complexes served as antigenic substrates to capture patients’ antibodies. Subsequently, 50 µL of serum or plasma samples diluted 1:100 in PBS–3% BSA were added to the microwells and incubated for 1 hour at room temperature. The microwells were washed 4 times with PBS–0.05% polysorbate 20 between steps. Diluted horseradish peroxidase–conjugated antihuman IgG antibody was added to the microwells and incubated for 1 hour. Color was developed with tetramethylbenzidine–hydrogen peroxide, and the reaction was stopped with 0.36N sulfuric acid. Optical density was read at a wavelength of 450 nm (650 nm reference).

The intra-assay precision (coefficient of variation percentage) ranged from 7.4% to 12.6% for weakly and 5.5% to 9.9% for moderately and strongly reactive samples. EY2C9 is an IgM monoclonal antibody against β₂-GPI of the antigenic substrate, to select strongly reactive samples to be used as control samples. However, the IgG oxLig-1/β₂-GPI antibody concentration of patients’ samples (expressed in units per milliliter as stated earlier) was calculated against a standard curve prepared with a selected positive sample. A normal cutoff value for the assay was established by testing serum samples from healthy blood donors (mean + 3 SD).

**ELISA for aCL and Anti-β₂-GPI Antibodies**

All APS samples were tested for IgG aCL and anti-β₂-GPI antibodies by using commercially available ELISA test kits (Corgenix, Westminster, CO) according to the manufacturer’s instructions. The IgG aCL ELISA test requires exogenous bovine β₂-GPI, thus measuring β₂-GPI-dependent antiphospholipid antibodies. The anti-β₂-GPI ELISA uses purified human β₂-GPI as the antigen and detects anti-β₂-GPI antibodies in the absence of exogenous phospholipids.

**Statistical Analysis**

Statistical analysis was performed with a SigmaStat program (SPSS Science, Chicago, IL). The Student t test was performed to compare the results between different groups and the Fisher exact test to assess the relationship between antibodies and clinical manifestations. Sensitivity, specificity, positive predictive value (PPV), and odds ratio of anti–oxLig-1/β₂-GPI antibodies were calculated by 2 × 2 contingency table analysis. We also calculated 95% confidence intervals for the odds ratios. The Pearson product moment correlation was performed to assess the association of individual values between variables. A P value of .05 or less was considered significant.

**Results**

**Serum oxLDL/β₂-GPI Complexes in Consecutive Patients With SLE**

Of 97 consecutive patients with SLE, 67 (69%) had positive serum levels of oxLDL/β₂-GPI complex with a mean level of 73.2 ± 78.1 U/mL, which was significantly higher than the mean level for healthy control subjects (1.3 ± 0.4 U/mL; \( P = 7.2 \times 10^{-15} \)). Only 3 (2.5%) of 120 consecutive patients with RA had positive results for oxLDL/β₂-GPI with a mean of 1.4 ± 0.7 U/mL, which was statistically different from that for healthy control subjects (\( P = .09 \)). In contrast with the RA group, 13 (65%) of 20 patients with syphilis had positive results for oxLDL/β₂-GPI with a mean of 56.2 ± 71.5 U/mL, which was significantly higher than the level for the healthy control subjects (\( P = .001 \)). The mean level for the patients with syphilis was lower than for patients with SLE, but this difference was not statistically significant (\( P = .174 \)). These results indicate that oxidation of LDL and its complex formation with β₂-GPI are common in SLE and syphilis. Although RA is a systemic autoimmune disease, oxLDL/β₂-GPI complexes were not detected in our RA patient population.
Serum IgG Anti–oxLig-1/β2-GPI Antibodies in Consecutive Patients With SLE

Of 97 consecutive patients with SLE, 31 (32%) had positive serum levels of IgG anti–oxLig-1/β2-GPI antibodies (mean, 72.3 ± 215.2 U/mL; median, 13.4 U/mL with 25% and 75% quartile ranges of 7.6 and 30.9 U/mL, respectively) that were significantly higher (P = .002) than the mean level for healthy control subjects (8.9 ± 4.4 U/mL; median, 7.8 U/mL with 25% and 75% quartile ranges of 6.5 and 8.5 U/mL, respectively). Of 120 consecutive patients with RA, 7 (5.8%) had results positive for IgG anti–oxLig-1/β2-GPI antibodies (mean, 11.8 ± 11.3 U/mL; median, 8.9 U/mL with 25% and 75% quartile ranges of 6.7 and 11.4 U/mL, respectively; P = .02 vs healthy control subjects), and 5 (25%) of 20 patients with syphilis had positive results (mean, 25.4 ± 30.3 U/mL; median, 11.6 U/mL with 25% and 75% quartile ranges of 9.6 and 24.8 U/mL, respectively; P = .01 vs healthy control subjects). The mean antibody level for the patients with syphilis was significantly lower than that for patients with SLE (P = .02) Figure 2B. The results indicate a higher prevalence of IgG anti–oxLig-1/β2-GPI antibodies in patients with SLE than in patients with RA or syphilis and in healthy control subjects.

Time Course Studies of Serum oxLDL/β2-GPI Complexes and IgG Anti–oxLig-1/β2-GPI Antibodies

For 6 patients with SLE, at least 4 serum samples were obtained at different intervals within a 12-month follow-up period. The oxLDL/β2-GPI complexes and IgG anti–oxLig-1/β2-GPI antibodies were measured in these samples. Table II summarizes the relationship of IgG anti–oxLig-1/β2-GPI antibodies with various clinical and serologic measures of disease activity in these patients. Four patients were classified as having moderate or high disease activity (by the SLE-DAI) during the follow-up period, 3 had elevated levels of IgG anti–oxLig-1/β2-GPI antibodies, and all 4 had a positive anti–double-stranded DNA antibody titer and a low C4 level. A high erythrocyte sedimentation rate was found in 3 of 4 patients with active disease and in 1 with inactive disease. Serum levels of oxLDL/β2-GPI complexes for each patient were mostly positive, while IgG aCL and anti-β2-GPI antibody levels were normal or borderline. Except for the anti–oxLig-1/β2-GPI antibodies, oxLDL/β2-GPI complexes and antiphospholipid antibodies (aCL and anti-β2-GPI) showed no relationship with disease activity. These results suggest a possible association of IgG anti–oxLig-1/β2-GPI antibody levels with disease activity in patients with SLE. Figure 3 depicts results for 4 of 6 patients with SLE, 2 with active disease and 2 with inactive disease as representative cases. Serum levels of oxLDL/β2-GPI complexes fluctuated widely without a partic-
Table 1
Relationship Between IgG Anti–oxLig-1/β₂-GPI Antibodies and Disease Activity in Patients With SLE*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Mean IgG Anti–oxLig-1/β₂-GPI (U/mL)</th>
<th>SLE-DAI†</th>
<th>Anti-dsDNA‡</th>
<th>C3 Level</th>
<th>C4 Level</th>
<th>ESR</th>
<th>Mean IgG aCL Level (GPL U)</th>
<th>Mean IgG Anti-β₂-GPI Level (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.8</td>
<td>Inactive</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
<td>High</td>
<td>6.5</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>234.5</td>
<td>Moderate</td>
<td>Positive</td>
<td>N</td>
<td>Low</td>
<td>N</td>
<td>23.2</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>38.3</td>
<td>High</td>
<td>Positive</td>
<td>N</td>
<td>Low</td>
<td>High</td>
<td>11.5</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>9.9</td>
<td>Moderate</td>
<td>Positive</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>4.4</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>24.5</td>
<td>Moderate</td>
<td>Positive</td>
<td>N</td>
<td>Low</td>
<td>High</td>
<td>7.4</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>9.1</td>
<td>Inactive</td>
<td>Negative</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>5.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

aCL, anticardiolipin; β₂-GPI, β₂-glycoprotein I; DAI, disease activity index; dsDNA, double-stranded DNA; ESR, erythrocyte sedimentation rate; GPL, IgG phospholipid units; N, normal; NA, not available; oxLig-1, oxidized low-density lipoprotein–derived specific ligand; SLE, systemic lupus erythematosus.

* Bold type indicates an abnormal value. Reference ranges are as follows: C3, 60-220 mg%; C4, 20-40 mg%; ESR (Westergren method), <20 mm/h; IgG aCL, <23 GPL U; IgG anti-β₂-GPI, <20 U.

† Scoring for the SLE-DAI is as follows: 0, inactive; 1-5, minimal; 6-10, moderate; >10, high.

‡ By immunofluorescence crithidia assay; a positive titer was defined as >1:10.

Figure 3
Serum levels of oxidized low-density lipoprotein (oxLDL)/β₂-glycoprotein I (β₂-GPI) complexes (A, C, E, G) with corresponding IgG anti–oxLDL–derived specific ligand (anti–oxLig-1)/β₂-GPI antibodies (B, D, F, H) in 4 of 6 patients with systemic lupus erythematosus (SLE) tested over time. According to the SLE disease activity index (see text), patients 1 (A and B) and 6 (G and H) had inactive disease and patients 2 (C and D) and 3 (E and F), active disease. (cont next page)
ular pattern regardless of the disease activity of the patient. In contrast, IgG anti–oxLig-1/β2-GPI antibody levels were positive only in patients with active disease.

Serum oxLDL/β2-GPI Complexes in Selected Patients With APS

Of 40 selected patients with SLE with APS, 25 (63%) had positive serum levels of oxLDL/β2-GPI complexes with a mean of 13.5 ± 24.4 U/mL, which was significantly higher than the mean for 60 healthy control subjects (0.7 ± 0.1 U/mL; \( P < .001 \)). Of 50 selected patients with SLE without APS, 30 (60%) had positive levels with a mean of 10.6 ± 19.8 U/mL, which also was significantly higher than the mean for healthy control subjects (\( P = .001 \)). The mean level for patients with SLE with APS was not statistically different (\( P = .279 \)) from that for patients with SLE without APS Figure 4A. These results indicate that oxidation of LDL and its complex formation with β2-GPI is common in patients with SLE, regardless of the presence of APS.

Serum IgG Anti–oxLig-1/β2-GPI Antibodies in Selected Patients With APS

Of 40 selected patients with SLE with APS, 26 (65%) had positive serum levels of IgG anti–oxLig-1/β2-GPI antibodies compared with 6 (19%) of 32 selected patients with SLE without APS. The mean level for patients with SLE

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**Figure 3** (cont) The numbers on the x-axis represent the month of the year when the samples were collected. All samples were tested simultaneously in duplicate for oxLDL/β2-GPI complexes and for anti–oxLig-1/β2-GPI, anticardiolipin (aCL), and anti-β2-GPI antibodies. The oxLDL/β2-GPI complex and anti–oxLig-1/β2-GPI antibody cutoff values (horizontal broken line) were established by testing healthy subjects (mean + 3 SD). The cutoff values for IgG aCL and anti-β2-GPI antibodies had been established by the kit manufacturer at 23 GPL U and 20 U/mL, respectively.
with APS (24.4 ± 28.4 U/mL) was significantly higher than the mean for SLE control patients without APS (9.1 ± 5.1 U/mL; \( P = .0008 \)) and the mean for 43 healthy control subjects (5.7 ± 1.4 U/mL; \( P = 8.6 \times 10^{-5} \)). The results indicate a higher prevalence and serum levels of IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies in patients with SLE with APS patients compared with patients with SLE without a history of APS, which suggests a possible pathogenic role for these antibodies in APS.

**Relationship of IgG Anti–oxLig-1/\( \beta_2 \)-GPI With aCL and With Anti-\( \beta_2 \)-GPI Antibodies**

Owing to the prominent presence of \( \beta_2 \)-GPI in the antigenic mixture used to detect IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies, the relationship of these antibodies with \( \beta_2 \)-GPI-dependent aCL and anti-\( \beta_2 \)-GPI antibodies was evaluated in 40 selected patients with SLE with secondary APS. The distribution of IgG anti–oxLig-1/\( \beta_2 \)-GPI vs anti-\( \beta_2 \)-GPI antibodies followed a pattern different from that of aCL antibodies. This pattern suggests the presence of antibodies with at least 2 different reactivities: to \( \beta_2 \)-GPI and to oxLig-1/\( \beta_2 \)-GPI. Although preliminary, these results might indicate that IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies represent a distinct subset of antiphospholipid antibodies.

**Comparative Clinical Performance**

The clinical performance (relative sensitivity, specificity, and PPV) of IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies for clinical manifestations of APS in 90 selected patients with SLE was evaluated by \( 2 \times 2 \) contingency table analysis. IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies were 45% sensitive and 93.7% specific, with a PPV of 90% for APS (\( P < .001 \)). Table 2 shows the comparison of the results of IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies with those of IgG aCL and anti-\( \beta_2 \)-GPI antibodies. IgG aCL antibodies were 62.5% sensitive, compared with 35% for anti-\( \beta_2 \)-GPI and 45% for anti–oxLig-1/\( \beta_2 \)-GPI antibodies. This is due to more false-positive results observed in the SLE control group as evidenced by the lower specificity and PPV for IgG aCL antibodies. Thus, IgG anti–oxLig-1/\( \beta_2 \)-GPI antibodies are better predictors of APS.

**Discussion**

Oxidation of LDL occurs in vivo and has an important pathogenic role in atherogenesis. More than 60% of the patients with SLE in the present study had elevated serum levels of oxLDL/\( \beta_2 \)-GPI complexes as compared with 2.5% in RA and 0% in healthy control subjects (Figure 2A). However, the prevalence and the mean level of oxLDL/\( \beta_2 \)-GPI complexes in patients with SLE without a history of APS were similar to those for patients with APS (Figure 4A). The chronic inflammation of the vasculature frequently seen in

![Figure 4](https://example.com/figure4.png)

**Figure 4** Serum levels of (A) oxidized low-density lipoprotein (oxLDL)/\( \beta_2 \)-glycoprotein I (\( \beta_2 \)-GPI) complexes and (B) IgG anti–oxLDL–derived specific ligand (anti–oxLig-1)/\( \beta_2 \)-GPI antibodies in healthy control subjects (A, n = 60; B, n = 43), selected patients with systemic lupus erythematosus (SLE) (without antiphospholipid syndrome [APS]; A, n = 50; B, n = 32) and with secondary APS (A and B, n = 40). The cutoff (horizontal broken line) was established by testing 60 healthy subjects (mean + 3 SD). The horizontal solid lines indicate the mean for each group. A, Healthy subjects vs patients with SLE and secondary APS, \( P = .001 \); patients with SLE and no APS vs patients with SLE and secondary APS, \( P = .279 \). B, Healthy subjects vs patients with SLE and secondary APS, \( P = 8.6 \times 10^{-5} \); patients with SLE and no APS vs patients with SLE and secondary APS, \( P = .0008 \).
patients with certain autoimmune diseases might account for the increased oxidative modification of LDL and its interaction with β₂-GPI. These results are in agreement with recent reports that oxidation of LDL and interaction with β₂-GPI are common in patients with these diseases. Circulating oxLDL/β₂-GPI complexes have been implicated as atherogenic autoantigens, and their presence might represent a risk factor or an indirect but significant contributor to thrombosis and atherosclerosis. The presence of autoantibodies against oxLDL/β₂-GPI in patients with SLE with secondary APS (Figure 4B), together with the common occurrence of oxLDL/β₂-GPI complexes, might help explain the accelerated (or premature) development of vascular complications, including atherosclerosis, seen in some of these patients.

In contrast with SLE, oxLDL/β₂-GPI complexes were not found in RA (Figure 2A). To our knowledge, autoantibodies against oxLDL have not been reported in this disease. However, a high incidence of atherosclerosis has been described in patients with RA. The lack of circulating oxLDL/β₂-GPI complexes might help to explain the low prevalence of autoantibodies to the complex (Figure 2B) and the low incidence of thromboembolic complications seen in RA. It is possible that other mechanism(s), different from oxidation of LDL, might contribute to the development of atherosclerosis in RA. A recent review proposed several potential proatherogenic mechanisms, including dyslipidemias such as Lp(a), medications (steroids, methotrexate), proinflammatory cytokines (interleukins 1 and 6, tumor necrosis factor α), acute phase reactants (C-reactive protein, serum amyloid A), and newer risk factors such as hyperhomocysteinemia.

The oxLig-1/β₂-GPI complexes (Figure 2A) were detected in 13 (65%) of a small group (n = 20) of patients with syphilis. The presence of these complexes suggests that the infectious process also might result in vascular inflammation leading to oxidation of LDL and β₂-GPI complex formation, perhaps similar to SLE. About 25% of the patients with
syphilis showed low positive levels of IgG anti–oxLig-1/β2-GPI antibodies (Figure 2B). These antibody titers had a stronger correlation with IgG aCL (r = 0.889) compared with anti-β2-GPI (r = 0.229) antibodies (data not shown). These results not only show that anti–oxLig-1/β2-GPI antibodies might be different from anti-β2-GPI antibodies, but also suggest that oxLDL/β2-GPI complexes can induce the production of low levels of “infectious” anti–oxLig-1/β2-GPI antibodies. Unlike patients with APS, no “autoimmune” anti-β2-GPI antibodies were detected in this group of patients with syphilis. The exact mechanism(s) and possible pathogenic effect of these antibodies in thrombosis and atherosclerosis remain to be elucidated.

Kobayashi et al recently demonstrated that magnesium chloride dissociates intermediate forms of oxLDL/β2-GPI complexes bound by electrostatic interactions. Our ELISA system included magnesium chloride in the sample diluent to permit the detection of “stable” nondissociable oxLDL/β2-GPI complexes bound by covalent interactions. It can be hypothesized that these stable complexes might be clinically relevant. The wide fluctuation in serum levels of oxLDL/β2-GPI complexes in some patients with SLE (Figure 3) suggests that oxidation of LDL and/or the formation of complexes with β2-GPI in circulation is an active process. It is possible that a dysfunction of some regulatory mechanism(s) might account for these findings. For example, the HDL-associated enzyme paraoxonase (PON) has antioxidant activity that protects LDL from oxidation. Decreased PON activity has been reported in patients with aCL antibodies. Furthermore, IgG anti-β2-GPI antibodies were associated with reduced PON activity in patients with SLE and patients with primary APS. Vascular injury (biochemical or immunologic) as seen in patients with autoimmune diseases might affect PON activity or any other antioxidant mechanism, triggering the oxidative aforementioned changes. In addition, it is possible that patients with APS have circulating immune complexes (antibody/oxLig-1/β2-GPI), as suggested by the coexistence of oxLDL/β2-GPI and autoantibodies. These immune complexes might be removed from circulation by macrophages via Fcγ receptors, a mechanism also relevant in atherogenesis.

High serum levels of IgG anti–oxLig-1/β2-GPI antibodies were demonstrated in 31 (32%) of 97 consecutive SLE patients (Figure 2B). The APS status of the consecutive patients with SLE with IgG anti–oxLig-1/β2-GPI antibodies was not known; however, the 32% prevalence corresponds with the expected general prevalence or risk of APS in SLE. High serum levels of these antibodies were found in 4 patients with SLE with persistent clinical and serologic measures of disease activity (Figure 3 and Table 1). Although this is a small group of patients, these results suggest that IgG anti–oxLig-1/β2-GPI antibodies might serve as indicators of disease activity in patients with SLE and might contribute to the pathogenesis of APS. Taken together, these findings provide additional support to the hypothesis that oxidative stress has an important role in antiphospholipid antibody production and the development of APS.

We found no correlation between serum levels of oxLDL/β2-GPI complexes and IgG anti–oxLig-1/β2-GPI antibodies in patients with SLE with APS (data not shown). It is possible that high complex levels might result in increased antibody binding and immune complex formation, depleting “free” autoantibodies. However, the possible relationship between oxLDL/β2-GPI complexes and the autoantibodies is illustrated by the presence of IgG anti–oxLig-1/β2-GPI antibodies only in patients with SLE with a history of APS and the report of a stronger association of anti–oxLig-1/β2-GPI antibodies with thrombosis in patients with high levels vs low levels of oxLDL/β2-GPI complexes.

Serum levels of IgG anti–oxLig-1/β2-GPI antibodies showed a stronger correlation with IgG aCL (r = 0.737) than with anti-β2-GPI (r = 0.325) antibodies in patients with SLE with APS (Figure 5). On the other hand, the PPVs for APS of IgG anti–oxLig-1/β2-GPI (90%) and anti-β2-GPI (93.3%) antibodies were better than IgG aCL (71.4%) antibodies in our study population (Table 2). These results suggest that IgG anti–oxLig-1/β2-GPI antibodies might represent a subset of antiphospholipid antibodies that coexist with other autoantibodies. Additional studies using purified antibodies and/or inhibition experiments are needed to further characterize these antibodies. The results of this study suggest that measuring oxLDL/β2-GPI complexes and IgG anti–oxLig-1/β2-GPI antibodies by ELISA might be useful in the serologic evaluation of APS and that they are possible contributors to the development of atherosclerosis in some patients with autoimmune diseases such as SLE.

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