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IgA Anti-Lipoprotein Antibodies in Autoimmune Dyslipidemias Are Restricted to IgA1 Subclass

To the Editor:

Autoimmune hyperlipidemia (AIH) associated with myeloma was first described in 1965 (1). There have since been several reports of autoantibodies to lipoproteins and to lipoprotein receptors that induce AIH (2–4). Furthermore, AIH has been described in patients with systemic lupus erythematosus (5). Autoimmune hyperchylomicronemia related to IgA autoantibodies directed against lipoprotein lipase and hepatic triglyceride lipase has been described in a patient with idiopathic thrombocytopenic purpura associated with Graves disease (6). Recently, IgA–lipoprotein complexes (IgA-Lp) have been reported in patients with AIH associated with coronary artery, ischemic disease, and tendon xanthomas (7). These autoantibodies were repeatedly detected bound to the target autoantigens during the course of the disease and never found as free antibodies in the patients’ sera.

We hypothesized that IgA-Lp might participate in the generation of atherosclerotic lesions by trapping and activating the monocyte/macrophage through the membrane IgA receptor recently described on these cells (8) or via activation of the alternative pathway of complement (9). Some fragments generated during complement cascade are able to activate the monocyte/macrophages after specific interaction with receptors. The IgA subclass probably influences these two IgA effector functions. We characterized the subclass of IgA bound to lipoprotein in 13 adult patients with AIH.

Two patients had IgA monoclonal gammopathy with anti-lipoprotein activity; the other 11 patients had high-titer IgA-Lp, as shown by an ELISA (7). We also studied 12 healthy adults. The IgA-Lp subclass was characterized by a modified ELISA (7, 10). Briefly, affinity chromatography-purified rabbit IgG directed against human high-density lipoprotein (HDL) and low-density lipoprotein (LDL) was used as capture antibody on Maxisorp microplates (Nunc, Roskilde, Denmark). Serum diluted 25 to 250-fold in phosphate-buffered saline containing Tween 20 (1 mL/L), were treated with monoclonal antibodies specific for either IgA1 (clone NIF1; Zymed, San Francisco, CA) or IgA2 (a mixture of clone 2E2; Zymed) and clone N1522 (Nordic Laboratory, Tillingburg, The Netherlands). Bound monoclonal antibodies were revealed with the use of biotinylated rabbit IgG antibody to mouse IgG (human IgG monospecific adsorbed; Zymed), followed by treatment with alkaline phosphatase–streptavidin (Zymed) and substrate. A standard curve was established by coating wells in each plate with rabbit IgG antibody to human IgA (mouse protein–adsorbed; Jackson Immunoresearch Labs., West Grove, PA) and using a pool of sera with normal IgA1 content (95% of total IgA) and IgA2 myeloma proteins (Nordic Laboratory). Free circulating IgA-subclass autoantibodies to lipoproteins were detected with an ELISA in which Maxisorp plates were coated with purified HDL or LDL (5 mg/L); subsequent steps were identical to the ELISA described above.

IgA1-bound lipoproteins (IgA-Lp) were detected in all the patients (median 0.25, range 0.25–1250 mg/L) at concentrations much greater (P < 0.005 by nonparametric Mann–Whitney test) than in the two healthy controls who were positive (median 0, range 0–0.25 mg/L). No lipoprotein-bound IgA2 was detected. As previously reported (1, 2, 7), no free circulating autoantibodies were detected. No secretory component–bound IgA was detected when the monoclonal antibody GA-1 instead of clone NIF1 was used in the ELISA in which the standard was purified secretory IgA (Jackson Immunoresearch Labs.). The IgA1 subclass was confirmed by means of affinity chromatography with jacalin, a lectin relatively specific for IgA1; serum from four patients with AIH but no IgA gammopathy, chromatographed on jacalin–Sepharose microcolumns (Vector, Burlingame, CA), gave two peaks, only one of which contained IgA1 (mellibiose elution). The two peaks obtained for each patient were tested at various dilutions, and only IgA1-Lp was detected in the mellibiose peak.

The IgA subclasses in the two gammapathies were quantified by a sandwich ELISA in which the capture antibodies were monoclonal antibodies specific for each subclass. The first patient had 9.5 g/L IgA1 and 0.075 g/L IgA2; in the second patient, the respective values were 5 and 0.065 g/L. Moreover, Western blotting with monoclonal antibodies specific for each IgA subclass of sera separated by isoelectric focusing on ampholine pH 3.5–9.5 polyacrylamide gel (LKB, Bromma, Sweden) with electrophoretic transfer to nitrocellulose sheets showed multiband patterns of only the IgA1 subclass within a restricted acidic pl range.

As previously suggested (1, 2), we have now demonstrated that only a portion of the monoclonal immunoglobulin in patients with gammopathy has anti-lipoprotein activity. This is probably related to subtle modifications of amino and carboxyl moieties in heavy and light chains that occur after translation and assembly, in such a way that only some of the IgA bands show antibody activity.

In conclusion, our study demonstrates that IgA autoantibodies to lipoproteins in AIH are restricted to the IgA1 subclass and bear no secretory component. The hypothetical link of these IgA1 anti-lipoprotein autoantibodies with the monocyte/macrophage activation in the atherosclerotic plaque (11) via the IgA receptor remains to be established.

References
6. Kihara S, Matsuzawa Y, Kubo M,
Problems of C-Reactive Protein Determination in Patients with Monoclonal Immunoglobulins

To the Editor:

Urdal et al. (1) remind us that high concentrations of bilirubin, lipids, or rheumatoid factors are known to interfere with many techniques of C-reactive protein (CRP) titration. We observed that certain monoclonal immunoglobulins known to induce false results in certain biochemical analyses (2–4) are also likely to alter CRP measurements in certain techniques.

CRP was measured in 190 patients whose sera contained a monoclonal immunoglobulin. Ninety-four patients had IgG monoclonal immunoglobulin, 22 had IgA, and 74 had IgM. For CRP titration we used two classic immunochimical techniques: nephelometry with the BNA\textsuperscript{a} analyzer (Berhingwerke, Marburg, Germany), using specific antibodies coating latex particles (detection threshold: 3 mg/L), and turbidimetry with a Turbitimetr\textsuperscript{b} analyzer (Berhingwerke), using a specific antiserum (detection threshold 5 mg/L).

In patients with monoclonal IgG or IgA, there were no discrepancies between results obtained with the two methods, regardless of the existence of an inflammatory syndrome (CRP \( \geq 10 \) mg/L). In contrast, of the 74 patients with IgM monoclonal component, 9 (12.2% of cases) showed marked discrepancies between the two methods: CRP concentration was measured as high by the nephelometric technique (\( >100 \) mg/L in 6 cases, between 10 and 100 mg/L in 3 cases), whereas readings were normal (\( <5 \) mg/L) with the turbidimetric technique. In these 9 patients, neither the clinical pattern nor acute-phase proteins titration revealed any inflammatory syndrome. This shows that the nephelometry results were inaccurate and artificially increased. The nine cases with the discrepant IgM monoclonal component were patients with Waldenström disease, who had a kappa-type IgM at high plasma concentration (mean 38 g/L, range 11–81 g/L).

However, if, as we showed, monoclonal IgM interferes in nephelometric titration of CRP, this interference does not appear to be caused by high IgM concentration. Indeed, of the 52 sera with monoclonal IgM concentration \( >11 \) g/L, only 9 were falsely titrated (17.3%). It therefore appears that the anomaly observed is due to a particular structure of the monoclonal component, which induces a nonspecific reaction between the IgM and the latex particles.

References

Autoantibody Specific for Lactate Dehydrogenase Isoenzyme 4

To the Editor:

There have been several reports of complexes between isoenzymes of lactate dehydrogenase (LD; EC 1.1.1.27) and autoantibodies, or macro-LD (1–3). Podlasek et al. (3) described a series of these complexes and classified them into three categories, based on the antibody specificity, as (a) reactive against only M subunits, (b) reactive against both H and M subunits, and (c) reactive against isoenzymes containing both M and H subunits. We report an autoantibody that apparently reacts only with the LD4 isoenzyme: we believe this is the first case of such antibody specificity.

The patient was a 61-year-old man who presented with testicular atrophy and the following biochemical and hematological abnormalities.

Electrophoresis of the LD isoenzymes on a Beckman Instruments (Brea, CA) Paragon electrophoresis system (LD Agarose Gels, Beckman cat. no. 655940; LD barbitol buffer and developing substrate, cat. no. 655795) is shown in Figure 1.

The investigation of the abnormal band migrating cathodally to LD4 involved immunofixation with human antiserum and electrophoresis of an equilibrium mixture of the patient's serum and a normal subject's serum. Immunofixation revealed both LD activity and an IgG kappa immunoglobulin at the position of the abnormal band. Electrophoresis of an equilibrium

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Fig. 1. Electrophoretogram of LD isoenzymes: (1) normal subject, (2) patient's sample, and results of immunofixation against (6) polyclonal antiserum, (7) anti-IgG, (8) anti-IgM, (9) anti-kappa, and (10) anti-lambda.

A point of application