Autoantibodies to Lactate Dehydrogenase in Serum Identified by Use of Immobilized Protein G and Immobilized Jacalin, a Jackfruit Lectin

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In this method for identifying autoantibodies to lactate dehydrogenase (anti-LDs) in serum, we used immobilized Protein G to bind IgG-complexed LD and immobilized jacalin to bind IgA-complexed LD, leaving non-complexed LD in solution. The non-complexed LD and total LD were kinetically measured. We report results as LD bound to immobilized Protein G and LD bound to immobilized jacalin. Using sera demonstrating IgG and IgA anti-LDs by immunoelectrophoresis (IEP), respectively, we optimized the method for incubation time and concentration of binding agents. We demonstrated coconit binding of LD and ≥98% of IgG and of LD and ≥92% of IgA. For LD bound to immobilized Protein G the detection limit was 10 U/L, within- and between-run CVs ranged from 2.9% to 9.1%, and values for normal sera were ≤3% of total LD. Results for LD bound to immobilized jacalin were similar. We tested 10 sera displaying aberrant LD electrophoretograms: In seven, LD bound to immobilized Protein G was increased (range: 26–99% of total LD), indicating IgG-complexed LD. This was confirmed by IEP, demonstrating IgG1, IgG1,2, or IgG3 anti-LDs in these sera. In the other three sera, LD bound to immobilized jacalin was increased (range: 38–72% of total LD), indicating IgA-complexed LD. This was confirmed by IEP, demonstrating IgA anti-LDs in these sera. Evidently this method is an alternative to IEP for identifying anti-LDs in serum.

Occasionally, electrometaphoresis patterns of the isoenzymes of lactate dehydrogenase (LD; L-lactate:NAD+ oxidoreductase, EC 1.1.1.27) in serum do not display the usual five LD bands but instead show smeared, shifted, fewer, or additional bands of LD activity (1, 2). Frequently, these aberrant electrometaphoresis patterns are caused by autoantibodies to LD (anti-LDs), involving IgG or IgA. Rarely, complexes of LD and IgM are formed (3). The IgG anti-LDs contain kappa light chains, lambda light chains, or both (4). The IgA anti-LDs show a high incidence of the kappa type (5). The immunoglobulins differ in isoenzyme specificity and complex formation involving the M subunit, the H subunit, or both (1, 4). The clinical significance of anti-LDs is unclear. Persistently circulating anti-LDs are not associated with a specific disease and have no diagnostic or prognostic value (1). This may be different for the anti-LDs that are light-chain restricted (5), and for the anti-LDs that transiently appear in serum after acute myocardial infarction (6) or after streptokinase therapy (7, 8).

Techniques commonly used to identify anti-LDs in serum are immunoelectrophoresis and immuno precipitation, involving monospecific antisera to human immunoglobulins. The anti-LD is identified immunoelectrophoretically, the antiserum producing a precipitin arc that stains for LD activity (9), and by immunoprecipitation by the antiserum, which effects a decrease of LD activity (10) or a change of the LD electrophoretic pattern (1). Presumably because of weak binding affinity of the anti-LD for LD, use of insufficient antiserum, or displacement of LD from the complex by the antiserum, these techniques frequently are unsuccessful (11, 12). Non-immune binding of complexed LD to immobilized Protein A has been advocated as a substitute for the immunochromatographic methods (13). Immobilized Protein A, however, has no affinity for IgA and IgG3, which are frequently demonstrated in IgG anti-LDs (14).

Here we describe a new method for identifying anti-LDs in serum. We use immobilized Protein G to bind IgG-complexed LD and immobilized jacalin (a lectin derived from jackfruit seeds) to bind IgA-complexed LD in serum, leaving non-complexed LD in solution. The non-complexed LD and total LD are kinetically measured. We report results as LD bound to immobilized Protein G and LD bound to immobilized jacalin. We compared results with those obtained by immunoelectrophoresis.

Materials and Methods

Reagents

Agarose was obtained from IBF Biotechnics, Villeneuve La Garenne, France. The colorimetric lactate dehydrogenase isoenzyme staining set was from Corning Medical Co., Palo Alto, CA. Agar was from Difco Laboratories, Detroit, MI. Antisera specific for human IgG, IgA, IgM, kappa light chains, and lambda light chains were from Dako Immunocyticals, Copenhagen, Denmark. Monoclonal antibodies specific for human IgG1, IgG2, IgG3, and IgG4 were from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. Protein G immobilized on Sepharose 4 Fast Flow (binding capacity 20 mg of IgG per milliliter of gel) was from Pharmacia Fine Chemicals, Uppsala, Sweden. Jacalin immobilized on agarose (binding capacity 1–2 mg of IgA per milliliter of gel) was from Pierce Chemical Co., Rockford, IL. The reagents and calibrator (Cal 1, lot no. M803200) for measurement of IgG and IgA in the "Array" Protein System, as well as the quality-control material ("Control 1," lot no. M807092), were from Beckman Instruments, Inc., Brea, CA. We measured LD activities in a Genesee centrifugal analyzer (Electro-Nucleonics, Inc., Fairfield, NJ), applying the reaction conditions recommended by the Scandinavian Society for Clinical Chemistry (15). NADH (grade I) and sodium pyruvate were from Boehringer Mannheim GmbH, Mannheim, F.R.G., and Tris and EDTA were from E. Merck, Darmstadt, F.R.G.

Serum Specimens

The serum samples with aberrant LD electrophoretograms that we selected for this study were obtained from 10 unrelated patients, none of whom had received streptoki-
ase or showed clinical evidence of acute myocardial infarction. Control sera, obtained from patients for whom routine laboratory tests had been ordered, showed increased LD activities or LD activities within the normal reference interval and displayed no aberrant LD electrophoreograms.

Procedures

Electrophoresis. We separated LD isoenzymes in 1 μL of serum on agarose gel (16) and stained as recommended by the manufacturer with a LD substrate containing L-lactate, NAD⁺, and nitroblue tetrazolium.

Immunelectrophoresis. For immunelectrophoresis we used 3 μL of patient's and control serum, respectively, on a gel (3 mL poured on a 76 × 26 mm microscope slide) of 10 g/L agar in barbital buffer (50 mmol/L, pH 8.6), run at 15 °C and 8 mA per slide (constant current) for 45 min (17). For immunodiffusion against IgG-subclass-specific antisera, 50 μL of polyethylene glycol 4000 was added per liter to this buffer. After immunodiffusion for 36 h against 20 μL of antisera specific for human IgG, IgA, IgM, kappa and lambda light chains, IgG1, IgG2, IgG3, and IgG4, the gels were washed with two changes of isotonic saline for 24 h. The gels were covered with 10 layers of dry Whatman 1 MM chromatography paper, then left for 60 min with a glass plate with a 0.2-kg weight on top of it, and incubated with the LD substrate reagent for 1 h at 37 °C.

LD bound to immobilized Protein G and LD bound to immobilized jacalin. We washed Protein G immobilized on Sepharose 4 Fast Flow and jacalin immobilized on agarose with Tris–EDTA buffer (53 mmol of Tris and 5.3 mmol of EDTA per liter, adjusted to pH 7.4 with HCl), then suspended each gel in an equal volume of this buffer.

We added 5 μL of serum to 200 μL of each gel suspension, incubated the mixtures for 10 min at room temperature with agitation, mixed each with 500 μL of the buffer, centrifuged the mixtures for 5 min at 500 × g, and used the supernates to assay residual LD. To assay residual LD we combined 500 μL of each supernate with 5 μL of a 15 mmol/L solution of NADH and started the reaction with 25 μL of a 25 mmol/L solution of sodium pyruvate. To measure total LD we combined these reagents with 500 μL of a 140-fold dilution of the untreated serum in the buffer. We monitored the decrease of the absorbance in these solutions in the same analytical run at 340 nm against the reagent blank at 30 °C for 3 min.

LD bound to immobilized Protein G (U/L) was calculated from: (U−T) × 197 × 1/ε × 1000, where U is the change per minute in absorbance (∆A/min) of the solution of the untrated sample, T is the ∆A/min of the solution of the sample treated with this agent, V is the volume fraction of serum in the LD assay (0.0067), and ε is the molar absorption coefficient of NADH (6.3 L × mmol⁻¹ × mm⁻³). Similarly, LD bound to immobilized jacalin was calculated.

Concomitant binding of IgG and LD to immobilized Protein G and of IgA and LD to immobilized jacalin. We incubated 20 μL of a serum showing IgG3 anti-LD with increasing amounts of immobilized Protein G on Sepharose 4 Fast Flow and 20 μL of a serum demonstrating IgA anti-LD with increasing amounts of jacalin immobilized on agarose. Each binding agent varied by 0–200 μL in 400 μL (total volume) of the Tris–EDTA buffer. We incubated all mixtures for 30 min at room temperature, with agitation. We then centrifuged the mixtures for 5 min at 500 × g and measured IgG and IgA concentrations in aliquots of the supernates in the "Array" Protein System, using the "A" CSF sample dilution. To determine LD bound to immobilized Protein G and LD bound to immobilized jacalin, we simultaneously measured LD in 100 μL of the supernates after diluting with 400 μL of the Tris–EDTA buffer.

To assess the specificity of the IgG and IgA assay under the conditions of the test, we tested analytical recovery. We incubated (without serum) suspensions of each of the binding agents in the buffer (mixed in the proportions of the test) for 30 min at room temperature and centrifuged the mixtures for 5 min at 500 × g. We then supplemented aliquots of the supernates with known amounts of IgG and IgA (using "Control 1") and measured the IgG and IgA concentrations as described above.

Detection Limits, Specificity, and Precision

To estimate detection limits, we measured (10 times each) LD bound to immobilized Protein G and LD bound to immobilized jacalin in a pool of normal sera. We then measured (10 times each) LD bound to immobilized Protein G in samples prepared by diluting a serum demonstrating IgG3 anti-LD with the serum pool, and LD bound to immobilized jacalin in similarly prepared dilutions of a serum demonstrating IgA anti-LD.

To assess the specificity of the assay of residual LD in the final test, we incubated (without serum) 200 μL of suspensions of immobilized Protein G and immobilized jacalin in the buffer, mixed each with 500 μL of the buffer, and centrifuged the binding agents. We then added 5 μL of control sera to 500 μL of the supernates and assayed for total LD. We compared these LD activities with total LD activities assayed under the conditions of the final test.

To assess within-run precision, we determined (seven times each) LD bound to immobilized Protein G and LD bound to immobilized jacalin in undiluted patients' serum containing IgG3 and IgA anti-LDs, respectively, and in dilutions of these sera with a normal control serum. Between-run precision was calculated by assaying aliquots of similar specimens three times in three consecutive days.

Interference Studies

We mixed 100 μL of two sera showing IgG3 and IgA anti-LDs, respectively, with 0, 20, 60, and 100 μL of an icteric, a lipemic, and a "hemolysed" serum. We prepared the hemolysed serum by diluting a cell-free hemolyte with normal control serum (1/9, by vol). We then adjusted the total volumes of the samples to 200 μL with normal control serum. The bilirubin, triglycerides, and hemoglobin concentrations of these samples ranged up to 252 μmol/L, 14.2 mmol/L, and 0.021 mmol/L, respectively. We then determined LD bound to immobilized Protein G in all samples containing IgG3 anti-LD, and LD bound to immobilized jacalin in all samples containing IgA anti-LD.

We mixed 100 μL of the two sera showing IgG3 and IgA anti-LDs, respectively, with 100 μL of a normal control serum and with 100 μL of sera demonstrating IgG (n = 5) and IgA (n = 6) paraproteins by immunofixation. The paraprotein concentrations, in grams per liter, determined by densitometry of the serum protein electrophoresis pattern ranged from 6.8 to 35.1 for IgG and 3.0 to 42.0 for IgA. We then determined LD bound to immobilized Protein G in all samples containing IgG3 anti-LD and LD bound to immobilized jacalin in all samples containing IgA anti-LD.
Results

Electrophoresis

The LD electrophoretograms of the sera we selected for this study showed smeared, broadened, shifted, or fewer LD bands (Figure 1).

Immunoelectrophoresis

Precipitin arcs of the 10 patients' samples produced by either anti-IgA or anti-IgG antiserum, and by either anti-kappa or anti-lambda light chain antiserum, stained for LD. Except for one sample (sample 6), sera demonstrating LD staining of IgG precipitin arcs showed LD activity in either IgG3 or IgG1 arcs, or in IgG1 and IgG2 arcs. Identification of the anti-LDs in the sera, based upon LD staining of arcs corresponding to a particular anti-immunoglobulin and anti-light chain antiserum, is shown in Table 1.

Evaluation of the Method

Concentrations of binding agents. To optimize the amounts of immobilized Protein G and immobilized jacalin, we studied binding of LD over a wide range of concentrations of these agents. We determined LD bound to immobilized Protein G in 10 μL of three sera demonstrating IgG1, IgG1,2, and IgG3 anti-LDs, respectively, using increasing amounts (0–100 μL) of this binding agent in the test. We found (Figure 2) that, on increasing the amount of binding agent, the LD bound to immobilized Protein G increased in the same proportions in the sera showing different IgG subclass anti-LDs. The LD bound to immobilized Protein G became constant for all these sera when 50–100 μL of immobilized Protein G was used. We used 100 μL of immobilized Protein G in the finally adopted test, to ensure binding excess. We determined LD bound to immobilized jacalin in 5 μL of two sera containing IgA anti-LDs, using increasing amounts (0–100 μL) of this binding agent in the test. Figure 2 shows identical binding curves of LD for the two sera. The LD bound to immobilized jacalin became constant when we used 75–100 μL of this agent. We use 100 μL of immobilized jacalin in the finally adopted test.

Concomitant binding of IgG and LD. We assessed concomitant binding of IgG and LD to immobilized Protein G by incubating 20 μL of a serum showing IgG3 anti-LD with increasing amounts of immobilized Protein G. We then simultaneously measured the IgG left in solution and the LD bound to immobilized Protein G in these mixtures as described in Materials and Methods. At zero amount of immobilized Protein G, the IgG concentration, in grams per liter, was 0.518 and gradually decreased (by increasing the amount of the binding agent in the test) to ≤0.012 (the lower limit of the analytical range of the IgG assay). This indicates that immobilized Protein G extracted ≥98% of the IgG.

We plotted the IgG bound to immobilized Protein G (as a percentage of the IgG concentration at zero amount of binding agent) and the LD bound to immobilized Protein G (as a percentage of the value using the highest concentration of the binding agent) against the log amount of binding agent. Figure 3 shows parallel binding curves for LD and IgG, and a plateau for LD bound to immobilized Protein G concomitant with complete binding (≥98%) of IgG. When we added known amounts of IgG to aliquots of serum-less supernatants, the mean analytical recovery over a 0.099–0.515 g/L range of added IgG was 105% (SD 2.7%), indicating a lack of matrix effect.

Concomitant binding of IgA and LD. We assessed concomitant binding of IgA and LD to immobilized jacalin by incubating 20 μL of a serum containing IgA anti-LD with increasing amounts of immobilized jacalin. We then simultaneously measured the IgA left in solution and the LD bound to immobilized jacalin in these mixtures as described in Materials and Methods. At zero amount of immobilized

<table>
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<tr>
<th>Sample</th>
<th>Anti-LD</th>
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<tbody>
<tr>
<td>1</td>
<td>IgG3 L</td>
</tr>
<tr>
<td>2</td>
<td>IgG1,2 L</td>
</tr>
<tr>
<td>3</td>
<td>IgG1,2 L</td>
</tr>
<tr>
<td>4</td>
<td>IgG3 L</td>
</tr>
<tr>
<td>5</td>
<td>IgG3 L</td>
</tr>
<tr>
<td>6</td>
<td>IgG1 K</td>
</tr>
<tr>
<td>7</td>
<td>IgG1 K</td>
</tr>
<tr>
<td>8</td>
<td>IgG1 K</td>
</tr>
</tbody>
</table>

* Identical sample numbering is used in Figure 1 and Table 3. © IgG subclass not detectable.
jacalin, the IgA concentration was 0.133 g/L and was gradually decreased (by increasing the amount of binding agent in the test) to ≤0.011 g/L (the lower limit of the analytical range of the IgA assay). This indicates that immobilized jacalin extracted ≥92% of the IgA.

Figure 3 shows a plot of IgA bound to immobilized jacalin (as a percentage of the IgA concentration at zero amount of binding agent) and the LD bound to immobilized jacalin (as a percentage of the value using the highest concentration of the binding agent) as related to the log amount of binding agent. This figure shows that binding of IgA paralleled binding of LD and demonstrates a plateau for LD bound to immobilized jacalin concomitant with ≥92% binding of IgA. When we added known amounts of IgA to serum-less supernates, the mean analytical recovery over a 0.030–0.189 g/L range of added IgA was 98% (SD 5.1%), indicating a lack of matrix effect.

Incubation time. Varying incubation time from 2 to 30 min, we determined LD bound to immobilized Protein G and LD bound to immobilized jacalin in two sera demonstrating anti-LDs. The results (Figure 4) indicate that binding of LD to immobilized Protein G in a serum containing IgG3 anti-LD was complete within 2 min. Binding of LD to immobilized jacalin in a serum containing IgA anti-LD was complete within 10 min (Figure 4). We chose an incubation time of 10 min for both binding agents in the finally adopted test.

Detection limits. The limits of detection for LD bound to immobilized Protein G and LD bound to immobilized jacalin, defined as the lowest LD activities absorbed by these agents that distinguished (at 95% confidence) the sera with anti-LDs from the pool of normal sera, were 9 and 10 U/L, respectively.

Specificity and precision. The total LD activities of 15 sera determined after diluting with serum-less supernates from suspensions of immobilized Protein G and with serum-less supernates from suspensions of immobilized jacalin averaged 99% (SD 2.1%) and 100% (SD 1.7%), respectively, of the values measured under the conditions for total LD in the final test (range: 216–1155 U/L). This indicated a lack of matrix effect for the assay of residual LD activity. Results for within- and between-run precision (Table 2) indicate CVs to range from 2.3% to 9.1%.

For normal control sera and for sera with increased LD activities (range: 815–1020 U/L), LD bound to immobilized Protein G and LD bound to immobilized jacalin were ≥3% of total LD activities.

Interference studies. Bilirubin, triglyceride, and hemoglobin concentrations up to 252 μmol/L, 14.2 mmol/L, and 0.021 mmol/L, respectively, did not interfere with the method. The same was true for IgA paraproteins (1.5–21.0 g/L) and IgG paraproteins (3.4–17.5 g/L).

Results for Sera with Aberrant LD Electrophoretograms

We determined LD bound to immobilized Protein G and LD bound to immobilized jacalin in the sera displaying the abnormal LD electrophoretograms shown in Figure 1. The results (Table 3) show that the LD bound to immobilized Protein G (range: 26–99% of total LD) was increased in seven samples and the LD bound to immobilized jacalin (range: 38–72% of total LD) in three. These ranges represent the immunoglobulin-bound LD activities (as percentage of total LD activities) in the sera.

Variability of Results

Variability of LD bound to immobilized Protein G between sera of different patients (as shown in Table 3) was also demonstrated in two sera from a single patient. In serum 10, LD bound to immobilized Protein G was 99% of the total LD, indicating that all LD activity was complexed. This was also suggested by the LD electrophoretogram of the serum, which displayed only one band of complexed LD activity (Figure 5). In sample 11, collected from the same patient one year later, LD bound to immobilized Protein G constituted 88% of the total LD, indicating non-complexed LD in addition to complexed LD activity. To demonstrate the non-complexed LD by another method, we performed LD electrophoresis. The LD electrophoretogram of this serum displayed a band of isoenzyme LD1 activity that was not seen in the serum collected a year earlier (Figure 5).
Table 2. Precision of LD Binding to Immobilized Protein G and to Immobilized Jacalin

<table>
<thead>
<tr>
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<th>Within run</th>
<th></th>
<th>Between run</th>
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<tr>
<td></td>
<td>Mean ± SD,</td>
<td>CV, %</td>
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<td>CV, %</td>
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<tr>
<td></td>
<td>n = 7</td>
<td></td>
<td>n = 9</td>
<td></td>
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<tr>
<td>LD bound to immobilized Protein G, U/L</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Low concn</td>
<td>65 ± 6 (210)*</td>
<td>9.1</td>
<td>105 ± 7 (306)</td>
<td>6.7</td>
</tr>
<tr>
<td>High concn</td>
<td>439 ± 13 (472)</td>
<td>2.9</td>
<td>368 ± 22 (394)</td>
<td>5.9</td>
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<tr>
<td>LD bound to immobilized jacalin, U/L</td>
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<td></td>
</tr>
<tr>
<td>Low concn</td>
<td>73 ± 4 (207)</td>
<td>5.2</td>
<td>159 ± 11 (390)</td>
<td>7.1</td>
</tr>
<tr>
<td>High concn</td>
<td>429 ± 10 (677)</td>
<td>2.3</td>
<td>476 ± 10 (672)</td>
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</table>

LD bound to immobilized Protein G and LD bound to immobilized jacalin were measured in sera containing IgG and IgA anti-LDs, respectively, as described in Materials and Methods. * Mean total LD activity, U/L, listed in parentheses.

Table 3. Total LD and LD Bound to Immobilized Protein G and to Immobilized Jacalin in the Patients’ Sera

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Total LD, U/L</th>
<th>LD bound to immobilized Protein G</th>
<th>LD bound to immobilized jacalin</th>
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<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>96%</td>
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<tr>
<td>2</td>
<td>256</td>
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<td>0</td>
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<tr>
<td>3</td>
<td>307</td>
<td>56%</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
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<td>6</td>
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<td>64</td>
</tr>
<tr>
<td>8</td>
<td>550</td>
<td>2%</td>
<td>72</td>
</tr>
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* Identical sample numbering is used in Figure 1 and Table 1. Normal reference interval for LD is 67–287 U/L. For control sera, LD bound to immobilized Protein G and LD bound to immobilized jacalin were <3% of total LD.

Fig. 5. LD electrophoreograms of serum 10, serum 11 (collected one year later from the same patient), and control C.

Comparison of Results

The LD bound to immobilized Protein G (as a percentage of total LD) was increased in seven samples (Table 3; sera 1–4, 6, 9, and 10), indicating IgG-complexed LD. This was confirmed by immunoelectrophoresis, demonstrating IgG-anti-LDs in these samples (Table 1). The LD bound to immobilized jacalin was increased in three samples (Table 3; sera 5, 7, and 8), indicating IgA-complexed LD. These sera demonstrated IgA anti-LDs by immunoelectrophoresis (Table 1).

Discussion

Chronically circulating anti-LDs are suggested when persistent unexplained increased values for LD in serum are measured. To confirm complex formation of LD, electrophoresis of LD in serum is recommended, because anti-LDs typically alter the electrophoretic pattern of LD isoenzymes (18). However, aberrant LD electrophoretograms may be caused by conditions not related to immunoglobulin binding to LD (19, 20).

By immunoelectrophoresis, we identified the anti-LD immunoglobulin class (IgG/IgA) and light chain type (kappa/lambda) in all 10 sera showing aberrant LD electrophoretograms. This compares favorably with reports (1) identifying anti-LDs in only 17% (two cases) of such sera. In agreement with an earlier report (14), we detected no additional IgG subclasses with anti-LD activity in samples demonstrating IgG3-complexed LD. In contrast with suggestions that IgG-complexed LD involves almost exclusively IgG3 (14), we detected IgG1 and IgG2 anti-LDs in 43% (three samples). Immunelectrophoresis repeatedly failed to detect the IgG subclass in sample 6, as did the Ouchterlony double-immunodiffusion method (not described here).

Protein G and Protein A are bacterial proteins that specifically bind to the Fc region of IgG by a non-immune mechanism. Protein G binds to IgG1–4, Protein A only to IgG1, 2, and 4. Because of the high incidence of IgG3 anti-LDs, we selected immobilized Protein G for binding IgG-complexed LD. Jacalin specifically binds to human IgA1 and IgD, but not to IgA2, IgM, or any of the IgG subclasses. This non-immune mechanism involves a unique oligosaccharide structure located in the hinge region of IgA1 and IgD, which is lacking in the other immunoglobulins (21). Because complexes of IgD and LD have never been reported, immobilized jacalin is probably specific for IgA1-complexed LD.

Because of the poorly defined structures of the immunoglobulin–LD complexes, we were unable to assess analytical recovery by adding known amounts of analyte to serum. Therefore, we assessed concomitant binding of LD and IgG/IgA to the binding agents and extraction of IgG and IgA under the conditions of the test. We demonstrated parallel binding of LD to immobilized Protein G over a wide range of concentrations of this agent in three sera showing
different IgG subclass anti-LDs. This suggests that binding of complexed LD is independent of the IgG subclass specificity of the anti-LDs. For one of these sera we showed parallel binding of LD and IgG, indicating concomitant extraction of IgG and LD-complexed IgG. We found complete binding of IgG (≥98%), which suggested to us that complexed LD (independent of the IgG subclass) is completely extracted by immobilized Protein G. Similarly, we demonstrated parallel binding of IgA and LD, and we found ≥92% extraction of IgA, suggesting adequate extraction of IgA1-complexed LD. We did not expect complete extraction of IgA, because immobilized jacalin is specific for IgA1, and IgA2 constitutes ~10% of the total serum IgA. In addition, we demonstrated a plateau for the LD bound to immobilized Protein G and the LD bound to immobilized jacalin during incubation (Figure 4), excluding the possibility that desorption of complexed LD invalidated the measurement of the non-complexed LD activity in the test. These data suggest satisfactory accuracy to identify anti-LDs. The variability of the LD bound to immobilized Protein G and the LD bound to immobilized jacalin between the different patients' sera (range: 26–99% of total LD) suggested to us that these samples had different anti-LD titers. A high titer of anti-LD, complexing all the LD in the serum, was suggested in sample 10 (Figure 1 and Table 3), and, to exclude the possibility that anti-LD excess interfered with the evaluation of the method, we used sera demonstrating non-complexed LD in addition to complexed LD. This method demonstrated IgG- and IgA-complexed LD in sera showing aberrant LD electrophoretograms. Results agreed with those obtained by immunoelectrophoresis. We conclude that binding of LD to immobilized Protein G and immobilized jacalin is an alternative for immunoelectrophoresis to identify anti-LDs in serum.

References