Immunoglobulin A (λ Chains) Conjugated with Lactate Dehydrogenase in Serum

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An atypical pattern for lactate dehydrogenase (EC 1.1.1.27) isoenzymes in a patient with bladder neck obstruction was describable to complexes between lactate dehydrogenase and IgA. This complex formation was also replicable "in vitro." We determined that the IgA bound to lactate dehydrogenase was of the λ type, a very unusual occurrence.

There are several reports of lactate dehydrogenase (LD; lactate:NAD⁺ oxidoreductase, EC 1.1.1.27) forming complexes with IgG or IgA to produce unusual LD isoenzyme patterns in serum (1, 2). In all cases the investigators found that normal LD isoenzymes were bound to an anomalous immunoglobulin fraction: IgG–LD complexes characterized all five isoenzyme fractions (3), whereas most IgA–LD complexes involved a specific isoenzyme (4). The light chain of Ig involved in the complex formation is mainly the κ type (5). Previous reports (6, 7) described that in this situation the LD activity in serum is normal or moderately increased, or, less often, low; the pathophysiological meaning of these lactate dehydrogenase–immunoglobulin complexes is uncertain.

We encountered a patient with markedly increased LD activity and an atypical pattern of LD isoenzymes. Immunofixation followed by assay of LD activity demonstrated the presence of the LD–IgA complex involving a light chain of the λ type. Here we report the chemical data of this case and evaluate the chemical features and diagnostic significance of the complex.

Case History

The patient, a 45-year-old man, was admitted to the Department of Urology of Padua University with sclerosis of the bladder neck. He later underwent an endoscopic resection of the bladder neck posterior side. The most striking laboratory finding was an increased LD activity in serum, ranging from 1482 U/L at admission to 823 U/L, a value that persisted through six months of observations. The electrophoretic pattern of serum proteins and the total concentration of immunoglobulins in serum were both within normal limits.

Materials and Methods

Immediately after collection, we stored 0.5-mL aliquots of the patient's serum in hermetically closed microtubes at −20 °C.

Blood samples, collected into glass tubes containing K₂EDTA, were promptly assayed for LD activity in the erythrocytes.

We determined total LD activity at 37 °C (6) with a commercial kit (Roche Diagnostics, Milano, Italy) and an Eppendorf ACP 5040 automatic analyzer (Leitz Italiana, Milano, Italy). In our laboratory the reference interval for normal subjects is 200–400 U/L.

The LD isoenzymes were determined by electrophoresis

(9) on cellulose acetate (Titan III; Helena Laboratories Italia, Milano, Italy).

Serum immunoglobulins were quantified with the Immunochemistry System (Beckman Instruments Inc., Brea, CA). Immunofixation was performed according to Saga and Kano (10) with antisera to IgG, IgA, IgM, λ, and κ chains (Gelman Sciences Inc., Ann Arbor, MI).

We precipitated the immunoglobulin fractions with 55% saturated ammonium sulfate, by modifying the method of Podlasek et al. (11). After mixing equal volumes of the patient's serum and cold aqueous saturated ammonium sulfate solution, refrigerating (4 °C) for 30 min, then centrifuging at 10 000 × g for 5 min, we dissolved the pellets in de-ionized water and precipitated as before in saturated ammonium sulfate. Finally, we redissolved the second pellets in appropriate volumes of serum from a healthy voluntary subject and incubated at 37 °C overnight.

Results

The patient's LD isoenzyme profile was characterized by a broad band migrating between the LD₂ and LD₄ isoenzymes, with an apparent absence of LD₃. The LD₁ and LD₅ isoenzymes appeared to have normal mobility (Figure 1B). The patient's erythrocytic LD electrophoretic pattern (Figure 1C) was normal.

This atypical isoenzyme pattern suggested the probable presence of a macromolecule. The immunological nature of the complex was confirmed after immunofixation by demonstrating LD activity in the precipitate produced by anti-IgA and anti-λ chain antisera, but not in those produced by anti-IgG or anti-IgM antisera.

No activity was observed with antiserum to κ chain (Figure 2).

Treatment of patient's serum with 55% saturated ammonium sulfate did precipitate the fractions containing the anti-LD activity. Mixing the separated and redissolved precipitate with normal serum produced an atypical isoenzyme pattern similar to that of patient's serum alone. A broad band showing the electrophoretic mobility of a LD₃ isoenzyme without evident electrophoretic modifications of the other fractions was observed (Figure 3).

Discussion

The presence in serum of macromolecular lactate dehydrogenase has been noticed in several occasions, both in

![Fig. 1. LD isoenzyme patterns of a control serum (A), the patient's serum (B), and a hemolysate of the patient's erythrocytes (C)](image-url)
healthy subjects and in patients with various apparently unrelated diseases. Most of these have been attributed to the formation of complexes between enzyme molecules and immunoglobulin in serum. The increased half-life of these complexes often results in an unexpected and constant above-normal activity of LD in serum.

In the case we report, the responsible complexing factor was a patient's IgA molecule with special affinity for normal LD, capable of altering normal serum in vitro as well as in vivo. The reported investigation seems to demonstrate that the LD sub-isoenzyme is involved in the binding to immunoglobulin. The normal LD isoenzyme profile in the patient's erythrocytes negated the possibility of a genetic variant of LD.

The anti-LD activity of IgA is apparently not related to the patient's specific clinical conditions, and he has so far presented no definite signs of autoimmune diseases.

References