Unusual Variant of Lactate Dehydrogenase Isoenzymes

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Thirteen electrophoretic bands of lactate dehydrogenase isoenzyme activity were detected in the serum of a 69-year-old Negro woman who died of pseudomonas pneumonia. No evidence of tumor was found at necropsy, and additional studies of the enzymatic activity of crude extracts of liver, lung, spleen, kidney, brain, skeletal muscle, lymph node, and heart revealed 1, 2, 3, 4, and 5 separate isoenzyme bands for lactate dehydrogenase isoenzymes 1, 2, 3, 4, and 5, respectively (a total of 15 isoenzyme bands). Serum and erythrocyte hemolysate from one of two healthy daughters displayed a similar pattern of multiple isoenzyme bands in isoenzymes 2 and 3. The observed pattern is consistent with the heterozygous form of a mutation of the genetic locus controlling synthesis of the M monomer such that two differently charged monomeric proteins are produced, each of which apparently combines with H chains with equal facility, leading to the multiband enzymatic specificity seen.

Although determination of total serum lactate dehydrogenase (LD; EC 1.1.1.27) activity is frequently diagnostically useful, additional information can be obtained as to the tissue sources of LD in disease states by separating and measuring the activity of the isoenzymes. Each isoenzyme is a tetramer composed of monomeric subunits, which have been designated H or M (7). LD-1 moves most anodally during electrophoresis and is composed of four H chains (HHHH). LD-2, -3, and -4 migrate less rapidly (in that order) and are tetramers represented by HHHM, HHMM, and HMMM, respectively. The remaining isoenzyme, LD-5, is composed only of M monomers (MMMM).

We recently observed the presence of more than the usual five isoenzyme bands in a Negro patient with pseudomonas pneumonia. Electrophoresis of her serum showed the presence of one band of LD-1, two bands of LD-2, three bands of LD-3, and multiple bands of LD-4 and -5 activity (Figure 1, A and B). The patient died several days after admission to the hospital. No evidence of tumor was found at autopsy.

To document the tissue sources of the multiple forms of the enzyme, we prepared extracts from several tissues obtained at autopsy and determined their isoenzyme activity. Although genetic variants of LD isoenzymes in sera and in hemolysates of erythrocytes have been reported, to our knowledge the anomaly has not previously been demonstrated in various organs.

Materials and Methods

Tissue specimens were obtained after death and were immediately placed in phosphate buffer (0.1 mol/liter, pH 7.4) and refrigerated until extracted. Each tissue was dissected free from fat and connective tissue, cut into small pieces, and washed three times in cold buffer to remove as many erythrocytes as possible. A crude extract was prepared by homogenizing 4 g of wet tissue in 20 ml of phosphate buffer (50 mmol/liter, pH 7.4) at 4 °C. The extract was then centrifuged at 5000 × g for 5 min and the bottom layer of cellular debris discarded. Erythrocytes were lysed in 5.0 ml of the buffer after thorough washing in isotonic saline. All crude extracts were kept at 4 °C until electrophoresis; a portion of each extract was also inactivated at 65 °C for 30 min before electrophoresis.

Electrophoretic separation was performed on glass slides with use of agarose (5 g/liter) in barbital buff-
er, pH 8.6; enzymatic activity was identified by a modification of the method of Wright et al. (2). The zymograms were developed at 37 °C for 10 min and the reaction was then stopped and the gel fixed with dilute (5 ml/dl) acetic acid.

Results

Except for LD-1, zymograms for each of the tissue extracts contained more than one band of activity. Figure 2 shows the bands seen in extracts of liver, lymph node, spleen, kidney, heart, skeletal muscle, brain, and lung. Although the fourth component of LD-4 and the first component of LD-5 are not clearly visible in Figure 2, they could be demonstrated by two-dimensional electrophoresis of liver and skeletal muscle extracts. Heat-inactivated extracts produced the expected loss of activity in LD-4 and LD-5 (all components) and resulted in diminished activity in LD-2 and LD-3. Figure 1C and D, shows isoenzyme activity in serum from the patient’s two daughters, one of whom demonstrated a similar multiple band pattern of LD-2 and -3 (1-C).

Discussion

Boyer et al. (3) first reported the presence of more than five isoenzyme bands in a healthy 25-year-old Nigerian man, who was initially examined during the course of a hemoglobin survey. For both serum and extracts of erythrocytes five bands of LD-1, four LD-2, three LD-3, and two LD-4 could be demonstrated; LD-5 was not detected. These authors postulated a mutant allele involving the genetic locus producing the H monomer, such that tetramers comprised of both the normal H and mutant H' subunits were possible in a heterozygous person. LD-1 could thus be comprised of HHHH, HHHH', HHH'H', HH'H'H', and H'H'H'H' to account for the five bands of activity observed. LD-2 could consist of four isoenzyme bands—HHHM, HHH'M, HH'H'M, and H'H'H'M—and a similar arrangement could account for the multiple bands seen in LD-3 and LD-4.

The alternative mutation, that involving the M subunit, has also been reported (4–6) but is difficult to fully demonstrate in normal persons, because LD-4 and -5 activities usually account for only a small percentage of the total LD activity of serum or lysed erythrocytes. The multiple bands of expected activity were easily detected in both serum and tissue extracts from the patient described here, who demonstrated a probable mutation of the M monomer. An apparently hereditary pattern was demonstrated by the finding of a similar LD-2 and LD-3 band pattern in one of two daughters; in both the patient and her daughter the least rapidly migrating band of each fraction displayed the mobility of the corresponding normal isoenzyme. Similar mutants that result in a slower mobility of the substituted tetramers have also been reported (4, 5).

The frequency of mutation involving either the H or M subunit is low and appears to be more frequent in Negroes. Kraus and Neely (5) studied hemolysates of erythrocytes from 610 Negroes and 330 Caucasians and found isoenzyme patterns that differed from normal in seven Negroes and one Caucasian. Davidson et al. (4) found two of 1015 English who were presumably heterozygous for the M subunit mutation, and family studies subsequently revealed 16 males and 15 females with the abnormality. Vesell (6) screened 600 Caucasians and 600 Negroes in the United States and found three with variant forms similar to the case described here. A fourth person was presumably homozygous for the mutation and while the usual number of bands were found, abnormal rates of isoenzyme migration were noted as com-
pared with normal serum. Although other extra bands of LD activity have been described in patients with malignancy (7–9) and in apparently normal persons (10), the abnormalities have not affected all four of five bands, which suggests a mechanism different from the monomeric mutation postulated in the present instance. The multiple bands of activity observed are consistent with the suggestion that the mutation involved a gain, loss, or substitution of amino acids that led to a net increase in negative charge on the abnormal subunit as compared with the normal monomer.

As increasing clinical use is made of isoenzyme quantification, similar unusual patterns owing to anomalous protein monomers may be observed and should not be interpreted as being necessarily diagnostic of disease.

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References