Macro Creatine Kinase Type 1 with Electrophoretic Mobility Identical to That of the MB Isoenzyme

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We describe the case of an elderly woman whose symptoms and electrocardiographic pattern initially suggested acute myocardial infarction. The value for total serum creatine kinase (EC 2.7.3.2; CK) was 737 U/L (reference interval: 22–269 U/L), and electrophoresis for CK isoenzymes demonstrated two bands, the more anodal migrating to the CK-MB region and the second migrating between the CK-MB and CK-MM regions. The above-normal total CK and electrophoretic pattern persisted during her 11-day hospital course. The QuikCK-MB (International Immunoassay Labs.) and Tandem-E CK-MB (Hybritech) immunoassays, however, showed CK-MB mass measurements within the normal range. In further investigation with a mixture of patient’s serum and human-serum-based control containing all CK isoenzymes, the electrophoretic mobility of only CK-BB was altered, proving that the patient had antibody to the B unit of CK in her serum. Immunofixation revealed the more anodal band to be a CK–IgA lambda complex, and the more cathodal band, a CK–IgG kappa complex. Mixing the patient’s serum with polyclonal antibody specific for CK-B slowed the electrophoretic mobility of only the more anodal band. Polyclonal antibody specific for CK-M had no effect on either band. Evidently, this patient had two different types of macro CK type 1, both containing CK-BB. We conclude that macro CK type 1 can mimic CK-MB and be a source of confusion.

Additional Keyphrases: heart disease · electrophoresis, agarose · isoenzyme-specific immunoassays · immunofixation

Previous reports (1–12) have described variants of creatine kinase (EC 2.7.3.2; CK) having atypical electrophoretic mobility and appearing between the CK-MM and CK-MB regions. These variants have been termed atypical CK (8), creatine kinase Z (7), CK-X 1–3 (5), and (most commonly) macro CK type 1 (1–4, 6, 9–11). Macro CK type 1 is most often a complex of CK-BB isoenzyme with another serum constituent (1–4, 6, 9–11), and is found in sera of 1 to 2.9% of hospitalized patients (2, 8); rarely, CK-MM has been the isoenzyme involved (5). The CK is most often complexed to IgG (1, 3, 10), via the Fab sites of the immunoglobulin (11), but complexes of CK with IgA (4, 5) or β-lipoprotein (12) have also been reported. Whether the macro CK type 1 is the result of a specific antigen–antibody reaction (9, 11), implying autoimmunity, or a nonspecific complex (4) remains controversial.
Materials and Methods

Enzyme assay. We measured total CK at 37 °C in a Multistat III microcentrifugal analyzer (Instrumentation Laboratory, Lexington, MA) with Statzyme CK n-1 reagents (Worthington Diagnostics Systems, Inc., Freehold, NJ).

Electrophoresis of isoenzymes. For the 20-min electrophoresis of 1 μL of serum, we used "Electro-Trace Special Purpose Electrophoresis Film" agarose in Mops (sodium 4-morpholinepropane sulfonate) buffer (50 mmol/L, pH 7.8), both from Corning Medical, Medfield, MA. After electrophoresis, we incubated the gels with a CK-isoenzymes substrate set (Corning) for 20 min at 37 °C. To exclude interference from activity of adenylyl kinase (EC 2.7.4.3), we also incubated the patient’s serum without creatine phosphate after electrophoresis. After drying the gels at 50 °C, we scanned them at 356 nm with a Corning Model 720 fluorometer/densitometer, which automatically integrated and calculated the percentage of each isoenzyme. With each gel we included a human-serum-based control containing all CK isoenzymes (Corning "3-in-1" electrophoresis control).

Immunoassays of CK-MB mass. CK-MB in the patient’s serum was also determined with two immunoassays: the QuiCK-MB (International Immunoassay Laboratories, Santa Clara, CA) and the Tandem-E CK-MB (Hybritech, Inc., San Diego, CA).

The QuiCK-MB assay (13) is a solid-phase, two-site, sandwich-type immunoradiometric method. First, the patient’s serum is mixed with antibody specific for the CK-B unit, which is bound to the solid phase. Then, the CK-BB and CK-MB that become attached to the solid phase are mixed with 125I-labeled antibody specific for the CK-M unit. We quantified the radioactivity bound with an Auto-gamma Scintillation Spectrometer (Model 5130; Packard Instrument Co., Rockville, MD) as a measure of the CK-MB in the patient’s serum.

The Tandem-E CK-MB assay (14) is a solid-phase, two-site, sandwich-type immunoenzymometric method. The patient’s serum is mixed simultaneously with monoclonal antibody specific for the CK-M unit, which is fixed to a plastic bead, and with monoclonal antibody specific for the CK-B unit, which is linked to alkaline phosphatase (EC 3.1.3.1). After separation of the solid phase, p-nitrophenyl phosphate (substrate) is added and the p-nitrophenol cleaved by the enzyme is quantified at 405 nm in the Photon immunoassay analyzer (Hybritech), reflecting the amount of CK-MB present in the patient’s serum.

Mixture of the patient’s serum with a human control serum. We also assayed by electrophoresis and incubation with substrate (as described above) mixtures of the patient’s serum and a human control serum—in 1/1 and 1/2 by vol proportions.

Addition of antisera specific for the CK-B unit or CK-M unit to the patient’s serum. Electrophoresis and incubation with substrate (as described above) was also done after we mixed both the patient’s serum and the electrophoresis control with antibody specific for either the CK-B unit or the CK-M unit (International Immunoassay Laboratories).

Immunofixation. After electrophoresis (described above), we applied rabbit anti-sera to human IgG, IgA, IgM, kappa, and lambda (Dako Corp., Santa Barbara, CA) directly to the gels for 5 min. The gels were then washed twice in saline, pressed dry (three times) with "Sta-Moist" Filter Paper (Corning), and incubated with the CK-isoenzyme substrate set for 20 min at 37 °C. We dried the gels at 50 °C, as described above.

Electrophoresis for serum proteins. For the 20-min electrophoresis of serum proteins we used "High-Resolution Protein Electrophoresis Film" agarose in Mops buffer (50 mmol/L, pH 7.3) containing calcium lactate (1 mmol/L) and N-(tris(hydroxymethyl)methyl)glycine (Tricine). We fixed the proteins with 50 mL acetic acid and stained with Acid Violet 17.

Quantification of serum immunoglobulins. We quantified serum immunoglobulins by fluorescent nephelometry in a Multistat III. The monospecific antisera for this procedure were obtained from International Immunology Corp., Murrieta, CA.

Results

The values of total serum CK in the patient’s serum samples we studied (collected on the sixth and tenth hospital days) were 478 and 458 U/L. Electrophoresis of both samples gave identical results and revealed two bands (Figure 1). The more anodal band (27.1% of total CK activity) migrated identically to CK-MB. The more cathodal band (72.9%) migrated between CK-MM and CK-MB. Neither band was visible when the same samples were incubated with the CK-isoenzyme substrate without creatine phosphate after electrophoresis; therefore, the bands represented creatine kinase activity.

The QuiCK-MB and Tandem-E CK-MB immunoassays demonstrated normal mass of CK-MB, inconsistent with a CK-MB band of 27.1% by electrophoresis. CK-MB by the QuiCK-MB was <2 EU/L (normal, <2.9 EU/L) (13), and CK-MB by the Tandem-E CK-MB was 2.2 μg/L (normal, <9 μg/L) (14). This inconsistency between the results of electrophoresis and the immunoassays prompted our further investigation.

Mixing the patient’s serum with human-serum-based control containing all CK isoenzymes completely restricted the electrophoretic mobility of the CK-BB in the control (Figure 1). The CK-BB region contained no fluorescence. The CK-MM of the control was unaffected by the patient’s serum.

Mixing antibody specific for the CK-B unit with the patient’s serum dramatically changed the electrophoretic pattern of the patient (Figure 2). The band migrating to the CK-MB region disappeared and much more fluorescence appeared at the origin. The more cathodal band persisted. Incubating the patient’s serum with antibody specific for the CK-M unit did not change the electrophoretic mobility of
Fig. 2. Electrophoretic patterns of (1) CK control (diluted threefold), (2) patient's serum (undiluted), (3) patient's serum mixed with antibody specific for CK-B unit, (4) patient's serum mixed with antibody specific for CK-M unit.

The more cathodal band is unaffected. The mobility of the anodal band is slowed by antibody specific for the CK-B unit, showing that this band contains CK-B either band. The antisera did alter the mobility of the isoenzymes in the CK control (data not shown).

Immunofixation of the patient's serum (Figure 3) revealed IgG kappa bound to the more cathodal band and IgA lambda bound to the band within the CK-MB region. There was no fixation of IgM.

Electrophoresis for serum proteins revealed two discrete, faint bands within the beta–gamma region. Immunoglobulin quantification demonstrated an above-normal concentration of IgA, 3750 mg/L (reference interval: 400–3500 mg/L), and normal values for IgG and IgM.

Discussion

This patient had excess antibody specific for the CK-B unit in her serum, as shown by mixing her serum with the CK control, which altered the electrophoretic mobility of only CK-BB, not CK-MM. The more anodal band that migrated to the CK-MB region was a CK–IgA lambda complex that consisted, at least in part, of CK-B unit. Although this complex could contain CK-MB, we think this possibility is not probable, for the following reasons: (a) CK-MB complexed to IgA lambda is unlikely to have electrophoretic mobility identical to CK-MB; (b) the electrophoretic mobility of this complex was not altered by antibody specific for the CK-M unit; and (c) no evidence suggests that the patient had antibody specific for the CK-M unit that would bind to and "mask" the M antigenic sites of this complex. Instead, one can reasonably conclude that the more anodal band was a complex of CK-BB and IgA lambda. Although CK-BB complexed to IgA is distinctly unusual, one similar example has been reported (4).

The more cathodal band in this patient's serum was also creatine kinase, complexed with IgG kappa. However, we have been unable to determine definitively which isoenzyme of CK was bound to the IgG kappa; antibodies specific for the CK-B and CK-M units did not affect the electrophoretic mobility of this complex. We cannot exclude that this band represented mitochondrial CK–IgM complex, but mitochondrial CK usually migrates cathodal to the point of application. We instead hypothesize that this CK was CK-BB, for the following reasons: (a) the patient had antibody specific for the CK-B unit in her serum; (b) the electrophoretic mobility of the cathodal band was unaffected by antibody specific for the CK-M unit; and (c) there was no evidence of excess antibody specific for the CK-M unit in the patient's serum. Although the more cathodal band could have contained CK-MM or CK-MB, it seems unlikely that the patient would have had antibody specific for the CK-M unit in enough quantity to "mask" all of the M antigenic sites but not affect the CK control. However, this hypothesis does not explain why the antibody specific for the CK-B unit did not alter the electrophoretic mobility of the cathodal band. Perhaps the IgG kappa antibody has greater affinity for CK-B unit than the IgA lambda antibody such that the IgG kappa "masked" the antigenic sites of the CK-BB in the cathodal band [similar "masking" of CK within macro CK type 1 has been described by Kanemitsu et al. (5)]. We conclude that the hypothesis that CK-BB is the isoenzyme bound to both the IgG kappa and IgA lambda is the only one consistent with all the data, thereby indirectly proving its identity.

Although uncommon, similar patients have been described. Jockers-Wretou and Plessing (4) described 12 patients with macro CK type 1; one patient's serum contained a CK-BB–IgA complex that migrated to the CK-MB region. Bohnet et al. (2) described two patients' sera with similar electrophoretic patterns: both contained two types of macro CK type 1—one migrating between the CK-MM and CK-MB regions, the second migrating to the CK-MB region.

This case illustrates one of the disadvantages of using electrophoresis to quantify CK isoenzymes: because electrophoretic mobility is nonspecific, fluorescence in the MB region may not be due to CK-MB activity. If electrophoresis were the sole method for quantifying CK isoenzymes, patients such as the one we describe and others in the literature would have been mistakenly considered to have CK-MB. Furthermore, the isoenzyme bands in these patients came to the attention of the investigators—i.e., the bands were persistent—whereas Sax et al. (8) have described atypical CK forms that appear and disappear over a few days. Perhaps additional cases of (transient) macro CK type 1 have been misinterpreted as CK-MB.

Our results also suggest that measuring CK-MB mass by immunoassay is helpful in patients with macro CK type 1.
This use of antibodies against the B and M units of CK allows specific quantification of the mass of CK-MB. During the past few months, we have used QuiCK-MB and Tandem-E CK-MB assays with four patients whose sera contained macro CK type 1 and with two patients with macro CK type 2. None of these sera contained CK-MB, and all gave negative results for these immunoaassays.

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References