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Review

Electrophoretic analysis of enzyme-linked immunoglobulins and their clinical significance

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ABSTRACT

The appearance of circulating enzyme-linked immunoglobulin complexes (E–Ig) is common for most enzymes used in clinical biochemical tests. The presence of E–Ig may result in altered enzyme activity in serum and interfere with the measurement of isoenzymes, and is thus of diagnostic importance. E–Ig can be identified by confirming that the binding protein is an immunoglobulin by its reaction with specific anti-human immunoglobulin antibodies. Currently, the presence of E–Ig in an individual is regarded as a benign phenomenon, not indicative of any particular disease process. However, it is becoming clear that E–IgG are closely associated with autoimmune states.

CONTENTS

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMYL	Amylase
AST	Aspartate aminotransferase
CA	Cellulose acetate
CK	Creatine kinase
E–Ig	Enzyme-linked immunoglobulin complexes
Ig	Immunoglobulin
LD	Lactate dehydrogenase

1. INTRODUCTION

A biochemical alteration ascribed to the complexing of normal amylase (AMYL, EC 3.2.1.1) and immunoglobulin (Ig) in the circulation was first demonstrated as a cause of hyperamylasemia by Wilding *et al.* in 1964 [1]. Similar types of complexes occur with lactate dehydrogenase (LD, EC 1.1.1.27) [2], alkaline phosphatase (ALP, EC 3.1.3.1) [3], aspartate aminotransferase (AST, EC 2.6.1.1) [4], alanine aminotransferase (ALT, EC 2.6.1.2) [5], creatine kinase (CK, EC 2.7.3.2) [6], acid phosphatase (EC 3.1.3.2) [7], leucine aminopeptidase (EC 3.4.11.1) [8], lipase (EC 3.1.1.3) [9] and elastase (EC 3.4.21.11) [10]. These complexes are called enzyme-linked Ig complexes (E–Ig). AMYL–Ig is included in macro-AMYL but they are not identical. The naturally occurring type is an AMYL–Ig. A second type is produced by interaction of AMYL with hydroxyethyl starch following infusion of the latter compound into the circulation [11–14]. Macro-CK also occurs in two forms: type 1 is a CK–Ig and type 2 is an oligomeric mitochrondrial CK [15,16].

The understanding of E–Ig is important in the evaluation of biochemical results. E–Ig is generally catalytically active, but rarely inactive. E–Ig may cause a reduced rate of clearance of enzyme from serum. Consequently, E–Ig may lead to a high enzyme level in serum and thus interfere with measurements of isoenzymes. Thus, poor understanding of E–Ig may lead to misdiagnosis and unnecessary diagnostic tests [11,12,16–19]. The presence of E–Ig can be confirmed by two principal methods, namely identification of the binding Ig in E–Ig and detection of the macromolecular mass of an enzyme due to complex formation. No common pathological conditions are associated with the presence of any E–Ig; ac-

cordingly the presence of E–Ig in a patient is regarded as a bening phenomenon at this time [11,17,19]. So far, many immunological studies on E–Ig have shown that the Ig of E–Ig may be an autoantibody to the enzyme, however, its pathological role is not clear.

This paper reviews the detection, identification, and clinical significance of E–Ig, with emphasis on the electrophoretic analyses.

2. CLINICAL LABORATORY FINDINGS INDICATING POSSIBLE PRESENCE OF ENZYME-LINKED IMMUNOGLOBULINS

2.1. Total enzyme levels in serum

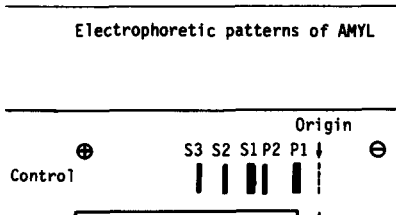
Patients with E–Ig often have a persistently increased enzyme level in their serum that is not associated with clinical signs and symptoms. The mechanism of the increased enzyme activity are understood to be as follows: for AMYL–Ig, a macromolecular form of serum AMYL precludes its excretion in the urine and results in the accumulation of AMYL in the serum; for the other E–Igs it has been proposed that the antibody binding to the enzyme interferes with the reticuloendothelial system clearance mechanism [11,19,20]. However, an increased level of serum enzyme is insensitive and is not specific for diagnosing E–Ig, because it has been noted that many patients with E–Ig have normal levels of total enzyme in their serum [11,16,19]. There have even been reports of some patients with LD–Ig and decreased LD activity in their serum [17,21–23].

2.2. Isoenzyme electrophoretic patterns in serum

Isoenzyme electrophoresis is a good predictor of the presence of E–Ig. Igs capable of complexing with isoenzymes can alter the electrophoretic migration of normal isoenzymes, and E–Ig results in anomalous isoenzyme electrophoretic patterns on various types of supports such as agar, agarose and cellulose acetate (CA). Minor variations in electrophoretic patterns caused by E–Ig depending on electrophoretic conditions and supports may be present.

2.2.1. Amylase

AMYL can be separated into two principal isoenzymes, pancreatic and salivary AMYL, and several isoforms [13]. On electrophoresis AMYL–Ig migrates as an atypical band. The band is characteristically broad, but in some cases it is seen as a relatively sharp band [24–34]. Forsman [33] classified 249 specimens of macro-AMYL into three groups depending on the location of the atypical bands on agarose (Special Purpose Agarose Film, Corning ACI, Palo Alto, CA, USA) electrophoresis using 0.05 *M* phosphate buffer (pH 6.7): (1) cathodal bands faster than pancreatic AMYL (10%), (2) cathodal bands between pancreatic and salivary AMYL (30%) and (3) anodal bands (60%). We classified 127 specimens of



Electrophoretic patterns of AMYL	Classes of immunoglobulin in AMYL-Ig			
	IgA	IgG+ IgA	IgG+IgA+ IgM	IgG
A	64	5	0	0
B	18	1	0	0
C	11	1	0	1
D	1	0	0	3
E	0	0	0	4
F	0	1	0	3
G	0	0	1	7
H	0	0	1	5
Total	94	8	2	23

Fig. 1. Diagrammatic representation of different migrations of atypical AMYL bands on CA (TITAN III Lipo plate) electrophoretic patterns of 127 specimens of AMYL–Ig, and number of cases in every class(es) of the binding Ig(s) for each migration. P = pancreatic AMYL. S = salivary AMYL. Origin = serum application point. Control shows typical AMYL bands. Open rectangles express atypical AMYL bands: A, anodal band faster than pancreatic and salivary AMYL; B and C, anodal bands faster than salivary AMYL; D and E, anodal bands between pancreatic and salivary AMYL; F, G and H, cathodal and anodal bands directly extended from serum application point.

AMYL–Ig into eight groups and four further general groups depending on the location of the atypical bands on CA (TITAN III Lipo plate, Helena Labs., Beaumont, TX, USA) electrophoresis using 0.34 *M* tris-glycine (pH 8.9–9.3) as anodal buffer and 0.05 *M* barbital–sodium–boric acid (pH 8.7–9.1) as cathodal buffer (Helena Labs.), as shown in Fig. 1; (1) anodal bands faster than pancreatic and salivary AMYL (55%), (2) anodal bands faster than salivary AMYL (25%), (3) anodal bands between pancreatic and salivary AMYL (6%) and (4) cathodal and anodal bands directly extended from the sample application point (14%). AMYL–IgA samples were found in group 1 or 2, and AMYL–IgG samples were in group 3 or 4. In this electrophoretic system the direction of typical AMYLs was anodal, as shown in Fig. 1, while in that used by Forsman [33] it was cathodal, with the pancreatic AMYL and the salivary AMYL being the faster- and the slower-migrating bands, respectively. Accordingly groups 1 + 2, 3 and 4 by our classification correspond, respectively, to groups 3, 2 and 1 in Forsman's classification.

2.2.2. Lactate dehydrogenase

LD isoenzymes are demonstrated as five equally spaced bands. LD1 migrates most anodally, and LD2, LD3, LD4 and LD5 migrate less anodally in that order. The presence of LD–Ig causes various anomalous patterns characterized by additional bands of LD, broadening of bands, smearing of LD activity between the usual bands, alteration of the position of usual bands and/or alteration of the intensity of the usual bands [2,35–59], as shown in Fig. 2. The patterns characteristic of LD–IgA-κ in serum show one or more abnormalities around LD3, together with normally migrating LD1 and LD5, while there are no patterns characteristic of the other classes of Ig in LD–Ig. With regard to the anomalous patterns for patients with catalytically inactive LD–Ig, two different types have been reported. The first displays unusually decreasing activity of LD isoenzymes

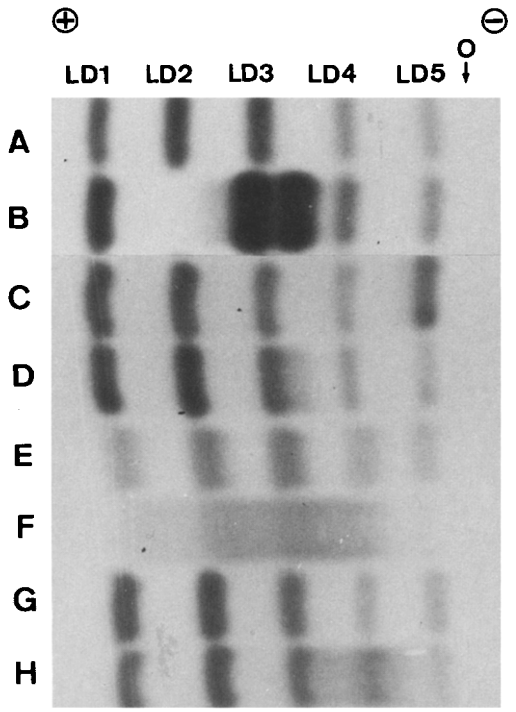


Fig. 2. Some examples of different anomalous LD electrophoretic patterns characteristic of LD–Ig in serum. Electrophoresis was performed on TITAN III Lipo plates, and LD was stained with tetrazolium. A, C and G, serum from subjects with the typical five LD bands; B, serum containing LD–IgA-κ shows two atypical bands, one slightly anodal to LD3 and one between LD3 and LD4, together with absence of LD2; D, serum containing LD–IgA-κ shows an anomalous band cathodal to LD3; E, serum containing LD–IgG-κ shows five diffuse broad bands with anomalous migrations; F, serum containing LD–IgK-κ,λ shows a diffuse broad band, spread over which typical LD isoenzymes migrate; H, serum containing LD–IgG-κ,λ shows smearing of LD activity between LD3 and LD5.

containing the LD-M subunit [60,61]. The second displays only one band, which is located at the site of the sample application point [62,63] or around LD4 [21–23].

2.2.3. Alkaline phosphatase

ALP isoenzymes in serum are derived from liver, bone, intestine and placenta. Of these, the isoenzymes of liver and bone origin appear commonly in serum. ALP–Ig is manifested as a slow-moving band on polyacrylamide gel electrophoresis [3,64–69], while the band can often be confused with intestinal ALP on CA and agarose electrophoresis. Most ALP–IgG show a broader band at the position of intestinal ALP [3,65,66,70,71], and rarely between intestinal and placental ALP [72]. The broader band and the reduction in both liver and bone ALP indicate the presence of ALP–IgG. ALP–IgA migrates slightly more slowly than bone ALP [69] or close to intestinal ALP [68], and the mobilities make the detection of ALP–IgA difficult.

2.2.4. Aspartate aminotransferase

AST has two isoenzymes. Cytoplasmic AST migrates to the α_2-globulin region and mitochondrial AST to the slow γ-globulin region. Only the former appears commonly in serum. AST–IgG migrates as an atypical band between the two isoenzymes [4,52,72–78], and AST–IgA migrates slightly cathodally to the cytoplasmic AST [79].

2.2.5. Creatine kinase

CK in intracellular fluid has three isoenzymes termed BB, MB, MM. CK-BB migrates to the prealbumin region, CK-MB to the α_2-globulin region and CK-MM to the fast γ-globulin region. Mitochondrial CK, known as macro-CK type 2, migrates cathodally to CK-MM [80]. Of these, only CK-MM appears commonly in serum. CK-BB–IgG migrates as an atypical band between CK-MM and CK-MB, closer to CK-MM than CK-MB [6,12,80–87]. CK-BB–IgA migrates slightly cathodally [12,83,86] or identical [86,88] to CK-MB. Mitochondrial CK–IgA migrates between CK-MM and the sample application point [89]. CK-MM–IgA migrates slightly anodally to CK-MM on CA electrophoresis [90,91], or cathodally to CK-MM on agarose [92] and agar [93] electrophoresis. The CK-BB–IgA that co-migrates with CK-MB cannot be distinguished from it by position alone. A persistent band in the CK-MB region may be due to CK-BB–IgA [88,94,95].

When the presence of CK-BB–Ig is suspected, an immunoinhibition test for measuring CK-MB is available. The test depends on immunological inhibition of the CK-M subunit and measurement of residual CK-B subunit activity. Accordingly, CK-BB–Ig results in an unusually high ratio of CK-MB to total CK activity (>20–25%), as does macro-CK type 2 also [12,19,81,82,84,96–101].

3. METHODS FOR IDENTIFICATION

The technique commonly used to identify E–Ig is precipitin reactions using specific antisera against human Ig. E–Ig is finally identified by determination of either precipitated enzyme or non-precipitated enzyme. The precipitated enzyme is mostly visualized by colorimetric techniques. The visualization is performed by the same procedure used to vizualize isoenzymes separated by electrophoresis, except that the incubation time is longer. Electrophoresis may be carried out on agar, agarose and CA. An advantage of CA is that non-precipitated enzyme can be eluted in a short time. An advantage of agar/agarose over CA is that the precipitates can be visualized without staining. The non-precipitated enzyme is determined by measuring the acitivity of enzyme or by electrophoretic separation of isoenzymes in supernatants of the liquid phase reactions. The use of antiserum containing activity of the enzyme in E–Ig can lead to misinterpretation of the enzyme results. High AMYL activities are often detected, even in commercially available antibody solutions. The AMYL can be removed by gel permeation. In regard to sensitivity, liquid-phase reaction is more sensitive, because a larger amount of precipitate can be obtained. Moreover, electrophoretic techniques are insensitive for identification of AMYL–Ig and LD–Ig, because both frequently dissociate during an electrophoretic run [36,102]. Actually, the frequency of each E–Ig with immunoelectrophoresis in specimens which were found to be positive for the E–Ig by immunoprecipitin reaction in free liquid media was as follows: 97% for ALP–Ig, 88% for CK–Ig, 72% for AMYL–Ig and 55% for LD–Ig. All LD–IgA-κ was identified by immunoelectrophoresis, but the other LD–Igs were not.

The presence of IgG in E–Ig can be verified by absorption onto immobilized protein A or protein G [58,103], and that of IgA in E–Ig can be verified by adsorption onto immobilized jacalin [58]. Protein A and protein G are bacterial proteins that specially bind to IgG by a non-immune mechanism [104], and jacalin is a lectin derived from jackfruit seeds that binds to IgA1 and IgD by a non-immune mechanism [105].

3.1. Immunoelectrophoresis

Since 1970, beginning with the study of Biewenga and Thijs [37], the technique described by Scheidegger [106] has been used for identification of E–Ig. About 4 μl of serum are electrophoresed on a 1–2% agar/agarose gel plate in barbital buffer (μ = 0.06, pH 8.2–8.6) for 45–60 min at a constant current of 1–2 mA/cm [50,58], and each antiserum is loaded into the troughs as shown in Fig. 3. The gel is incubated in a moist chamber for 20 h at 4°C, washed in several changes of saline over two days and stained for enzyme.

The same method but using CA has also often been used to identify AMYL–Ig [34]. A 2-μl sample of serum is electrophoresed on CA membrane in barbital

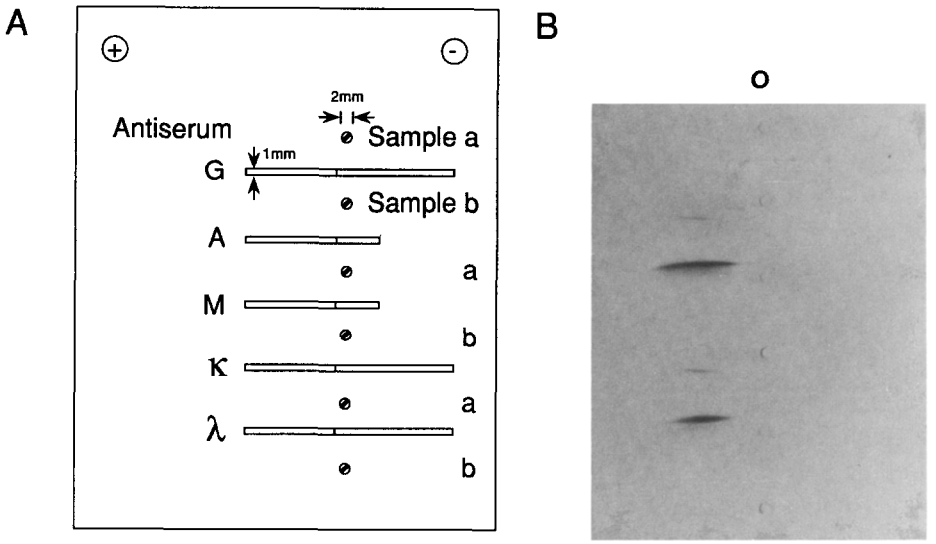


Fig. 3. (A) Configuration for immunoelectrophoresis on an agar–agarose gel plate. (B) Result of immunoelectrophoresis study of LD–Ig. LD was stained with tetrazolium. The LD activity on the precipitations indicates the Ig in either serum (a) or serum (b) to be IgA-κ.

buffer (μ = 0.06, pH 8.6) for 30 min with a 1 mA/cm current, and then filter paper strips (1–2 mm wide) impregnated with each antiserum are placed on the membrane according to the same pattern as for immunoelectrophoresis in gel. After completion of the diffusion, the membrane is washed in several changes of saline with agitation overnight, and then stained. One disadvantage of the method is that a longer incubation time for the diffusion is needed. This method has been used for identification of LD–Ig [20,37,46,48,50,53,58,107], ALP–Ig [3,64,66–68], AMYL–Ig [25,26,34,102,108–110], CK–Ig [83,92] and AST–Ig [73,76].

3.2. Immunofixation electrophoresis

In 1979, the following technique [111] was introduced by Sudo *et al.* [27] for identification of E–Ig. A 4-μl sample of serum is electrophoresed on CA (Cellogel, Chemetron, Milan, Italy) in barbital buffer (μ = 0.06, pH 8.6) for 45 min with a 5 mA/cm current. After the electrophoresis, CA strips (0.7 × 5 cm) soaked in each antiserum are placed on the Cellogel to cover the zones occupied by Igs and incubated for 1 h at 27°C. The Cellogel is then washed in the optimal buffer with agitation overnight at 4°C and stained for enzyme. This method using agarose gel is also successful (Fig. 4). The success of the method depends on the optimum content of fixed precipitate; accordingly concentrated commercially available antisera are often necessary. This method equals immunoelectrophoresis in sensitiv-

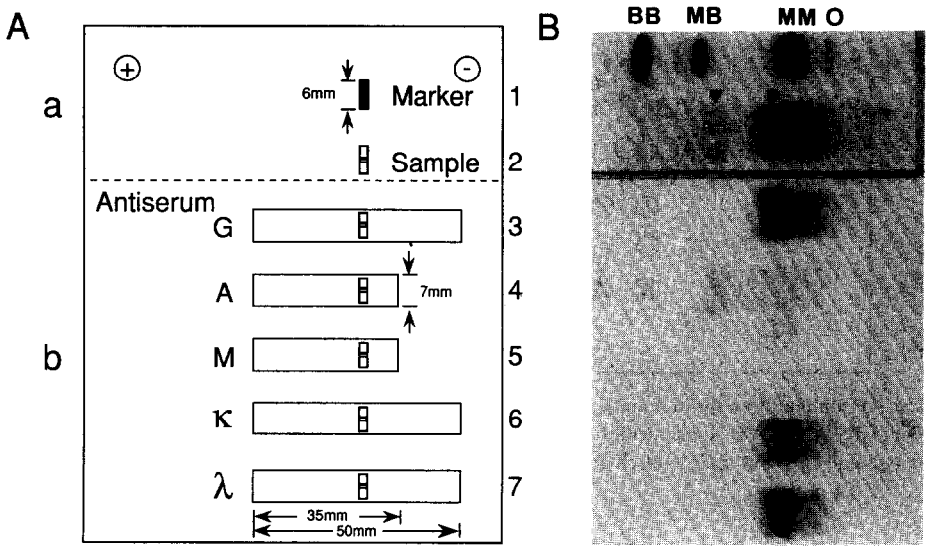


Fig. 4. (A) Configuration for immunofixation electrophoresis on an agarose film. Electrophoresis is performed in barbital buffer (μ = 0.06, pH 8.6) at 90 V for 60 min under an ice pack, and then the film is cut out according to the dashed line in diagram. The segment (a) of the film is stained for enzyme. The CA strips are impregnated with each antiserum and placed on the gel surface of the segment (b) as in the diagram. After incubation, the CA strips are removed and discarded. The segment is directly overlaid with an adsorbent pad for 3 min, soaked in the buffer for 5 min, overlaid with another adsorbent pad once more and stained for enzyme. (B) Result of immunofixation electrophoresis study of CK–Ig. Electrophoresis was performed on Special Purpose Electrophoresis Film (Ciba Corning, Palo Alto, CA, USA) and CK was stained with tetrazolium. The CK activity on the fixed Igs corresponding to the bands (▼) and (●) enables the identification of CK–IgA and CK–IgG-κ,λ, respectively. O = Serum application point.

ity. The method has been used for identification of CK–Ig [8,52,86,89,90,92], LD–Ig [8,27,47,49,55,56], AMYL–Ig [8,27–29, 34], ALP–Ig [8,27,69,71,72] and AST–Ig [52,76].

3.3. Immunoelectrosyneresis

This technique [112] was used first in 1983 for identification of E–Ig by Nagamine and Okochi [74]. Slit-shaped wells in three parallel rows are prepared in a 0.8% agar–agarose (3:5, w/w) gel plate containing 0.3% polyvinylpyrrolidone and 5 g of sucrose. The anodal and cathodal wells are 10 and 8 mm from the central wells, respectively. An 8-μl sample of serum is applied to the central well, and 12 μl of each antiserum are applied to the anodal and cathodal wells. For anti-IgA and anti-IgM, application to the cathodal well is not necessary.

Electrophoresis is performed in 0.025 *M* sodium pyrophosphate–0.01 *M* barbital buffer (pH 8.7) at 105 V for 60 min. After the electrophoretic run, a filter paper is moistened with the same buffer and applied to the entire gel plate. This is

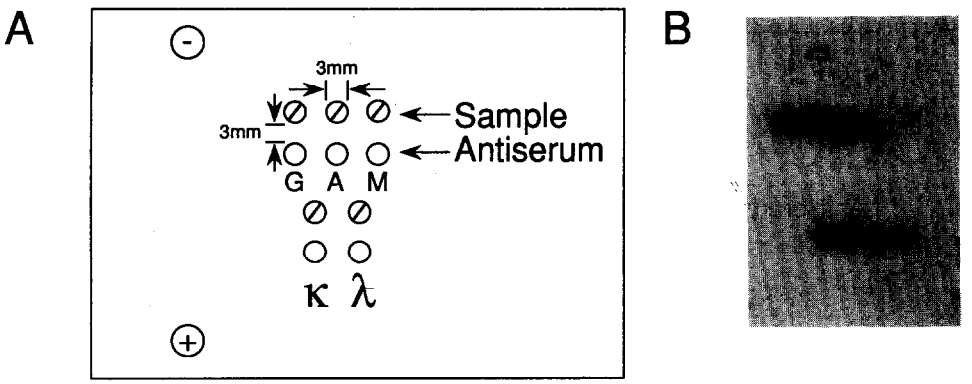


Fig. 5. (A) Configuration for immunoelectrosyneresis as used by us. (B) Result of immunoelectrosyneresis study of CK–Ig. CK was stained with tetrazolium. The CK activity on the precipitations indicates the Igs to be IgG and IgA, but their light-chain types cannot be determined.

overlaid with three adsorbent pads (Nihonshoji, Osaka, Japan) and loaded with a weight of 3 kg for 15 min. The procedure is repeated once more, and the thinned gel is stained for enzyme. In this electrophoretic system, the serum application position is located in the middle of the γ-globulin region, and precipitation lines of IgG can be formed on the anodic and cathodic sites of the central well.

We [113] have used a 1.2% agar–agarose (1.4:4.6, w/w) gel plate and another pattern of wells, as shown in Fig. 5, for identification of ALP–Ig and CK–Ig. A 10-μl sample of serum is applied to the cathodal well and 10 μl of antiserum to the anodal well. Electrophoresis is performed in barbital buffer (μ = 0.06, pH 8.6) at 2 mA/cm for 60 min. In this electrophoretic system, serum is applied in the γ-globulin region one third of the way from its anodal end, but all of ALP–IGs can be identified. Every ALP–IgG migrates anodally, even if the binding IgG migrates in the most cathodic region. The sensitivity of this method is higher than that of either immunoelectrophoresis or immunofixation electrophoresis.

Horii and Kano [114] have used another modification. Their procedure is the same as for isoenzyme electrophoresis except for the application of antisera, as shown in Fig. 6. To obtain a sufficient intensity of the precipitations, concentrated antiserum is often needed. The advantages of this method are its rapidity and simplicity.

3.4. Immunoprecipitin reactions in free liquid media

The first AMYL–Ig [115] was identified in 1968 by using a very simple technique, which consists of constructing a "precipitin curve" based on the principle decribed by Heiderberger and Kendall [116]. Increasing volumes of serum are incubated with a constant volume of antiserum overnight at 4°C. The quantitiy of

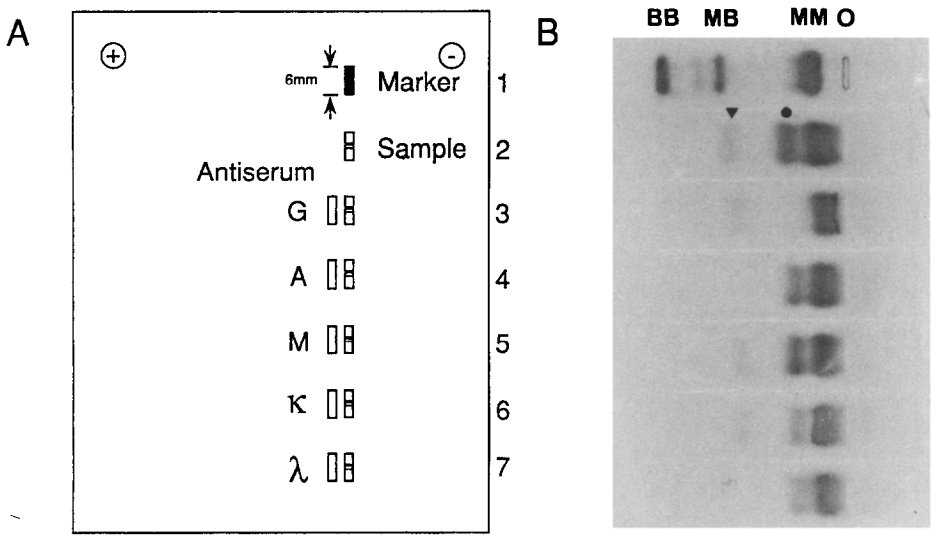


Fig. 6. (A) Configuration for immunoelectrosyneresis as used by Horii and Kano [114]. (B) Identification of CK–Ig. Serum (1 μl) and antisera (3 μl) were applied on an agarose film (Special Purpose Electrophoresis Film) as in the diagram, electrophoresed in barbital buffer (μ = 0.06, pH 8.6) at 90 V for 60 min under an ice pack, and stained with tetrazolium. Anti-IgG abolished the band (●), and anti-κ chains decreased the intensity of the band (●). This indicates the Ig giving the band (●) to be IgG-κ,λ. Anti-IgA and anti-λ chains abolished the band (▼). This indicates the Ig giving the band (▼) to be IgA-λ. O = Serum application point.

enzyme activity removed with the precipitate is determined by measuring the activity remaining in the supernatants. This early method requires a large amount of antiserum. Since 1979, the appropriate ratio of antiserum to serum has been incubated, and then saline added to give an equal final volume of the mixtures [6,17]. In another method [117], an excess of antiserum is incubated with serum and the complexes formed in the mixture are precipitated by incubation with 10% polyethylene glycol. E–Ig is finally identified by measuring enzyme activity or by electrophoretic analysis of isoenzymes in supernatants. The sensitivity of the methods is insufficient because of the dilution of the enzyme activity. CK–Ig [6,12,47,85,88,117], LD–Ig [47,50,54,59] and AST–Ig [4,75] have been identified by these methods.

Since 1982 we have identified E–Ig by measuring enzyme activity in the precipitates. A 25-μl sample of serum is mixed with an equivalent amount of each antiserum for 3 h at 4°C. After removing the supernatant by centrifugation, the precipitate is resuspended in 3 ml of 0.06 *M* phosphate buffer (pH 7.4). This cycle is repeated three times, and then 0.5 ml of the staining reagent for the enzyme are mixed with the last precipitate for 2 h at 37°C and centrifuged. The enzyme activity is determined by measuring absorbance in the supernatants of the mix-

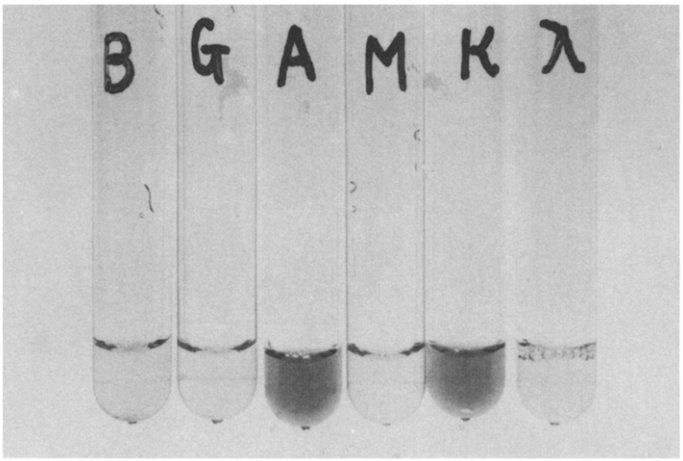


Fig. 7. Identification of AMYL–Ig by our method using immunoprecipitin reaction in free liquid media. Precipitate of Igs was obtained by adding an equivalent amount of anti-IgG (G), anti-IgA (A), anti-IgM (M), anti-κ chains (κ) or anti-λ chains (λ) (Dakopatts, Glostrup, Denmark) to serum (25 μl). AMYL was stained with blue starch suspension. From the AMYL activity in the tubes, it is clear that the Ig is IgA-κ. B = Reagent blank tube.

tures (Fig. 7). Significant advantages of the method are its highest sensitivity and reliability. The method is recommended for the identification of AMYL–Ig and LD–Ig [113]. Recently, elastase 1–Ig was identified by this method using radiolabeled enzyme [10]. Serum is preincubated with the radiolabeled enzyme and mixed with an equivalent amount of each antiserum. E–Ig is finally identified by determination of the radioactivity in the precipitates. By using a method essentially the same as this, ALT–Ig [5] and CK-BB–Ig [118] have been identified.

3.5. Others

One-dimensional electroimmunodiffusion [119] was used for identification of the first LD–Ig [2] in 1967. Double immunodiffusion [120] was used for identification of CK–Ig [80–83] and AMYL–Ig [28,108]. The techniques have not been used for identification of E–Ig during the last ten years. A significant disadvantage of the former technique is that it is troublesome to prepare the gels containing each antiserum. A disadvantage of the latter technique is that it is not sufficiently sensitive.

4. FREQUENCIES

The data reported so far on the frequency of occurrence of E–Ig in patients and healthy individuals are shown in Table 1. Higher frequencies are found in particular disease categories and lower frequencies in healthy populations. The frequencies in patients are widely distributed, while those in healthy populations are similar. The different results among patients may be due to the differences in

TABLE 1

FREQUENCIES OF ENZYME-LINKED IMMUNOGLOBULINS

E–Ig	Reference	Method[a]		Frequency (%)	Population
		Screening	Identification		
AMYL–Ig	29	EP and TLG	IFE	0.78	4826 Mixed patients: 4665 patients slected for assay of isoAMYL and 181 patients with hyperAMYLemia
	113	EP	IPR	0.18	44 985 Patients selected at random
				0.03	10 020 Healthy subjects
MacroAMYL	121	MiniCG		1.1	891 Mixed population: 440 patients selected at random, 251 normal subjects and 200 newborns
	122	TLD	CG	5.9	51 Patients with hyperAMYLemia
	123	TLG	MiniCG	1.5	1052 Mixed patients: 932 patients selected at random and 120 patients with diabetes mellitus
	124	MiniCG		1.6	190 Patients with hyperAMYLemia
	29	EP	TLG	0.85	4826 Mixed patients: 4665 patients selected for assay of isoAMYL and 181 patients with hyperAMYLemia
	125	PEGP	MiniCG	4.5	66 Patients with hyperAMYLemia
	126	PEGP	TLG	2.7	74 Patients with hyperAMYLemia
	33	EP	CG	9.6	2900 Specimens for assay of isoAMYl
LD–Ig	43	EP	IEP	1.0	100 Patients with rheumatic arthritis
				0.0	19 Patients with lupus erythematosus
	46	EP	IEP	10.0	20 Patients with ulcerative colitis
				0.0	25 Patients with Crohn's disease
				0.0	104 Patients with other types of intestinal deseases
	127	EP	IFE and IES	0.15	10 000 Normal population
	107	EP	IEP	0.03	21 800 Patients selected at random
	113	EP	IPR	0.32	48 829 Patients selected at random
				0.15	10 020 Healthy subjects
ALP–Ig	64	EP	IEP	0.4	2000 patients selected for assay of ALP isoenzymes
	65	EP	EID	0.1	3000 patients selected for assay of ALP isoenzymes
	66	EP	IEP	1.0	500 Patients selected for assay of ALP isoenzymes
	46	EP	IEP	15.0	20 Patients with ulcerative colitis
				0.0	25 Patients with Crohn's disease
				0.0	104 Patients with other types of intestinal disease
				0.1	25 000 Patients selected at random

(Continued on p. 360

TABLE 1 *(continued)*

E–Ig	Reference	Method[a]		Frequency (%)	Population
		Screening	Identification		
	128	EP	IES	0.61	4289 Hospitalized patients
				0.03	3015 Healthy subjects
	113	IES	IES	0.26	48 829 Patients selected at random
				0.03	10 020 Healthy subjects
AST–Ig	78	EP	IES	0.0	798 Healthy subjects
	79	EP	IPR and IES	13.1	260 Patients selected for high serum AST activity and a high AST/ALT ratio
CK–Ig	6	EP	IPR	1.6	310 Patients selected for assay of enzymes
	92	EP	IFE	0.8	356 Patients selected at random
	93	EP	IEP and IFE	0.9	550 Patients selected for assay of CK isoenzymes
	80	EP	REP	1.2	1253 Patients selected at random
	84	EP	Protein A	0.8	1686 Patients with acute myocardial infarction
	129	EP	Protein A	0.4	2345 Patients undergoing cardiac surgery
	130	Ion-ex. CG and EP	Protein A	4.3	3000 Patients selected at random
				13.8	556 Patients with angina pectoris
	89	EP	IFE	11.5	234 Patients with malignant diseases
				0.0	121 Healthy subjects
				0.9	215 Patients with benign deseases
	113	IES	IES	0.61	42 068 Patients selected at random
				0.23	10 020 Healthy subjects

[a] EP = electrophoresis; TLG = thin-layer gel permeation; MiniGC = minicolumn gel permeation; CG = standard column gel permeation; PEGP = polyethylene glycol precipitation; IFE = immunofixation electrophoresis; IPR = immunoprecipitin reaction in free liquid media; IEP = immunoelectrophoresis; IES = immunoelectrosyneresis; Ion-ex. CG = ion-exchange gel permeation; EID = one-dimensional electroimmunodiffusion; REP = radio-electrophoresis; Protein A = absorption to protein A–Sepharose.

disease and age distribution of the screened subjects. In regard to the age distribution, the incidence of every E–Ig is age-related, generally increasing with age [113,128,130]

5. CLINICAL SIGNIFICANCE

As yet there is no causative association between any common disease and the

presence of any E–Ig, but there are some diseases in which E–Ig are found more often. This indicates that E–IgG may be formed as a result of autoimmune reactions, but some E–IgA may not.

5.1. Ulcerative colitis

Autoimmune phenomena may be involved in the etiology of ulcerative colitis [131]. Leroux-Roels *et al.* [46] detected ALP–IgG in three out of twenty patients with this disease, and LD–Ig in two others. We measured four different E–Igs in a group of 59 patients with this disease and found the frequency of each E–Ig to be as follows: 29% for ALP–Ig, 19% for LD–Ig, 12% for CK–Ig and 2% for AMYL–Ig. The frequency of co-occurrence of E–Ig with different enzymes was 16% in the E–Ig-positive cases. Regardless of the binding enzyme, except for AMYL, the class of the Ig in E–Ig was IgG. The concentration of E–IgG in the patients fluctuated with the disease activity, and the E–IgG in some cases disappeared as their clinical states improved. The persistence of ALP–IgG, even after proctocolectomy, indicates that the inflammatory reaction in the bowel wall may be not the sole cause of the occurrence of ALP–IgG [46].

5.2. Coronary heart disease

Delanghe *et al.* [130] detected CK-BB–IgG in 13.8% of 556 patients with coronary heart disease. This frequency is significantly higher than that found in reference hospital patients ($p<0.01$). They concluded that CK-BB–IgG in the patients may have resulted from an autoimmune reaction to vascular wall CK-BB.

5.3. Malignant neoplasms

Kanemitsu [89] detected mitochondrial CK–IgA in 11.5% of 234 patients with malignant tumors. This frequency is significantly higher than that found in either patients with benign diseases ($p<0.01$) or healthy adults ($p<0.001$). They also detected CK-MM–IgA in 3.8% of 127 other patients with cancer [92]. The occurrence of CK–IgA in patients with malignant tumors appears to be a poor prognostic sign [89,92], but the interaction between CK–IgA and malignant tumors is obscure.

5.4. Progressive muscular dystrophy

CK–IgA has been detected in 44% of 61 patients with progressive muscular dystrophy. Serum total CK levels in the positive cases were significantly higher than those in the negative cases ($p<0.05$) [132]. Itagaki and Nishitani [90] detected CK–IgA in eight patients with this disease and in ten patients with an increased serum level of CK suffering from other neuromuscular diseases. CK–IgA

in patients with neuromuscular diseases is thought to be formed by non-specific reactions when there are increased serum levels of CK [90,132].

The occurrence of CK–IgA in this situation has also been observed in patients with either acute myocardial infarction or severe accidental injuries [113]. These observations suggest that some CK–IgAs, but not all, are formed by non-specific protein–protein reaction when serum CK activity is high.

5.5. Some considerations of clinical significance (conclusions)

There is no reason to doubt that most E–Ig complexes form as the result of a specific reaction between circulating autoantibodies and serum enzymes. However, why the antibodies form is not fully understood. The various explanations that have been proposed will not be mentioned here, and the reader is referred to the review by Remaley and Wilding [19] for details.

There is no evidence that the antibodies cause disease, therefore they probably are not pathogenic. Nevertheless, some clinical observations of E–Ig are noteworthy. The frequency of E–Ig has been ovserved to be age-related, generally increasing with age. The frequency of autoimmune diseases in subjects with E–IgG has been noted to be relatively high, but this is not the case with E–IgA. Many patients with several types of E–Ig, particularly E–IgG have pathophysiological conditions that are characterized by autoimmune diseases or ageing or both [113]. These observations suggest that autoimmune diseases or ageing are common pathophysiological conditions for the occurrence of E–IgG. The close relation between ulcerative colitis and E–IgG confirms autoimmune diseases as the common pathophysiological condition for the occurrence of E–IgG. Many well defined autoantibodies are found more commonly in asymptomatic aged than in young individuals, suggesting a breakdown of self-tolerance with ageing [133]. Consequently, the presence of E–IgG in individuals may reflect autoimmune states, and the potential for the development of pathogenic autoantibodies may exist in individuals with E–IgG. Either patients with E–IgG suffering from diseases without any known relationship to autoimmunity or apparently healthy individuals with E–IgG need to be studied for many years to determine if they have any clinical or serological abnormalities of autoimmune diseases in common.

In contrast, no specific diseases have been associated with the occurrence of E–IgA, however, it has been suggested that some CK–IgA, but not all, are formed by non-specific protein–protein interaction when serum CK activity is high. Further study on E–IgA formation and clinical states in subjects with E–IgA is necessary to understand the clinical significance of E–IgA.

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