Comparison of molecular weight of two cases of alkaline phosphatase-immunoglobulin G complexes in relation to complement fragment C3c

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INTRODUCTION

Nagamine and Ohkuma reported the presence of alkaline phosphatase-like immunoglobulin G in the serum of a patient with multiple epiphysial dysplasia in 1975.1) Since that time, numerous studies have dealt with alkaline phosphatase-immunoglobulin complexes (ALP-Ig). The heterogeneous molecular weight of these complexes have been described as 550 kD,2) 280 kD,3) 500 kD4) or 900 kD.5) These results have been discussed from the view that the complex consisted of one or more enzymes against one immunoglobulin molecule.

On the other hand, some immunoglobulins which associated with the complexes of serum enzyme and immunoglobulin have been considered as autoantibodies.6,7) In such cases, these complexes have been accepted as immune complexes. Fragments of complement component C3, such as C3c and/or C3d bind to many kinds of immune complexes, and many circulating immune complexes carry these fragments.8,9) Therefore, ALP-Ig may bind other substances such as complement components.

In the present study, ALP-Ig from two patients were analyzed by counterimmunoelectrophoresis using anti-human antibodies, in order to determine the association of complement components.

MATERIALS AND METHODS

1) Materials

Serum samples were obtained from two patients at our facility: one, a 70-year-old male with pancreatic cancer, and the other, a 72-year-old male with lung cancer. These were stored at 4°C, and used within one week.

2) Electrophoretic studies of ALP isozymes

ALP isozymes were examined by cellulose acetate membrane electrophoresis and counterimmunoelectrophoresis (CIE) as described previously.10) ALP activity on the membrane was visualized by incubation with 5-bromo-3-indolylphosphate. Rabbit anti-human IgA, IgG, IgM, kappa light chain, lambda light chain, and anti-complement C1q and anti-complement C3c were purchased from Dako-Immunoglobulins Co. Ltd. (Denmark).

3) Gel filtration

Gel filtration was carried out on a Sephacryl
S-300 (2.2 × 84 cm, Pharmacia, Sweden) in a buffer consisting of 0.05 mol/l Tris/HCl, pH 7.4, 0.15 mol/l NaCl and 0.1% NaN3. The protein concentrations of each fraction were measured by the absorbance at 280 nm. ALP activity in the fractions was assayed, using a 1 ml solution composed of 5 mmol/l p-nitrophenylphosphate, 0.5 mmol/l MgCl2 and 1 mol/l diethanolamine, pH 10.3, at 37°C, and expressed by the absorbance at 405 nm. Ferritin and catalase were obtained from Boehringer Manheim (Germany) and used as molecular weight markers. In addition, IgM position was determined by the measurement of the concentration of IgM in the elutes by BNA Boehringer nephelometer analyzer (Hoe-chst Japan Ltd., Japan).

4) Amino acid inhibition assays
The final concentrations of amino acids were as follows: L-phenylalanine, 5 mmol/l; L-leucine, 0.4 mmol/l; L-homoarginine, 5 mmol/l.
Amino acid inhibition and heat inactivation assays were performed using 5 mmol/l p-nitrophenylphosphate described in the assay of ALP activity.

5) Heat inactivation
Sera were transferred into glass tubes and heated in a water bath at 56°C or 65°C for 5 min.

6) Placental ALP assay by monoclonal antibodies
Placental ALP was assayed by enzyme immunoassay (EIA) using monoclonal antibodies against human placental ALP (Sigma Chemical Company, U.S.A.).

RESULTS

1) Electrophoretic analyses of ALP isozymes
In one case, ALP activity was observed in the form of a diffuse belt at the alpha 2-gamma globulin region (Fig. 1, 1C). Through the use of CIE, ALP activity was observed on the precipitin lines formed by anti-IgG or anti-C3c (Fig. 1, 1C, 1C3c). These findings indicated that ALP-IgG was associated with C3c. But, the light
chain of this bound IgG was not detected.

In the other case, the highest level of ALP activity was observed at the beta-gamma globulin region (Fig. 1, 2C). By CIE, ALP activity was detected on the precipitin lines formed by anti-IgG or anti-lambda light chain (Fig. 1, 2G, 2L), but not on the precipitin line formed by anti-C3c, indicating ALP-IgG was not associated with C3c. Although data were not shown, none of these two cases were associate with C1q.

2) Gel filtration profile of ALP on Sephacryl S-300

By gel filtration, two peaks of ALP activity were seen in both cases (Fig. 2). In one case, ALP having an approximate molecular weight of 900 kD (ALP-900kD) was separated from normal sized ALP having molecular weight of approximate 160 kD (Fig. 2-1). In the other case, ALP with a molecular weight of approximate 450 kD (ALP-450kD) was seen as a macro ALP (Fig. 2-2).

3) Other studies

Inhibition of ALP activity in ALP-900kD and ALP-450kD by L-homoarginine was 70% and 65% of the total, respectively. These macro ALPs showed little sensitivity to L-phenylalanine or L-leucine (inhibition ratio of less 5% each). Ninety percent of ALP activity level of ALP-900kD, and 15% of that of ALP-450kD were inactivated by heating at 56°C for 5 min. ALP activity in ALP-900kD was completely inactivated by heating at 65°C for 5 min, but 36% of ALP activity in ALP-450 kD was inactivated by the same treatment. Neither macro ALP reacted to the monoclonal antibodies against human placental ALP.

DISCUSSION

With regard to the molecular weight of ALP-Ig, Crofton described an ALP-Ig of 550 kD, and suggested that the complex might consist of two IgG molecules associated with one ALP molecule. The 280 kD ALP-Ig, reported by Hattori et al. was proposed to consist of one molecule of IgG and one molecule of ALP. However, Umeki et al. reported an ALP-Ig of 900 kD where two classes of Ig (IgA and IgG) and two types of light chains were detected. Reviewing other serum enzyme-Ig complexes, the authors have suggested that these complexes might consist of enzyme and Ig.

We studied ALP-Ig from the viewpoint of the immune complex (IC) or circulating immune complex (CIC). The IC is metabolized by reticuloendothelial systems or macrophages, depending upon its size or solubility. Since macrophages have C3b receptors on their surfaces, the IC associated with C3b is eliminated from the circulation rapidly than IC without C3b. However, C3c receptors have not been demonstrated on any phagocytes, the IC with C3c may remain in the circulation for an extended period.

We studied ALP-Ig using anti-C3c, and demonstrated the ALP-IgG from a pancreatic cancer patient associated with C3c. From the evidence that IgG is a bivalent antibody, and the molecular weight of C3c is 143 kD, ALP-900 kD was proposed to consist of two molecules of ALP, two molecules of IgG and two molecules of C3c or other combinations. Here, no light chains of IgG
were detected, possibly as a result of two molecules of ALP being associated with one molecule of IgG. However, it remains to be elucidated whether ALP-IgG complex has other substances than complement. ALP-450 kD was proposed to consist of two ALP molecules and one IgG molecule or vice versa.

Results from amino acids inhibition assays, heat inactivation tests and EIA using monoclonal antibodies against human placental ALP, it was indicated that the ALP associated with both macro ALPs were tissue non-specific ALP. Heat stability was lower in ALP-900 kD, but higher in ALP-450 kD, compared to tissue non-specific ALP. Umeki _et al._ reported an increase in heat stability in the ALP-Ig of 900 kD. But, the mechanisms responsible for the changes in that stability are unclear.

In the future, clinical significance of the interaction of ALP-Ig and complement should be investigated.

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**REFERENCES**