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Development of Specific Immune Complex to Basic Fetoprotein Using Solid- Phase Anti-C3 Enzyme Immunoassay and Survey of Specific Immune Complex in Sera of Cancer Patients

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SUMMARY

We developed the assay to detect the specific immune complex (IC) to tumor marker. The assay was performed using F $(ab')_2$ anti-C3; after interaction to matrix-fixed F$(ab')_2$ anti-C3 with C-fixing IC, the bound tumor marker was detected with alkaline phosphatase linked F$(ab')_2$ antitumor marker. We detected Basic Fetoprotein (BFP) specific IC in sera of patients with various cancers by this method. Fifteen of 112 (13.4%) patients with malignant tumor were positive in BFP-specific IC level. The combination of BFP-specific IC and BFP assay resulted in an increased sensitivity of tumor detection (40.21%) compared with BFP assay alone (30.93%).

The results show that antibody to BFP emerges in sera of some of patients with cancer. In addition, this may suggest the presence of tumor specific immunological determinant in BFP.

Key words: Basic fetoprotein (BFP), Immune complex

INTRODUCTION

Numerous tumor markers have been found by development of various immunochemical methods. Tumor markers are unquestionably useful for diagnosis of cancer, but not necessarily specific to tumor; therefore the studies of human tumor marker have been taken notice of specific marker to tumor or the presence of tumor specific immunological determinants of those already known such as CEA.

The aim of present study is to demonstrate the presence of tumor specific immunological determinants of Basic Fetoprotein (BFP) (Ishii, 1978). For this purpose the assay was developed to detect the specific immune complex (IC) to BFP in sera. We present here a simple, sensitive, and reproducible solid phase enzyme immunoassay (EIA) for the detection of C-fixing BFP-specific IC. Our

attemption was to interact matrix-fixed $F(ab')_2$ anti-C3 with C-fixing BFP-specific IC after that the bound BFP was detected with alkaline phosphatase linked $F(ab')_2$ anti-BFP.

MATERIALS AND METHODS

BFP was purified according to methods of Ishii (Ishii, 1978). Ten m*l* of the concentrated ascitis of a patient with primary hepatocellular carcinoma were applied on a column (5×25 cm) of QAE-Sephadex A-50 equilibrated with 0.02 M phosphate buffer, pH 7.0 and passed through the column in the same buffer. BFP fractions from QAE-Sephadex A-50 column were passed through a column (2.5×40 cm) of Sepharose 4B coupled with anti-normal human serum-γ-globulin and anti-hepatoma ascites containing no BFP-γ-globulin. BFP rich pooled fractions obtained with affinity chromatography were further purified by isoelectric focusing. A 0 to 50% sucrose density gradient containing 1% carrier ampholytes pH 6 to 10 was prepared in a 440-LKB electrofocusing column. The pure BFP was obtained with gel filtration of BFP fractions from isoelectrofocusing using a Sephadex G-150 column.

Purification of C3 complement compornent

C3 was purified with modified method of Tack and Prahl (Tack & Prahl., 1976). A plasminogen depletion serum of 5~12% PEG fraction was put on DEAE-Sephacel (Pharmacia Co.) column was equilibrated with 0.01 M Tris-HCl, pH 7.8 containing 0.1 M ε-aminocaproic acid, 1 mM PMSF and 5 mM EDTA. After application and washing, a linear NaCl gradient was followed. The gradient mixer contained 400 m*l* of starting buffer and the second chamber contained 400 m*l* of 0.3 M NaCl in the same buffer. C3 fractions from DEAE-Sephacel column were furthermore purified with a Sepharose 6B (Pharmacia Co.) and Hydroxylapatite (Bio-Rad) column.

Preparation of $F(ab')_2$ anti-BFP and C3

For this purpose, female rabbits were injected subcutaneously with 0.1 mg of BFP or C3 mixed with an equal volume of Freund's complete adjuvant. A booster injection was given in each of two subsequent weeks, using 0.1 mg BFP or C3. Anti-BFP or C3 rabbit IgG were obtained by the column chromatography of DEAE-Sephacel after 50% saturated ammonium precipitation of antisera. The BFP specific anti-BFP rabbit IgG was prepared by passing through a column of Sepharose 4B coupled with the purified BFP. $F(ab')_2$ anti-BFP or C3 were purified by pepsinization (1% by weight, in 0.1 M acetate buffer pH 4.0) and followed by gel filtration on Sephadex G-150 column.

Radioimmunoassay of BFP

The radioiodinated BFP was prepared by method of Hunter and Greenwood

(Hunter & Greenwood, 1962). The radioimmunoassay was based on a coprecipitation-inhibition technique using the two antibody methed (Morgan & Lazalow, 1963). The first reaction mixtures consist of 0.1 m*l* of test serum or standard BFP, 0.1 m*l* of a 1: 40,000 dilutions of anti-BFP rabbit antiserum, 0.1 m*l* of ^{125}I-BFP (10,000 cpm) and 0.5 m*l* of 0.05 M phosphate buffered saline, pH 7.4 containing 1% bovine serum albumin in each tube. The above mixtures were incubated for 24 hrs at 4℃. At the end of this period, 0.1 m*l* of a 1 : 50 dilutions of normal rabbit serum and 0.1 m*l* of a 1 : 10 dilutions of anti-rabbit γ-globulin goat serum were added to these mixtures in each tubes and then a second reaction was proceeded for 24 hrs at 4℃. After this process, each tube was cetrifuged at 3,000 r. p. m for 30 min, and then the radioactivity of precipitates was measured.

Detection of BFP-specific IC

This assay was performed according to modified method of Pereira (Pereira, *et al.*, 1980). Wells of U-shaped flexible microplate were coated with 0.1 m*l* of F(ab')$_2$ anti-C3 in Borated buffered saline, pH 7.5 (BBS). After aspiration of F (ab')$_2$ anti-C3 solution, 0.2 m*l* of 1% human serum albumin (HSA)-BBS was added to each well. After standing for 1 hr at room temperature, the wells were washed once with BBS. To these wells, 0.1 m*l* of sample sera were added and incubated at 37℃ for 2 hrs. After incubation, the wells were washed five times with BBS, and then 0.1 m*l* of alkaline phosphatase linked F(ab')$_2$ anti-BFP were added. After incubation for 2 hrs at 37℃, wells were again washed five times with BBS, and 0.1 m*l* of substrate for alkaline phosphatase was added. After further incubation for 1 hr at 37℃, the reaction was terminated by adding 0.1 m*l* of 1 N-NaOH. Amount of BFP-specific IC was determined spectrophotometrically at 405 nm.

RESULTS

Dilution test of standard serum

Because we determined an absorbance at 405 nm as an amount of BFP-specific IC, we needed a standard serum to compare with amount of BFP-specific IC in sample serum. We used a high level in BFP-specific IC as a standard serum that was preliminary measured. In Figure 1, right side shows that when the microplate was not coated with F(ab')$_2$ anti-C3, the absorbance was 0 and left side shows the results of the dilution test of standard serum. The dilution test showed that indeed the absorbance diminished linearly in relation to the dilution of standard serum.

Detection of BFP-specific IC in serum

Results of the determination of BFP-specific IC were shown in Figure 2. Twenty-one human serum samples obtained from healthy donors were analyzed with the anti-C3 EIA and the value was distributed from 0.01 to 0.1. In

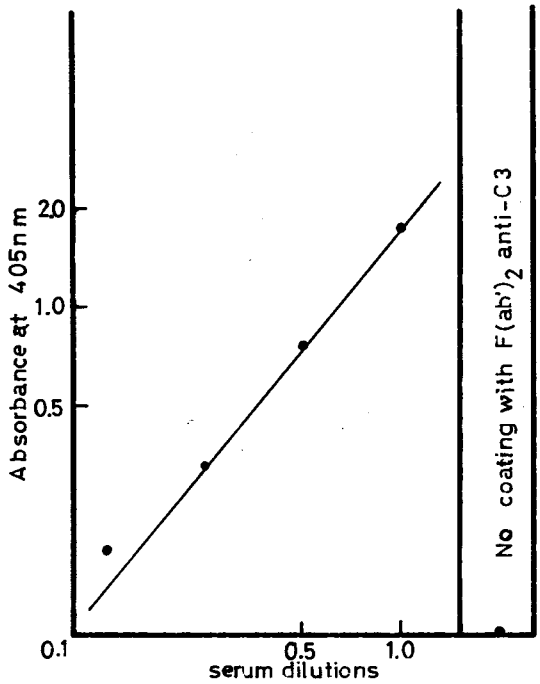

Fig. 1 Dilution test of standard serum.

comparison, high levels (above 0.15) and incidences of BFP-specific IC were found in sera of patients with hepatoma, gastric cancer and hepatitis. Fifteen of 112 sera from patients with malignant tumor revealed BFP-specific IC levels higher than 0.15. Five of 16 (31.25%) patients with hepatoma, 8 of 45 (17.8%) patients with gastric cancer, 1 of 15 (6.7%) patients with lung cancer and 1 of 36 (2.8%) patients with colon cancer were positive cases in BFP-specific IC level.

Correlation of BFP-specific IC with BFP levels in gastric cancer

Figure 3 shows a correlation of BFP-specific IC with BFP levels in gastric cancer. No correlation was found between BFP-specific IC and BFP levels. Ten of 45 (22.2%) cases of gastric cancer were positive in BFP level (100 ng/m*l*) and 8 of 45 (17.8%) cases were positive in BFP-specific IC level. On the other hand, 17 of 45 (37.8%) patients with gastric cancer were detected by the combined BFP, BFP-specific IC assay compared with 10 of 45 (22.2%) for BFP alone.

Correlation of BFP-specific IC with BFP levels in hepatoma

Results were shown in Figure 4. In the case of hepatoma, 11 of 16 (68.75%) patients had the BFP concentration higher than 100 ng/m*l* in serum. In the group of patients with highly elevated BFP levels (above 100 ng/m*l*), 3 of 11 (27.3%) patients had positive levels of BFP-specific IC in serum. However, 2 of 16 (12.

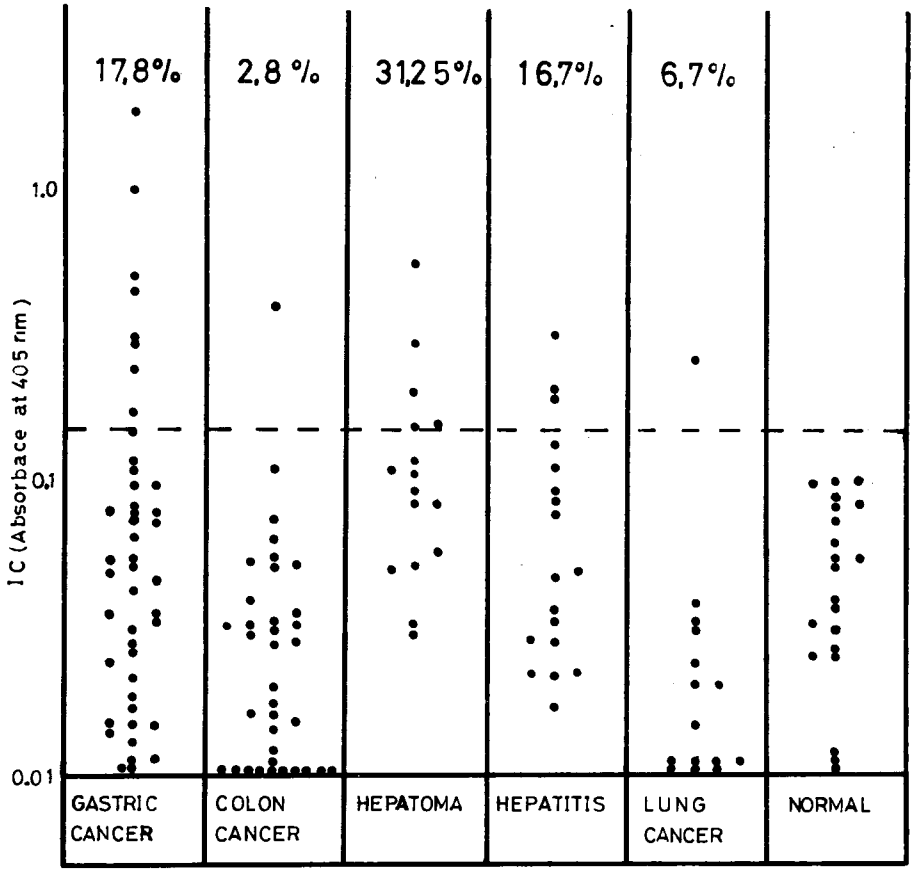

Fig. 2 BFP-specific IC levels in sera

5%) patients had positive levels of BFP-specific IC even if BFP levels were lower than 100 ng/m*l* in serum. Thirteen of 16 (81.25%) patients with hepatoma were detected by the combined BFP, BFP-specific IC assay compared with 11 of 16 (68.75%) for BFP alone.

Correlation of BFP-specific IC with BFP levels in colon cancer

Figure 5 shows correlation of BFP-specific IC with BFP levels in colon cancer. No correlation was found between BFP-specific IC and BFP levels, and also BFP-specific IC could be detected in only one case (2.9%) with colon cancer.

DISCUSSION

The many assays are currently available to detect IC. These methods take advantage of certain physicochemical characteristics of IC as well as their interactions with cellular Fc and C3 receptors and Clq, rheumatoid factor (RF) and

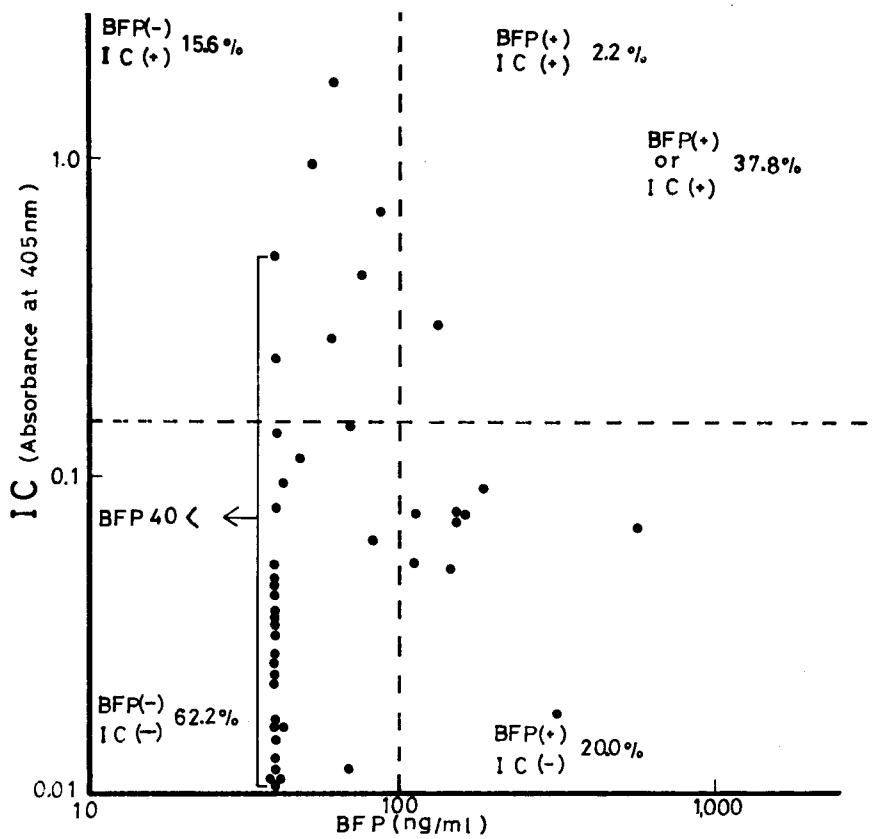

Fig. 3 Correlation of BFP-specific IC with BFP in gastric cancer.

conglutinin. Recently many researchers have reported the detection of IC in sera of patients with malignant diseases such as leukemia, breast cancer and lung cancer by methods above described. Although IC value has provided much useful information in regard to immunopathology, prognosis and follow-up of immunologic disorders in patients with tumor, several limitations are in existence.

First, some procedures are technically difficult and also detect non-IC substances. Second, these methods detect IC including non-specific IC to tumor.

We described in this paper the use of $F(ab')_2$ anti-C3 antibody in a solid phase EIA for detection of specific IC to BFP which increased in serum of patients with malignant tumor. This method is sensitive and simple to prepare, and not affected by non-IC substances such as polyanion, endotoxins, nucleic acid, RF, and anti-lymphocyte antibodies. we measured BFP-specific IC in sera of patients with various cancer by this method. High levels of BFP-specific IC were found in sera

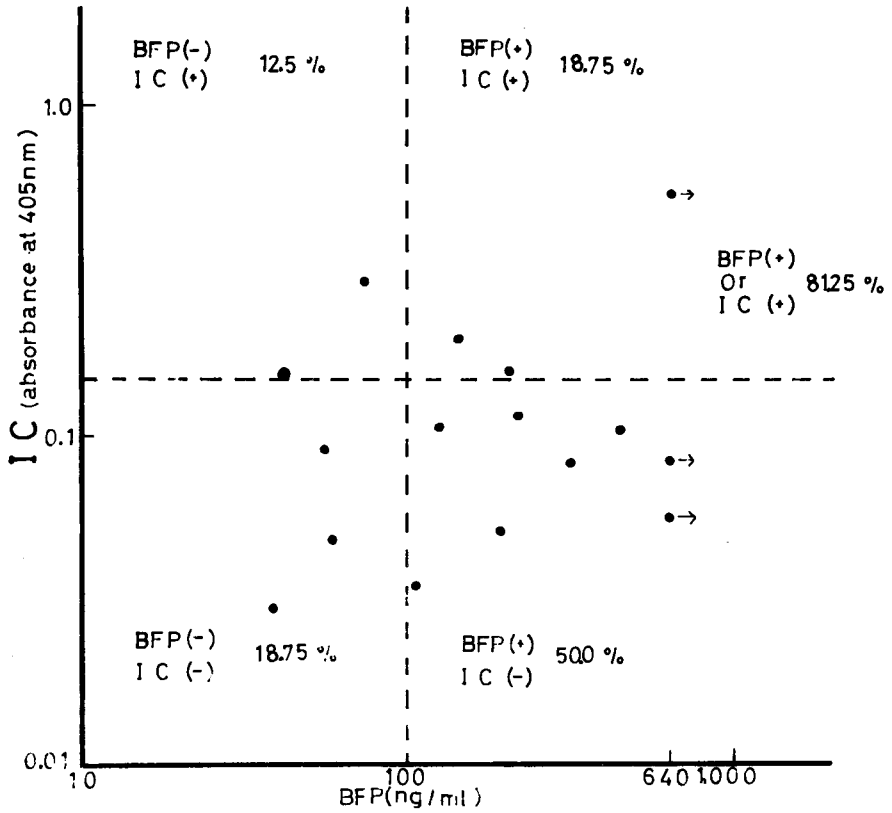

Fig. 4 Correlation of BFP-specific IC with BFP in hepatoma.

of patients with hepatoma, gastric cancer. Fifteen of 112 (13.4%) patients with malignant tumor were positive cases in BFP-specific IC level. No correlation was found between BFP-specific IC and BFP levels in sera. The combination of BFP-specific IC and BFP levels assay resulted in an increased sensitivity of tumor detection (40.21%) compared with BFP levels alone (30.93%). The combination assay resulted in an especially increased sensitivity of diagnosis of gastric cancer and hepatoma.

We now presented here that BFP-specific IC could be detected in sera of patients with malignant tumor. Although several studies of Ishii showed that BFP levels in sera of patients with cancer increased depending on the stage of tumor and BFP was produced by tumor cells, it is not obvious whether BFP produced by tumor cells has the different immunological determinants from that come from normal cells or not. One may consider that antibody to BFP bring forth in patients with cancer, because BFP produced by tomor cells posseses the distinct immunological

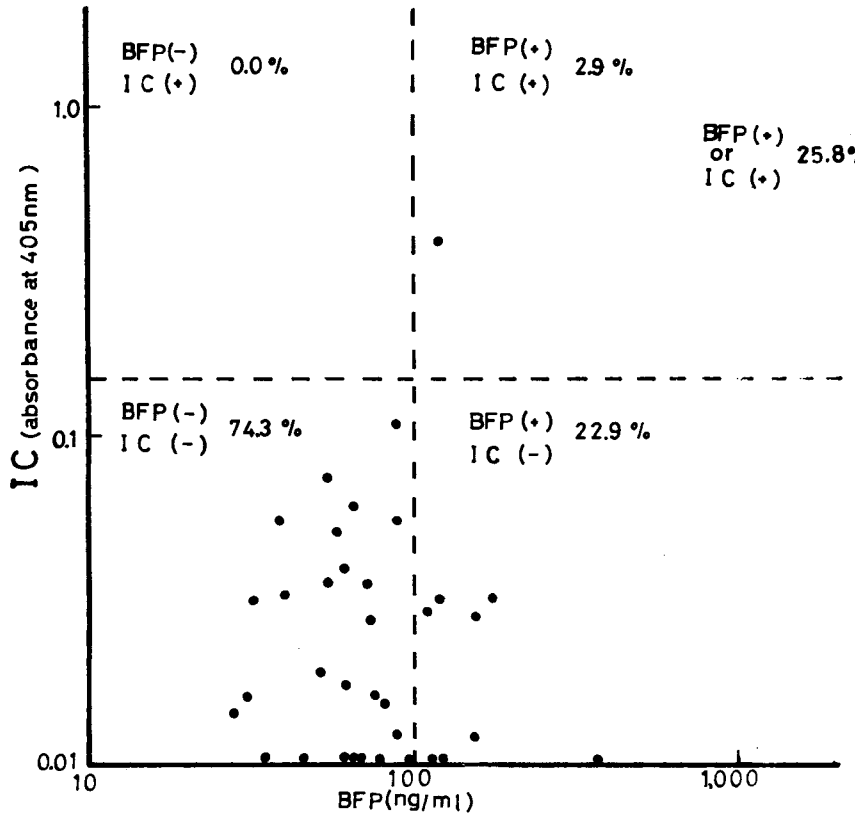

Fig. 5 Correlation of BFP-specific IC whth BFP in colon cancer.

determinants from self, or because of mis-recognition of BFP to escape from the immunological surveillance mechanism in patients with cancer. We investigate about the immunological determinants of BFP by using monoclonal antibodies to clarify this problem.

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