Detection of a Serum DNA-binding Protein Associated with Cancer

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ABSTRACT

An accompanying report describes the purification and partial characterization of a unique DNA-binding protein (M, 64,000; pi 5.9) that is present in human sera. This report gives the results of assays of sera from patients for the bleomycin inhibitor protein (BIP) using the Pseudomonas bacterio-phage covalently closed circular DNA fluorescence technique standardized for DNA breakage induced by bleomycin. The results of the BIP assays were expressed by values of specific activity of inhibition. One arbitrary unit of inhibitory activity was defined as equivalent to the amount of serum protein required to cause 50% inhibition of DNA degradation using standard conditions of the DNA breakage assay. The mean values of specific activity of inhibition (SAI) for groups of healthy individuals (n = 26), patients with nonmalignant diseases (n = 33), and patients with malignant diseases (n = 88) were 12.60 ± 4.69 (S.E.), 12.53 ± 3.17, and 2.40 ± 0.84 units/mg, respectively. Mean SAI values for patients with cancers of various types were: solid tumors (n = 46), 2.44 ± 0.86; leukemias (n = 24), 2.59 ± 0.96; and lymphomas (n = 18), 2.07 ± 0.64. The decrease in BIP activity was not correlated with sex, age, or prior chemotherapy. Mean SAI values of male (n = 29) and female (n = 59) patients with cancer were 2.61 ± 0.87 and 2.30 ± 0.83 units/mg, respectively. Mean SAI values for different age groups were: 0 to 40 years (n = 21), 2.05 ± 0.68 units/mg; 41 to 70 years (n = 56), 2.59 ± 0.68 units/mg; and >70 years (n = 11), 2.12 ± 0.67 units/mg. Cancer patients with and without prior chemotherapy had mean SAI values of 2.97 ± 0.85 (n = 23) and 2.20 ± 0.86 units/mg (n = 65), respectively. Linear regression analysis comparing SAI values and serum protein levels showed no correlation (r = 0.21). These results suggest the decrease of the BIP is associated with malignant disease. Additional controlled studies are required before the significance of this association can be adequately assessed.

INTRODUCTION

A number of potential biological markers for malignant disease including α-fetoprotein (1-4, 19), carcinoembryonic antigen (12, 13), human chorionic gonadotropin (6, 14-17), DNA-binding proteins, and nucleolar antigens (5, 7, 10, 20) have been described. The serum DNA-binding proteins which have been associated with human cancers of various types offer the distinct advantage of not requiring surgery to obtain specimens to be diagnostically tested. The presence of several apparently different DNA-binding proteins appears (5, 8, 10, 20) to be correlated with cancer in humans. The relationship among these proteins and the possible role they might have in the etiology of the neoplasms remain unknown.

We have described previously the presence of a unique DNA-binding protein in human serum (Ref. 10 and the accompanying report, this volume). The protein has been termed the BIP because it inhibits the degradation of DNA by bleomycin. The inhibitory activity exerted by the serum protein on the bleomycin-induced breakage of PM-2 DNA forms the basis of the diagnostic method used to detect the presence of the protein in serum (accompanying report, this volume). The BIP has been purified to homogeneity and has a molecular weight of 64,000 and a pI of 5.9. It binds to calf thymus DNA and PM-2 DNA and inhibits the DNA degradation induced by other antitumor agents including nocazarinostatin and macromycocin. Evidence of DNA binding by BIP has been obtained by several methodologies including affinity column chromatography, fluorescence quenching, circular dichroism, and agarose gel electrophoresis (see accompanying report, this volume). The present report describes the PM-2 DNA fluorescence method used to detect the BIP. The results of assay of sera obtained from groups of healthy individuals and patients with nonmalignant and malignant diseases of various types are given.

MATERIALS AND METHODS

The chemicals were reagent grade quality obtained from local sources. Blenoxane was supplied by Bristol Laboratories, Syracuse, N. Y. PM-2 DNA was isolated and purified by the procedures described previously (11). Sera from the patients and normal individuals were donated voluntarily after informed consent had been obtained. The patients were from Upstate Medical Center, State University of New York, Syracuse, N. Y., and the Veterans Administration Hospital, Houston, Texas, where documentation of the medical diagnosis for each patient was maintained. The study was performed in a blind fashion. The serum assays were performed on samples identifiable by coded numbers, the identities of which were known only to the oncologists at the respective hospitals.

The PM-2 DNA Fluorescence Technique. The PM-2 DNA fluores-
ence technique used in these experiments has been described in a previous report (11).

Assay of the BIP. The inhibitory activity of serum and subfractionations obtained in the course of the purification procedure was determined by use of the PM-2 DNA fluorescence technique (standardized for DNA cleavage by bleomycin). The assay consisted of addition of the test samples (50 μl) to solutions containing 35 nm bleomycin, 1.0 A260 unit PM-2 DNA, and 25 mm 2-mercaptoethanol in 50 mm sodium borate buffer (pH 9.5). The mixture was incubated 30 min at 37°. Aliquots (100 μl) were added to 0.9 ml denaturation buffer (0.09 M Na3PO4:0.01 M EDTA:0.01 M NaCl adjusted to pH 12.1 with 0.15 M NaOH) followed by the addition of 0.1 ml ethidium bromide (56 μM in denaturation buffer).

The fluorescence of the ethidium bromide-DNA mixture was determined with an Amino-Bowman spectrophotofluorometer (American Instruments Co., Silver Spring, Md.) at 530 nm excitation and 590 nm emission. The change in concentration of PM-2 DNA was determined by the percentage of decrease in fluorescence relative to control reactions not containing the DNA-degradative drug. The percentage of inhibition was calculated on the basis of the DNA degradation induced by bleomycin in the absence of the inhibitor.

Protein Determination. The protein concentrations of the sera were determined by the method of Lowry et al. (18). All assays were performed in triplicate. Assays were repeated after storage of sera for several months, and no evidence of instability of the BIP was observed. The IC50 values were obtained from probit analyses of at least 5 concentrations resulting in inhibition within the range of 10 to 80%.

RESULTS

The amount of inhibitory activity in the various sera was determined in terms of protein concentration. IC50 values were obtained from probit-logarithm plots of the percentage of inhibition of DNA degradation induced by bleomycin as a function of serum protein concentration. Chart 1 shows a representative probit-logarithm plot of the percentage of inhibition versus the concentration of serum protein. The IC50 values varied between different individuals and were independent of the total serum protein levels. Patients who had high serum protein levels did not necessarily have low IC50 values. Linear regression analyses comparing values for the SAI with the serum protein concentrations revealed the 2 parameters were not correlated (r = −0.21). The IC50 value was plotted against the log of the serum protein concentration determined by the method of Lowry et al. From this graph, IC50 values were obtained. The IC50 is the protein concentration required to cause 50% inhibition of DNA degradation.

Chart 1. Probit analysis of inhibitory activity obtained in sera from normal individuals and cancer patients. Inhibitor assays were performed on serially diluted sera from patients with various cancers, and normal individuals. This chart shows the results of a representative assay. The percentage of inhibition was plotted against the log of the serum protein concentration determined by the method of Lowry et al. From this graph, IC50 values were obtained. The IC50 is the protein concentration required to cause 50% inhibition of DNA degradation.

Chart 2. Scattergram of mean SAI values of sera from patients with malignant and nonmalignant diseases. SAI values were obtained from probit-logarithm plots of results from assays of the BIP (see “Materials and Methods”). The types of nonmalignant and malignant diseases are described in the text.

1 shows the mean SAI values and the p values obtained for the various groups. The mean SAI value of 26 healthy individuals was 12.6 ± 4.69 units/mg. The mean SAI value of 33 patients having nonmalignant diseases was 12.53 ± 3.17 units/mg. The mean SAI values for the groups of patients having malignant diseases were: breast carcinomas (n = 20), 2.26 ± 0.83 units/mg; lung carcinomas (n = 7), 2.82 ± 1.01 units/mg; gastrointestinal carcinoma (n = 5), 3.25 ± 0.9 units/mg; testicular carcinomas (n = 11), 2.12 ± 0.86 units/mg; one case of melanoma, 1.56 unit/mg; synovial cell carcinoma, 4.17 units/mg; leukemias (n = 24), 2.59 ± 0.96 units/mg; lymphomas (n = 10), 2.23 ± 0.62 units/mg; and multiple myeloma (n = 9), 1.87 ± 0.65 unit/mg. The difference in SAI values for the various groups of cancers and the control groups were statistically significant (p < 0.05). Statistical analyses were performed by Student’s t test for groups having n < 5. The
The mean SAI value obtained from the sera of 88 patients was 2.40 ± 0.84 units/mg. The group of patients with ages between 40 and 70 years (n = 56) had a value of 2.59 ± 0.92 units/mg. Patients older than 70 years (n = 11) had a value of 2.12 ± 0.67 units/mg. Similarly, the SAI was not significantly affected by prior chemotherapy. Patients with prior chemotherapy (n = 23) had a specific activity value of 2.97 ± 0.85 units/mg. Patients having had no previous chemotherapy (n = 65) had an SAI value of 2.20 ± 0.82 units/mg. These results showed the decrease in inhibitory activity of sera from the cancer patients was not due to the possible effects of chemotherapy on the BIP.

**DISCUSSION**

The results of the present study show the BIP is decreased approximately 5-fold in sera of patients with cancer compared to sera from patients having nonmalignant diseases or healthy individuals. The levels of the protein in the various sera can be determined by the use of the PM-2 DNA fluorescence technique standardized for assaying the DNA degradation induced by bleomycin. The IC50 values, obtained by measuring the serum protein required to cause 50% inhibition of the bleomycin-induced degradation of PM-2 DNA, represent an easy and reproducible monitor for the BIP. Numerically small SAI values indicate the BIP is present in decreased amounts in the serum. The mean SAI value obtained from assays of the inhibitor in sera from 26 healthy individuals was 12.6 ± 4.69 units/mg. The mean SAI value from sera of 33 patients having nonmalignant diseases was 12.53 ± 3.17, and 2.40 ± 0.84 units/mg. These results indicate that the BIP was decreased by greater than 5-fold in the sera of patients with cancer and that this decrease was not a function of variations in serum protein concentration.

Table 3 shows the results of these assays expressed in terms of age, sex, and whether the patients had received chemotherapy prior to sampling of the serum. The mean SAI values of the group of patients with cancer were 2.61 ± 0.87 units/mg for the males (n = 29) and 2.50 ± 0.83 units/mg for the females (n = 59). The classification on the basis of age of the patients showed no significant correlation of the SAI values with age. Patients whose age was 40 years or less had an SAI of 2.61 ± 0.87 units/mg. The group of patients with ages between 40 and 70 years (n = 56) had a value of 2.59 ± 0.92 units/mg. Patients older than 70 years (n = 11) had a value of 2.12 ± 0.67 units/mg. Similarly, the SAI was not significantly affected by prior chemotherapy. Patients with prior chemotherapy (n = 23) had a specific activity value of 2.97 ± 0.85 units/mg. Patients having had no previous chemotherapy (n = 65) had an SAI value of 2.20 ± 0.82 units/mg. These results showed the decrease in inhibitory activity of sera from the cancer patients was not due to the possible effects of chemotherapy on the BIP.

**DIFFERENCE OF MEAN SAI VALUES BETWEEN THE COMBINED GROUP OF MALIGNANT DISEASES AND THE CONTROL GROUPS**

The difference of mean SAI values between the combined group of malignant diseases and the control groups had a p < 0.01.

Table 2 shows the summary of the SAI values obtained from assays of the healthy individuals and the patients having nonmalignant and malignant diseases. The mean serum protein determined by the method of Lowry et al. for the groups of healthy individuals and those with nonmalignant and malignant diseases was 83.8 ± 7.6, 82.2 ± 8.3, and 87.3 ± 13.3 mg/ml, respectively. The mean SAI values for the respective groups were 12.60 ± 4.69, 12.53 ± 3.17, and 2.40 ± 0.84 units/mg. These results indicate that the BIP was decreased by greater than 5-fold in the sera of patients with cancer and that this decrease was not a function of variations in serum protein concentration.

The mean SAI value obtained from assays of the inhibitor in sera from 26 healthy individuals was 12.6 ± 4.69 units/mg. The mean SAI value from sera of 33 patients having nonmalignant diseases was 12.53 ± 3.17 units/mg. The mean SAI value obtained from the sera of 88 patients having carcinomas of various tissues, leukemias, and lymphomas was 2.4 ± 0.84 units/mg. SAI values were characteristically low for patients with cancer of various types including a variety of different solid tumors, leukemias, and lymphomas.

**DISCUSSION**

The results of the present study show the BIP is decreased approximately 5-fold in sera of patients with cancer compared to sera from patients having nonmalignant diseases or healthy individuals. The levels of the protein in the various sera can be determined by the use of the PM-2 DNA fluorescence technique standardized for assaying the DNA degradation induced by bleomycin. The IC50 values, obtained by measuring the serum protein required to cause 50% inhibition of the bleomycin-induced degradation of PM-2 DNA, represent an easy and reproducible monitor for the BIP. Numerically small SAI values indicate the BIP is present in decreased amounts in the serum. The mean SAI value obtained from assays of the inhibitor in sera from 26 healthy individuals was 12.6 ± 4.69 units/mg. The mean SAI value from sera of 33 patients having nonmalignant diseases was 12.53 ± 3.17 units/mg. The mean SAI value obtained from the sera of 88 patients having carcinomas of various tissues, leukemias, and lymphomas was 2.4 ± 0.84 units/mg. SAI values were characteristically low for patients with cancer of various types including a variety of different solid tumors, leukemias, and lymphomas.
Whether the decrease in the serum of the BIP in patients having malignant disease represents a secondary manifestation of the disease or is somehow involved in the initial developmental stages of the disease is not known. Clearly, the decrease in the BIP is not due to changes in the levels of serum proteins since the SAI values did not correlate with changes in serum protein concentrations (Table 2). Nevertheless, a number of DNA-binding proteins have been reported to be decreased in neoplastic tissue. Thus, it is conceivable that changes in the DNA-binding proteins are manifestations of a state that is susceptible to tumorigenic transformation. The decrease of DNA-binding proteins could be related to the circulatory processes involved with the maintenance of low cholesterol levels associated with cancer as reported by Rose and Shipley (21).

Current investigations are directed to establishing the correlation between the BIP in serum with the clinical status of the malignant disease and the possible role of the protein in the process of neoplasia transformation. Numerous other factors need to be studied to show the association is significant and to demonstrate that a BIP assay is a potential diagnostic tool for cancer. Clarification is needed with respect to source of the protein, its correlation with state of disease and hereditary factors, and its changes prior to the clinical manifestation of the disease.

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REFERENCES


Table 3

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