Isolation and Characterization of an Immunosuppressive Acidic Protein from Ascitic Fluids of Cancer Patients

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ABSTRACT

Analytical isoelectric focusing in polyacrylamide gel at pH 2.5 to pH 5 was used successfully to detect an acidic protein which can be found in large quantities in the sera of cancer patients but in only small amounts in the sera of healthy persons. The main peak of this acidic protein when obtained from cancer patients revealed a pi of 3.0, while the pi of that obtained from normal subjects was 3.1. The results obtained also demonstrated that qualitative and quantitative differences of such serum acidic proteins discriminate between sera of normal persons and cancer patients.

Purification of this acidic protein (pl 3.0) was attempted with cancer ascitic fluids by successive ammonium sulfate precipitation, diethylaminoethyl cellulose column chromatography, Sephadex G-100 gel filtration, and preparative isoelectric focusing. When the purified acidic protein was characterized for physicochemical properties, it was found to have an isoelectric point of 3.0, to have a molecular weight of 50,000, and to contain 31.5% carbohydrate. Its behavior in immunodiffusion and immunoelectrophoresis was indistinguishable from that of normal human ai-acid glycoprotein. However, in gel isoelectric focusing, in molecular weight, and also in carbohydrate content, the acidic protein isolated from cancer ascitic fluid differed from normal ai-acid glycoprotein. Furthermore, this acidic protein was found to suppress both phytohemagglutinin-induced lymphocyte blast formation and mixed-lymphocyte reaction in vitro. The acidic protein, which we called "immunosuppressive acidic protein," and its biological activities were discussed regarding its possible role in the impairment of the immunological surveillance mechanism in the tumor-bearing host.

INTRODUCTION

Changes in the serum levels of glycoproteins, such as acute-phase proteins, have been reported in various disease processes; varying degrees of increase in infectious, degenerative, and malignant diseases; and as a decrease in a few clinical conditions, e.g., in Marfan’s syndrome. It has been suggested that these changes may represent nonspecific responses to disease, since they have been observed in a wide number of often unrelated disease processes (2).

Koj (16) and Synder and Ashwell (33) showed that the levels of ai-AG were frequently abnormal in cancer patients. Although the physical and biochemical properties of ai-AG of normal human origin are well established (29), there are relatively few detailed studies on the qualitative analysis of ai-AG in cancer patients. Rudman et al. (25, 26) found that plasma and effusions of cancer patients frequently contained a glycoprotein similar to the protein moiety of ai-AG, but with an abnormal carbohydrate content. Bacchus (2) pointed out that the amount of several glycoproteins increases in cancer patients, some exhibiting a very high correlation to the disease process. He also insisted that qualitative alterations in the seromucoid demonstrated patterns that may be used in the differential diagnosis of patients. Bradley et al. (5), however, suggested that serum protein-bound carbohydrates may be of adjunctive value in assessing tumor burden and immune reactivity in cancer patients.

In the present study, we developed a new method to analyze abnormalities of the serum acidic protein of cancer patients by gel isoelectric focusing with a low, narrow pH range. We will also discuss the characterization of an acidic protein, which was isolated from cancer ascitic fluid, in comparison with ai-AG. Clinical evidence suggests that a definite increase of this protein can be detected in 67% of all cancer patients. Furthermore, the possibility of using this method to monitor the prognosis of cancer patients is suggested.

MATERIALS AND METHODS

Serum Collection

Serum specimens from healthy donors, 13 to 72 years old (30 samples), and from pregnant women (29 samples) were obtained from Tohoku University Hospital. Sera from benign diseases (50 samples) and malignant diseases (90 samples) were obtained from Sendai National Hospital. The diagnoses for all cancer patients were confirmed by serological and pathological reports and reviews of hospital charts. Cancer serum was obtained before the patients received some form of radiation and/or chemotherapy. The collected sera were stored at -20°C until use.

Collection of Cancer Ascitic Fluid

The ascitic fluids (1 to 2 liters per person) obtained from stomach cancer patients with peritonitis carcinomatosa were pooled and used for the purification of IAP. The IAP content in each ascitic fluid was determined by single radial immunodiffusion assay. Fluids containing a higher concentration of IAP were pooled to make a 10-liter lot. Starting with the 10-liter pool, the purification study was repeated almost 30 times. This pool was first centrifuged at 7000 x g for 20 min to remove cell debris, and the supernatant obtained was frozen and stored at -20°C until use.

1 This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: ai-AG, ai-acid glycoprotein; IAP, immunosuppressive acidic protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PHA, phytohemagglutinin; PBS, 10 mM phosphate-buffered saline, pH 7.4.

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Gel Isoelectric Focusing

IAP in the sera and IAP at each purification step of the ascitic fluids were identified by the isoelectric focusing technique as follows. Slab gels (2 mm x 12.5 cm x 26 cm) consisting of 5.0% polyacrylamide and 12.5% sucrose were applied to the concentration of 2% carrier ampholites (Pharmacia Fine Chemicals AB, Uppsala, Sweden) to form a linear gradient in the pH range of 2.5 to 5. Riboflavin was added as a photochemical polymerizing agent. The run was increased from 400 to 850 V after an initial 30-min run at a constant current of 40 ma. The run continued for 70 min at 850 V. Focusing was carried out under cool running water at 10°. After focusing, protein bands were stained by Coomassie Brilliant Blue R-250 at 60° for 30 min, followed by decolorization in ethanol:acetic acid:distilled water (5:2:13) as described by Vesterberg (36). The gel was scanned at 540 nm with a densitometer (Asuka Mfg. Co., Ltd., Tokyo, Japan).

Isolation of IAP

Step 1. A pool of cancer ascitic fluids (10 liters) received solid ammonium sulfate to make a 60% saturation at pH 7.0; from the supernatant, IAP was precipitated by the addition of solid ammonium sulfate at 90% saturation. The resulting precipitate was dialyzed against distilled water and against a 20 mM sodium acetate buffer (pH 4.0) at 4°.

Step 2. The product after dialysis (98 g protein) was applied to a DEAE-cellulose column (6.0 x 54 cm) previously equilibrated with 20 mM sodium acetate buffer (pH 4.0), and the column was eluted successively with 0.1, 0.2, 0.3, 0.4, and 0.5 mM sodium acetate buffer (pH 4.0). Over 80% of the IAP was eluted by 0.4 mM sodium acetate buffer. Fractions containing IAP were pooled, dialyzed, and lyophilized.

Step 3. DEAE-chromatographed IAP (70 mg) was dissolved in 2 ml of 10 mM PBS, and the solution was passed through a Sephadex G-100 column (2.3 x 78 cm) previously equilibrated with PBS at a constant flow rate of 13.2 ml/sq cm/hr. Five-ml fractions were collected.

Step 4. The Sephadex G-100 fraction was further purified by preparative isoelectric focusing. All experiments were conducted at 10° in a 440-ml LKB electrofocusing column using carrier ampholites of a gradient mixer. Isoelectric focusing in a sucrose gradient (5 to 25%) and centrifuged for 18 hr at 165,000 x g at 4°. The bottoms of the tubes were punctured, and 31 fractions were collected and assayed for absorbance at 280 nm. Bovine serum albumin (M.W. 67,000), ovalbumin (M.W. 43,000), and chymotrypsinogen (M.W. 25,000) were used as marker proteins.

Chemical Analysis of IAP

To estimate the molecular weight of purified IAP, a 50- to 200-μg sample was dissolved in 1% sodium dodecyl sulfate containing 1% 2-mercaptoethanol, heated in boiling water for 2 min, and analyzed by the SDS-PAGE method as described by Segrest and Jackson (31). The S20,w value for IAP was determined by the sucrose gradient method of Martin and Ames (20). The 0.1-ml samples were placed on 4.8-ml gradients (5 to 25%) and centrifuged for 18 hr at 165,000 x g at 4°. The bottoms of the tubes were punctured, and 31 fractions were collected and assayed for absorbance at 280 nm. Bovine serum albumin (M.W. 67,000), ovalbumin (M.W. 43,000), and chymotrypsinogen (M.W. 25,000) were used as marker proteins.

Immunoassay

Serological properties of purified IAP were examined by both immunoelectrophoresis and double immunodiffusion methods. The antisera to human plasma proteins used were from Behringwerke AG (Marburg-Lahn, W. Germany) and Miles Laboratories (Elkhart, Indiana). These were prealbumin, α1-AG, albumin, α2-antitrypsin, α1-fetoprotein, Gc-globulin, ceruloplasmin, α1-antichymotrypsin, α1-lipoprotein, α1-B-glycoprotein, α1-T-glycoprotein, α2-macroglubulin, α2-HS-glycoprotein, Zn-α1-glycoprotein, α1-SP1-glycoprotein, haptoglobin, hemopexin, transferrin, α-PA-glycoprotein, C-reactive protein, β-lipoprotein, C1 esterase inhibitor, plasminogen, ferritin, fibrinogen, IgG, IgM, IgA, Ig (λ chain), Ig (k-chain), IgG/Fab, IgG/Fc, and whole human serum. The α1-AG used in this study was kindly provided by Dr. Karl Schmid (Department of Biochemistry, Boston University School of Medicine).

Lymphocyte Culture and Mitogen Treatment

Lymphocytes obtained from a healthy donor using Ficoll-Hypaque (Pharmacia) were distributed 5 x 10⁶ cells to 0.25 ml of Roswell Park Memorial Institute Medium 1640 supple-
mented with 10% fetal calf serum in plastic microplates (No. 3042; Falcon, Oxnard, Calif.). They were incubated in the absence or presence of various concentrations of IAP and human serum albumin, the latter being used as a control. PHA (PHA-P; Difco Laboratories, Detroit, Mich.) was added at an optimal concentration (15 μg/ml), and the cultures were incubated for 72 hr at 37° in humidified 5% CO₂ atmosphere. [³H]Thymidine (New England Nuclear, Boston, Mass.), 0.5 μCi/well, was added to the cultures for the final 7 hr.

Mixed-lymphocyte reaction was conducted at a final concentration of 1 × 10⁶ lymphocytes/well, in the presence and absence of IAP, in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum for 5 days at 37°. [³H]Thymidine, 0.5 μCi/well, was added for the final 7 hr. The harvested cells were counted for [³H]thymidine incorporation by a scintillation counter.

RESULTS

Detection of IAP in Serum of Cancer Patients

In a preliminary study, a single broad protein band was detected in the acidic pH range (pH 3.0 to 3.3) only when the sera from cancer patients were analyzed by gel isoelectric focusing at a pH range of 2.5 to 6 (Fig. 1A, Samples 1, 2, 4, 5, and 11). This protein band was scarcely visible in specimens obtained from healthy donors (Fig. 1A, Samples 3, 7, and 8) and benign patients (Fig. 1A, Sample 6) except for the rheumatoid arthritis patients (Fig. 1A, Samples 9 and 10). Further resolution of such acidic proteins was achieved by using a narrow pH range, pH 2.5 to 5. As shown in Fig. 1B, several distinct protein bands, ranging from pH 2.6 to 3.4, were discriminated in cancer serum. From the intensity of the staining,
it was evident that the bands of such acidic proteins in the sera from cancer patients (Fig. 1B, Samples 3, 4, and 5) and in body fluids (Fig. 1B, Samples 6 and 7) were higher than that of normal serum (Fig. 1B, Samples 1 and 2). Among the 9 distinct bands in the sera and effusions from cancer patients (Fig. 1B), 4 major bands (pl 2.8, 2.9, 3.0, and 3.1) were most distinct in intensity. Scanning densitometric analysis suggested that among these 9 bands, ranging from pl 2.6 to 3.4, the isoelectric point of the most intensely stained band was 3.0 (Chart 1, type III). In contrast to this, bands obtained from healthy subjects were distributed between pl 2.9 and 3.4 with 5 distinct bands, and the major acidic protein had a pl of 3.1, different from that of cancer serum (Chart 1, type I).

Results of Analytical Isoelectric Focusing

When 50 µl of serum obtained from 199 subjects were analyzed by the above gel isoelectric focusing, 4 patterns were discernible. Representative examples of each type are shown in Chart 1.

Type I. This is the normal pattern. In all 30 normal sera and the sera of 29 pregnant women, Coomassie Brilliant Blue-stained bands were detected throughout the pl range of 2.9 to 3.4. Among them, a major peak was always visible at pl 3.1.

Type II. All 9 patients with gastroduodenal ulcers, all 4 patients with liver cirrhosis, and all 5 patients with aplastic anemia were shown to have a major peak at 3.0. Some non-advanced cancer sera (27 samples) were also included in this type.

Type III. This type was encountered in the serum of all 8 patients with leukemia and 67% of the cancer patients: 27 of 43 stomach cancer patients (62%); 8 of 10 pancreas cancer patients (80%); 10 of 18 colorectal cancer patients (55%); 5 of 6 esophagus cancer patients (83%); and all 5 lung cancer patients (100%). All 6 ascitic fluids from metastatic cancer were included as well. Fourteen of 16 rheumatoid arthritis patients (87%) were also included in this type. They showed a major peak at pl 3.0 and a 2- to 3-fold increase in density was evident.

Type IV. Pleural effusion of all 4 lung cancer patients showed a major peak at pl 2.9, and a 3- to 4-fold increase in total density was noticeable. However, this type was very rarely found.

These results indicate that a significant increase of the acidic proteins was evident in cancer serum and effusion and that there was a qualitative difference between normal and cancer serum. The isoelectric focusing in polyacrylamide gel at the pH range of 2.5 to 5 provided one of the most sensitive methods for detecting such a difference in the composition of acidic proteins.

Purification of Human IAP from Cancer Ascitic Fluids

IAP was purified from the ascitic fluids of stomach cancer patients. Over 80% of the IAP from a 10-liter pool of ascitic fluid was recovered by 90% saturated ammonium sulfate precipitation (Step 1). Through DEAE-cellulose column chromatography (Step 2), IAP was eluted with 0.4 M sodium acetate buffer, pH 4.0 (Fraction 4), with 80% recovery (Chart 2). Vicinity fractions, i.e., Fraction 3 eluted with 0.3 M acetate buffer and Fraction 5 eluted by 0.5 M acetate buffer, contained 5 and 8% of the starting IAP concentrations (Chart 2). Fraction 4 (1.2 g protein) was further purified by Sephadex G-100 column chromatography (Step 3). Three fractions were obtained from the effluent, and over 80% of the IAP was detected in the second fraction, where albumin as a marker protein appeared. Finally, IAP was purified by column isoelectric focusing at pH 3.0.
2.5 to 6 (Step 4). IAP was eluted as a series of 3 overlapping peaks, but the major protein corresponded exactly to the one with an isoelectric point of 3.0 (Chart 3A). A narrow fraction was collected from the major protein region and refocused at the same pH gradient (Chart 3B), showing a relative pH gradient homogeneity.

For comparison, when Fraction 4, prepared from normal serum by DEAE-cellulose column chromatography (Step 2), was purified by isoelectric focusing (Step 4) under the same conditions (Chart 4), one major peak at pl 3.1 was found with 3 minor peaks in the pl range of 3.0 to 3.2.

These results demonstrate that the elution profiles of column isoelectric focusing are very similar to those obtained from the scanning pattern of gel isoelectric focusing at the pH range of 2.5 to 5 (Chart 1). Therefore, the possibility of detecting artificial components by proteolytic digestion during preparation is excluded.

Serological and Physicochemical Properties of IAP

Serological Properties. Purified IAP and α₁-AG, kindly provided by K. Schmid, were compared by both immunoelectrophoresis and double immunodiffusion. The purified IAP, a single protein with pl 3.0, reacted neither with antiserum to human whole serum nor with antiserum to 31 purified plasma proteins, as listed in "Materials and Methods," except for antiserum directed to α₁-AG or IAP (Fig. 2). Immunoelectrophoresis showed that the mobility of IAP was the same as that of α₁-AG, which showed precipitin arcs with both anti-α₁-AG and anti-IAP (Fig. 3). Both IAP and α₁-AG formed a precipitin arc slightly behind that of human serum albumin (Fig. 3). These results may indicate that these 2 proteins cannot be discriminated from each other by serological means.

pl Values. α₁-AG and purified IAP were compared by gel isoelectric focusing with a pH range of 2.5 to 5. IAP gave only one protein peak at pl 3.0, while α₁-AG had a broad band with a peak at pl 3.1 (Chart 5).

Molecular Weights. Repeated SDS-PAGE analysis with different lots of IAP showed that the molecular weight of purified IAP is almost 50,000, whereas that of α₁-AG is about 48,000 (Chart 6A), although a slightly higher value than that reported (11, 28) was obtained. This difference was confirmed by 5 to
**Table 1**

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>IAP (mol/mol)</th>
<th>a,,-AG (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>22.6</td>
<td>22.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>12.5</td>
<td>13.4</td>
</tr>
<tr>
<td>Serine</td>
<td>6.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>30.6</td>
<td>27.3</td>
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<tr>
<td>Proline</td>
<td>7.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>7.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Valine</td>
<td>6.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Methionine</td>
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<td>0.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>9.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>13.3</td>
<td>14.1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.8</td>
<td>7.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>11.9</td>
<td>13.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Tryptophan</td>
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<td>ND</td>
</tr>
<tr>
<td>M.W.</td>
<td>30,000</td>
<td>48,000</td>
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</table>

**Table 2**

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<th>Carbohydrates</th>
<th>a,,-AG</th>
<th>IAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexoses</td>
<td>16.2</td>
<td>10.1</td>
</tr>
<tr>
<td>Glucose</td>
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<td>0</td>
</tr>
<tr>
<td>Galactose</td>
<td>7.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Mannose</td>
<td>9.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Hexosamines</td>
<td>12.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>12.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Galactosamine</td>
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<td>0</td>
</tr>
<tr>
<td>Fucose</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>12.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Total</td>
<td>41.5</td>
<td>31.5</td>
</tr>
</tbody>
</table>

25% (w/v) sucrose density gradient ultracentrifugation, showing that the sedimentation coefficient of purified IAP was 3.8S (M.W. 50,000), whereas that of a,,-AG was 3.7S (M.W. 47,000) (Chart 6B). Quantitative amino acid compositions of both proteins are summarized in Table 1 with established data of a,,-AG for comparison (29). The sugar content of IAP was 31.5%, whereas that of a,,-AG was 41.5%, and the most significant difference was found in the sialic acid content (Table 2). Taken together, the physicochemical properties of IAP were slightly different from those of a,,-AG, but no immunological difference was found between the two.

**DISCUSSION**

Analytical isoelectric focusing in a thin layer of polyacrylamide gel was successfully used not only to detect an increased amount of acidic proteins in the serum of cancer patients but also to show the molecular difference between those in cancer patients and healthy people (Fig. 1). The finally chosen pH range of the gel was between 2.5 and 5, and IAP in cancer serum was distinctly developed as 9 protein bands distributed between pH 2.6 and 3.4, with the main band at an

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Inhibitory Effect of IAP on Lymphocyte Blast Formation in Vitro

Following the results obtained by Chiu et al. (6) with a,-AG, the inhibitory effect of IAP on PHA-stimulated lymphocyte proliferation was examined. Purified IAP, 5 and 10 mg/ml, significantly suppressed the incorporation of [3H]thymidine into DNA (Table 3). This suppression was dose dependent without toxicity even at a high dosage (20 mg/ml), which induced 100% suppression. A similar suppressive effect of IAP was also found in the mixed-lymphocyte reaction system, but with a lower concentration of 2 to 4 mg/ml (Table 4).
Each result was compared with the PHA-stimulated control and was evaluated by Student's t test.

Sample Nonstimulated PHA-stimulated IAP

serum was found in the pH range of 2.9 to 3.4, with 5 distinct bands and a main band at pi 3.1 (Charts 1 and 5). An initial isoelectric point of 3.0. On the other hand, α-AG in normal serum acidic proteins, as was used for the detection of genetic differences in the composition of α-AG between the sera of cancer patients and those of healthy persons. The use of acrylamide gel electrophoresis at pH 2.7 resolved normal α-AG into 4 to 8 components. Each of these components was shown to be the same in its amino acid compositions, but different in its carbohydrate compositions. Rudman et al. (26) reported that acrylamide gel electrophoresis at pH 2.7 resolved normal α-AG into 4 bands which corresponded to the 5 to 8 isomers found by Schmid et al. Furthermore, Rudman et al. (26) observed other distinct protein bands of α-AG from the plasma of cancer patients. Thus, the results obtained here are further confirmation of Rudman’s work.

For the isolation of α-AG, several procedures have been reported, including precipitation by perchloric acid, phosphotungstic acid, and ammonium sulfate, or ion-exchange chromatography. In the present study, α-AG was repeatedly purified under the name of IAP from cancer ascitic fluids. Such a pool contained almost 5 times more α-AG than normal. By means of ammonium sulfate precipitation, DEAE-cellulose chromatography at pH 4.0 followed by Sephadex G-100 chromatography, and finally by column isoelectric focusing at a pH range of 2.5 to 6, purified IAP was obtained in a relatively homogeneous form when examined immunologically or electrophoretically (Chart 5). Purified IAP contains 31.5% carbohydrate and has a molecular weight of 50,000 and an isoelectric point of 3.0. It revealed a sedimentation coefficient (s20,w) of 3.8, almost concordant to the molecular weight described above. In a parallel run, α-AG obtained from normal persons contained 41.5% carbohydrate and had a molecular weight of 48,000, a s20,w of 3.7, and a pl of 3.1. These results may suggest minute but distinct differences between these 2 α-AG acid glycoproteins, although the value obtained from SDS-PAGE could be incorrect. Glycoproteins are known to show erroneous molecular weights on sodium dodecyl sulfate gel (31).

Human α-AG is a well-characterized glycoprotein which is known to be an acute-phase protein present in human serum. Although the carbohydrate content of human α-AG examined by many investigators (11, 29) varied, the official value is about 45%. Its molecular weight is 40,000 to 44,000 and the pl is 2.7. However, the ionic strength and buffer composition at the time of determination are known to affect pl values. Because the amino acid composition of IAP was found to be almost identical to that of normal α-AG, the greatest difference between these 2 glycoproteins appears to be their carbohydrate content and compositions. These results confirm the report of Rudman et al. (25). Because several physicochemical and immunological characteristics of glycoproteins are known to be influenced by the nature of their carbohydrate units, both chemical and immunological studies of glycoproteins were warranted. Along the same lines, Rostenberg et al. (24) suggested that the cancer cell may alter the glycosylation of α-AG antitrypsin and that this abnormal glycosylation is a consequence of the malignant process.

Quantitative changes in the level of serum seromucoid have been reported in various neoplastic and nonneoplastic diseases (2). Moschides et al. (21) showed, on the basis of hexose:protein and hexosamine:protein ratios, that it is probable that qualitative changes also occur in seromucoid in several malignant diseases. Bradley et al. (5) showed that the levels of protein-bound carbohydrates reflect the sum of all the changes in the serum glycoprotein, primarily the changes in the acute-phase proteins found in the α-globulin fraction of the serum. In their opinion, serum protein-bound carbohydrates of
glycoproteins may be of adjunctive value in assessing tumor burden and immune reactivity in cancer patients. However, several types of neoplastic transformation are accompanied by alterations in the composition of cell glycoproteins which are a major structural component of the cell surface (1). Bosman and Hall (4) reported a distinct increase in sialyltransferase in breast and colon tumor tissue as compared with adjoining normal tissue. Silver et al. (32) reported that monitoring of plasma sialyltransferase may be of value in measuring tumor progression, metastatic involvement, or the success of therapeutic programs.

The synthesis of $\alpha$-AG has previously been known to occur in the liver (37) and, after the removal of terminal sialic acid, it is cleared from the circulation by binding to a receptor protein on liver cell plasma membrane (35). Sarcione (27) observed that the isolated rat liver has been shown to incorporate $[^14C]$leucine into the protein of plasma $\alpha$-AG. Increased amounts of circulating glycoproteins and their biosynthesis in tumor-bearing animals has been the subject of many discussions (18, 37). However, Gahmberg and Andersson (8) have recently demonstrated the presence of $\alpha$-AG, with an apparent molecular weight of 52,000, on normal leukocytes and suggested that this membrane protein is synthesized by lymphocytes, granulocytes, and macrophages. It is subsequently cleaved and released in soluble serum form with a molecular weight of 41,000. We found that IAP was released in the culture medium of normal macrophages and granulocytes by microassay of IAP.4

The present study demonstrated that human IAP, isolated from cancer ascitic fluids, suppressed the mitogenic effects of PHA on human lymphocytes and inhibited allogeneic lymphocyte stimulation in a 2-way mixed-lymphocyte reaction, although a 2-mg/ml dose was required to induce a 50% inhibition of the latter (Table 4). Thus, activity of IAP is not very strong, but it may cause the nonspecific immune suppression reported in many diseases, including cancer. For such suppression, IAP showed twice the activity when compared with normal $\alpha$-AG (data not shown), and the results reported by Costello et al. (7), which indicated that asialo-$\alpha$-AG has a higher suppressive effect than that of the original, seem to support this.

The immunosuppressive effect of certain serum protein fractions was demonstrated first by Kamurin (15) and later by Mowbray (22), who described an $\alpha$-globulin fraction of both human and bovine serum which could suppress antibody response in animals. More recently, inhibition of the in vitro response of lymphocytes to specific antigens or mitogens by such fractions has been studied (10). The majority of these reports described the active fractions as being $\alpha$-globulin in nature but did not identify the active components in the mixtures, described as immunoregulatory $\alpha$-globulins, except for the report of Occhino et al. (23). Immunoregulatory $\alpha$-globulin has been shown to diminish rejection of skin allografts, tumor transplants, and allograft organtransplant and to impair PHA-induced lymphocyte blastogenesis. IAP exhibits almost all of these activities (data not shown). The inhibitory effect of cancer patients' sera and ascitic fluid on lymphocytes has been reported with various tumors (3, 9). These factors are not unique to cancer, inasmuch as serum factors which inhibit lymphocyte transformation in vitro in response to nonspecific mitogens and/or specific antigens have been described in a number of pathological conditions (5, 14). Hanna et al. (13) reported that normal immunosuppressive protein isolated from human plasma or Cohn Fraction IV was found to inhibit both humoral and cellular immune responses and that normal immunosuppressive protein was a glycoprotein or a glycopeptide. However, there have not yet been any reports on the physicochemical properties of normal immunosuppressive protein. Thus, we believe that the IAP, or modified $\alpha$-AG, characterized here is one of these immunosuppressive glycoproteins.

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Unpublished data.
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Isolation and Characterization of an Immunosuppressive Acidic Protein from Ascitic Fluids of Cancer Patients

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