Anemia-inducing Substance from Plasma of Patients with Advanced Malignant Neoplasms

Ken-ichi Honda, Osamu Ishiko, Ichiro Tatsuta, Masaaki Deguchi, Kouzo Hirai, Shin-ichi Nakata, Toshiyuki Sumi, Tomoyo Yasui, and Sachio Ogita

ABSTRACT

Patients with advanced malignant neoplasms develop anemia and immunosuppression. During an attempt to identify the causes, we have found that plasma from such patients makes RBCs more fragile in hypotonic buffer, according to results obtained with a coil planet centrifuge. Plasma from these patients suppresses mitogen-stimulated lymphocyte proliferation. In this study, we identified the substance with these effects as a protein. During two-dimensional gel electrophoresis, two isomers with $M_r$ 50,000 and slightly different isoelectric points near 6.0 were found. Cell fractionation showed that these proteins were in both the cytosol and the nuclear fraction of cells in neoplasms. Another protein with the same antigenicity and a $M_r$ 100,000 was found in the nuclear fraction of cells in neoplasms.

INTRODUCTION

As early as the 1950s, humoral factors were suspected to cause the anemia and cachexia in patients with advanced neoplasms who show no signs of bleeding or myelosuppression. When RBCs from healthy subjects are transfused into patients with advanced cancers, the life span of these cells is shortened (1). Increased frequency of hemolysis seems to be a factor in the development of anemia in such patients.

Since the 1980s, tumor necrosis factor and other cytokines have been isolated and many of their effects have been identified, including impairment of hematopoiesis in cultured bone marrow cells (2, 3). A high level of tumor necrosis factor or other cytokines contributes to hematopoietic disturbances in patients with cancers (4, 5), but hematopoietic disturbances are also observed in patients with chronic inflammatory disease (6–8).

Our previous study suggested that osmotic fragility of RBCs is more common at an advanced stage of malignant neoplasm, and that the fragility increases as the disease progresses (9); the patients had somewhat enlarged spleens and a significantly higher mean level of serum bilirubin than healthy subjects, although within reference limits (10). These findings suggested that increased hemolysis is one factor contributing to the anemia of cancer.

Plasma of patients with an advanced malignant neoplasm seems to contain some substance that decreases the osmotic resistance of RBCs (9, 10). Osmotic resistance can be measured by a CPC, in which RBCs pass through a gradient of NaCl concentrations below the physiological osmotic pressure, causing gradual inflow of water into the cells. Normal RBCs incubated in plasma from a patient with an advanced malignant neoplasm begin hemolysis at a higher osmotic pressure than RBCs incubated in plasma from a healthy subject. We named the substance that causes this fragility AIS. Plasma containing AIS also suppresses lymphocyte proliferation stimulated by PHA. The purpose of this study was to separate AIS from plasma of patients with advanced malignant neoplasms and to characterize its physical characteristics.

MATERIALS AND METHODS

Phenyl-Sepharose Column Chromatography of Plasma. Blood was sampled with a syringe coated with heparin from two patients (one with uterine leiomyoma and one with uterine leiomyosarcoma, which was at an advanced stage), and was centrifuged at 1000 × g for 10 min to obtain 10 ml plasma from each patient. The plasma was diluted 2-fold in 10 mM phosphate buffer (pH 7.4), 1.5 mM NaCl, 0.1 mM EDTA, and 0.1 mM dithiothreitol (final concentrations), and the mixture was put on a phenyl-Sepharose CL4B column (1.5 x 5 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) and equilibrated with 10 mM phosphate buffer (pH 7.4) containing 1.5 mM NaCl, 0.1 mM EDTA, and 0.1 mM dithiothreitol. After the nonadsorbable fraction passed through, the NaCl concentration of the buffer was lowered gradually from 1.5 to 0 mM. Eluted fractions were dialyzed against PBS (0.01 M sodium phosphate and 0.15 mM NaCl, pH 7.4) and tested to determine whether they could change the osmotic pressure at which RBCs started to lyse in a CPC coil with a NaCl gradient from higher to lower osmotic pressure. The dialyzed fractions were tested also to determine whether they could change the extent of lymphocyte proliferation stimulated by PHA.

Measurement of Osmotic Pressure at Which RBCs Started to Lyse. Blood was sampled with a syringe coated with heparin from a healthy subject whose ABO blood type was the same as the patient to be tested, and centrifuged at 1000 × g for 10 min to prepare packed RBCs and plasma. Packed RBCs (250 μl) and plasma (500 μl) were incubated with 50 μl of a 5-ml fraction of plasma separated by phenyl-Sepharose column chromatography or with 50 μl PBS for 30 min at 37°C. After the incubation, the osmotic pressure at which RBCs started to lyse in the CPC coil (Sanki Engineering, Ltd., Kyoto, Japan) was measured as described elsewhere (11, 12). The CPC had a rotating coil holder that carried coiled polyethylene tubes that undergo centrifugal force during rotation. RBCs were put in the area with high osmotic pressure (150 m0sm/kg) of a gradient of NaCl in one of the tubes (inside diameter, 0.7 mm; 240-cm long), and the cells were stimulated to move toward the area with low osmotic pressure (30 m0sm/kg) by centrifugal force at 300 × g for 15 min. The osmotic pressure at which hemolysis started for RBCs incubated earlier with various fractions of plasma was compared with that when RBCs were incubated with PBS, and the differences between osmotic pressure at which hemolysis started were calculated. The difference was taken to be positive if RBCs started to lyse at higher osmotic pressure than the osmotic pressure at which hemolysis for RBCs incubated with PBS started.

Measurement of Lymphocyte Proliferation Stimulated by PHA. Blood was sampled with a syringe coated with heparin from a healthy subject and centrifuged in a Leukoprep tube (Becton Dickinson and Co., Oxnard, CA) to obtain lymphocytes. The lymphocytes (2 × 10^7 cells/ml) were cultured in a multiwell plastic dish with 200 μl RPMI 1640 medium containing 10% fetal bovine serum and 2 μg PHA, to which 20 μl of a plasma fraction or PBS (as a control) were added. After 72 h of culture in a CO2 incubator at 37°C, 0.5 μCi [3H]thymidine in 10 μl RPMI 1640 medium was added, and the culture was continued for 4 h. The uptake of [3H]thymidine into lymphocytes was measured by harvesting of cells on a glass filter and counting of the scintillation rate in scintillation liquid (Emulsifier-Safe; Packard Instruments B.V., Groningen, the Netherlands). Effects of various fractions of plasma on lymphocyte proliferation stimulated by PHA were expressed as the percentage of inhibition: [(cpm after culture with PBS - cpm after culture with the fraction)/ cpm after culture with PBS] × 100.

One- or Two-dimensional Gel Electrophoresis. Proteins in a fraction of plasma were analyzed using one- or two-dimensional gel electrophoresis. For...
one-dimensional gel electrophoresis, a slab gel of 10% polyacrylamide-SDS was used as described by Laemmli (13). Two-dimensional gel electrophoresis was done as described by O’Farrell (14). An isoelectric focusing gel containing 1.6% ampholytes (pH 6–8) and 0.4% ampholytes (pH 3.5–10) was used for the first dimension, and a 10% polyacrylamide-SDS slab gel was used for the second dimension. After electrophoresis, the gel was stained with Coomassie blue or processed for Western blotting.

Production of Polyclonal Antibodies. After two-dimensional gel electrophoresis of the plasma fraction, the protein spots stained with Coomassie blue were cut from the gel and stored at −20°C until use. For the first immunization, 10 gel spots were both neutralized and homogenized together in PBS with a Teflon-glass homogenizer, emulsified with Freund’s complete adjuvant, and injected into Japanese White rabbits s.c. in several places on the back. For booster injections, homogenates from 10 gel spots were emulsified with Freund’s incomplete adjuvant and injected in the same way and 4 weeks after the first injection. Antiserum obtained 8 days after the second booster injection was used in this study. The IgG fraction of the antiserum was separated on a Teflon-glass homogenizer, emulsified with Freund’s complete adjuvant, and injected into Japanese White rabbits s.c. in several places on the back. For booster injections, homogenates from 10 gel spots were emulsified with Freund’s incomplete adjuvant and injected in the same way and 4 weeks after the first injection. Antiserum obtained 8 days after the second booster injection was used in this study. The IgG fraction of the antiserum was separated on a protein A-Sepharose column (Sigma Chemical Co., St. Louis, MO).

Western Blotting. Proteins separated by one- or two-dimensional gel electrophoresis were transferred to a nylon membrane (Clear Blot P, Atto, Tokyo, Japan) at 1.5 mA/cm² for 1 h in a Trans Blot apparatus (Atto) with a buffer of 0.1 M Tris-glycine (pH 8.7) containing 20% methanol (v/v). The nylon membrane was immersed in blocking solution (20% fetal bovine serum in PBS) for 20 min and treated for 1 h with antiserum diluted 100-fold with blocking solution. After being washed with 0.5% Tween 80 in PBS and then with PBS alone, the membrane was incubated with goat antiserum against rabbit IgG conjugated with peroxidase. After the membrane was washed, the immune complexes were made visible by incubation of the membrane in 50 mM acetic buffer (pH 5.0) containing 0.04% 3-amino-9-ethylcarbazole and 0.015% H₂O₂.

Immunofinity Column. The IgG fraction of the antiserum was coupled to tressyl-activated Sepharose (Pharmacia) in 100 mM sodium carbonate buffer (pH 8.0). The Sepharose gel was decoupled with 100 mM Tris-HCl buffer (pH 8.0) and equilibrated with PBS. The plasma fraction that was eluted from the phenyl-Sepharose column was dialyzed against PBS and its ability to decrease the osmotic resistance of RBCs was tested. The adsorbed protein was eluted from the column with 0.1 M glycine-HCl buffer (pH 2.5), neutralized with 1 M NaCl, and dialyzed against PBS. The dialyzed solution was sampled and assayed for its ability to decrease the osmotic resistance of RBCs or the extent of lymphocyte proliferation stimulated by PHA. The dialyzed solution that remained was concentrated 10 times, and the proteins in it were separated by SDS-gel electrophoresis and stained with Coomassie blue.

Immunoadsorption Study. Blood was sampled with a syringe coated with heparin from a patient with ovarian carcinoma at an advanced stage or from a healthy subject and centrifuged for plasma separation. The plasma was dialyzed against PBS at 4°C overnight. Then 200 μl dialyzed plasma were incubated with 100 μg IgG from a rabbit not immunized or anti-AIS rabbit IgG at 4°C overnight. One ml of protein A-Sepharose CL4B (Sigma) was added, and incubation was continued for 2 h to precipitate IgG complexes. The supernatant was tested for its ability to change the osmotic resistance of RBCs or the extent of lymphocyte proliferation stimulated by PHA.

Cell Fractionation. One g ovarian tumor tissue from a patient with adenoma or adenocarcinoma was minced in 20 ml ice-cold hypotonic buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 1.5 mM MgCl₂, and 1 mM PMSF) and homogenized with a Teflon-glass homogenizer. The homogenate was centrifuged at 600 × g for 5 min, at 10,000 × g for 5 min, and then at 100,000 × g for 1 h at 4°C to give a supernatant that was used as the cytosol fraction. SDS was added to the cytosol fraction to a final concentration of 1.0%. After centrifugation at 600 × g, the pellet was dispersed in 20 ml hypotonic buffer. Then NP-40 and sodium deoxycholate were added to the final concentrations of 0.2% each, and the mixture was homogenized with 10 up-and-down strokes. The homogenate was centrifuged at 12000 × g for 5 min, and the pellet was dispersed in 10 ml of a mixture of 0.25 M sucrose, 3.3 mM CaCl₂, and 1 mM PMSF; layered over 10 ml of a mixture of 0.88 M sucrose and 1 mM PMSF; and centrifuged at 12000 × g for 10 min to obtain a pellet of purified nuclei (15). SDS was added to this nuclear fraction at a final concentration of 0.2%. Proteins in the cytosol and the nuclear fractions were analyzed using one-dimensional SDS-gel electrophoresis and Western blotting.

RESULTS

Plasma fractions obtained from a patient with uterine leiomyoma had no effect on the osmotic resistance of RBCs (Fig. 1A). Plasma fractions obtained from a patient with uterine leiomyosarcoma at an advanced stage and eluted at NaCl concentrations of slightly less than 0.5 M decreased the osmotic resistance of RBCs (hemolysis occurred at higher osmotic pressure; Fig. 1B). This patient had normochromic anemia, with 8.2 g/dl hemoglobin, and a hematocrit of 24.1%, but had not had blood transfusions until after blood sampling for this study. Plasma fractions obtained from the patients with uterine leiomyoma or leiomyosarcoma at an advanced stage and eluted from a phenyl-Sepharose column suppressed lymphocyte proliferation stimulated by PHA, but the suppression was greater with the plasma fractions from the patient with leiomyosarcoma. AIS seemed to be in the plasma fractions of the patient with uterine leiomyosarcoma at an advanced stage that were eluted at NaCl concentrations of slightly less than 0.5 M, and proteins in the plasma fractions were examined by two-dimensional gel electrophoresis (Fig. 2). Because of the capacity of the isoelectric focusing gels, proteins with isoelectric points between 5.0 (right side) and 7.5 (left side) were separated from proteins with other isoelectric points which did not enter the isoelectric focusing gel. Two specific proteins with Mᵦ 50,000 and slightly different isoelectric points near 6.0 were found in these fractions (arrows, Fig. 2B); these proteins were not found in the corresponding fractions of plasma from the patient with uterine leiomyoma (Fig. 2A). When these two proteins, taken from the gel separately, were used to immunize two rabbits so that polyclonal

<table>
<thead>
<tr>
<th>Protein concentration (A)</th>
<th>NaCl concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein concentration (A)</th>
<th>NaCl concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 1. Phenyl-Sepharose column chromatography of plasma from a patient with uterine leiomyoma (A) and a patient with uterine leiomyosarcoma at an advanced stage (B). Open arrows, fractions were tested for their effects on the osmotic pressure at which RBCs incubated with the fraction lysed (expressed by the difference in the osmotic pressure at which RBCs started to lyse in the CPC coil, upper graphs; the upward direction indicates higher osmotic pressure and 0 is the value for the control RBCs incubated in PBS), or for their effects on lymphocyte proliferation stimulated by PHA (expressed by percentage of inhibition, middle graphs; the upward direction indicates increased inhibition). Lower graphs, the protein concentration is shown by the thick line and the concentration of NaCl is shown by the thin line.

3624
ANEMIA-INDUCING SUBSTANCE IN PLASMA

Fig. 2. Two-dimensional gel electrophoresis of plasma fractions. Plasma from a patient with uterine leiomyoma (A) and a patient with uterine leiomyosarcoma at an advanced stage (B and C) was eluted from a phenyl-Sepharose column at NaCl concentrations between 0.5 and 0.2 M, separated by two-dimensional gel electrophoresis, and stained with Coomassie blue (A and B), or were treated using Western blotting and reacted with rabbit antiserum against AIS (C). Arrows, isomers of AIS; the one on the right, acidic isomer.

antibodies would be produced, both antisera cross-reacted with the other protein. Fig. 2C shows the results with the antiserum against the protein with the lower isoelectric point. We considered these proteins to be isomers, and refer to them as a single protein below.

In a study conducted to determine whether this protein was present in the plasma of patients with other advanced malignant tumors, plasma of a patient with cervical adenocarcinoma at an advanced stage was fractionated using phenyl-Sepharose column chromatography and analyzed using Western blotting (Fig. 3). The antiserum raised against this protein from a plasma fraction of the patient with uterine leiomyosarcoma at an advanced stage (above) reacted with a protein of the same molecular weight in the plasma fractions of this patient with cervical adenocarcinoma at an advanced stage that were eluted at NaCl concentrations of 0.5 or 0.2 M (Fig. 3B, Lanes 4 and 5). The antibodies did not react with any plasma protein of the patient with uterine leiomyoma (Fig. 3B, Lanes 6–9).

The protein was purified from the plasma fractions of the patient with uterine leiomyosarcoma at an advanced stage on a column.

Fig. 3. AIS in plasma of patients with malignant neoplasm. Plasma proteins that passed through the phenyl-Sepharose column (Lanes 2 and 6) or that were eluted at NaCl concentrations of 1.0 M (Lanes 3 and 7), 0.5 M (Lanes 4 and 8), or 0.2 M (Lanes 5 and 9) were separated by one-dimensional gel electrophoresis, stained with Coomassie blue (A), or treated with rabbit antiserum against AIS after Western blotting (B). AIS was detected in the plasma of a patient with cervical adenocarcinoma at an advanced stage (Lanes 2–5), but was not detected in the plasma of a patient with uterine leiomyoma (Lanes 6–9). Plasma proteins of a patient with uterine leiomyosarcoma at an advanced stage eluted at NaCl concentrations of 0.5 M or less (Fig. 1, Lane 1) were separated on a SDS slab gel in the same way. Lane 10, molecular weight markers: phosphorylase b, M, 92,500; bovine serum albumin, M, 69,000; amylase, M, 55,000; and ovalbumin, M, 45,000.
ANEMIA-INDUCING SUBSTANCE IN PLASMA

LYMPHOCYTES STIMULATED BY PHA INCUBATED IN THE PRESENCE OF THE PROTEIN WAS RELATED SOMewhat TO THE CONCENTRATION OF THE PROTEIN (FIG. 5B). WE CONSIDERED THE PURIFIED PROTEIN TO BE AIS, AND AN IMMUNOADSORPTION STUDY WAS DONE TO TEST THE EFFECT OF THE ANTISERUM. IMMUNOADSORPTION OF AIS FROM PLASMA OF A PATIENT WITH OVARIAN CARCINOMA AT AN ADVANCED STAGE BY INCUBATION WITH ANTI-AIS ANTIBODY AND PRECIPITATION WITH PROTEIN-A SEPHAROSE HAD TWO EFFECTS. WITHOUT THE ANTIBODY TREATMENT, RBCS INCUBATED WITH THIS PLASMA BEGAN TO LYSE AT AN OSMOTIC PRESSURE OF 7.0 mOsm/kg HIGHER THAN THAT AT WHICH CONTROL RBCS BEGAN TO LYSE. TREATMENT WITH THE ANTI-AIS ANTIBODY COMPLETELY PREVENTED THIS SHIFT. SUCH IMMUNOADSORPTION ALSO DECREASED THE INHIBITION OF LYMPHOCYTE PROLIFERATION STIMULATED BY PHA FROM 24% (WITHOUT ANTISERUM) TO 8% (WITH ANTISERUM). THE SUBCELLULAR DISTRIBUTION OF AIS IN CELLS OF OVARIAN ADENOMA AND ADENOCARCINOMA TISSUE WAS IDENTIFIED BY USE OF THE ANTISERUM IN WESTERN BLOTTING (FIG. 6). AN IMMUNOREACTIVE PROTEIN WITH A $M_r$ 50,000 WAS DETECTED IN BOTH THE CYTOSOL AND THE NUCLEAR FRACTIONS OF CELLS IN OVARIAN ADENOCARCINOMA TISSUE (FIG. 6, LANES 7 AND 8). ANOTHER IMMUNOREACTIVE PROTEIN WITH A $M_r$ 100,000 WAS FOUND IN THE NUCLEAR FRACTION OF THESE CELLS (FIG. 6, LANE 8). IMMUNOREACTIVE PROTEINS WERE NOT DETECTED IN THE CYTOSOL FRACTION OR THE NUCLEAR FRACTION OF CELLS IN OVARIAN ADENOMA TISSUE. PROTEINS WITH $M_r$ 100,000 OR HIGHER WERE MORE ABUNDANT IN THE NUCLEAR FRACTIONS OF CELLS IN OVARIAN ADENOMA TISSUE (FIG. 6, LANE 2) THAN IN THE NUCLEAR FRACTION OF

![Fig. 4. SDS-gel electrophoresis of AIS separated by an immunoaffinity column. AIS in plasma fractions from a patient with uterine leiomyosarcoma was eluted from the immunoaffinity column, which was coupled with anti-AIS antibody, concentrated 10-fold (Lane 1) or 5-fold (Lane 2), separated by SDS-gel electrophoresis, and stained with Coomassie blue. Molecular weight markers shown in Lane 3 are the same as those in the legend to Fig. 3.](image)

coupled with the anti-AIS antibody. The purified protein had a $M_r$ 50,000 on SDS-gel electrophoresis (Fig. 4). Minor smears with $M_r$ 60,000–70,000, probably arising from proteins abundant in the fractions, eluted from the phenyl-Sepharose column and nonspecifically bound to the immunoaffinity column. In Western blotting, the anti-AIS antiserum showed no reaction to the proteins with $M_r$ 60,000–70,000 (Fig. 3B, Lane 1).

The purified protein had a concentration-dependent effect on the osmotic pressure at which RBCs began to lyse (hemolysis occurred at higher osmotic pressure; Fig. 5A). The osmotic pressure at which RBCs began to lyse shifted about 7 mOsm/kg toward higher osmotic pressure after incubation of the cells in the presence of the AIS protein (about 0.2 μg/ml); shifts caused by 2-fold and 10-fold diluted solutions were smaller, in a concentration-dependent way.

The protein also suppressed the extent of lymphocyte proliferation stimulated by PHA. Percentage of inhibition of the proliferation of lymphocytes stimulated by PHA incubated in the presence of the protein was related somewhat to the concentration of the protein (Fig. 5B).
ANEMIA-INDUCING SUBSTANCE IN PLASMA

Fig. 6. Subcellular distribution of AIS. Cells from ovarian adenoma tissue (Lanes 1, 2, 5, and 6) or ovarian adenocarcinoma tissue (Lanes 3, 4, 7, and 8) were fractionated. The proteins of the cytosol fraction (Lanes 1, 3, 5, and 7) and the nuclear fraction (Lanes 2, 4, 6, and 8) were separated by SDS-gel electrophoresis and stained with Coomassie blue (Lanes 1-4), or were treated using Western blotting and reacted with rabbit antiserum against AIS (Lanes 5-8). Molecular weight markers are the same as those in the legend to Fig. 3.

DISCUSSION

Some immunosuppressive substances are increased in plasma or ascites of subjects with advanced cancers (16, 17). Plasma fractions of healthy women (one of whom was pregnant, data not shown) eluted from a phenyl-Sepharose column also suppressed lymphocyte proliferation stimulated by PHA, as did those of the patient with uterine leiomyoma, although the suppression rates were smaller than those of plasma fractions from the patient with uterine leiomyosarcoma at an advanced stage. Osmotic resistance of RBCs did not change by incubation with the plasma fractions of the healthy women or the patient with uterine leiomyoma. On the other hand, RBCs started to lyse at osmotic pressures of 4–5 mOsm/kg higher after incubation with plasma fractions of patients with gynecological neoplasms at advanced stages (cervical, endometrial, and ovarian carcinomas, data not shown) eluted from phenyl-Sepharose columns, as did RBCs after incubation with plasma fractions of the patient with uterine leiomyosarcoma at an advanced stage.

In rabbits given a transplant of VX2 carcinoma cells, the life span of RBCs is reduced from the normal mean of 170 h to a mean of 95 h (10). In rabbits with disseminated VX2 carcinoma, the life span of RBCs is further reduced to a mean of 30 h. The osmotic resistance of RBCs is reduced with the growth of VX2 carcinoma, suggesting that some substance that shortens the life span of RBCs is released from the tumor.

RBCs incubated with plasma of patients with malignant tumors at an advanced stage had decreased glucose influx, pyruvate kinase activity, and ATP concentration (9). Normal RBCs incubated at 37°C with the plasma fraction of a patient with ovarian carcinoma at an advanced stage had increased osmotic fragility, but the RBCs incubated with the plasma fraction at 4°C for 30 min and washed at 37°C with a plasma fraction of a healthy subject had no osmotic fragility (data not shown). Therefore, the mechanism causing increased osmotic fragility seemed not to depend on receptors, but on inhibition of metabolism in RBCs.

An anti-AIS antibody almost completely prevented plasma of a patient with ovarian carcinoma at an advanced stage from causing osmotic fragility of RBCs. The suppression of lymphocyte proliferation by PHA decreased after incubation of plasma of the patient with the anti-AIS antibody. Immunosuppression can be caused by many factors, one of which is AIS.

Zucker et al. (18) extracted with detergent a cytotoxic protein with a Mr 62,000 from the plasma membrane of mouse NIH-3T3 cells transformed with ras oncogene. RBCs incubated with the protein at 37°C for 18 h lyse at a lower concentration than fibroblasts. Some membrane components of normal blood cells also lyse RBCs, but detergent is needed for extraction of the components (19–21). A protein with mild hemolytic properties and a Mr 66,000 has been purified from the culture medium of NIH-3T3 cells transformed with ras oncogene (22). Such hemolysis of RBCs is metal dependent and occurs after 2 days of incubation. These substances seem to be different from AIS, the extraction of which does not require detergent and the hemolysis by which does not require metal ions or long incubation.

Nuclear matrix proteins have been detected in the incubation medium of dying cancer cells and in serum from cancer patients (23). These proteins may be released from the nuclei of cancer cells. AIS was detected in both the cytosol and the nuclear fraction of cells in cancer tissue, but another protein with the same antigenicity and a Mr 100,000 was detected in the nuclear fraction of cells in cancer tissue.

We examined plasma of a patient with rheumatoid arthritis, but no plasma fraction had AIS-like effects on RBCs. AIS seems to be specific to malignant neoplasms and not to be associated with chronic inflammatory disorders.

ACKNOWLEDGMENTS

We thank Professor Emeritus of our department, Dr. Tadashi Sugawa, for his guidance. We acknowledge the technical aid of Dr. Keizo Naka. We thank Caroline Latta for reading the manuscript.
REFERENCES

Anemia-inducing Substance from Plasma of Patients with Advanced Malignant Neoplasms

Ken-ichi Honda, Osamu Ishiko, Ichiro Tatsuta, et al.


Updated version  Access the most recent version of this article at:  [http://cancerres.aacrjournals.org/content/55/16/3623](http://cancerres.aacrjournals.org/content/55/16/3623)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.