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Tumour Regression after Extracorporeal Affinity Chromatography of Blood Plasma across Agarose Beads Containing Staphylococcal Protein A*

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Abstract—*The therapeutic effect of absorbing plasma from tumour patients with immobilized staphylococcal protein A was tested. Plasma prepared by centrifugation was passed over protein A–Sepharose and then reinfused into the patient. Five patients were thus treated. One with malignant melanoma and one with renal adenocarcinoma showed measurable regression of metastatic lesions. In another with malignant melanoma a subcutaneous metastasis showed histopathological changes compatible with a therapeutic effect. In two patients, one with malignant melanoma and one with renal adenocarcinoma, no signs of regression were found. No severe adverse effects of the treatment were observed.*

INTRODUCTION

THE IMMUNE response towards malignant tumours in man only rarely controls the disease when it has become clinically manifest. Inhibition of specific lymphocyte-mediated cytotoxicity by serum-blocking factors can be partly responsible for this [1–3]. Blocking factors have been identified as antibodies [4–6], free antigens [7] and antigen–antibody complexes [8, 9]. Steele and co-workers [10, 11] reported that blocking factors of human- and rat-tumour-bearer sera could be removed with killed *Staphylococcus aureus*, strain Cowan I (SAC), carrying immunoglobulin-binding protein A. This principle has been used in the treatment of human [12–14] and animal tumours [15–19]. An antitumour effect was also reported when purified protein A bound to agarose [20], silica gel [21] or fixed to collodion charcoal was used [22, 23]. Cytosine arabinoside was also given [22, 23] in some of the treatments in the latter studies.

The risk of leakage of bacterial substances [15, 24] and of adverse effects of treatments employing SAC prompted us to use protein A bound to agarose (protein A–Sepharose®). Feasibility experiments involving reinfusion of protein A–Sepharose-treated autologous citrate plasma in rabbits, dogs and pigs resulted in no untoward effects [Jonsson and Schantz, unpublished data]. We now report clinical effects of treating tumour patients with plasma absorbed with protein A–Sepharose.

MATERIALS AND METHODS

Only patients with metastatic disease resisting all other therapy were included. The series comprises three patients with malignant melanoma and two with adenocarcinoma of the kidney. Detailed data and treatment plans are given in the case reports and in Table 1.

Investigations done before treatment included computerized tomography (CT) of the brain, CT of the abdomen, pulmonary X-ray, bone- and liver-scintigraphy, and determinations of serum or plasma concentrations of sodium, potassium, calcium phosphate, urea, creatinine, total bilirubin, alkaline phosphatase and immunoglobulins. The biochemical tests were repeated after each treatment session. The circulating immune complexes (CIC) were determined by a C_{1q}-binding technique [25].

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Table 1. Off-line treatment procedure for affinity chromatography of blood plasma from tumour patients

Preparation of blood plasma by centrifugation in a discontinuous procedure where the blood cells are returned to the patient immediately
↓
Recentrifugation of the plasma to eliminate contaminating blood cells
↓
Affinity chromatography of plasma over protein A-Sepharose CL-4B. Flow rate approx. 10 ml/min
↓
Infusion of treated plasma to the patient within 24 hr. Flow rate approx. 10 ml/min

Preparation of plasma

Most treatments were given off-line: plasma was collected by intense plasmapheresis using a Haemonetics® Cell Separator. Blood was aspirated from a cubital vein (in patient 2 also from the subclavian vein) at a rate of 40–60 ml/min. Heparin, 3000 IE to 1000 ml of blood, was used as anticoagulant during the first seven plasmaphereses in patient 1 and the first five in patient 2. In all subsequent treatments acid-citrate-dextrose solution (NIH, formula A) was used.

The cell separator operates on an intermittent flow principle, whole blood being separated into plasma and blood corpuscles by centrifugal force. When plasma corresponding to 225 ml of packed blood corpuscles has been removed, the corpuscles are returned to the patient together with some plasma substitute; the cycle can be repeated. In this study each plasmapheresis comprised 5–10 cycles, and a total of 2000–2500 ml of plasma was obtained. As a rule the temporary loss of plasma was made up with saline; if the pre-run albumin value was low, 200–300 ml of 20% albumin (Kabi, Sweden) was given during each run.

To avoid repeated damage to the platelets in the centrifuge (possibly resulting in activation of coagulation even in the presence of heparin), platelets from each cycle were collected in separate bags until the plasmapheresis procedure was complete, when all the platelets were returned.

Before transferring the plasma to the protein A-Sepharose column, the plasma was centrifuged at 4200 rev/min for 5 min to free it from any remaining platelets and erythrocytes.

In six sessions in patient 3 treatment was made on-line using a plasmapheresis filter (Plasmaflo 0.65 m^2, ASAHI Medical Co., Japan). The filter was prepared as specified by the manufacturer. The patient was given 5000 IE of heparin before starting plasma preparation, and subsequently 2000 IE/hr. Blood was aspirated from a Thomas femoral shunt (Physio-Control, Washington, DC, U.S.A.) at a rate of 150–200 ml/min, giving about 20 ml plasma/min. The plasma was pumped through the protein A-Sepharose column and then reunited with the blood before being returned to the patient. The extracorporeal circulation was established and monitored by a modified blood unit from a dialysis monitor (Ak 10, Gambro, Sweden).

Protein A-Sepharose columns

Protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Sweden) was handled aseptically when swollen in saline as directed by the manufacturer. The gel was sedimented and thoroughly washed with saline in columns previously sterilized with ethylene dioxide. Three types of columns, with capacities of 45, 150 (Gambro AB, Sweden) and 250–500 ml (K 50/30 Pharmacia Fine Chemicals, Sweden), were used. Before absorption of patient plasma for the first time each column was washed once with 0.20 M glycine adjusted to pH 2.8 with HCl and then neutralized with 1000 ml of sodium-acetate-buffered saline pH 7.0.

Samples for bacteriological culture were taken from swollen gel. In addition, the first 100–200 ml of saline which passed the column was concentrated on sterile filters which were then incubated on culture plates. Samples of saline passing the column were also tested for the presence of pyrogenes. Between treatments the columns were stored at +4°C with merthiolate 0.01% as preservative. When columns were re-used their sterility was repeatedly tested before each run after thoroughly washing away the merthiolate, 200–300 ml saline being concentrated on sterile filters and incubated as described above. The sterility of the columns was preserved throughout, even when columns were re-used 10–20 times.

Absorption of plasma

About 2000 ml of the previously prepared plasma (for details see Table 2) passed the column (containing 90–500 ml gel; see Table 2) at a flow rate of 10 ml/min. The treated plasma was then returned to the patient within 24 hr as an intravenous infusion over about 3 hr. In the treatment of the last three patients (in Table 2) the first portion of plasma, containing no IgG after absorption, was not returned to the patient.

The column was then regenerated as follows. It was thoroughly washed with acetate-buffered saline. The bound proteins were eluted with a continuous pH gradient buffer which was achieved by gradually mixing 0.2 M dibasic sodium phosphate solution and 0.1 M citric acid

Table 2. Patient data and treatment plan

Patient	Age	Sex	Diagnosis	Frequency of treatment sessions	Amount of protein A-Sepharose CL-4B	Amount of treated plasma
1	57	M	malignant melanoma; primary site, dorsum. Metastasis to skin, abdominal lymph nodes and cerebrum	session* 1–5 twice weekly 6–9 once weekly	session 1 45 ml 2–6 90 ml 7–9 220 ml	1300 ml 2300 ml 2500 ml
2	53	F	malignant melanoma; primary site, right leg. Metastasis to regional lymph nodes, skin, abdominal lymph nodes and cerebrum	session† 1–4 twice weekly 5–18 once weekly 19–22 twice weekly	session 1–7 90 ml 8–18 250 ml 19–22 450 ml	2200 ml 2200 ml 2200 ml
3	45	M	adenocarcinoma of the kidney. Metastasis to skin, bone, lung and pleura	session 1–6 once weekly 7–9 three per month 10–13 once weekly	session 1–13 250 ml	2500 ml
4	55	F	left hypernephroma. Bilateral pulmonary metastasis	session 1–8 once weekly	session 1–8 300 ml	2500 ml
5	71	M	malignant melanoma; primary site, right foot. Metastasis to skin of right leg	session 1–14 once weekly	session 1–5 150 ml 6–11 300 ml 12–14 500 ml	3000 ml

Heparin was used as anticoagulant in sessions *1–6 and †1–5.

solution. The total volume of the gradient was 600–1000 ml, and it reached a minimum pH of 3.0. Finally the column was washed with 500–1000 ml glycine-HCl, pH 2.8, and neutralized with acetate-buffered saline, 0.01% of merthiolate being added as preservative. This procedure retains the binding capacity of CIC fairly well for at least 15 treatment cycles [26]. All buffers and solutions were standards set out by the Nordic Pharmacopoeia.

Each patient had his own column which was eluted and re-used in all treatments (see Table 2). In the first treatment of patient 1 1300 ml of blood plasma passed through a column containing 45 ml of protein A-Sepharose with an IgG-binding capacity of 900 mg. As no adverse effects were observed 90 ml of protein A-Sepharose was used in the following five treatment sessions with this patient and the initial seven treatments with patient 2. Between 2000 and 2500 ml of plasma was absorbed. As no certain antitumour effect occurred with 90 ml of protein A-Sepharose the gel volume was increased to 220–250 ml, and this was subsequently used in most cases. In the treatment of patients 2 and 5 the gel volume was further increased to 450–500 ml as no therapeutic effect was obtained with the smaller volume.

Histological examination

Subcutaneous and lymph-node metastases from three of the five patients were examined several times during the course of treatment (Table 3). The primary tumours had been diagnosed many years earlier, but were re-examined for comparison and confirmation of reactive changes. In patient 2 the primary tumour was examined during the course of therapy. The tissue samples were carefully inspected with the naked eye for size, spread and appearance of the tumour. Five to ten sections were taken from different parts. Routine fixation-, dehydration- and embedding techniques were used and the sections were stained with van Giesons's and Masson's stains (for detection of melanine pigment), haematoxylin and eosin, and periodic acid-Schiff.

Special consideration was taken for the presence of: necrosis, usually coagulation necrosis, seen as an amorphous mass of granular debris; hydropic degeneration, reflecting serious injury to the tumour cells and seen as extensive vacuolization of the cytoplasm; pyknosis, an early indication of irreversible cell injury and appearing as hyperbasophilia and shrinkage of the nucleus; and inflammation, such as mononuclear

Table 3. Histological findings

Patient No.	Diagnosis	Time of biopsy: Last session	Time of biopsy: Interval after last session	Tissue	Histological findings: Necrosis	Hydropic degeneration	Pyknosis	Inflammation
1	malignant melanoma	–	–	ScM	+	–	–	–
		6	192 hr	LM	+++ (1.5 cm in diam.)	++	+	+ (F,M)
2	malignant melanoma	untreated (5 yr)		LM	–	–	–	–
		untreated (10 yr)		LM	+	+	+	(+)
		9	168 hr	LM	+	+++	+	+ (M)
		15	2 hr	LM	+	+++	++	+ (M)
3	adenocarcinoma of the kidney	–	–	ScM	–	–	–	+ (P)
		1	1 hr	ScM	–	–	–	+ (P)
		3	24 hr	ScM	+	++	+	+++ (P)
		7	14 hr	ScM	+	++	+	+++ (P)
		11	16 hr	ScM	+	++	++	+++ (P)
		11	134 hr	ScM	++	+++	++	+++ (P)
		9	48 hr	PT	–	–	–	+ (M)
5	malignant melanoma	–	–	ScM	–	–	–	–
		5	18 hr	ScM	–	–	–	+ (M)
		7	18 hr	ScM	–	–	–	–
		10	18 hr	ScM	–	–	–	(+)(M)

The number of treatment sessions and the interval between the last session and biopsy are given. The predominant inflammatory cell is classified as mononuclear (M) or polymorphonuclear (P). The degree of damage or inflammation was scored as: missing (–), weak (+), moderate (++), or intense (+++). ScM, subcutaneous metastases; LM, lymph-node metastases; PT, primary tumour.

or polynuclear cells in direct relation on the tumour cells; the focal and diffuse patterns were estimated.

RESULTS

Five patients were treated in the present series. In two of them (Nos 4 and 5) no effect on the tumour was observed. The others are described below.

Case reports

Patient 1: a 57-yr-old man in whom a malignant melanoma on the left side of the dorsum was excised in June 1973. Metastases appeared repeatedly, starting in August 1974. They were excised as they appeared, and after each operation chemotherapy was given. During spring 1979 extensive retroperitoneal metastases developed and the patient experienced increasing nausea and vomiting soon after meals. Frequent severe pain in the upper abdomen necessitated treatment with ketobemidon (Ketogin®, Lundbeck, Denmark). Before starting immune absorption therapy, examination showed extensive retroperitoneal spread of the disease, numerous subcutaneous metastases, one brain metastasis and suspicion of central liver metastasis, but no skeletal or pulmonary metastases. Biochemical investigations were normal, except for increased serum lactic dehydrogenase.

The first five treatment sessions were given twice weekly (Table 2). The intention was to continue with one treatment per week. Increasing food intolerance necessitated parenteral nutrition, however, and treatment No. 7 was delayed for 1 week. After six sessions moderate progression of the disease was noted. The metastases at the right zygomatic process measured 20 × 20 mm, that at the tip of rib XII measured 30 × 30 mm and a new one at the tip of the right scapula measured 10 × 10 mm. After a further three sessions the clinical status of the patient was markedly improved. The food intolerance, nausea and vomiting had almost disappeared, even after stopping 2 weeks' treatment with metoclopramide (Primperan®, Lundbeck, Denmark). Regular medication with analgesics was no longer necessary. The metastases showed regression: that in the right zygomatic process measured 7 × 7 mm and that at the tip of rib XII 12 × 20 mm. CT of the brain showed regression of the metastasis (Fig. 1).

Just before the start of the 10th session the patient developed a bleeding gastric ulcer. At operation a prepyloric ulcer with no sign of malignancy was found. The abdominal tumour masses did not seem to have progressed, on

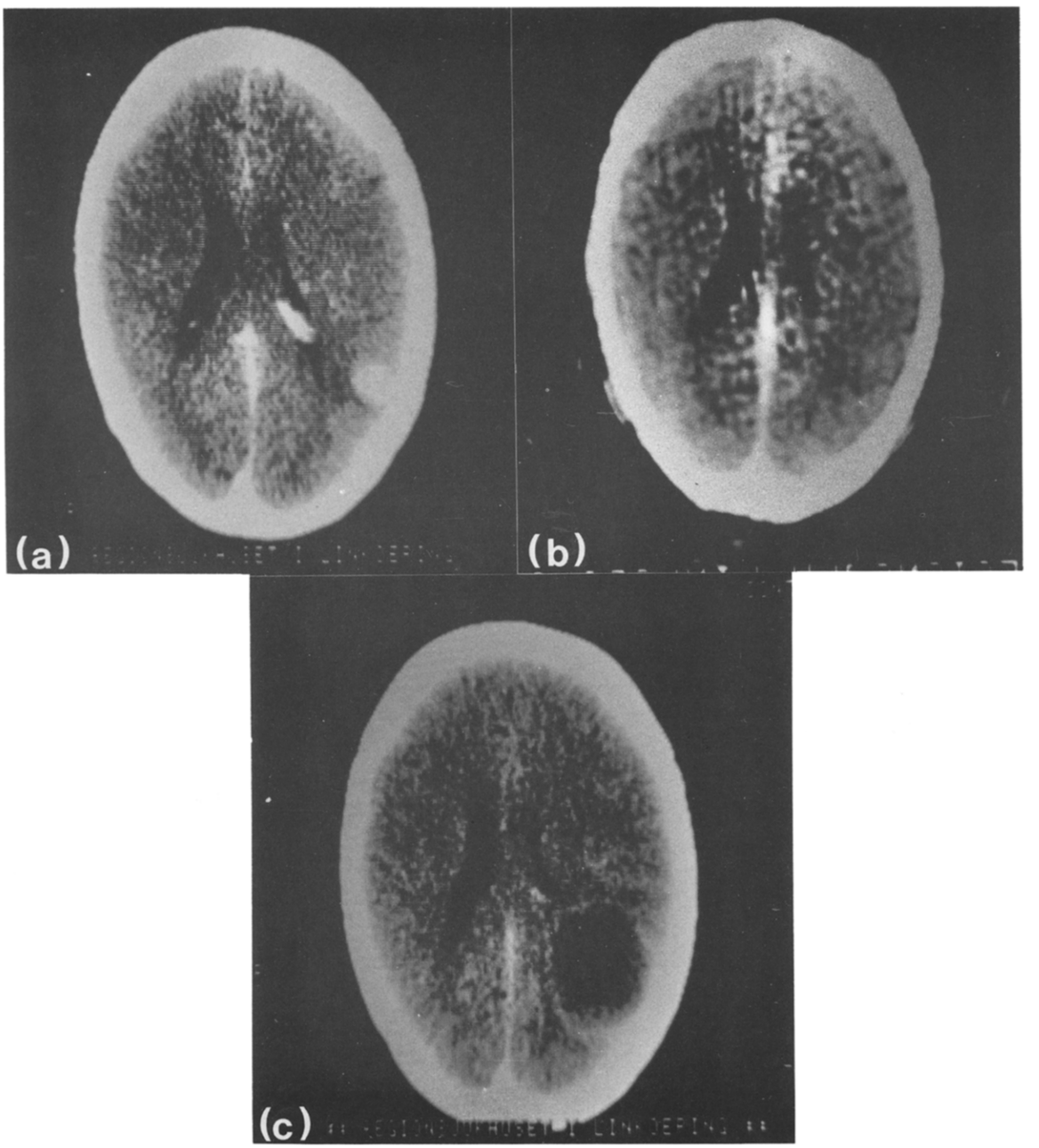

Fig. 1. *Computerized tomography of brain, patient* 1. (*a*) *Before treatment;* (*b*) *regression after* 6 *treatment sessions;* (*c*) *recurrence of metastases* 5 weeks *after the* 9*th treatment session.*

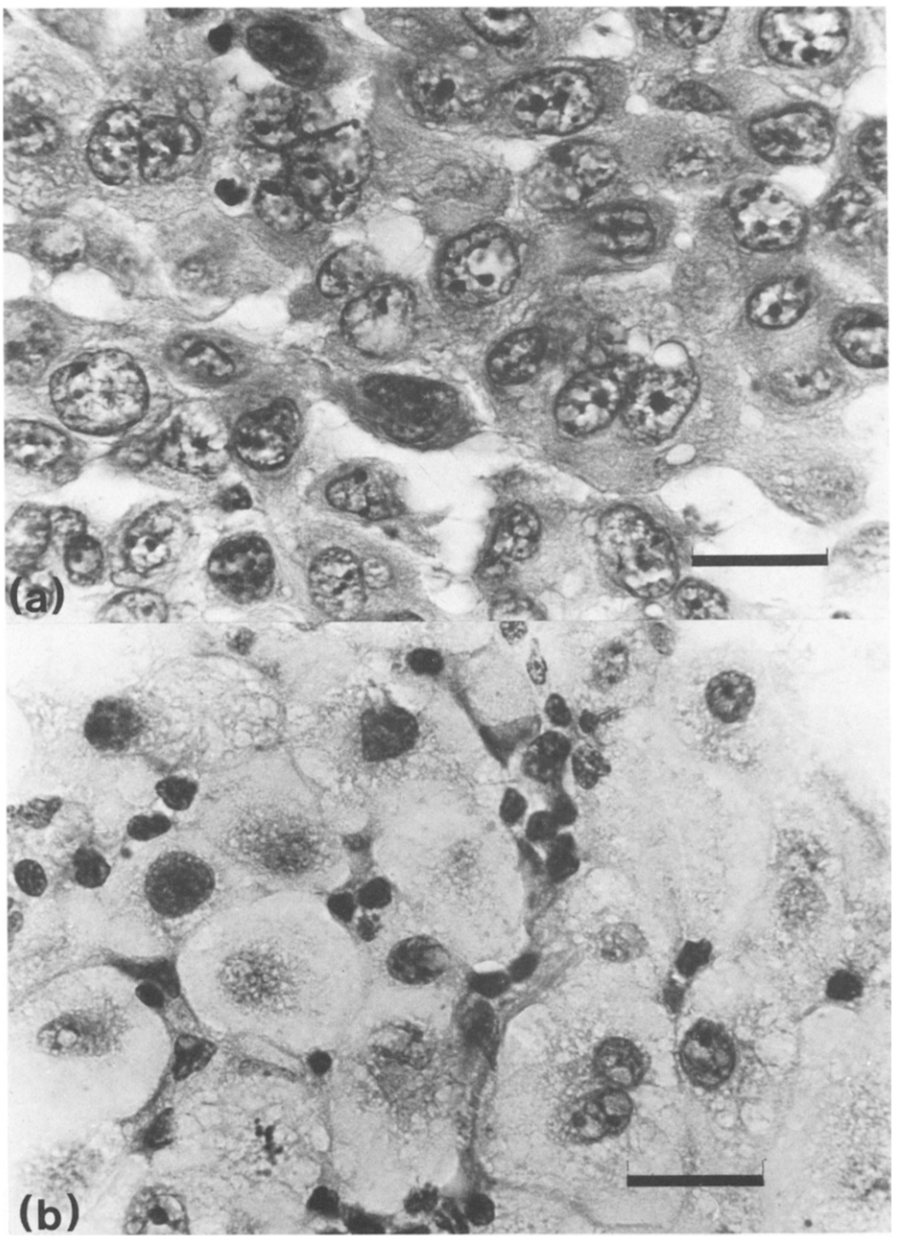

Fig. 2. Patient 2. Lymphonodular metastases from malignant melanoma. van Gieson. (a) Untreated. Dense, heavily stained cytoplasm. Bar = 25 μm; (b) 2 hr after the 15th treatment session. Marked signs of hydropic degeneration of cytoplasm. Bar = 25 μm.

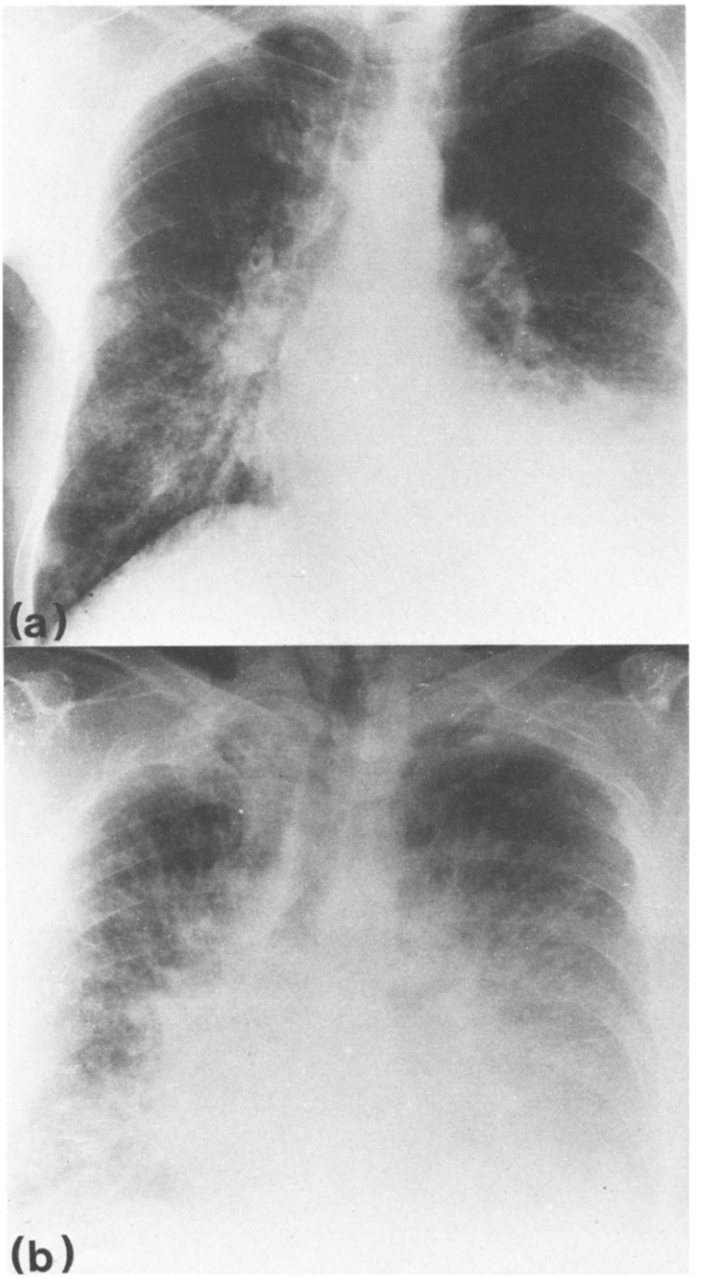

Fig. 3. Lung metastases from renal adenocarcinoma, patient 3. (a) Before starting treatment; (b) 2 hr after infusion of absorbed plasma, 1st treatment session.

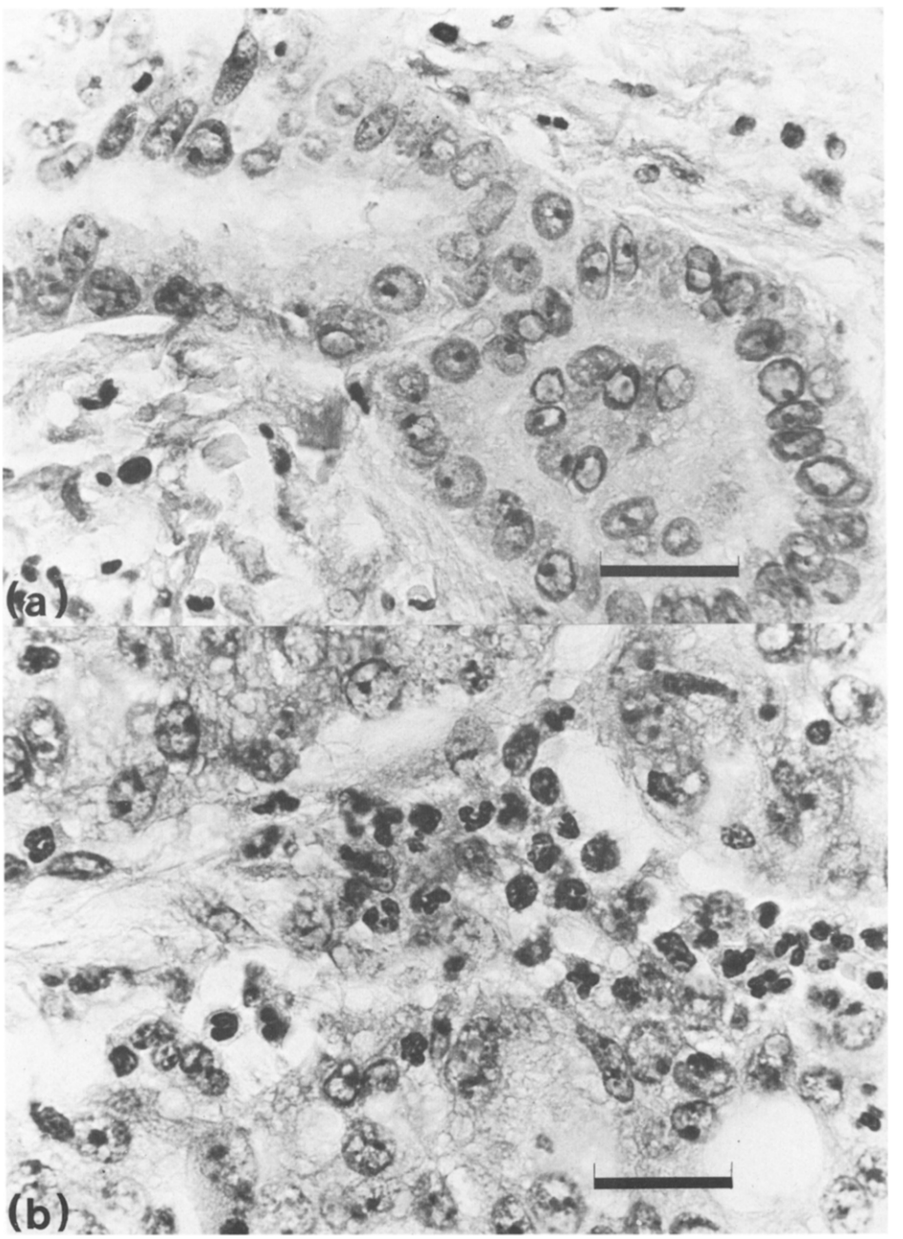

Fig. 4. Patient 3. Subcutaneous metastases from renal adenocarcinoma. van Gieson. (a) Before therapy. Note the well-maintained epithelial cells in tubular arrangement. Bar = 25 μm; (b) 16 hr after the 11th treatment session. The cellular structure is deranged and shows signs of hydropic degeneration. A marked inflammatory reaction is also seen. Bar = 25 μm.

comparison with the initial CT of the abdomen. The postoperative period was complicated by septic shock and bronchopneumonia, and treatment had to be abandoned for a month. During this period general progression of the malignant disease occurred, and only two more sessions could be given before the patient died.

Patient 2: a 53-yr-old woman who in 1968 was operated on for malignant melanoma on the left leg. Inguinal metastases were excised in 1973, and in 1978 extensive para-aortal metastases were incompletely removed. Cytostatic therapy was given after each operation. In May 1979 investigation showed large retroperitoneal metastases but no further spread of the tumour. After seven treatment sessions using 90 ml of protein A–Sepharose metastases appeared bilaterally in the inguinal lymph nodes and subcutaneously in the paraumbilical area. The absorption of plasma was then intensified by increasing the amount of protein A–Sepharose to 250 ml (see Table 2). Two hours after the 15th session a lymph node metastasis was excised. This showed histological changes compatible with a favourable effect of treatment (see below and Table 3). Because of slight progression, the subsequent treatment sessions (Nos 19–22) were further intensified (see Table 2). After session 22 the patient developed a fatal cerebral haemorrhage. At necropsy the bleeding was found to have started in a previously unidentified necrotic cerebral metastasis.

Patient 3: a 44-yr-old man suffering from metastatic carcinoma of the kidney. Before treatment there were extensive subcutaneous, muscular, skeletal and pulmonary metastases. The possibility of a cerebral metastasis could not be excluded.

About 1 hr after starting the infusion of absorbed plasma at the first treatment session the patient developed severe respiratory distress (see below). The infusion was stopped and the symptoms subsided after giving hydrocortisone, furosemide and theophyllamine. This episode was attributed to an inflammatory reaction in disseminated pulmonary metastases. The patient recovered completely by the next day. The remaining absorbed plasma was now infused at a slow rate and caused only mild respiratory distress. The patient complained of frequent attacks of moderate to fairly intense skeletal pain after infusion of treated plasma. After six sessions 50% regression of six measurable subcutaneous metastases was recorded. Session 7 was delayed for 1 week owing to the development of pneumothorax. During this time slight progression occurred. Because of life-threatening haematuria, nephrectomy had to be done after the 9th treatment session. After another four sessions the subcutaneous metastases again regressed by about 25%. Blood access for further treatment was obtained via a Thomas femoral shunt in the right thigh. Subsequently the plasma was prepared by means of filtration for sessions 14–19. During this period rapid growth of the metastases took place, and the patient died of respiratory failure.

Histological findings

For dates of biopsy in relation to treatment sessions, see Table 3. Before treatment no metastases showed marked signs of degeneration or inflammation.

Morphological changes (necrosis, hydropic degeneration, pyknosis and inflammation) in the metastases during the treatment period are presented in Table 3.

Moderate changes were noted in patient 1, who had a malignant melanoma. The lymph-node metastasis showed a large necrotic area, but otherwise tumour-cell damage was moderate and mononuclear infiltration only slight after six sessions. This is apparently in contrast to the clinical regression which took place later. However, only one biopsy was done 8 days after ending treatment. Other histological signs of therapeutic effect therefore could already have disappeared.

Pronounced signs of tumour-cell damage were found in two lymph-node metastases from patient 2 and five subcutaneous metastases from patient 3. Marked increase in tumour-cell degeneration and subsequent irreversible changes were noted during the course of therapy. Patient 2 (Fig. 2) showed a mononuclear-cell reaction which was most pronounced 2 hr after the 15th session. In patient 3 (Fig. 4) signs of tumour-cell damage were first seen 24 hr after the third session. In this and later specimens obtained between 14 and 134 hr after ending treatment marked polymorphonuclear-cell infiltration was found. No such changes were found in the primary tumour, which was removed only 48 hr after treatment. However, the three sessions preceding this operation were given within a 1-month period, a clearly inadequate intensity of treatment because slight progression of the subcutaneous metastases took place. There were no signs of tumour-cell damage in patient 5, who had malignant melanoma, and only a very slight inflammatory reaction was noted.

The tumour morphology evidently differs with regard to signs of tumour-cell damage appearing during treatment. In patients showing objective regression of tumour metastases, histological signs of tumour-cell damage were demonstrated.

Biochemical findings

All patients developed slowly progressive anaemia, probably owing to the extensive blood sampling. Except for a transient fall in the platelet count immediately after the first treatment in patient 1, the platelet and leukocyte counts were unchanged.

Two out of five patients showed abnormal lactic dehydrogenase values at the start of treatment. All tests of liver and kidney function were normal 1 day after the 1st, 6th and 12th sessions. The alkaline phosphatases were raised after 12 sessions in patients 1 and 3, probably owing to progression of the disease.

The immunoglobulins were normal in 3/5 patients at the start of therapy. After six and 12 sessions the IgG concentration in two of the three were reduced; in the 3rd patient, as in the remaining two, both of whom had a considerable tumour burden and who showed initially elevated IgG concentrations, no fall in IgG concentration was found. The IgA level tended to fall in the two patients in whom the IgG concentrations decreased and in one with unaltered high IgG.

The concentrations of C_{1q}-binding circulating immune complexes in these patients were largely within normal limits, and no consistent changes were found in connection with the treatment sessions.

Adverse effects

No serious circulatory effects were noted when absorbed plasma was infused. A slight fall in blood pressure occurred only occasionally during plasmapheresis.

In patient 3, with extensive pulmonary metastases, severe respiratory distress occurred during the first treatment session. There was no history of heart or pulmonary disease. The episode was not accompanied by chest pain, and no electrocardiographic or laboratory signs of myocardial infarction were found. The respiratory distress was therefore considered to be due to an acute inflammatory reaction in the pulmonary metastases (Fig. 3). All subsequent treatment sessions were accomplished with no such reactions. Respiratory distress did not occur in any other patients, in whom pulmonary metastases were less extensive or absent.

Two patients who at the start of treatment complained of pain localized to the tumour area experienced intensification of this during and the day after infusion of protein A–Sepharose-treated plasma. The accentuation of the pain appeared irregularly, which makes interpretation difficult; the cause could have been an inflammatory reaction in the tumour.

Fever occurred during and sometimes the day after a treatment session. The irregular timing makes it seem unlikely that it resulted from exposure of plasma to the protein A–Sepharose, but could instead have been caused by material released from the tumour.

Patients 1, 2 and 3 complained of progressive muscular weakness which became apparent after 5–6 sessions and progressed until walking was limited to only 25–50 m. Electromyography showed peripheral neurogenic damage. The histopathological picture in muscle from one of these patients showed no muscular damage. The muscular weakness varied in degree, but was steadily progressive. No such symptoms were present in the other two patients, who had received exactly the same treatment. These two, however, had a considerably smaller tumour burden. The muscular weakness was therefore considered to be a paramalignant phenomenon rather than an adverse effect of treatment.

Haemorrhage occurred in patients 1, 2 and 3. Patient 1 developed a bleeding gastric ulcer with no malignant change. Patient 2 died from cerebral haemorrhage caused by regressive changes in a metastasis, possibly a result of treatment. Patient 3 had intermittent haematuria before starting treatment. Although 3/5 patients showed bleeding, in none of them could it be attributed to the treatment.

DISCUSSION

The rationale of the form of treatment used in this study is binding to protein A of serum-blocking activity exerted by circulating immune complexes [10, 11]. Tumour regression has been induced in animals [15–19] and human patients [12–14] after repeated infusion of autologous plasma absorbed with *Staph. aureus* carrying protein A. Considerable adverse effects have occurred and it cannot be excluded that these and the antitumour effect, at least to some extent, were due to release of protein A or other bacterial substances [12, 15, 24]. Activation of complement must also be considered as a possible cause of some of the unwanted effects. None of our five patients suffered from any serious complications of treatment.

In two of our patients tumour regression was obtained when purified protein A covalently linked to agarose beads was used to absorb the plasma. Similar results have been reported by others using purified protein A either covalently linked to agarose [20], silica gel [21] or immobilized in collodion charcoal [22, 23]. Terman *et al.* [22, 23], adding cytosine arabinoside in some of the treatments, obtained tumour regression with a remarkably small amount of protein A on the column.

Nevertheless, the mode of action of protein A requires elucidation, because it can interact with the immune system in several different ways. After infusion of SAC-absorbed plasma Bansal *et al.* [12] reported hyperplasia of lymphatic tissue in dogs and Ray *et al.* [13, 14] found the natural killer cell (NK-cell) activity, the mitogenic stimulation of lymphocytes and the reactivity in skin tests to be increased. Studied *in vitro*, purified protein A was found to increase interferon production [27], to activate NK-cells [28] and to be mitogenic for lymphocytes [29, 30]. Furthermore, injection of purified protein A can induce regression [31] or reduce the progression of animal tumours [32, 33]. To produce such effects in the type of immune adsorption treatments described here protein A has to be released from the gel. Although the protein is covalently linked to the gel, small amounts might leak but not enough to induce antitumour effects [15, 31, 32]. Thus it seems unlikely that leakage of protein A is of importance for the tumour regressions that have been observed in this investigation.

It is therefore reasonable to assume that one basic principle of protein A in the present type of treatment is to bind CIC. However, if removal of CIC were the only effect of protein A absorption, it could be accomplished much more easily by simple exchange plasmapheresis. We suggest that the binding of immune complexes to protein A causes a change in their composition so that relatively more antigen becomes bound, resulting in generation of free antibody. This view is supported by the appearance of complement-dependent cytotoxic activity [12] or cytotoxic antibodies [13] in serum after treatment of patients. Steele *et al.* [10] found after absorption of rat-tumour-bearer sera with SAC not only disappearance of blocking activity but also generation of unblocking activity and of complement-dependent cytotoxic antibodies. The same treatment of human-tumour-bearer sera resulted only in absorption of blocking activity, possibly owing to almost complete binding of IgG; in the rat experiments, however, only about 20% of the IgG was bound. These results suggest that complete or near-complete absorption of IgG should be avoided in the treatment of patient plasma in order not to bind generated tumour-specific antibodies.

The mechanism by which tumour cells are killed is still not quite understood. Inflammatory histological reactions are described at different times after treatment in this and other studies [12, 16, 19], but whether they are the cause or the result of tumour cell destruction is not clear. The rapid increase in tumour temperature, the occurrence of tumour pain during treatment [12, 14] and the appearance of cytotoxic antibodies [10, 12, 13, 18, 19] could be evidence that humoral rather than cell-mediated immunity is of importance in this type of immune absorption treatments.

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