the disease. We have not yet assessed this form of treatment more widely in the disease.

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Gastric carcinoma and thrombotic thrombocytopenic purpura: association with plasma immune complex concentrations

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Abstract

A patient with metastatic adenocarcinoma of the stomach developed microangiopathic haemolytic anaemia, thrombocytopenia, renal insufficiency, and fluctuating neurological abnormalities in association with appreciably raised plasma concentrations of immune complexes. This syndrome, similar to thrombotic thrombocytopenic purpura, occurred while the tumour was in sustained objective remission after successful treatment with fluorouracil, doxorubicin, and mitomycin. Reversal of the syndrome was achieved with plasmapheresis, azathioprine, corticosteroids, and antiplatelet treatment; this response was paralleled by a reduction in immune complex concentration, suggesting an immune aetiology for the syndrome. Antibodies eluted from the immune complexes reacted with 50% of cells from the gastric cancer but less than 10% of cells from normal gastric mucosa. There was no reactivity with either carcinoembryonic antigen or mitomycin. A 17S immune complex reacted with a glycoprotein from the patient's autologous platelets and produced platelet aggregation.

It is postulated that reducing the tumour and the pre-existing state of antigen excess by chemotherapy allowed soluble antigen-antibody complexes to form and the syndrome to develop.

Introduction

Microangiopathic haemolytic anaemia has been described in association with disseminated malignancies, most commonly adenocarcinoma of the breast or stomach.¹⁻³ Recently a syndrome

resembling thrombotic thrombocytopenic purpura, manifested by microangiopathic haemolytic anaemia, thrombocytopenia, and renal insufficiency, has been reported in patients with adenocarcinomas, particularly of the stomach,^{4,5} and in two patients with epidermoid carcinoma.⁶ Microangiopathic haemolytic anaemia is usually a late and fatal complication of advanced metastatic carcinoma.² In contrast, most patients with the syndrome similar to thrombotic thrombocytopenic purpura have had only microscopic or no evidence of active tumour after effective treatment with chemotherapy.⁴⁻⁶

The association of thrombotic thrombocytopenic purpura with disorders of the immune system such as lupus erythematosus^{7,8} in addition to the findings of raised platelet-associated IgG concentrations^{9,10} and the presence of immunoglobulin and complement in the characteristic vascular lesions¹¹ have supported an immune aetiology. Although the role of circulating immune complexes in producing vascular injury, with the resultant clinical syndrome, has been widely speculated,¹²⁻¹⁵ only recently has the presence of such complexes in a patient with thrombotic thrombocytopenic purpura been documented.¹⁶ We report on a patient with metastatic adenocarcinoma of the stomach who developed microangiopathic haemolytic anaemia, thrombocytopenia, renal insufficiency, and fluctuating neurological abnormalities in association with considerably raised plasma immune complex concentrations.

Case report

In July 1979 a 48-year-old woman was found to have unresectable gastric carcinoma. Histology showed a mucin-producing adenocarcinoma of the linitis plastica type affecting the entire thickness of the gastric wall, and lymph node and mesenteric metastases were noted. A gastrojejunostomy was performed with no attempt at tumour resection. Before chemotherapy with the FAM regimen (fluorouracil, doxorubicin (Adriamycin), and mitomycin)¹⁷ was started in August abdominal sonography was performed, which showed a mass in the left upper quadrant and ascites. Until March 1980 she received four full cycles of treatment with appropriate dose modification during the fourth cycle for mild thrombocytopenia (platelet count of $128 \times 10^9/l$). During this time she showed symptomatic improvement, with an increased appetite and weight gain of 0.7 kg; repeat abdominal sonography showed complete disappearance of the mass and no evidence of ascites.

In April she was readmitted to hospital for evaluation of progressive anaemia, thrombocytopenia, episodic fever, intermittent dysphasia,

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and weakness of the left arm. Evaluation before admission had included computed tomography of the brain, which yielded a normal scan, and abdominal sonography and chest x-ray examination, which failed to show evidence of recurrent tumour. On admission she was alert, oriented, and in no apparent distress. There was no palpable lymphadenopathy, petechiae or ecchymoses, abdominal masses, or detectable ascites. Neurological examination was remarkable only for episodic stuttering of speech.

Packed cell volume was 0.21, haemoglobin concentration 9.0 g/dl, reticulocyte count 0.9%, white cell count $9.1 \times 10^9/l$, and platelet count $70 \times 10^9/l$. A peripheral blood smear showed numerous burr cells, schistocytes, and helmet cells. A bone-marrow aspirate and biopsy specimen showed mild hypocellularity with an adequate number of megakaryocytes. Serum haptoglobin was not detectable. Blood urea nitrogen concentration was 11.4 mmol/l (32 mg/100 ml), creatinine concentration 115 $\mu\text{mol/l}$ (1.3 mg/100 ml), and creatinine clearance 42 ml/min. Activities of lactic dehydrogenase, serum aspartate transaminase, and serum alanine transferase and bilirubin concentrations were within normal limits. Urine analysis showed 10-12 red blood cells/high power field, eight to 10 white blood cells/high power field, 4+ proteinuria, and numerous granular and hyaline casts. The prothrombin time was 11.5 s (control 11.4 s) and partial thromboplastin time 33.2 s (control 30.5 s). The thrombin time was slightly prolonged at 27.3 s (control 20.6 s). Fibrinogen concentration was 3.85 g/l (385 mg/100 ml). Fibrin degradation products determined by latex agglutination were 20 mg/l (normal 10 mg/l). A direct Coombs test and tests for rheumatoid factor and antinuclear antibody yielded negative results, and total haemolytic complement was normal. X-ray examination of the upper gastrointestinal tract, liver and spleen scans, and abdominal sonography showed no evidence of progressive tumour with chest.

Immune-complex analysis on the initial plasma sample showed 43% binding inhibition by the Clq assay¹⁸ (normal range 1-6% binding inhibition) and an immune complex concentration of 324 mg/l by the Raji cell assay¹⁹ (normal range 10-65 mg/l). Polyethylene glycol sedimentation of the plasma followed by double-crossed immunoelectrophoresis²⁰ showed two medium bands (17S, 22S) and one heavy band (44S). The 17S band was found to contain an IgG antibody, which was examined by immunodiffusion against its antigen as well as against prepared dissolved autologous and allogeneic platelet membranes. A line of identity between the antigen and a glycoprotein from the patient's autologous platelets was found. This 17S immune complex also produced greater than 80% platelet aggregation in five minutes. The 22S band contained IgG and an unidentified protein; the 44S band contained only IgG reacting with

IgG, with no identifiable antigen. Neither the 22S nor the 44S immune complex produced platelet aggregation.

The eluted antibodies from the immune complexes were also tested for reactivity against mitomycin by double diffusion in agarose gel. The antibodies were concentrated to 1 g/l by negative-pressure dialysis and reacted against a 1 g/l solution of the drug. Diffusion in agarose plate wells was allowed to take place over 24 hours and the plates read in diffuse light after being soaked in 8% saline to intensify the reaction. No reactivity with mitomycin was seen with any antibody eluted from the complexes. In addition, all of the complexed antibodies were tested for reactivity to carcinoembryonic antigen by an enzyme-linked immunoassay and failed to show reactivity.

Reactivity of the separated antibodies with the tumour was determined by immunofluorescence. Sections of the tumour were cut from paraffin-embedded material, dewaxed in cold xylene, and equilibrated by passage through cold alcohols. The sections were stained by the indirect immunofluorescence method of Phillips and Lewis²¹ using 1/4 and 1/8 dilutions of the patient's antibody dissociated from the medium band complexes, and goat antihuman IgG conjugated to fluorescein isothiocyanate. The tumour specimen from the original operation showed staining of greater than 50% of the cells, whereas normal gastric tissue from necropsy specimens had less than 10% staining. Normal gastric tissue obtained during the second abdominal exploration, which contained no microscopic tumour, failed to cross-react with the antibody.

A tumour-associated syndrome similar to thrombotic thrombocytopenic purpura was diagnosed, and treatment with corticosteroids (prednisone 80 mg daily) and dipyridamole (400 mg daily) was started. After the demonstration of circulating immune complexes plasmapheresis was begun using an Aminco cell separator and was performed on five occasions, with exchange of four to five litres of plasma on each occasion. Within 25 days of the start of treatment the immune complex concentrations gradually declined from 324 to 32 mg/l as measured by the Raji method, and from 43% to 5% by the Clq-binding technique (figure). Azathioprine (3 mg/kg/day) was added after the third plasmapheresis, and the platelet count progressively rose from $40 \times 10^9/l$ to $110 \times 10^9/l$ by day 32, which corresponded to the progressive decrease in immune complex concentrations. Prednisone was gradually stopped and chemotherapy with the FAM regimen resumed. Azathioprine was subsequently stopped because of contributing haematological toxicity.

She was maintained on the FAM regimen for six additional months, with appropriate dose modifications for haematological toxicity. Plasma immune complex concentrations remained in the normal range. The peripheral blood smear returned to normal;

Clinical course in patient from onset of syndrome similar to thrombotic thrombocytopenic purpura to clinical resolution and reinstitution of chemotherapy.

P=Plasmapheresis. PC=Transfusion with packed red blood cells. Stippled areas indicate normal ranges.

packed cell volume, haemoglobin concentration, white cell count, and platelet count remained normal. Haptoglobin concentration was greater than 1.0 g/l (100 mg/100 ml) (normal 0.5-1.0 g/l; 50-100 mg/100 ml). In December 1980, with no evidence of malignancy on clinical evaluation, she underwent abdominal re-exploration. No gross tumour was detected, and multiple full-thickness biopsy specimens of the stomach failed to show residual adenocarcinoma. Chemotherapy was stopped, and she remained in complete tumour remission and with no evidence of a recurrence of thrombotic thrombocytopenic purpura.

Discussion

The patient described in this case report developed a syndrome similar to thrombotic thrombocytopenic purpura without evidence of disseminated intravascular coagulation while in a complete response after chemotherapy with fluorouracil, doxorubicin, and mitomycin. The finding of immune complexes, whose concentration decreased in parallel with clinical improvement, suggests an immune mechanism. This was further supported by the finding that one of the antibodies in the circulating immune complexes showed a strong cross-reaction to tumour antigen. We hypothesise that a pre-existing state of antigen excess decreases as the tumour is reduced by chemotherapy, thus allowing for the formation of soluble antigen-antibody complexes. Antigen-antibody complexes have been found in high titres in patients with malignancy²²⁻²⁴ and can induce release of platelet substances *in vitro* that can potentially initiate intravascular coagulation.²⁵⁻²⁷ Such immune complexes could accumulate in the blood vessels, causing local vascular injury as well as initiating deposition of platelets and fibrin, leading to the clinical syndrome described. Certainly the presence of a platelet-aggregating immune complex in this case lends support to this theory. In addition, the finding of platelet glycoprotein as the antigen in one of the immune complexes identified in this patient indicates that there is an immune reaction to the platelets, due either to an antibody previously formed reacting with normal, intact platelets or to an antibody response to platelet injury.

Combination chemotherapy, and in particular mitomycin, has recently been implicated as the factor responsible in two patients with gastric carcinoma who developed this syndrome,⁶ but the evidence is only speculative. Although nephrotoxicity associated with mitomycin is said to occur in as many as 10% of patients, it appears to be dose related.²⁸ Our patient received 10 mg mitomycin/m² every eight weeks, giving a total dose of 40 mg/m² at the time of onset of the syndrome, which is less than is usually reported for nephrotoxicity.²⁹ The renal histology in our previous cases⁴ and those of Jones *et al*⁵ is not consistent with that described with mitomycin nephrotoxicity²⁸ but conforms with the histopathology of thrombotic thrombocytopenic purpura. We have attempted to identify mitomycin as an antigen in the antigen-antibody complex but cannot find a line of identity. While we have observed this syndrome in patients with gastric cancer treated with a mitomycin-containing combination, we have not observed it in patients with adenocarcinoma of the lung treated with the same regimen.³⁰

As treatment of gastric cancer improves and increasing numbers of patients achieve the striking response observed in the present case this syndrome, similar to thrombotic thrombocytopenic purpura, may become more common. Patients should be monitored carefully for haemolytic anaemia and renal impairment, and signs of pancytopenia should not be automatically ascribed to myelosuppressive treatment. Plasma samples should be obtained for determination of immune complex concentrations in patients who develop this syndrome, and empiric treatment with plasmapheresis and azathioprine should be started.

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