The clinical manifestations and immunologic features of a patient with plasma cell leukemia who produced k, IgG half-molecules are described. His serum contained both 7S myeloma protein and 4.3S half-molecules, whereas his urine contained predominantly half-molecules. The half-molecules were discovered because the serum and urine formed double precipitin lines when analyzed by commercially available IgG radial immunodiffusion plates that contained antibodies to determinants on both the Fab and Fc fragments. Immunoelectrophoresis also revealed double precipitation lines with such antisera. In contrast, when antisera specific for the IgG Fc fragment were used, the serum showed only a single line formed by intact IgG, and the urine failed to react, indicating that the half-molecule was antigenically deficient in the Fc fragment. The half-molecule consisted of one covalently linked heavy and light chain, both having about normal molecular weights, suggesting that they did not have a large deletion which could have caused the half-molecule production. Comparison of the clinical manifestations of the patient with those of four other known patients who produced half-molecules suggested that half-molecule formation is not associated with a distinct clinical syndrome.

Patients with plasma cell dyscrasias usually form large quantities of monoclonal immunoglobulins (paraproteins, M-components). In structure, most of these immunoglobulins are similar, if not identical, to their normal counterparts. On rare occasions, however, paraproteins are formed which seem to be abnormal or, at least, cannot easily be detected in normal serum and urine. The best known examples of abnormal proteins are those found in heavy-chain disease and myeloma proteins with relatively large deletions in the polypeptide chains. Immunoglobulin half-molecules, another
rare type of paraprotein, may also represent an abnormal immunoglobulin. In man, they were first reported by Hobbs and collaborators in three patients with extramedullary soft-tissue plasmacytoma.6,7 One of these patients, who was described in detail,8 excreted a large amount of k type IgG half-molecules into his urine. The half-molecules were composed of one covalently linked heavy and light chain of about normal molecular weight. Two additional patients forming half-molecules have since been detected.8,9 The clinical manifestations and laboratory findings of our patient are described in this report.

CASE REPORT

History

Patient KN, a 72-yr-old retired naval officer was admitted to the San Diego Naval Hospital in November 1971 because of pain, tenderness, and swelling of the left lower extremity. He had thrombophlebitis which was recurrent since 1964 and had, at times, involved the deep saphenous veins of both legs. A routine white blood cell differential count revealed a large percentage of plasma cells and led to evaluation by the Hematology Service.

When first seen, the patient's only complaints were those related to his admission problem. There had been no undue weakness, bone pain, or symptoms of anemia.

The past medical history included an abdominal aortoendarterectomy in 1956 with bilateral aortofemoral grafts for occlusive disease. In 1962 he had an acute myocardial infarction, and in 1965 a left bundle branch block was discovered by electrocardiography during hospitalization for mild congestive heart failure. Before admission, he lived a relatively normal life, played golf, swam, etc.

Physical Examination

The patient was a well developed, well nourished white male who appeared to be his stated age. Significant findings included bilateral arcus senilis, grade II atherosclerotic changes of the eyegrounds, an S4 heart sound associated with normal sinus rhythm, and cardiomegaly. The liver edge was palpable; however, the over-all expanse was 13 cm. The spleen was not palpable. Other than for well healed surgical scars, the abdomen was normal. The femoral pulses were present bilaterally, but peripheral pulses were absent in the lower extremities. The remainder of the physical examination was within normal limits.

Laboratory Data

The hematocrit was 24 volumes/100 ml, the white count 12,000/cu mm, the hemoglobin 7.7 g/100 ml, and the reticulocyte count 1.6%. The differential white count revealed 17% band neutrophils, 33% segmented neutrophils, 5% monocytes, 13% lymphocytes, 1% myelocytes, 3% metamyelocytes, 10% immature and 18% mature plasma cells. The total serum protein was 6.5 g/100 ml, and the blood urea nitrogen and creatinine were 57 mg/100 ml and 2.7 mg/100 ml, respectively. Serum electrolytes and the acute phase reactants were normal. A bone marrow aspiration showed 91% immature or mature plasma cells. The cells of the erythropoietic system appeared normal, the cells of the granulopoietic system showed a slight shift to the right, and the number of megakaryocytes was reduced. The patient has proteinuria, but tests for Bence Jones protein were negative. A liver/spleen scan revealed an enlarged liver and slightly enlarged spleen. No bone lesions were seen on x-rays of chest and skeleton.

Hospital and Subsequent Course

The patient was treated for his thrombophlebitis with heat and elevation and responded favorably. Phenylalanine nitrogen mustard (Alkeran) therapy, 2 mg/day, was initiated; digoxin, furosemide (Lasix), and potassium chloride treatment was continued. After 10 days, the patient was discharged to the Hematology Clinic, where he was seen at weekly intervals. The number of circulating plasma cells decreased markedly by late December 1971. Therapy was continued until
February 1972. At that time he had a total white count of 2000 cells/cu mm, which were predominantly plasma cells. Small dosages of prednisone were then substituted for Alkeran, and in March, cyclophosphamide (Cytoxan), 50 mg every other day, was administered. A herpess zoster infection had developed in February involving the right thorax and abdomen but resolved over a few weeks. In August 1972, he began to develop increasing signs and symptoms of worsening cardiac reserve and evidence of renal failure. Because of neutropenia, the Cytoxan treatment was decreased to 50 mg every third day. The prednisone was also decreased in view of his cardiac status. In December 1972, the Cytoxan was discontinued because of persistent low white count. In January 1973, he was readmitted to the hospital because of marked increase in his cardiovascular problems. On the second hospital day he developed a fever of 103°F associated with signs of septicemia. He died shortly thereafter without responding to therapy.

**Autopsy**

At autopsy a 15 x 12 x 5-cm tumor mass encompassing the distal aorta and bilateral iliac vessels was found. This tumor appeared to be a confluent mass of lymph nodes having a gray-tan, bulging cut surface with focal areas of hemorrhage and necrosis. Microscopically, there was complete effacement of nodal architecture by monotonous sheets of primitive plasma cells that infiltrated the retroperitoneal fat. There was no evidence of other extramedullary involvement. Sections of vertebral, iliac, rib, and sternal marrow displayed extensive replacement of the medullary space by sheets of similar plasma cells with scattered islands of normal-appearing hemopoietic elements.

Other findings were cardiomegaly with biventricular hypertrophy (700 g), minimal coronary atherosclerosis, and minimal diffuse interstitial myocardial fibrosis. There were functionally potent aorto-femoral bypass grafts. Chronic passive congestion of the liver with minimal centrilobular necrosis and congestive splenomegaly (400 g) with marked depletion of the white pulp were seen. Passive congestion of the lungs with pulmonary edema and focal atelectasis was noted. There was arteriolar nephrosclerosis with numerous sclerotic glomeruli, tubular dilatations and interstitial renal fibrosis. No amyloidosis was present.

The cause of death was attributed to plasma cell myeloma with bone marrow failure associated with arteriosclerotic renal dysfunction and congestive heart failure.

**MATERIALS AND METHODS**

Serum and urine samples were stored at -20°C. The urine was dialyzed against 0.005 M phosphate buffer, pH 8.0, containing toluene as a preservative, and lyophilized. The paraproteins were isolated from the serum and concentrated urine by DEAE-cellulose chromatography employing a 0.015 M phosphate buffer, pH 8.0, followed by Sephadex G-200 gel filtration. Cellulose acetate electrophoresis was performed with a Beckman microzone electrophoresis apparatus. Immunoglobulin concentrations were determined with commercially available immunodiffusion plates (Hyland Laboratories, Costa Mesa, Calif.). Immunoelectrophoresis was carried out according to the micro method described by Scheidegger, employing antisera specific for the different classes of human immunoglobulins and for κ and λ light chains. One rabbit, one goat, and one commercial (Hyland Laboratories) antisera to IgG Fc fragments were used. All three antisera formed strong precipitin lines in double gel diffusion analyses with isolated Fc fragments of normal human IgG and with intact IgG. The goat antisera to normal human IgG was absorbed with κ and λ Bence Jones proteins. It precipitated isolated Fab and Fc fragments of normal IgG but did not react with immunoglobulins of the other classes. Analytical ultracentrifugation was performed using a Beckman Model E analytic ultracentrifuge equipped with Schlieren optics. The sedimentation rate was determined by the standard procedure to give an extrapolated S_{20,w} value.

**RESULTS**

The diagnosis of plasma cell leukemia was established by the presence of 28% plasma cells (3300/cu mm) in the peripheral blood. Bone marrow aspirations showed 60%-90% of either well or poorly differentiated plasma cells. No
Fig. 1. Electrophoretic patterns of serum and concentrated urine of patient KN. Specimens obtained December 10, 1971.

Fig. 2. Radial diffusion with IgG immuno-plates containing antibodies to determinants on both Fab and Fc fragment (top) and only to Fc fragment (bottom). (1) 15 mg/ml IgG; (2) 7.5 mg/ml IgG; (3) 3.75 mg/ml IgG; (4) serum KN obtained December 10, 1971; (5) concentrated urine KN obtained in March 1972; (6) normal human serum.
osteolytic lesions but general osteoporosis was found upon survey of the skeleton.

The electrophoretic analyses of the serum and concentrated urine at the time of admission in December 1971 are shown in Fig. 1. A monoclonal paraprotein spike of medium γ-electrophoretic mobility was seen in both the serum and urine. The quantities were 1.5 g/100 ml in the serum and 309 mg/100 ml in the urine. When the serum and urine were analyzed for immunoglobulin concentrations by commercially available immunoplates, the serum and urine showed a double ring in immunoplates specific for IgG (Fig. 2, top). These immunoplates contained antibodies to determinants specific for IgG localized on both Fab and Fc fragments. In contrast, when the more recently available immunoplates were used which contain an antiserum specific for the IgG Fc fragment, a single precipitin ring was observed with serum and urine (Fig. 2, bottom). The quantities of IgG in the serum and urine calculated from either the inner or outer precipitin rings or from the single ring obtained with the Fc specific plate did not correlate to the quantities of IgG determined by electrophoresis. The concentrations of the other immunoglobulins in the serum were low: IgA, 36 mg/100 ml; IgM, 10 mg/100 ml; IgD, less than 0.5 mg/100 ml. No double rings were seen in these plates. The serum and urine of the patient were further analyzed by immunoelectrophoresis employing antisera specific for IgG (Fab and Fc fragment), for the IgG Fc fragment for κ and λ light chains. As seen in Fig. 3, the serum showed a double precipitin line and the urine a single precipitin line with the antiserum to IgG. A single faint precipitin line was observed with the anti-Fc fragment antiserum and the patient's serum, but not with the urine (Fig. 4). The antiserum to κ-chains gave a single line with both the serum and urine. The paraproteins were isolated by DEAE-cellulose chromatography and analyzed by ultracentrifugation (Fig. 5). The urinary paraprotein showed a single protein peak with a sedimentation rate of 4.3S.
significantly higher than 3.5S characteristic for Bence Jones protein dimers. The serum IgG fraction showed two protein peaks, one having a sedimentation rate of 7.0S, slightly higher than normal IgG analyzed under the same conditions, and the other of 4.3S like the urinary protein.

The 7S and 4.3S proteins were separated by Sephadex G-200 gel filtration and the isolated protein preparations further analyzed. In immunoelectrophoresis, the 7S protein formed a single precipitin line with both antisera to IgG and Fc fragment, whereas the 4.3S protein formed a single precipitin line...
only with the antiserum to IgG. When tested in the IgG immunoplates, both preparations showed a single precipitin ring, and in the plates containing antiserum to the Fc fragment, the 7S protein formed a single sharply delineated ring, whereas the 4.3S protein did not react. Following reduction and alkylation, both the 7S and 4.3S paraproteins separated into heavy and light polypeptide chains as shown by elution from Sephadex G-100 columns equilibrated with 1 N acetic acid. The heavy and light chains eluted at positions similar to normal heavy and light chains, indicating that they did not have a large deletion. The mass ratio of the heavy and light chain peak was 71%–74% and 26%–29%, respectively, in different experiments.

Both the 7S myeloma protein and the half-molecule had the genetic marker Gm(f) which is characteristic for IgG1 Fab fragments (kindly determined by Dr. H. Kunkel).

**DISCUSSION**

IgG half-molecules consisting of one heavy and one light chain appear to be a very rare type of paraprotein. To our knowledge, they have only been found in four additional patients. Three suffered from extramedullary soft-tissue plasmacytoma,6,7 one from classic multiple myeloma (Seligmann, personal communication), and this patient had plasma cell leukemia. The occurrence in these three different manifestations of myeloma suggests that half-molecule production is probably not associated with a distinct clinical syndrome. The extramedullary tumor mass found in the retroperitoneum at the autopsy of patient KN was unlikely to have been the primary tumor because such extramedullary tumor masses are often found in the retroperitoneum in terminal stages of myeloma.13 However, both plasma cell leukemia and extramedullary myeloma are uncommon expressions of plasmacytoma, and it is of interest that four of the five known patients producing half-molecules did not have classic multiple myeloma.

The IgG half-molecules appeared to be antigenically deficient in the Fc fragment. They formed a precipitin reaction with antiserum to IgG containing antibodies to determinants on both the Fab and Fc fragment and with antiserum to κ-light chains. In contrast, the half-molecules failed to precipitate with anti-Fc fragment antiserum, a finding which has also been observed by Seligmann et al.9 The serum of the patient formed a double precipitin line with anti-IgG antiserum when tested either by radial immunodiffusion or immunoelectrophoresis. This double line was probably the result of a reaction of the anti-Fab antibodies with the half-molecules and the anti-Fc antibodies with the 7S myeloma protein and normal IgG, since the half-molecules apparently did not precipitate with the anti-Fc antibodies. The reason for the lack of precipitation of the half-molecules with anti-Fc fragment antiserum is unknown. It is possible that most antigenic determinants of the Fc fragment depend on the tertiary structure formed only by the “double-stranded” normal Fc fragment, whereas the “single-stranded” Fc fragment of the half-molecule does not express these determinants. The lack of reaction with the anti-Fc fragment antiserum could have been interpreted as indicating that the 4.3S protein was related to the pepsin F(ab')2 fragment. However, the fact that the 4.3S protein separated into
heavy and light chains that eluted from Sephadex G-100 columns at a position and a mass ratio similar to normal IgG heavy and light chains indicated that the 4.3S protein was a half-molecule rather than a F(\(ab\'))\(_2\)-like fragment. Hobbs and Jacobs reported that the half-molecule precipitated with anti-\(\gamma\)-chain antisera. This is not in contrast to our findings, since the antiserum was not absorbed with Fab fragments (personal communication), and it appears from the present analysis that the IgG determinants on the Fab fragment are normal in the half-molecule.

The structural features of the IgG half-molecule of patient KN were similar to the other reported half-molecules. Both the heavy and light chains had about normal molecular weights, suggesting that half-molecule formation was not the result of a large deletion in the polypeptide chains. Protein KN and the protein reported by Seligmann et al.\(^9\) had the genetic marker Gm(f), indicating that they were related to the IgG\(_1\) subclass.\(^{14}\) The presence of monoclonal 7S myeloma protein besides the half-molecules had previously not been reported; however, subsequent studies by Hobbs (personal communication) also revealed 7S monoclonal myeloma protein in one of their patients. The reason for the half-molecule formation is presently unknown. Structural studies under investigation in our laboratory indicate that the myeloma protein KN lacks the noncovalent bonds that are normally present between the Fc portions of the two heavy chains. Following mild reduction and alkylation in aqueous solutions at neutral pH, the 7S myeloma protein dissociated into half-molecules, a phenomenon which has never been observed with normal human immunoglobulins of any class or subclass. Whether the cells secreted only 7S molecules which were reduced in vivo to half-molecules could not be determined because no cell culture experiments could be performed that could answer this question.

Whether the myeloma IgG half-molecules are abnormal immunoglobulins or monoclonal representatives of a rare type of immunoglobulin is presently unknown. To our knowledge, two-chain antibodies have not been reported in man, but a careful search has not been made. In rabbits, nonprecipitating anti-ovalbumin antibodies having a sedimentation rate of 4.3S, like KN's half-molecule, have been reported by Sutherland and Campbell.\(^{15}\) Furthermore, the serum of colostrum-deprived piglets contains small quantities of IgG half-molecules.\(^{16,17}\) Several lines of mineral oil-induced mouse myelomas produce IgA half-molecules.\(^{18,19}\) However, these appear to differ from the human IgG half-molecules in that they have a large deletion in the \(\alpha\)-chains.\(^{20}\) In view of the occurrence of half-molecules in a number of mammalian species, it is possible that in the future half-molecules will be detected in normal human serum.

NOTE ADDED IN PROOF

Experiments performed since submission of the manuscript indicate that the heavy chain of this IgG half-molecule has a significant deletion which was not detected when the heavy and light chains were separated by Sephadex G-100 gel filtration but was clearly demonstrable by analytical ultracentrifugation in 6\(\text{M}\) guanidine.
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IgG half-molecules: clinical and immunologic features in a patient with plasma cell leukemia

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