Coexistence of Polycythemia Vera and Biclonal Gammopathy (γGK and γAL) with Two Bence Jones Proteins (BJK and BJL)

By Klaus Dittmar, Shaul Kochwa, Dorothea Zucker-Franklin and Louis R. Wasserman

Polycythemia Vera is not ordinarily associated with abnormalities in the serum proteins, more particularly in the immunoglobulins. Reports of immunologic aberrations in the myeloproliferative disorders, however, although relatively few, have been increasing. The occurrence of a myeloma-like picture or myeloma itself has been noted in a number of patients with polycythemia.1,12

Multiple myeloma is almost always associated with a monoclonal type of gammopathy, although a few studies describe the presence of more than one type of abnormal immunoglobulin.1,13,20

The present report concerns a patient with a nine-year history of polycythemia vera, who, in the last year of life, developed severe anemia, azotemia, a very rapid sedimentation rate, proteinuria with Bence Jones protein, and two abnormal γ globulin peaks in the serum. The bone marrow showed about 15 per cent plasma cells. Further studies disclosed two types of Bence Jones protein in the urine.

Since the cellular origin of two abnormal γ globulins with different immunological characteristics arising in the same individual has not been studied so far, the present case offered an opportunity to determine whether one or both abnormal proteins were synthesized by the same or different plasma cells. The results of these and related studies are the subject of the present report.

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CASE REPORT

S. S., a 72 year old white Russian-born male was in good health until 1956 when he developed severe vertigo. The diagnosis of polycythemia vera was eventually established. The blood findings were: hemoglobin 19.3 g per cent, hematocrit 63 per cent. RBC 8,740,000, WBC 20,200, platelets 200,000. The sedimentation rate was 1 mm. per hour and the uric acid 9.7 mg. per cent. Therapy consisted of phlebotomies, intermittent chlorambucil and P32 administration for the next seven years. A total dose of 20 mc. P32 in five divided doses was given, the last one three months prior to the patient's first hospital admission. During this time, the red cell count ranged from high normal to polycythemic values.

In November 1962, he had an episode of gout. Shortly thereafter he developed severe generalized pruritis. Because of congestive heart failure, digitalis and diuretics were administered.

In June, 1964, increasing weakness and anorexia developed and one month later a marked anemia (Hgb 6.8 g per cent, hematocrit 23 per cent) was noted together with an elevation of the blood urea nitrogen to 125 mg. per cent. At this time, the patient was admitted to The Mount Sinai Hospital.

The patient complained of exertional dyspnea, nocturia, intermittent claudication, marked weakness and a 20 pound weight loss. On physical examination, he was normotensive and afebrile, appeared pale and chronically ill. There were moist rales at both bases. The heart was enlarged and there was a systolic murmur at the apex. The liver was palpated two finger breadths below the right costal margin but the spleen was not enlarged. The left wrist and the right ankle were swollen, hot and painful.

Laboratory examinations on admission were as follows: hemoglobin 6.6 g per cent, PCV 20 per cent, white blood count 8,650/mm3, segmented polymorphonuclear leukocytes 66 per cent, lymphocytes 31 per cent, and monocytes 3 per cent. The platelet count was 103,000 per mm3. The erythrocytes showed mild anisocytosis. The ESR was 156 mm. per hour (Westergren method). The leukocyte alkaline phosphatase score was 311 using the azo-dye-technique (normal score 25-100).

Several bone marrow aspirations showed erythroid hyperplasia with an increase in plasma cells ranging to 15 per cent, but no sheets of plasma cells were seen.

The latex fixation test was positive at a titer of 1:320. The anti-globulin test, several L.E. preparations, the Sia, cryoglobulin, formolgel, and Wassermann tests, serum haptoglobin, serum B12 and serum folate levels, the serum iron and TIBC, serum histamine and serotonin level were all normal.

The urine specific gravity was 1.020, with a pH of 5.0, and 2+ protein. The 24 hour protein excretion was 1.1 g. per liter and Bence Jones protein was present. The urinary output varied between 850 and 1200 ml. per 24 hours. Several urine cultures were negative. The blood urea nitrogen was 112 mg. per cent, creatinine 2.5 mg. per cent. Fasting glucose, sodium, chloride, CO2 and phosphorus were normal. The serum calcium (6.1 mg. per cent) and potassium (1.8 mEq.) were low, presumably due to previous Diuril therapy and azotemia. The blood uric acid was 9.0 mg. per cent, total cholesterol 175 mg. per cent, esters 148 mg. per cent, total protein 8.7 g. per cent with albumin 3.0 and globulin 5.7 g. per cent. Serum electrophoresis showed peaks in the mid γ and in the β region (Fig. 1); a bone survey revealed diffuse demineralization without any definite lytic lesions.

The patient was transfused with seven units of packed red blood cells and was given oral potassium supplements. Within two weeks, the hematocrit had risen from 20 per cent to 35 per cent and the potassium had become normal. The BUN fell to 80 mg. per cent. Both the pruritis and arthritis gradually subsided.

The hemoglobin was maintained at levels between 11 and 12 g. per cent without additional transfusions. Repeated bone marrow aspirations revealed findings similar to the original picture. The highest plasma cell count was 15 per cent. A few months later, the patient was readmitted because of an episode of gout, which was treated with colchicine. The laboratory data showed no significant changes except for transient hypercalcemia.

Two months after discharge, the patient presented with a short history of increasing confusion and involuntary twitching and was readmitted to the hospital. He was dis-
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oriented, breathing deeply, and had spontaneous twitching. Marked hypotension and acidosis were present. The blood urea nitrogen was 204 mg. per cent, CO$_2$ 11 mEq. per liter, Cl 103 mEq. per liter, Na 126 mEq. per liter, K 3.8 mEq. per liter, uric acid 13 mg. per cent, creatinine 9.2 mg. per cent. Within 24 hours of admission, the patient's temperature rose to 105 F and despite intravenous fluids, sodium bicarbonate, calcium gluconate and antibiotics, the patient became hypotensive and expired two days after admission. Permission for autopsy was not obtained.

**SPECIAL STUDIES**

**Methods**

Total proteins were determined by refractometry (Atago Serum-Protein Refractometer, Atago, Tokyo, Japan). Zone electrophoresis on acetate strips was performed with the model R-101 micro electrophoresis cell and quantitated with the model RB Analytrol (Beckman Instruments, Palo Alto, California).

Immunoelectrophoresis and immunodiffusion were performed with LKB equipment (LKB Produktor, Stockholm, Sweden). Rabbit antisera were prepared in our laboratories (anti-normal human serum, anti-$\gamma$G globulin (Fraction II), anti-$\gamma$A myeloma protein, anti-K and anti-L Bence Jones protein). Antisera to single immunoglobulins were absorbed to give only one precipitin line with the corresponding antigens. Goat anti-$\gamma$M serum was obtained from Hyland Laboratories, Los Angeles, California.

Quantitation of immunoglobulins was performed on Hyland immunodiffusion plates. The precipitin rings were photographed on transparencies with a MP-3 camera (Polaroid Corporation, Cambridge, Mass.) and then measured with a two dimensional comparator (Shadowgraph, Nikon, Japan). Column chromatography of sera was performed on DEAE cellulose according to Sober et al. and using the cone-sphere gradient of Fahey et al. Carefully adjusted flow rates, usually with the assistance of micropumps, permitted a simultaneous separation of serum and urine with a single buffer gradient.

Analytical ultracentrifugation was performed at 59,780 rpm and 18 C in a Spinco Model E instrument. The calculated $s_{20,w}$ values were not corrected for concentration.

**Fluorescence Studies**

Two contrasting fluorochromes were used to tag rabbit antisera to human $\gamma$A and $\gamma$G globulins. The anti-human $\gamma$A serum was prepared against normal $\gamma$A globulin isolated by Hereman's procedure. It was absorbed with Bence Jones and human cord sera. The anti-$\gamma$G serum was prepared against the isolated $\gamma$-globulin from a patient who produced only the $\alpha$-Fc fragment of $\gamma$G globulin. Both antisera were shown to be monospecific on immunoelectrophoresis. The $\gamma$A antiserum was conjugated with fluorescin isothiocyanate by a modification of the method of Coons and the anti-$\gamma$G serum was conjugated with tetramethylrhodamine isothiocyanate, as described by Hiramoto. Both conjugated antisera were purified by DEAE column chromatography which eliminated non-specific staining.

Bone marrow buffy coats were washed with phosphate buffered saline (PBS) at pH 7.4 to which 3 mg. per cent bovine albumin had been added to protect cellular morphology. Smears of this marrow were fixed with absolute ethyl alcohol for five minutes and rinsed with PBS without added albumin. Rhodamine-conjugated anti-$\gamma$G serum (red) was applied for 45 minutes. Following three rinses with PBS over a period of one half hour, the slides were flooded with fluorescein-conjugated anti-$\gamma$A globulin (green) for 45 minutes. Washing with PBS was repeated as before. The specimens were examined with a Leitz Labolux fluorescence microscope, equipped with an Osram 200 mercury burner, a UC 1 excitor and a blue-absorbing barrier filter.

Photographs were taken with high speed Ektachrome (ASA 160), using exposures ranging from one to three minutes.

**RESULTS**

On acetate strip electrophoresis, two abnormal serum components were noted, one in the $\beta$ region and the second in the $\gamma$2 region (Fig. 1). Little
change was found in the total protein as well as in the relative concentrations of these abnormal components over a period of more than seven months. On immunochromatographic quantitation, all immunoglobulins were found to be elevated and remained unchanged during the course of observation. γG was 2,600 mg./100 ml. (normal 1206 ± 319), γA 1700 mg./100 ml. (normal 288 ± 121) and γM 230 mg./100 ml. (normal 80 ± 29).

Serum immunoelectrophoresis showed a distinct homogeneous increase in the middle of the γG precipitation line (Fig. 2). This was identified as type K by the parallel increase seen with anti-type K serum. The curve of the γA line corresponded to the one seen with the anti-type L serum, identifying the γA as type L.

Further information on the protein composition of this serum was obtained from the chromatography on DEAE cellulose columns (Fig. 3). A small amount of γG appeared in the fall-through fraction (Fraction 20). This probably represented the normal γG because it typed as both K and L. The bulk of the γG type K and γA type L was eluted in a narrow range of increasing
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Fig. 2.—Immunoelectrophoresis of serum. Anti WNHS represents rabbit anti-normal human serum. The straight arrows point to the corresponding precipitin lines with anti-γG and unit-type K serum. The oblique arrows identify the γA as type L.

buffer concentration. When individual fractions were tested by immunoelectrophoresis and double gel diffusion, several tubes containing only one component were found.

A small amount of protein with kappa antigenic determinants was eluted after the albumin peak (Fraction 80).

Ultracentrifugation of the serum revealed a small quantity of protein sedimenting as 9.1S consistent with an intermediate component of γA globulins.

Immunoelectrophoresis of concentrated urine revealed albumin, γG and γA globulins, and small quantities of other proteins (Fig. 4). The precipitin lines obtained with both anti-Bence Jones K and anti-Bence Jones L sera were heavy in comparison with the γG and γA precipitin arcs, suggesting the presence of additional protein molecules with kappa and lambda antigenic determinants.

On DEAE column chromatography of concentrated urine (Fig. 5), γG and γA globulins were demonstrated in fractions 35–45 with specific precipitin
Fig. 3.—Chromatography of serum on DEAE cellulose columns. 4 ml. serum was applied to the 20 × 400 mm. column. After equilibration, a gradient from 0.005M pH 8 phosphate buffer to a 0.3M pH 5.6 phosphate buffer was applied at the arrow. Fractions were collected every 30 minutes at a flow rate of 15 ml. per hour.

reactions, but anti-Bence Jones K and anti-Bence Jones L provided more strongly positive tests than did anti-γG and anti-γA sera. Additional proteins with kappa and lambda antigenic determinants were eluted after the albumin.

Separation of urine on starch block confirmed the presence of γG, γA and BJ, K and L but in addition degradation products of BJ protein were found. Fractions eluted from starch block were resolved on acrylamide gel electrophoresis into distinct components which could be correlated with ultracentrifugal and immunochemical analysis (Fig. 6).

Fluorescent antibody studies were done on the bone marrow cells (Fig. 7). The number of cells which stained with the conjugated antisera generally agreed with the total number of mature and immature plasma cells seen on routine Jenner-Giemsa-stained marrow smears. Fluorescence could be blocked completely with the corresponding unconjugated antisera. The incidence of cells which stained with the green fluorochrome, presumably the cells containing γA globulin was seven to eight times greater than the number of cells which stained red and presumably contained γG globulin. The sequence in which the antisera were applied did not influence these results nor did simultaneous application of the two antisera alter the outcome. At no time were cells with mixed fluorescence encountered, and the cells containing γG globulin could not be distinguished morphologically from the cells producing γA globulin.
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Fig. 4.—Immunoelectrophoresis of concentrated urine. The specimens were applied the same way as described in Fig. 2.

The immunocytological findings paralleled the immunodiffusion measurements of these immunoglobulins. The $\gamma G$ globulin level of 26 mg. per ml. was at least twice the usual normal value whereas the $\gamma A$ globulin level of 17 mg. per ml. was about seven times normal.

DISCUSSION

The diagnosis of myeloma is strongly supported by the development of marked anemia in this patient with previously stable polycythemia vera in association with a very rapid sedimentation rate, hypergammaglobulinemia, spikes of $\gamma G$ and $\gamma A$ globulins, proteinuria of moderate degree with Bence Jones protein, azotemia and generalized osteoporosis. It is of course possible that the recent treatment with P₃₂ contributed to the rather rapid development of the anemia. There was no evidence of myelofibrosis. The presence of only 15 per cent plasma cells in the marrow does not rule out the diagnosis of myeloma in view of the associated findings in this case, notably the gammapathies, Bence Jones proteinuria and the typical appearance of the plasma cells on electron-microscopy.
Fig. 5—Chromatography of urine on DEAE cellulose columns. 4 ml. concentrated urine (25 times) was applied to the 20 x 400 mm. column. After equilibration, a gradient from 0.005M pH 8 phosphate buffer to a 0.3M pH 5.6 phosphate buffer was applied at the arrow. Fractions were collected every 30 minutes at a flow rate of 15 ml. per hour.

Fig. 6—Concentrated urine was electrophoretically separated into 6 fractions on starch block. These tractions were arbitrarily numbered from 1 to 6 with number 1 starting at the cathodic side. After elution, Fractions 2, 3 and 4 were analyzed by ultracentrifugation, acrylamide gel electrophoresis and immunoelectrophoresis.
Fig. 7.—Fluorescent antibody stain of plasma cells from the bone marrow. Anti-
\( \gamma_A \) serum was conjugated with fluorescin isothiocyanate (green); anti-\( \gamma_0 \) serum was conjugated with tetramethyl rhodamine isothiocyanate (red).

Coexistence of multiple myeloma and polycythemia has been described in a number of instances.\(^{1,2,11} \) In reviewing these reports, one has to bear in mind that the criteria for the diagnosis of myeloma have changed with the advent of newer methods of investigation. The presence of Bence Jones protein in the urine alone is not sufficient to establish the diagnosis.\(^2,3 \) A histological confirmation of the diagnosis of myeloma was not obtained in the cases described by Perla and Biller\(^4 \) and by Arnholdt.\(^5 \) Even in some of the more recently described cases, the diagnosis has at times been based on insufficient evidence.\(^6,8,10 \)

Well-documented evidence of polycythemia and myeloma in the same patient have been presented by Giertsen,\(^9 \) Spickard\(^11 \) and Brody.\(^12 \) Autopsy confirmed the diagnosis on two other patients reported by Lawrence and Rosenthal.\(^6 \)

Electrophoretic patterns were reported by Spickard\(^11 \) and Brody.\(^12 \) Hill\(^1 \) described a patient with quadriplegia, who had questionable polycythemia and an elevated \( \beta \) and \( \gamma \) globulin on electrophoresis, but no further studies were done to characterize the abnormal protein found on serum electrophoresis. In addition, this patient had evidence of lues and tuberculosis, which may have contributed to the bizarre findings in this case.
Multiple gammopathies have been reported in multiple myeloma and at times in association with other disorders such as macroglobulinemia and other neoplastic conditions. Bachmann's study would indicate that this condition is not quite as rare as previously suspected. The number of cases of multiple gammopathies reported so far is too small to relate those findings to a distinct clinical entity, although the first few cases all had clinical evidence of myeloma. There is a marked variability in the type of protein pattern reported. Vaerman's case can still be explained as a monoclonal paraproteinemia. The two peaks on electrophoresis were shown to be aggregations of immunologically identical proteins. Waldenström described two patients as did Imhoff and Ballieux who had simultaneous macroglobulins and myeloma proteins. Engle and Nachmann's case had only one paraprotein in the serum, but two types of Bence Jones protein in the urine. In Costea's patient, the amounts of the two abnormal serum proteins varied considerably with time, while in the present case the serum protein values stayed practically constant throughout the patient's disease.

The results of the fluorescence studies support the impression gained by others that, as a rule, only one type of heavy chain is synthesized by each individual cell. Several investigators observed that the cells in the germinal centers of lymphoid follicles may synthesize both types of immunoglobulins. Since the cells in the germinal centers of lymphoid nodules are young and perhaps "uncommitted," it seemed possible that immature-appearing neoplastic cells might also produce more than one immunoglobulin. However, in the case under discussion, no doubly stained cells were seen in the marrow which makes it likely that even the precursors of the mature plasma cells were capable of synthesizing only γG and γA globulin.

This suggests that the neoplastic process, whatever its underlying stimulus, had given rise to two distinct clones of cells, each responsible for only one of the abnormal serum proteins present in this patient.

The cause of this unusual combination of findings is obscure. One can speculate that: (1) The occurrence of myeloma in a patient with polycythemia vera is rare and might be coincidental. The finding of a biclonal proliferation of plasma cells in polycythemia vera is of extreme rarity, only one possible case having been reported. It appears unlikely that the combination of these two unusual pictures can be attributed to chance alone. The moderate elevation of γM-globulin in this case lends further support to the thought that some unknown stimulus, possibly related to polycythemia vera itself, may have been responsible for the proliferation of plasma cells, which in turn produced the abnormal proteins.

(2) In a study of a group of polycythemia vera patients by Wasserman and colleagues, elevated immunoglobulins have been demonstrated in a number of cases and myeloma-like proteins have been found in a few. One can therefore postulate that one of the clonal proliferations of plasma cells was due to

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*A rare mutation of immunologically competent cells was offered by Costea et al. to explain simultaneous finding of γG and γA globulins in one cell line.*
myeloma and the other was related to the presence of polycythemia vera.

There may have been a common, although unknown stimulus which caused proliferation of both red cells and plasma cells. The finding of an elevation of all three immunoglobulins lends some support to this hypothesis.

Radiation (in the form of P32) may have incited proliferation of the plasma cells. Again, this is not too likely in view of the large number of patients with polycythemia treated with P32 and the relatively small number of patients reported to have both myeloma and polycythemia.

**SUMMARY**

A patient with long standing polycythemia vera, who was treated with P32, chemotherapy and phlebotomies recently developed a myeloma-like disease picture. Two paraproteins (γG type K and γA type L) were found in the serum together with two Bence Jones-like proteins in the urine (kappa and lambda). γM was increased to twice the normal value.

Immunofluorescence studies of the bone marrow showed two clones of plasma cells each of which produced only one type of heavy chain. No cells with double fluorescence were seen. The two types of immunologically different plasma cells were morphologically indistinguishable from one another.

**SUMMARIO IN INTERLINGUA**

Un patiente con polythemia ver de longe duration, tractate con P32, chimitherapia, e phlebotomias, disvello recentemente un myelomoide tableau clinic. Duo paraproteinas (γG tipo K e γA tipo L) esseva trovate in le sero insimul con duo proteinas urinari simile a proteina de Bence Jones (kappa e lambda). Le valor de γM esseva duo vices be norma.

Studios immunofluorescentic del medulla ossee mostrava duo clones de plasmocytos, cata-un producente solo un typo de catena pesante. Nulle cellulas con duple fluorescentia esseva incontrate. Le duo typos de immunobogicamente differente plasmocytos esseva morphologicamente indistinguibile.

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