"Myeloma Protein" in a Patient with Monocytic Leukemia

By M. Dave Poulk, Lawrence Berman* and Ananda S. Prasad

The purpose of this paper is to report the occurrence of "myeloma gamma globulin" in serum and hydrolytic fragments of gamma globulin in urine of a patient with monocytic leukemia. The case differs in certain respects from the other example of this association reported by Osserman and Lawlor. The isolation and characterization of the aberrant proteins and detailed morphologic findings are presented here.

Case Report

A 74 year old Negro male felt well until one month prior to hospitalization, at which time he experienced sore throat followed by swelling of both sides of the neck. He was found to have tonsillitis without exudate, small discrete enlarged cervical, axillary, and inguinal lymph nodes and slight enlargement of liver and spleen. The first leukocyte count was 30,800 per cu. mm. The differential count showed: eight per cent atypical monocytoid stem cells (Fig. 1), seven per cent atypical immature monocytes (Fig. 2), six per cent monocytes, and four per cent neutrophil promyelocytes. The cytoplasm of the monocytoid stem cells contained azurophilic granulations including occasional large irregular masses and often numerous small dust-like granules. Nuclear chromatin was generally light-

Fig. 1.—Atypical monocytoid stem cells in peripheral blood. The nuclear chromatin pattern is undifferentiated. Multiple small nucleoli are present. The cytoplasm is finely granular. The granules are small and dust-like and appear reddish-purple in the Leishman-stained dry film. There are clear vacuoles in the cytoplasm, some overlying the nuclei.

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Fig. 2.—Immature monocyte in peripheral blood. The cytoplasm contains very fine dust-like azurophilic granulation which imparts a grayish-blue color. The individual granules are too small to be resolved in the halftone engraving. The nucleus has deep indentations. A few of the small roundish light spots in the nucleus are nucleoli.

Fig. 3.—Example of bizarre nuclear shapes in the monocytoid stem cells of peripheral blood. A large nucleolus is visible in the nuclear lobule on the left and two small nucleoli are present along the edge of the lobule on the right.

Fig. 4.—Typical overlapped appearance of portions of the nucleus in the monocytic cells of peripheral blood.

staining and arranged in delicate strands or masses, without any tendency for clumping. Nuclear shapes varied from circular or oval to convoluted forms (Fig. 3). Frequently, parts of the nuclei appeared overlapped (Fig. 4). Deep indentations or narrow clefts gave many a lobulated appearance. Nucleoli of the size of those present in normal myeloblasts, and many that were smaller, appeared in the majority of these cells. The immature monocytes were of similar appearance except that the nuclear chromatin was slightly coarser and granulation of cytoplasm was more in evidence (Fig. 4).

The aspirated bone marrow was hypercellular. The myeloid:erythroid ratio was increased to 12:1. Occasional erythroblasts were dysplastic, or megaloblastoid. Megakaryocytes were normal in number and morphology. The hypercellularity of the specimen was due to the presence of 31 per cent atypical monocytoid stem cells and 11 per cent immature and atypical monocytes. Often these were very large (Fig. 5). There were one to three per cent plasma cells, among which there were large forms with irregular nucleoli (Fig. 6).
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Fig. 5.—Large monocytoid stem cell of bone marrow. The magnifications in this photomicrograph and the others of bone marrow are identical to those used for the blood cells in the preceding figures. A small nucleolus is present at the edge of the nucleus in the ten o’clock position.

Fig. 6.—A large plasma cell in bone marrow. In the center of the nucleus there is a pale area representing an irregularly-shaped nucleolus.

Fig. 7.—An unusual cell with a monocytoid nucleus and plasmacytic cytoplasm. In the Leishman-stained smear the cytoplasm was deeply basophilic and its edges were reddish in color.

A few bizarre cells had convoluted nuclei similar to those seen in monocytoid stem cells but having cytoplasm of the so-called “flame cell” type of plasma cells (Fig. 7).

The main feature of the monocytoid stem cells was that 10 per cent had extremely large nucleoli reaching or exceeding the size of nucleoli seen in Reed-Sternberg cells of Hodgkin’s lesions (Figs. 8 and 9).

Biopsy of a lymph node showed that its architecture was almost entirely obliterated by cellular tissue composed of large cells with vesicular nuclei and sometimes prominent nucleoli (Fig. 10). The initial opinion of the pathologist, who was not aware of the hematologic findings, was reticulum cell sarcoma, but the primitive cells of the lesion exhibited deep nuclear indentations that were observed in the blood and bone marrow smears.
Fig. 8.—A large monocytoid stem cell in bone marrow. The pale area at the upper edge of the nuclear lobule on the right is a large nucleolus which had a light blue color in the Leishman-stained dry film.

Fig. 9.—A huge stem cell in bone marrow. The cytoplasm contains a large amount of very fine azurophilic granulations and some vacuoles similar to those seen in the smaller monocytoid stem cells with lobulated and convoluted nuclei. A giant nucleolus is present. It is approximately as large as the erythrocytes in the field.

Fig. 10.—A section of lymph node. The field was selected to show a remnant of persisting normal lymphocytic tissue represented by the cells with small dark-staining nuclei. The larger cells with vesicular nuclei, often with deep clefts, make up the abnormal tissue which obliterated almost all of the normal lymph node architecture.

The cytologic and histologic findings were quite similar to those we find regularly in monocytic leukemia, except for the distinctive feature of a high incidence of enlarged nucleoli in the monocytoid stem cells, as compared with other examples of this form of leukemia. Corroborative evidence was the presence of lysozyme in the urine, in amounts typical of monocytic leukemia.9

Treatment with mercaptopurine-6 (6MP), 50 mg. per day, and Prednisone, 60 mg. per day, was followed in four weeks by a decrease in the leukocyte count to 9,200 per cu. mm. The dose of 6MP was then reduced to 25 mg. per day. Oral lesions thought to be due to leukemia appeared 10 weeks later. The patient felt better for two months. Thereafter, the lymph nodes enlarged considerably. The dosages of 6MP and steroid were increased (6MP 50 mg. per day, and Prednisone 40 mg. per day). At this time the blood findings were as follows: Hb 7.5 Gm. per cent, WBC 11,000 per cu. mm., platelets 290,000 per cu. mm. The differential count showed the following: neutrophils 95 per cent, myelocytes
1 per cent, lymphocytes 2 per cent, and only occasional atypical monocyteid stem cells. Extensive x-ray surveys of the skeleton were carried out, but no evidence suggestive of myelomatosis were found. Renal functions were normal except for proteinuria which ranged from 8–10 Gm./24 hrs.

Five months after admission, the patient developed fever and pneumonia. He did not respond to antibiotic therapy and expired. Permission for autopsy was not granted.

**MATERIALS AND METHODS**

Sera were obtained by venipuncture at different time intervals during the course of the disease. Urine was collected and stored in the cold room. The urine was exhaustively dialyzed against distilled water and lyophilized prior to use.

Immunoelectrophoretic analysis was performed on histologic slides by the method of Scheidegger. Starch block electrophoresis was carried out by the method of Kunkel. Half-inch segments were eluted with saline and protein was estimated by Lowry's method using the Technicon Autoanalyzer. Appropriate pools were made, dialyzed, and lyophilized prior to investigation. Lyophilized urinary proteins were dissolved in the desired buffer and centrifuged. The supernatant was used for the electrophoretic and gel filtration experiments. Starch gel electrophoresis was conducted in a discontinuous system of buffers as previously described. The methods of two dimensional zone electrophoresis and starch gel immunoelectrophoresis were essentially the same as reported earlier. Ultra-centrifugal analysis was carried out in a Spinco Model E analytical ultracentrifuge (Beckman Model Analytical Ultracentrifuge, Spinco Division, Palo Alto, California) with schlieren optics. The determinations were carried out in three different dilutions in each case in order to obtain the sedimentation rates for infinite dilution.

Gel filtration was performed in columns (Pharmacia) measuring 2.5 cm. × 100 cm. using Sephadex G-100. The eluting buffer was 0.1 M phosphate buffer (pH 7.8) containing 1 M NaCl. The flow rate did not exceed 10 ml./hr. and 5–6 ml. of the effluent was collected per tube. The elution patterns were registered by an automatic ultraviolet light absorption meter (LKB-Uvicord). Pools of the peaks were dialyzed against distilled water and lyophilized. Urinary proteins isolated by starch block electrophoresis as well as the whole serum were screened for the presence of gamma globulin genetic markers (Gm. and Inv. groups) and lysozyme. Immunologic screening of the light and heavy chains of gamma globulins was done with highly specific antisera prepared in rabbits in this laboratory. Additional testing of some of the materials was done in the laboratory of Dr. J. L. Fahey, National Cancer Institute, Bethesda, Maryland. Antiserum was prepared in rabbits against the isolated “myeloma gamma globulin.” This antiserum contained antibodies specific for Fab, Fc and Fc fragments of normal gamma globulin (Cohn Fraction II) digested with papain. In addition, it also contained antibodies specific for the patient's "myeloma protein gamma chains" and the hydrolytic fragments thereof. Rabbit antiserum against human lysozyme was obtained through the courtesy of Dr. J. Mestecky. Lysozyme levels were determined by an enzymatic method.

**RESULTS**

Serum taken three weeks prior to death contained 6.6 Gm. per cent of the total protein. Filter paper electrophoretic analysis revealed the following distribution of the serum components: Albumin, 2.9 Gm. per cent; alpha-1 globulin, 0.4 Gm. per cent; alpha-2 globulin, 0.7 Gm. per cent; beta globulin, 0.4 Gm. per cent; and gamma globulin, 2.2 Gm. per cent (Fig. 11A). Filter paper electrophoresis of urinary proteins is shown in Fig. 11B. The presence

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of high concentration of gamma globulin was confirmed by immunoelectrophoresis (Fig. 12A) and identified as IgG "myeloma gamma globulin" by using a specific anti-gamma chain antiserum (Fig. 12B). Two dimensional zone electrophoresis confirmed the presence of "myeloma gamma globulin" which was separated into several discrete protein zones (Fig. 12C). Starch gel electrophoresis of highly concentrated urine disclosed the presence of a
Fig. 13.—Separation of urinary proteins by starch block electrophoresis and gel filtration. (A) starch block electrophoresis (A-I-eluted ½’ starch block segments). (B) gel filtration elution pattern (I-VII-pooled peaks).

number of protein zones. More detailed information about the nature of the urinary proteins was obtained after starch block electrophoresis of the lyophilized urine. The fractions obtained are shown in Figure 13. The proteins in each fraction were analyzed by starch gel electrophoresis (Fig. 14). In segment A (Fig. 14), three protein zones were found, and of particular interest is a protein zone which has an elongated shape and migrates with highest mobility to the cathode. Proteins found in the remainder of the segments as shown in Figure 14, B-H. A, D, and F, were investigated further in the ultracentrifuge. The majority of the material of fraction A sedimented with a velocity of 1.6–1.7 \( S_{20w} \), that of fraction D with a velocity of 2.2 \( S_{20w} \), and that in fraction F with a velocity of 2.5 \( S_{20w} \).

Gel filtration of the lyophilized urine resulted in separation of seven major fractions (I-VII) as shown in Figure 13. Starch gel electrophoretic patterns of the materials in each chromatographic peak are shown in Figure 14, I-VI. A cathodally migrating protein was isolated in fraction V (Fig. 14, V). This component bears assimilarity in shape and migration to a protein component found in starch block fraction A (c.f. Fig. 14, A). Fractions VI and VII absorb strongly in ultraviolet light but do not stain with protein dye (Amido black 10B) as shown in Figure 14, VI and VII.
Fig. 14.—Starch gel electrophoresis patterns of fractions of urinary proteins separated by starch block electrophoresis and gel filtration. (a) starch block electrophoresis; A-H starch block segments; U-whole urine. (b) gel filtration fractions; I-VII-pools of emerging peaks; U-whole urine.

The “myeloma protein” was characterized immunologically as IgG \(\gamma\)-1, Kappa, and serologically as Gm. \((a+b-f-),\) Inv. (a) Genetic typing of the urinary proteins isolated from the starch block fractions A, D, and F was also attempted but with negative results. Immunelectrophoretic screening of the urinary proteins isolated by starch block electrophoresis have demonstrated the presence of Fab, Fc, and F\(\prime\)c and undigested gamma globulin, as shown in Figure 15. The presence of the F\(\prime\)c fragment was demonstrated in starch block fractions E and F as well as in gel filtration fraction IV by immunelectrophoresis using an antiserum containing antibodies specific for gamma chains and F\(\prime\)c fragment. The electrophoretic position of the urinary
Fig. 15.—Immunoelectrophoresis of fractions of urinary proteins isolated by starch block electrophoresis. A-F-individual starch block segments; p-papain digest of normal gamma globulin (Cohn Fr-II); u-whole urine; precipitin lines developed with antiserum (a) containing antibodies against the Fab, Fc and Fc' fragments of IgG gamma globulins. Arrows indicate position of Fc fragment.

Fc' fragment and Fc fragment produced by papain digestion of normal gamma globulin is shown in Figure 15 (p and u). The isolated urinary Fc' migrates in the same position as shown in Figure 15 E and F. Ultracentrifugal analysis of starch block fraction F ($S_{20w}^{0}=2.5$) and gel filtration results (fraction IV) are in close agreement with the accepted S-rate value and molecular weight of Fc' fragment. Investigation of fraction A by starch gel
immunoelectrophoresis showed two merging precipitin lines with anti-Kappa light chain antiserum over the two sharp protein zones. It should be stressed that no precipitin line could be demonstrated with the elongated cathodically migrating zone using a battery of antisera against a number of protein components. A similarly negative result was obtained by subjecting the peak V obtained by gel filtration to starch gel immunoelectrophoretic analysis. High amounts of light chains of type Kappa were found in starch block segments D and E and gel filtration peaks II and IV.

The lysozyme activity of the whole urine was found to be 33 μg./ml. (at an approximate concentration of 1 mg./ml.), and the activity of segment A was found to be 200 μg./ml. (also at an approximate concentration of 1 mg./ml.). These results were confirmed by demonstration of the lysozyme by immunoelectrophoresis using a rabbit antiserum prepared against human lysozyme. Strong precipitin lines were obtained with material in starch block segment A and gel filtration peak V.

**DISCUSSION**

The morphologic findings were adequate for a diagnosis of monocytic leukemia but because of the unusual association with serum and urine findings typical of myelomatosis, the demonstration of lysozyme in the urine of monocytic leukemia patients by Osserman and Lawlor added a new requirement for diagnosis which was satisfied in our case. Our case differed in certain respects from the other example of the association of myeloma proteins with monocytic leukemia reported by Osserman and Lawlor. Their patient represented a well-documented plasma cell myeloma with amyloidosis and Bence Jones proteinuria of at least four years duration prior to development of monocytic leukemia as a terminal event. The disease developed against a background of long-standing urinary tract infection. The authors commented on their experience with plasma cell dyscrasias by pointing out the frequency of long-standing inflammatory disease as an antecedent event in the development of plasma cell dyscrasias. On the other hand, in our patient, monocytic leukemia was present when he was first seen. There was no history of long-term inflammatory disease. Marrow studies showed a range of plasma cells from 1-3 per cent. There were rare atypical plasma cells (less than 0.1 per cent) but there was no demonstrable skeletal changes in extensive surveys of the skeleton.

Two additional patients were studied by Osserman. In both cases, only small amounts of "myeloma gamma globulins" were shown in the serum (Cases no. 5 and 7). A "narrow band" dysglobulinemia was also found in 15 cases out of 500 subjects studied who were older than 68 years.

Electrophoretic analysis of the serum has shown the presence of fast-migrating myeloma globulin which was resolved by starch gel electrophoresis into a number of discrete bands. This protein was found to be typical of "myeloma proteins" by all physico-chemical, immunologic and genetic criteria. All of the bands reacted equally well with specific anti-IgG antiserum.

The urine contained small amounts of albumin, α1-trypsin inhibitor, inhibitor, acidic α1-globulin and prealbumin I, as well as large amounts of
light chains type Kappa, and gamma globulin and fractions thereof. Enzymatic fragments Fab, Fc, and F’c identified by specific antisera were found to be present in large amounts. The F’c fragment\textsuperscript{13} was present in large quantity and contained antigenic determinants specific for this “myeloma protein” as revealed by antibodies obtained by immunization with the purified “myeloma protein.” This observation, which points to immunologic heterogeneity of the F’c fragment, will be published elsewhere.

Although one cannot rule out the possibility that this patient had two neoplastic proliferations, involving both the monocytes and plasma cells and that the “myeloma protein” was being formed by these abnormal immature plasma cells which were present in very small numbers, an alternative hypothesis should be considered. Inasmuch as occurrences of monoclonal gammopathies have been noted in the past in cases ranging from lympho- to myeloproliferative disorders of various kinds,\textsuperscript{3,5} the fundamental defect in protein synthesis may reside in undifferentiated reticulum cells of lymphoid and myeloid organs. A case reported by Okano, Azar and Osserman\textsuperscript{7} with lesions resembling both myeloma and reticulum cell sarcoma and protein findings of myeloma suggests a primary involvement of reticulum cells. In 20 per cent of Osserman’s patients with plasma cell dyscrasia there was a documented background of chronic inflammatory disease that could be regarded as instances of chronic and persistent stimulation of the reticuloendothelial system. It may be significant that a proliferation of large reticulum cells has been noted as one of the features of the bone marrow and lymph node lesions in heavy chain disease.\textsuperscript{8} In our patient, there was an unusually high incidence of monocytoid stem cells with giant nucleoli, as compared with our subsequent series of examples of monocytic leukemia in which gammopathy has not, as yet, been demonstrated. In the light of the presumed function of nucleoli, it is suggested that the enlarged nucleoli may be a morphologic expression of abnormal protein synthesis. Further studies are necessary in order to understand the role of the monocytoid stem cells with large nucleoli, with respect to synthesis of “myeloma proteins” as observed in our patient.

**SUMMARY**

A 74 year old man with monocytic leukemia was found to have serum and urinary protein findings typical of myelomatosis. Lysozyme excretion in the urine was in the range characteristic of monocytic leukemia. There were no x-ray findings indicative of myeloma. Aspirated bone marrow specimens contained one to three per cent plasma cells, and less than 0.1 per cent atypical plasma cells. The leukemia responded temporarily but without remission to treatment with 6MP and steroid, with no effect on the myeloma protein profile or excretion of the urinary proteins.

The leukemia was present without overt morphologic or x-ray findings of myeloma, and the case differs from a similar example of the association of myeloma protein with monocytic leukemia in which the leukemia occurred as a terminal event in a previously well-documented myelomatosis.

The serum contained a fast-moving “myeloma gamma globulin” which was classified as γ-1, Kappa; Gm. (a+b−f−) Inv. (a). This protein was
heterogeneous by starch gel electrophoresis. The urine contained large amounts of lysozyme, Fab, Fc and F'c fragments of the “myeloma gamma globulin,” light chains of type Kappa, as well as whole gamma globulin. Small amounts of other serum proteins were also found.

The possibility of two neoplastic proliferations involving both the monocytes and plasma cells has been considered. Possible role of monocytoid stem cells with giant nucleoli in the synthesis of “myeloma protein” has been discussed.

SUMMARIO IN INTERLINGUA

In un masculo de 74 annos de etate qui habeva leucemia monocytic Ic constatationes relative al proteinas seral e urinari esseva de un typo characteristic de myelomatosis. Le excretion urinari de lysozyma esseva del ordine de magnitude characteristic de leucemia monocytic. Le constatationes radiologic non suggestionava myeloma. Specimens aspirate de medulla ossee contineva inter un e tres pro cento de plasmocytos e minus que 0,1 pro cento de atypic plasmocytos. Le leucemia respondeva temporarimente (sed sin remission) al tracimento con 6MP e steroide, sin que un effecto esseva producite in le configuration myelomatic del proteinas o in le excretion urinari de proteinas.

Le leucemia esseva presente sin patente constatationes radiologic o morphologic de myeloma, e le caso difere ab un simile exemplo del association de proteina myelomatic con leucemia monocytic in le qual le leucemia occurreva como evenimento terminal in un previamente ben documentate myelomatosis.

Le sero contineva un “globulina gamma myelomatose” a motilitate rapide le qual esseva classificate como γ-1, Kappa; Gm (a + b − f −) Inv (a). Iste proteina esseva hetrogenee in electrophorese a gel de amylo. Le urina contineva grande quantitates de lysozyma, de fragmentos Fab, Fc, e F’c del “globulina gamma myelomatic,” catenas leve del typo kappa, e etiam intacte globulina gamma. Esseva etiam trovate micre quantitates de altere proteinas seral.

Le possibilitate ha essite considerate que il se tracta in iste caso de duo proliferaciones neoplastic afficiente tanto le monocytes como etiam le plasmocytos. Es commentate le rolo possibile de monocytoid cellulias primordial con nucleolos gigante in le synthese de “proteina myelomatic.”

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REFERENCES

5. Klemm, D., Grusnick, D., Weinreich, J., Hauswaldt, Ch. and Hunstein, W.: Paraproteineamie und plasmareticuläre Zellpro-


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