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Paranuclear Microfilaments in Multiple Myeloma Associated with the Presence of Free Light Chains

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ABSTRACT

Two cases of multiple myeloma are reported showing striking arrays of paranuclear microfilaments confirming a previous single case report done on autopsy material. Both cases showed free light chains in the urine. In one case, free light chains were in the serum, and there was some evidence of polymer formation. Although this suggests that the microfilaments are polymerized light chains, amyloid stains were negative. Whatever their origin, the structures are clearly abnormal and may serve as a neoplastic marker.

Introduction

Recently, Udoji and Frigy[20] reported a unique case of a plasmacytoma composed of plasma cells containing large numbers of intracytoplasmic paranuclear microfilaments. They described these microfilaments as solid, nonbranching and wavy having a width of 66 to 132Å. Tissue obtained for ultrastructural study was from formalin fixed autopsy material, raising the possibility that the findings were artifactual. Two cases are reported by us of multiple myeloma with findings similar to the above. Fresh tissue was examined in both cases, one from biopsy material, the other from peripheral blood (plasma cell leukemia), confirming the presence of these microfilaments in myeloma. In addition, both patients had free light chains in the serum suggesting a possible immunoglobin origin of the microfilaments.

Materials and Methods

ELECTRON MICROSCOPY

Samples were fixed with 2.5 percent gluteraldehyde, 2 percent paraformaldehyde in 0.2M sodium cacodylate buffer, pH 7.2, postfixed in 1 percent osmium tetroxide, block-stained with uranyl acetate and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with either a Zeiss 95-2 or Hitachi HU-11C microscope.

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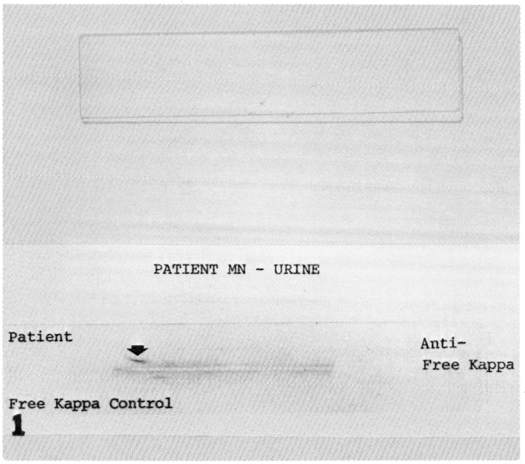

FIGURE 1. Urine Immunoelectrophoresis. Protein of restricted electrophoretic mobility (arrow) which reacts with antiserum specific for free kappa chains.

IMMUNOELECTROPHORESIS

Immunoelectrophoresis was performed using thin film agarose (Universal Electrophoresis Film, Corning Medical, Medfield, MA) and anti-sera specific for the Fc portion of heavy chains, bound and free light chains, or free light chains.[5] Amido black was used for staining.

Case Histories

Patient MN: This 78-year-old woman was hospitalized for progressive fatigue, dizziness, and confusion over a month's period. X-ray examination revealed a lytic lesion of the right ilium. Laboratory findings were: BUN 62 mg per dl; uric acid 12.9 mg per dl; creatinine 3.0 mg per dl; hemoglobin 11.9 g per dl; platelet count 83,000 per mm^3; total protein 6.5 g per dl; and globulins 2.5 g per dl. Although the serum protein electrophoresis appeared normal, immunoelectrophoresis showed monoclonal free Kappa chain in both the serum and urine (figure 1). Bone marrow aspiration and biopsy revealed diffuse replacement by young pleomorphic plasma cells consistent with multiple myeloma. Crystal violet and Congo red stains were negative for amyloid.

Patient MM: This 75-year-old woman entered the hospital after a two to three month history of fatigue, weight loss, severe back and hip pain. She had experienced recent pathologic rib fractures and was noted to have marked osteoporosis. The pertinent laboratory findings were: BUN 24 mg per dl; total protein 7.6 g per dl; globulin 4.2 g per dl; and hemoglobin 10.8 g per dl. Serum and urine protein electrophoresis showed two discrete monoclonal bands in the gamma region which on immunoelectrophoresis were demonstrated to be monoclonal IgG $_{l}$ and free λ chains (figure 2). Bone marrow biopsy and aspiration showed sheets of pleomorphic plasma cells consistent with myeloma. Crystal violet and Congo red stains were negative for amyloid. After a course of chemotherapy, the patient was discharged and returned with plasma cell leukemia (WBC of 9000 per mm^3 with 40 to 50 percent plasma cells). Her condition gradually deteriorated and she died within a month.

Results

Immunoelectrophoresis findings are shown in figures 1 (Patient MN) and 2 (Patient MM). Note that Patient MM has free light chains in the serum as well as the urine, and that the urine shows two distinct areas of restricted mobility. This suggests the presence of free lambda polymers.

Electron microscopic studies demonstrate paranuclear microfilaments in both cases as shown in figures 3 and 4.

Discussion

The ultrastructural findings in multiple myeloma and other plasma cell dyscrasias have been well defined in the past.[1,12,16,17,18] The neoplastic plasma cells show considerably greater than normal varibility in size and shape of the nuclei, nucleoli, and mitochondria. They often display nucleocytoplasmic dyssynchrony with the nuclear development lagging behing that of the cytoplasm. Other findings include multiple centrioles, intracytoplasmic and intranuclear dense bodies (Russell Bodies), and abnormalities of the rough surfaced endoplasmic reticulum (RER) such as marked dilation. These findings are nonspecific and are frequently appreciated by light microscopy alone.

Recently, sporadic cases have appeared in the literature showing more unusual electron microscopic findings in individual cases of myeloma. Kalderon and colleagues reported a myeloma patient with crystallizing cryoglobulinemia

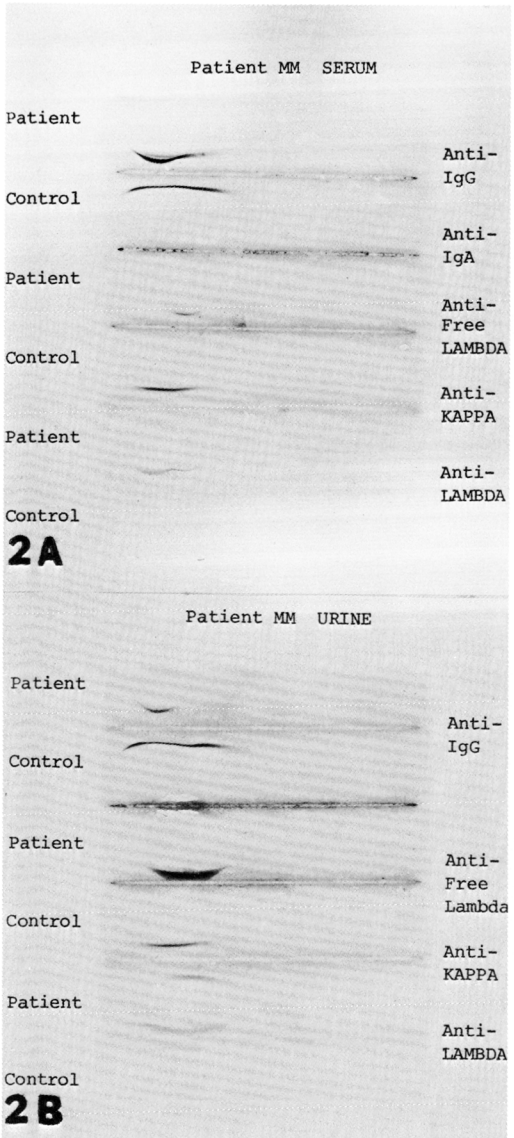

FIGURE 2. Immunoelectrophoresis-(A) Serum, restricted mobility of IgG with a corresponding abnormality in λ and an additional restricted area in λ which reacts with antiserum specific for free λ; (B) Urine, large quantity of λ of restricted electrophoretic mobility which reacts with antiserum specific for free light chains and an additional restricted area of IgG with a corresponding abnormality in λ.

whose plasma cells contained striking intracytoplasmic crystals.[9] The crystals were present within the RER and free in the cytoplasm, as well as extracellularly. On the basis of experimental data, the authors postulated that the free intracytoplasmic crystals were the product of unattached, free ribosomes, and that this aberrant synthesis indicated a neoplastic process. Another unusual case of a patient with a plasmacytoid lymphoproliferative disorder and free Kappa chains in the serum was described by Kjeldsberg.[10] The neoplastic plasma cells contained large masses of intracytoplasmic microfilaments which were bound by distinct membranes unlike the microfilaments described in our cases. The inclusions stained positively with routine amyloid stains.

The microfilaments reported by Udoji and Frigy[20] from autopsy studies on an intracardiac metastasis of a plasmacytoma originating in the humerus are similar in all respects to the two cases reported here. As in our case, the amyloid stains were negative. The patient's serum exhibited monoclonal IgG Kappa, hut there was no mention of free light chains. In addition to the cases reported here and by Udoji, Sorenson described similar paranuclear microfilaments in passing, in one of ten cases of myeloma he reviewed.[18]

Paranuclear microfilaments do not necessarily represent a single substance or specific organelle. They may be amyloid fibrils,[2,11,21] which take up amyloid stains,[11,15,21] or actin filaments[6] or they may be cytoskeletal structures which anchor the nucleus.[3,6] These structures are seen in benign as well as malignant hematopoietic cells.[19] They have been described in normal monocytes, macrophages[3] and activated lymphocytes[13] as well as in the neoplastic cells of acute lymphocytic leukemia[14] and acute myeloid leukemia.[4] In benign or reactive cells, the paranuclear microfilaments are scant and do not extend far beyond the nucleus. In neoplastic cells, on the other hand, including the cases described here, they are numerous and may occupy a sizeable portion of the cytoplasm. The overgrowth of what appear to be normal struc-

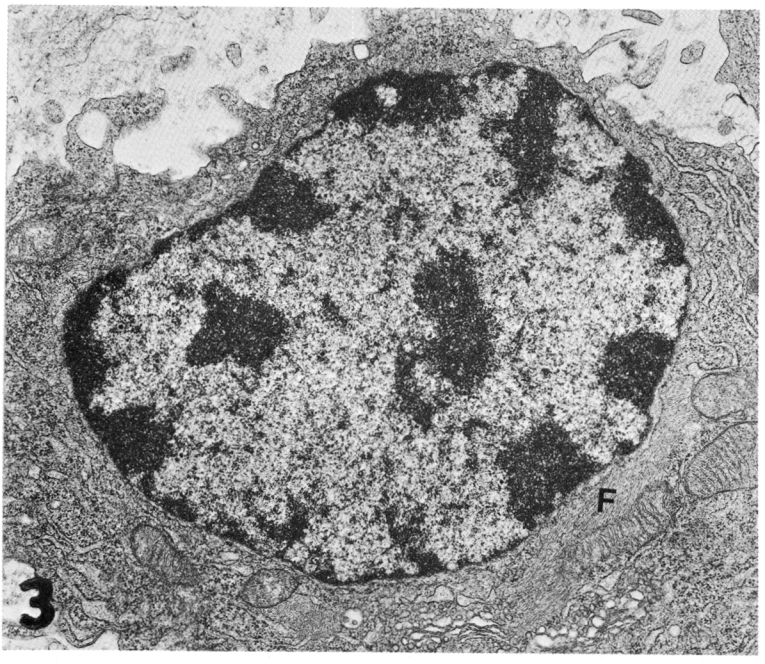

FIGURE 3A. Plasma cell of patient with free light chains. Note paranuclear filaments (F) × 12,000.

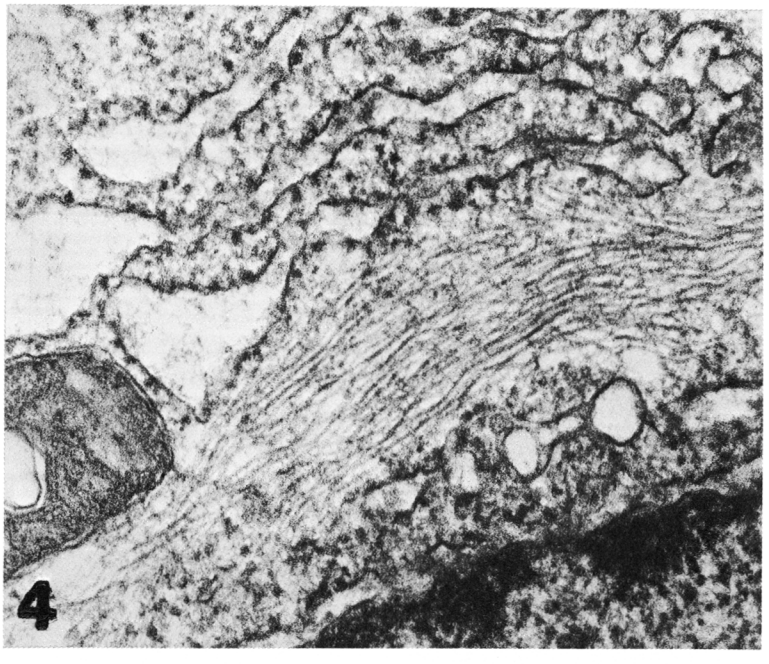

FIGURE 3B. Higher magnification of paranuclear filaments in plasma cell of patient with free light chains × 55,000.

tures in these malignant disorders may represent a neoplastic growth disorder and could prove to be a tumor maker.[19]

Since both cases reported here are associated with the elaboration of free light chains, it is tempting to speculate that the microfilaments are light chain polymers similar to amyloid synthesized intracytoplasmically as a consequence of a neoplastic process.[7,8,10] The suggestive evidence in Case MM that the free light chains tended to polymerize adds a bit to

this speculation (figure 2). On the other hand, the negative reaction to amyloid stains in both cases speaks against this interpretation.[11,15,21]

Regardless of whether the microfibrils described here represent an overgrowth of normal cytoskeletal structures or polymerized light chains or something else, they are clearly abnormal in both quantity and extent. Therefore, when present they may represent a convenient marker in differentiating benign from malignant plasma cells.

References

1. AZAR, H. A., ZAINO, E. C., PHAM, T. D., and YANNOPOULOS, K.: "Nonsecretory" plasma cell myeloma: Observations on seven cases with electron microscopic studies. Amer. J. Clin. Path. *58*:618–629, 1972.
2. COHEN, A. S. and CALKINS, E.: Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature *183*:1202–1203, 1959.
3. DEPETRIS, S., KARLSBAD, G., and PERNIS, B.: Filamentous structures in the cytoplasm of normal mononuclear phagocytes. J. Ultrastruc. Res. *7*:39–55, 1962.
4. FREEMAN, J. A. and SAMUELS, M. S.: The ultrastructure of a "fibrillar formation" of leukemic human blood. Blood *13*:725–731, 1958.
5. GERSON, B., LA BRIE, J. L., and COPELAND, B. E.: Performance comparison of the Corning thin-film agarose and the Hyland thick-film agar methods for immunoelectrophoresis. Clin. Chem. *24*:1634–1635, 1978.
6. GILBERT, D.: 10 nm filaments. Nature *272*: 577–578, 1978.
7. GLENNER, G. C., EIN, D., and TERRY, W. D.: The immunologic origin of amyloid (editorial). Amer. J. Med. 52:141–147, 1972.
8. ISOBE, T. and OSSERMAN, E. F.: Patterns of amyloidoses and their association with plasma cell dyscrasia, monoclonal immunoglobulins and Bence-Jones proteins. New Engl. J. Med. *290*:473–477, 1974.
9. KALDERON, A. E., BOGAARS, H. A., DIAMOND, I., CUMMINGS, F. J., KAPLAN, S. R., and CALABRESI, P.: Ultrastructure of myeloma cells in a case with crystal cryoglobulinemia. Cancer *39*:1475–1481, 1977.
10. KJELDSBERG, C. R.: Evidence for intracellular amyloid formation in myeloma. Blood *50*:493–504, 1977.
11. LINDER, E., LEHTO, V.-P., and VIRTANEN, I.: Amyloid-like green birefringence in cytoskeletal 10 nm filaments after staining with Congo red. Acta Path. Microbiol. Scand. *87A*:299–306, 1979.
12. MALDONADO, J. E.: Ultrastructure of the myeloma cell. Cancer *19*:1613–1617, 1966.
13. PARKER, J. W., WAKASA, H., and LUKES, R. J.: Cytoplasmic fibrils in mixed lymphocyte cultures. Blood 29:608–615, 1967.
14. ROSEN, N. R., DIFINO, S., NELSON, D. A.: Acute leukemia with unusual cytoplasmic inclusions: A cytochemical and ultrastructural study. Cancer *43*:2405–2409, 1979.
15. SHIRAHAMA, T., BENSON, M. D., COHEN, A. S., and TANAKA, A.: Fibrillar assemblages of variable segments of immunoglobulin light chains: An electron microscopic study. J. Immunol. *110*:21–30, 1973.
16. SMETANA, K.: A further note on the ultrastructure of myeloma plasmacytes. Neoplasma *18*:3–13, 1971.
17. SMETANA, K., GYORKEY, F., GYORKEY, P., and BASCH, H.: Ultrastructural studies on human myeloma plasmacytes. Cancer Res. 33:2300–2309, 1973.
18. SORENSON, G. D.: Electron microscopic observations of bone marrow from patients with multiple myeloma. Lab. Invest. *13*:196–213, 1964.
19. TANAKA, Y.: Fibrillar structures in the cells of blood forming organs. J. Nat. Cancer Inst. 33:467–485, 1964.
20. UDOJI, W. C. and FRIGY, A. F.: Cytoplasmic fibrils in plasma cells of a solitary myeloma. Amer. J. Clin. Path. *70*:836–839, 1978.
21. ZUCKER-FRANKLIN, D. and FRANKLIN, E. C.: Intracellular localization of human amyloid by fluorescence and electron microscopy. Amer. J. Path. 59:23–42, 1970.