Intranuclear Inclusions in Bence Jones Lambda Plasma Cell Myeloma

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A patient with plasma cell myeloma producing only Bence Jones lambda protein was found to have pale intranuclear inclusions in the majority of the bone marrow plasma cells. These inclusions, previously undescribed in myeloma patients producing only Bence Jones protein, contained Bence Jones lambda protein, were non-electron dense, bound by a single membrane, and contained no cytoplasmic structures. Intracytoplasmic inclusions were not present, and the perinuclear cistern was not dilated. Thus, the inclusions may represent intranuclear protein synthesis with anomalous release in the abnormal cells.

A variety of intracellular inclusions have been described in the plasma cells of patients with multiple myeloma by many authors as reviewed by Maldonado et al. and Bessis. Most often these are seen in the cytoplasm and have been designated Russell bodies, Mott cells, morular (grape) cells, and others. Intranuclear inclusions have been considered one of the characteristic findings in the lympho-plasma cells of Waldenström's macroglobulinemia, though a number of reports of intranuclear inclusions in cells from plasma cell myeloma have also appeared. These inclusions, which have generally been electron dense and similar in appearance to Russell bodies, have been found in only a small proportion of the abnormal plasma cells. Often they have been accompanied by typical intracytoplasmic inclusions. In those series of patients with myeloma demonstrating intranuclear inclusions, where the anomalous immunoglobulin type has been specified, IgA has been noted most often, though IgG myeloma has been seen as well. In this paper, we report detailed electron-microscopic and histochemical studies of the first case of non-electron dense intranuclear inclusions in plasma cells of a patient with multiple myeloma producing only Bence Jones lambda type protein. These findings will be discussed in relation to current thoughts regarding the pathogenesis of such inclusions.

CASE HISTORY

A 54-yr-old textile worker presented with a 2-mo history of anterior chest pain and low back pain, and a 15-lb weight loss with malaise and weakness. Initial physical examination revealed a chronically ill man with dorsal kyphosis. There was a depression in the sternum at the angle of Louis with marked bone tenderness over the sternum, adjacent ribs, and the thoracic spine. The remainder of the physical examination was generally unremarkable. Laboratory data in-

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included a hemoglobin of 12 g/100 ml, hematocrit 34%, and WBC 6000/cu mm. Urinalysis was negative for protein when performed with an albumastick but 4 plus with sulfosalicylic acid, and the heat precipitation Bence Jones test was strongly positive. The serum calcium was 11.9 mg/100 ml, uric acid 12.4 mg/100 ml, alkaline phosphatase 202 U, and BUN 20 mg/100 ml. X-ray studies revealed numerous compression fractures of the thoracic vertebrae, a fracture of the sternum, and numerous lytic lesions of the ribs and pelvis. The bone marrow aspiration established the diagnosis of multiple myeloma with over 60% abnormal plasma cells seen (see below). The total serum protein was 6.2 g/100 ml, and cellulose acetate electrophoresis showed that 7% was in the gamma range and 59% was albumin. No anomalous components were present by serum electrophoresis. Quantitative immunoglobulins were IgG, 1.2 mg/ml (normal 0.7-1.2 mg/ml); IgA, 0.07 mg/ml (normal 0.08-0.2 mg/ml); IgM, 0.01 mg/ml (normal 0.09-0.17 mg/ml). Electrophoresis of the urine revealed a sharp anomalous component in the beta region with little albumin present. Quantitative measurements of protein in the urine showed 6-10 g of Bence Jones protein per 24 hr. Immunoelectrophoresis studies performed on the serum and urine revealed free lambda light chains in the serum and Bence Jones lambda protein in the urine. No other abnormal components were demonstrated.

Treatment was initiated with BCNU, 120 mg intravenously, cyclophosphamide, 700 mg intravenously once a month, and prednisone, 75 mg a day for 7 days each month, and rapid relief of bone pain resulted. In July 1972, the urine Bence Jones protein had fallen to 0.34 mg/24 hr. Though considerable improvement in performance status was obtained, the patient continued to have some back pain, and in December 1972 there was 6 g of abnormal protein in the urine per 24 hr. There were 30% abnormal plasma cells in the bone marrow, and therapy was switched to melphalan, 14 mg/day for 4 days each month, and prednisone, 80 mg/day for 7 days each month. On this regimen the urine once again became negative for protein, but in June 1973 returned to 3 g/24 hr. Since that time the patient has continued to take melphalan and prednisone as described above with a performance status on the Karnofsky scale of 75%. Renal function has remained normal throughout the course of the disease.

MATERIALS AND METHODS

Light Microscopy

Bone marrow was aspirated from the sternum, and smears were immediately prepared, air dried, and stained with Wright's stain. Other air-dried films were used for histochemical staining and fluorescence studies and were stored at 4°C. Additional bone marrow material was allowed to clot and embedded in paraffin for histochemical studies. Immunofluorescence was determined using fluorescein isothiocyanate-conjugated antisera specific for immunoglobulin heavy and light chains (obtained from Meloy laboratories, Springfield, Va.).

Electron Microscopy

The cells were fixed in suspension by mixing 3 ml of bone marrow and 10 ml of a solution prepared by mixing equal volumes of 16% glutaraldehyde buffered with cacodylate and 4% osmium tetroxide buffered with S-collidine immediately prior to use. Fixation was carried out for 1 hr at 4°C, and the cells were then filtered through membrane filters (Millipore S.M. 5.0 ± 1.247 mm) forming an incomplete monolayer. Following dehydration with a graded series of alcohols, propylene oxide was used to dissolve the membrane filter and replace the ethanol. The cells were then embedded in a 0.5-mm layer of Epon by overlaying them with the unpolymerized resin and curing for 2 days at 56°C. Small rectangles were cut from the disc and embedded in the tips of Epon blocks. The preparations were stained en bloc with lead citrate and uranyl acetate, sectioned, and examined with an Hitachi HS-8 electron microscope.

RESULTS

Light Microscopy

The bone marrow was diffusely hypercellular, and abnormal findings were restricted to the large numbers of plasma cells which varied markedly in size.
and degree of maturation. Approximately 60% of the cells in the marrow were comprised of such abnormal plasma cells, at least half of which had intranuclear inclusions (Figs. 1 and 2). These cells were present in similar numbers and morphology on two different occasions within a 10-day period. In Wright-stained preparations the intranuclear inclusions were well circumscribed and stained a very pale blue, standing in sharp contrast to the deeply staining nucleus. The inclusions varied in size and occasionally occupied almost the entire nucleus. Often they abutted the nuclear envelope. From one to four such inclusions were present in the plasma cells (Figs. 1 and 2). The inclusions were clear and failed to stain with hematoxylin and eosin, giemsa, PAS, alcian blue, methyl green pyronine, feulgen reaction, Masson's trichrome, succinic dehydrogenase, glucose-6-phosphate dehydrogenase, and alkaline phosphatase. However, the inclusions were stained light blue with bromphenol blue and
Fig. 3. Electron micrograph of plasma cell. Two large intranuclear inclusions (I) are clearly separate from the nucleolus (Nu). Vesicles (V) are present within the inclusion, but no recognizable structures are seen. Many mitochondria (M), Golgi (G), profiles of endoplasmic reticulum (E) are present in the cytoplasm. Cytoplasmic secretory products (S) are present in small numbers. Lead citrate and uranyl acetate, x 6300.

demonstrated positive immunofluorescence with a polyvalent anti-immunoglobulin sera and specific anti-lambda-chain serum but not anti-kappa-chain serum.

Electron Microscopy

Cells with single and multiple intranuclear inclusions were frequently seen (Fig. 3). In most cases a single limiting membrane could be identified around each inclusion, the contents of which consisted of an amorphous granular proteinaceous material. No well-formed cytoplasmic or other structures were
Fig. 4. (A) Higher-power electron micrograph of cell shown in Fig. 3. Note amorphous proteinaceous material within the inclusion (I). The inclusion is separated from the nuclear membrane by an area of nuclear material (N). Endoplasmic reticulum (E) is seen in the cytoplasm. Lead citrate and uranyl acetate, × 12,000. (B) Electron micrograph of another plasma cell at higher power. In this case the intranuclear inclusion (I) abuts an intact nuclear envelope (solid arrows) which is clearly separate from the inclusion membrane (open arrows). Note the perinuclear cistern (C) which is not dilated. Endoplasmic reticulum (E) and mitochondria (M) are seen in the cytoplasm. Lead citrate and uranyl acetate, × 25,000.

present within these inclusions, although there were occasional round to oval membrane bound vesicles containing the same granular amorphous material. Several inclusions directly abutted the nuclear envelope, but no communication between the inclusions and perinuclear cistern was ever demonstrated (Fig. 4A and B) despite serial sectioning of a number of such cells. Nucleoli were clearly separate from these inclusions and were morphologically unremarkable. Rodlets, filaments, or viral-like structures were not present. Within the cytoplasm were many mitochondria, most of which contained matrix granules and were generally unremarkable. The Golgi apparatus was prominent, as were both the smooth and rough endoplasmic reticulum. Much of the endoplasmic reticulum was slightly dilated by clear material and occasionally contained faint amorphous proteinaceous contents. The perinuclear cisternal space contained material of a similar nature but was not dilated (Fig. 4). Free polyribosomes were abundant. No electron-dense inclusions were seen.
DISCUSSION

Plasma cell cytoplasmic inclusions have frequently been described in patients with plasma cell myeloma. Russell bodies, Mott cell, and grape cells (morular cells) have all been considered to represent condensations of protein within membranous structures, probably endoplasmic reticulum. Intracellular inclusions, on the other hand, have been noted less frequently: most are electron dense and are also considered to represent condensation of protein. These inclusions seem to have the same ultrastructural and histochemical composition as cytoplasmic Russell bodies. The intranuclear inclusions in the cells of patients with Waldenström's macroglobulinemia are usually of this type and contain PAS-positive material. On one occasion they were shown to contain IgM protein by immunofluorescence. In plasma cell myeloma such inclusions were at one time thought to be characteristic of IgA-secreting cells. However, further study makes this appear unlikely. Brittin et al. were able to demonstrate such inclusions in patients with Waldenström's macroglobulinemia, IgA, IgG, and in one case of Bence Jones protein variant of plasma cell myeloma. It should be noted that of the ten patients in that study only one had inclusions in 25% of the plasma cells, whereas nine had them in less than 2% of the plasma cells. In the cases studied more intensely the inclusions were PAS positive in cells producing Waldenström's macroglobulin and IgA protein but PAS negative in the cells producing IgG or Bence Jones protein only. One case was studied by immunofluorescent staining, and the vacuoles contained the same protein as the serum component. These authors concluded that such bodies are of intranuclear origin but that their presence does not indicate a specific type of serum protein abnormality.

The second type of intranuclear inclusion, that of low electron density, has been described much less frequently. Brittin et al. demonstrated two such cases in their study, one patient with Waldenström's macroglobulinemia and one with IgA multiple myeloma, and implied that others had been seen. The inclusions were PAS positive. Polli et al. reported one case with low-density inclusions in a patient with IgA myeloma; these vacuoles were also PAS positive. The intranuclear inclusions described by Fruhling et al. were somewhat less electron dense than the usual Russell bodies but otherwise had an identical appearance. The protein abnormality was not described in the report, but the cells were apparently obtained from a plasmacytoma. Another patient with IgA myeloma has been reported by Abbe et al. to have less strongly osmophilic intranuclear inclusions as well. Low-electron-density intranuclear inclusions of the type reported in this paper have not previously been described in patients with only Bence Jones protein production.

The origin of such low-electron-density intranuclear inclusions is uncertain. The electron-microscopic studies of the inclusions in our patient's cells, as well as the few others reported, demonstrate a proteinaceous material bound by a single well-defined membrane. The inclusions did not stain with PAS or other stains as noted above. However, their light- and electron-microscopic characteristics were virtually identical to the content of the perinuclear cisternal area and the endoplasmic reticulum. This appears to represent a somewhat dilute con-
centration of material and contrasts with the electron-dense inclusions reported elsewhere which have more concentrated proteinaceous matter. The positive staining with bromphenol blue and positive immunofluorescent staining for lambda light chains indicate that the material in the inclusions is of the same type as the protein demonstrated in the serum and urine.

Three main possibilities exist for the origin of such inclusions. First, they might simply represent invaginations of the cytoplasm into the nucleus with sectioning giving the appearance of an inclusion. Since many sections and many cells were studied without demonstrating such invaginations, this possibility appears unlikely. Moreover, cytoplasmic structures such as mitochondria or endoplasmic reticulum were never seen within these bodies. The second possibility suggests an origin of the inclusions in the perinuclear cisternal portion of the endoplasmic reticulum. Subsequent intranuclear invagination and pinching off would result in an inclusion bound by the inner nuclear membrane and containing material of the same nature as the perinuclear cistern. The third possibility is that the inclusions arise within the nucleus as the result of intranuclear protein synthesis. The material could subsequently become surrounded by a membrane and then discharged into the perinuclear cisternal space to continue in transport via the usual secretory mechanism.

The latter two suggestions both have support in the literature. Because of the similarity of the contents of the perinuclear cisterna and the inclusions, and because it was a membrane-bound structure with the contents similar to those of the endoplasmic reticulum, Polli et al. concluded that these inclusions resulted from the invagination and pinching off of the perinuclear cisterna as described above. However, no specific evidence was presented to support the conclusion as to the direction of flow of the secretory material. On the other hand, Thiery et al. performed phase-contrast and electron-microscopic studies on cells from lymph nodes and found intranuclear inclusions similar to those described here. They arose within the nucleus, enlarged, and apparently discharged their contents via direct connection with the perinuclear cisternal space. Fruhling et al. studied the bone marrow of a patient with IgA plasma cell myeloma and inclusions similar to those described in our patient except for PAS positivity. They also concluded that the contents of the inclusion were discharged into the cisternal space. However, in that instance the perinuclear cisternae were extremely dilated, and direct communications between these two structures were clearly demonstrated.

The inclusions we have described in this report are of similar density to the material within the perinuclear cisternae and the endoplasmic reticulum and are similar to the material described in the above reports. It is of significance, however, that the perinuclear cisterna was not dilated and that intracytoplasmic inclusions were never seen. Though direct communications were not seen, one cannot rule out the possibility that they exist and that these inclusions originated in the perinuclear cisterna and were the result of invaginations and pinching off of the membrane within the nucleus. However, the absence of dilatation of the perinuclear cisterna or other parts of the endoplasmic reticulum in these cells favors the possibility that these inclusions were formed within the nucleus and blocked from discharging their contents, thus allowing the inclusions to
reach an unusually large size and number. An analogous situation may exist in certain patients with "nonsecretory myeloma" in whom abundant immunoglobulin protein is found within the endoplasmic reticulum but secretion from the cell appears blocked. Furthermore, if the direction of protein secretion and hence inclusion formation had been from the perinuclear cisternae into the nucleus, one might have expected significant dilatation of this space as well as intracytoplasmic protein accumulation. These features were not present.

The existence of an intranuclear protein-synthesizing machinery or "nuclear ribosomes" has been a controversial issue. It seems clear that isolated nuclei can synthesize polypeptide material, though in most instances this has been nuclear protein. However, there is precedent for intranuclear formation of proteinaceous material later transported to the cytoplasm. Hemoglobin has been described in the nucleus of reticulocytes prior to its detection in the cytoplasm, and ferritin likewise has been noted in the nucleus of cells responding to iron loading. Our findings would seem to support this pathway, although intranuclear ribosomes were not seen, and the source of the membrane surrounding the inclusions remains unexplained. The frequency and importance of this occurrence in the normal pathway of secretory proteins is not clear. The presence of intranuclear material similar to that in the endoplasmic reticulum as we have described may thus represent a minor phase in the secretory cycle which is accentuated in the cells of some patients with anomalous production of protein. It is apparent that dynamic studies of protein secretion involving radioactive incorporation and localization in various cell structures will be needed before the issue of the site of origin of such nuclear inclusions is finally settled.

With this report of the low-density inclusions in a patient with Bence Jones lambda plasma cell myeloma, both low- and high-density inclusions have now been seen in all varieties of plasma cell myeloma and Waldenström's macroglobulinemia. PAS staining of the inclusions may provide further information with regard to protein type, since IgA and IgM inclusions are PAS positive while IgG and BJ inclusions appear negative. Ultimately, immunofluorescent staining of the inclusions with anti-immunoglobulin antisera provides the most specific assessment. It would thus appear that this phenomenon of low-density intranuclear inclusions is not specific for the secretion of any particular immunoglobulin type but depends more upon the secretory cycle of the plasma cell and the aberration unique to the particular cell involved.

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