A Study of Intracytoplasmic Inclusions in Myeloma Cells from Two Patients with Multiple Myeloma

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Two cases of multiple myeloma which showed inclusions within the cytoplasm of myeloma cells were reported. One contained crystalline inclusions which were not stained by May-Grunwald and Giemsa, and the other contained Auer rod-like spindle shaped inclusions which were stained by May-Grunwald and Giemsa and PAS. Both cases were stained intensively by acid phosphatase but they were not stained by Congo red or by thioflavine-T. Moreover, they reacted only with anti-κ sera in the immunofluorescent study and showed the same structure on electron microscopic observation. Although they showed different staining behaviors according to their compositions, they were considered to be essentially the same. It appears that light chains produced in excess are concentrated and form inclusions through the addition of sugar or digestion by lysosomal enzymes.

intradacytoplasmic crystalline inclusion; Auer rod-like spindle shaped inclusion; multiple myeloma; myeloma cells

It has long been known that various inclusions are contained in myeloma cells and plasma cells, and a number of papers have been published concerning such inclusions (Steinmann 1940; Bessis 1973; Graham and Bernier 1975). Intracytoplasmic inclusions such as crystalline inclusions, Auer rod-like spindle shaped inclusions, dense bodies, myeline-like structures, fibrillar formations, polysome lamellae complexes, virus-like particles and Russell’s bodies, etc., have been reported (Oikawa 1975). However, except for Russell’s body, the essential characteristics and mechanisms of these inclusions have not been elucidated. We have recently studied 2 patients with multiple myeloma in whom inclusions were
found in the cytoplasm of myeloma cells. One case had crystalline inclusions and the other had Auer rod-like spindle shaped inclusions. We performed cytochemical, ultrastructural and immunological studies to determine their characteristics.

**CASE REPORT**

**Case 1.** A 75 year-old woman was admitted to our hospital because of lassitude, anorexia and coughing on January 21, 1984. Physical examination revealed slight conjunctival anemia and rales in both lungs. Hemoglobin was 7.2 g/100 ml, with a reticulocyte count of 6.8%. The leucocyte count was 2,600/mm³ with 50% neutrophils, 44% lymphocytes, 4% monocytes, and 2% eosinophils. The hematocrit was 24.5% and the platelet count was 132,000/mm³. Bone marrow examination revealed the nuclear cell count to be 400,000/mm³, the megakaryocyte count to be 208/mm³, the M/E ratio to be 2.7 and plasma cells to be 14.8%, in about 60% of which crystalline inclusions not stainable with May-Grunwald and Giemsa were detected. Blood sedimentation rate was 107 mm/hr. Total protein was 10.0 g/100 ml, and γ-globulin was 50.3% showing a monoclonal peak. M protein was IgG-κ. Immunoglobulins were 4,161 mg 100 ml for IgG, 126 mg 100 ml for IgA and 156 mg/100 ml for IgM, showing an increase in IgG. No cryoglobulin or pyroglobulin was found. Urine was positive for protein, but no Bence-Jones protein was noted. Roentgenographic findings for the bone revealed no abnormalities such as punched out lesions. Rectal biopsy was negative for amyloid deposits.

**Case 2.** A 67-year-old man was admitted to our hospital with complaints of pain on the right side of the chest on February 15, 1982. Physical examination was unremarkable. Hemoglobin was 13.7 g/100 ml with a reticulocyte count of 2.6%. The leucocyte count was 5,800/mm³ with 47% neutrophils, 46% lymphocytes, 6% monocytes, 1% eosinophils. The hematocrit was 39.3% and the platelet count was 252,000/mm³. Bone marrow examination revealed the nuclear cell count to be 141,000/mm³, the megakaryocyte count to be 16/mm³, the M/E ratio to be 1.28 and plasma cells to be 24.0%, in about 60% of which Auer rod-like spindle shaped inclusions stained red with May-Gruwald and Giemsa were contained. The blood sedimentation rate was 20 mm/hr. Total protein was 8.2 g/100 ml, and γ-globulin was 29.9%, showing a monoclonal peak. M protein was IgG-κ. Immunoglobulins were 1,926 mg/100 ml for IgG, 57 mg/100 ml for IgA and 71 mg/100 ml for IgM, indicating a slight increase in IgG and a decrease in normal immunoglobulins. Bence-Jones protein was not revealed, nor was pyroglobulin or cryoglobulin found. Roentgenographic findings for the bone revealed punched out lesions in the cranial bone. Rectal biopsy was negative for amyloid deposits.

**MATERIALS AND METHODS**

In both cases smear samples collected from the bone marrow were examined by the following stainings and reactions.

**Cytochemical examinations**

1) May-Grünwald and Giemsa staining, 2) Peroxidase reaction (by 2,7-diaminofluorene method), 3) PAS staining (by a simplified method), 4) Alkaline phosphatase staining (by the Azo dye method), 5) Acid phosphatase staining (by the Azo dye method), 6) α-Naphthyl butyrate esterase staining (by the method described by Li et al. 1973), 7) Sudan Black B staining by Sheehan-Storey’s method (Sheehan and Storey 1947), 8) Congo red staining by a modified Highman’s method (Highman 1946) and 9) Thioflavine-T staining.
Immunofluorescent examinations

All immunofluorescent examinations were performed by the direct method using FITC labeled anti-human γ, α, μ, κ and λ sera (Dakopack Co., Ltd., Copenhagen, Denmark).

Electron microscopic examinations

Specimens were fixed with 2.5% gluteraldehyde, followed by double fixation with osmium acetate. Then, these specimens were dehydrated, embedded with epon resin and cut into super-fine slices. After they were doubly stained with uranium acetate sulfate, they were examined under an electron microscope.

RESULTS

The results of special staining tests were positive for May-Grunwald and Giemsa, PAS and acid phosphatase stainings in Case 2, while there was only a positive result for acid phosphatase staining in Case 1 (Figs. 1 and 2). In both cases negative results were obtained for peroxidase, alkaline phosphatase, Sudan Black B, α-NB esterase, Congo red and thioflavine-T stainings.

On immunofluorescent examination, almost all cells containing inclusions were stained positively by anti-γ and anti-κ sera in both cases, indicating that they were IgG-κ producing cells. Inclusions showed staining defects for anti-γ, while they were stained brightly by anti-κ sera in both cases. These findings were seen to be more prominent in Case 1 (Figs. 3 and 4).

Electron microscopic findings obtained in both cases revealed that inclusions were present independently from rough endoplasmic reticulum (RER). The contour of inclusion was irregular and surrounded by a monolayer of smooth membrane. They consisted of homogeneous substances of moderate density. According to the direction of the slices, there were a number of parallel bundles of fibrils showing regular arrangement. They were seen to be more regular in Case 1. These fibrils were about 20 Å in diameter, being clearly thinner than amyloid fibers (80 to 90 Å) (Figs. 5 and 6).

DISCUSSION

The presence of a variety of inclusions in cytoplasm and nuclei of plasma cells and myeloma cells has long been known. Not a few papers have been published concerning intracytoplasmic crystalline inclusions. However, their essential characteristics and etiology are not completely understood. As described by Kanoh et al. (1979), inclusions reported heretofore might be largely classified into four types: 1) Those considered to have originated from immunoglobulin, 2) those considered to be amyloid, 3) those considered to consist of lysosomal granules and 4) those which can not be classified into any of the above described three types.

Inclusions originating from immunoglobulin are often seen within RER and are called Russell’s bodies. They are formed through the concentration and
aggregation of immunoglobulin retained within RER and observed as circular granules of high electron density with diameters of 0.1 to 0.5 μm (Welsh 1960). However on rare occasions the presence of these inclusions in areas outside of RER has been reported (Jennette et al. 1981).

On the other hand, there are some inclusions which do not show reaction when

Fig. 1. Bone marrow smear from Case 1 showing plasma cell with crystalline inclusions.

a: May-Grünwald and Giemsa.
b: Acid phosphatase.
Fig. 2. Bone marrow smear from Case 2 showing plasma cell with Auer rod-like spindle shaped inclusions.

a: May-Gr"unwald and Giemsa.
b: Acid phosphatase.
tested by the immunofluorescent method, despite they are contained within RER. In such cases, it is highly probable that immunoglobulins have lost antigenicity through degeneration or destruction. It appears that inclusions originating from immunoglobulin are in general not stainable with May-Grünwald and Giemsa (Kanoh et al. 1979).

Kjeldsberg et al. (1977) reported a case which showed intracytoplasmic inclusions stained positively by Giemsa as well as Congo red and thioflavine-T
Fig. 5. Inclusion from Case 1 at higher magnification. It is composed of numerous fine fibrous bundles. ×66,000

Fig. 6. Inclusion from Case 2 at higher magnification. The structure of inclusion is the same as that of case 1. ×86,300
and consisted of fibrils 80 to 90 Å in diameter characteristic of amyloid fibers on electron microscopic observation. Moreover, Nomura et al. (1982) described a case in which inclusions stained positively with thioflavine-T yielding electron microscopic findings similar to those obtained by Kjeldsberg et al. (1977). These inclusions were considered to consist of amyloid fibers. They were stained positively by acid phosphatase and reacted only with anti-κ sera. It was thought that light chains produced in excess were transported to the Golgi apparatus to form crystalline inclusions, which became amyloid fibers through autophagosome by lysosomal granules. It is known that amyloid fibers seen in amyloidosis associated with myeloma consist of light chains of immunoglobulins or their decomposition products. These findings are interesting in that light chains and amyloid fibers are found to be closely related within cells, since amyloid fibers are usually formed between tissues.

There are other inclusions which do not show any reaction when examined by the immunofluorescent method and have no relation with RER. They are not stainable with Congo red or thioflavine-T. When these inclusions are stained positively by acid phosphatase or β-glucuronidase, they have been reported as being inclusions originating from lysosomal granules or lysosomes themselves (Kanoh and Uchino 1977; Nakamine et al. 1982; Raman and Van Slyck 1983). However, when lysosomal enzymes are found within the unit membrane, they may be considered to be secondary lysosomes except for cases in which they appear to be primary lysosomes morphologically (Weiss and Greep 1977). In these inclusions, there is a difference in structure according to the substances which are digested by lysosomal enzymes. From the above viewpoint, the inclusions found in the case reported by Nomura et al. (1982) cited above might be said to be secondary lysosomes. Thus, there is a possibility that there are various types of inclusions among those reported to have originated from lysosomes. Based on these findings, it may be more suitable to call this type of inclusions unidentified substances including lysosomal enzymes.

Finally, to our knowledge, there have been no reports regarding unidentified inclusions in studies in which acid phosphatase or β-glucuronidase staining was done.

As for our cases, the inclusions detected in Case 1 were not stained positively by May-Grünwald and Giemsa and showed crystalline morphology, while those from Case 2 were stained positively similar to Auer rods by the same staining method. Both cases were stained intensively by acid phosphatase but not by Congo red and thioflavine-T. Moreover, inclusions of both cases reacted only with anti-κ sera by the immunofluorescent method and showed the same structure on electron microscopic observation.

With respect to their essential nature, they resembled those detected in the case described by Nomura et al. (1982), in that they originated from light chains and were digested by lysosomal enzymes. However, since the inclusions of our
cases were not stained by Congo red and thioflavine-T, and fibrils were about 20 Å in diameter as observed by electron microscope, it is apparent that they are different from amyloid fibers. Akasaka (1981), who found a transitional form which might be classified between crystalline inclusions and dense bodies, discussed the origin of inclusions. He stated that it was most probable that myeloma protein retained within RER was transported into the Golgi apparatus, where it was accumulated and concentrated to form inclusions. In our two cases, it appears that light chains produced in excess were concentrated in the Golgi apparatus and formed inclusions through the addition of sugar or by digestion by lysosomal enzymes. Although they showed different staining behaviors, they were considered to be essentially the same. However, as to the cause for amyloid not being formed in our case, further study is required to determine whether it is related only to time or also to properties of immunoglobulin.

References


