protein[11]. On the other hand, the more basophilic component of the nucleolus seems to correspond to the granular areas[12].

Two kinds of vacuole-like structure have been described in vegetable nucleoli. The central vacuole[13] is not a constant feature, but represents rather some functional character[14], while the small vacuoles described within the fibrillar areas[13] seem to correspond to the intranucleolar chromatin (see review by LAFONTAINE[15]).

Our observations show that the components of the nucleolus have different staining affinities in plant cells and suggest that the argentophilic and the basophilic components correspond, respectively, to the following structures observed in animal cells: argentophilic spherules and interstitial matter[1]; argentophilic granules and fundamental substance[2]; nucleolonema and pars amorpha[3].

Resumen. La tinción con plata y con fucsina básica del nucleolo en células meristemáticas de *Allium cepa* permite detectar la localización de dos componentes de distinta afinidad tintorial. El componente de mayor argentofilia aparece en forma de gránulos, distribuídos en el interior de una matriz contínua que presenta marcada basofilia.

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Ultrastructure of Human Myeloma Cells Studied by Peroxidase Conjugated Antibodies Directed to Human Immunoglobulin Component Chains

Multiple myeloma is a malignant disease characterized by neoplastic proliferation of cells of plasmacytic line. The clinical observation that a pathological protein can be demonstrated in the serum and urine of 90–95% of patients with plasma cell neoplasm is well known[1,2].

In general, morphology of myeloma cell is very similar to that encountered in non-pathological plasma cells. There are no pathognomonic cytological features which permit accurate differentiation of neoplastic from normal plasma cells, or of cells responsible for the production of the various pathological proteins[3–6].

In 1961, BESSIS[6] described paracrystalline structure with periodicity in apparently pathological plasma cells obtained from a patient with prolonged multiple myeloma. Although the authors suggested that these structures were formed of polymers of gamma globulin molecules produced in myeloma cells, actual nature of these crystalline structures and their functional significance were not elucidated.

Recently, a case of multiple myeloma was reported by Dr. S. ITO and his co-workers of the Anjo Hospital, Aichi (Japan), in which similar fine crystalline inclusions were demonstrated in the cytoplasma of plasmacytic cells of sternal bone marrow specimens and within kidney tubules of a 50-year-old female patient with advanced multiple myeloma.

In an attempt to elucidate the origin of these crystalline structures and their functional significance, we introduced in the present study a combination of electron microscopy and immunocytochemistry. Sternal bone marrow specimen was obtained by aspiration from this patient and treated with peroxidase-conjugated antibodies each monospecific for immunoglobulin heavy chain class (anti-α, anti-γ, anti-μ) or light chain type (anti-$\varkappa$, anti-λ).

Antibodies were specifically purified by exclusive use of solid immunoadsorbent conjugated with an appropriate antigen as described previously[7,8]. Conjugation of purified antibodies with horseradish peroxidase was performed by closely following the method of NAKANE and PIERCE[9]. Antibody-peroxidase complexes thus prepared were found to retain the reactivity of both antibody and peroxidase, because precipitin line produced between antigen and enzyme-labelled antibody in agar exhibited brownish colour when placed in enzyme substrate.

When glutaraldehyde-fixed cell sediment was treated with peroxidase-conjugated antibody directed to $\varkappa$-type light chain, the intracellular site of immunoglobulin accumulation can be cytochemically revealed under electron microscope as conspicuous and discrete electron dense precipitate[10,11]. The unresponsiveness of these myeloma cells to the peroxidase label of other antibody specificity agreed with the serological observations that the increase of only free $\varkappa$-type light chain level was detected in the serum and urine of the patient.

Immunoglobulin light chain of $\varkappa$-type was localized to ergastoplasm, its membrane, ribosomes lining the ergastoplasmic membrane and nuclear membrane (Figure

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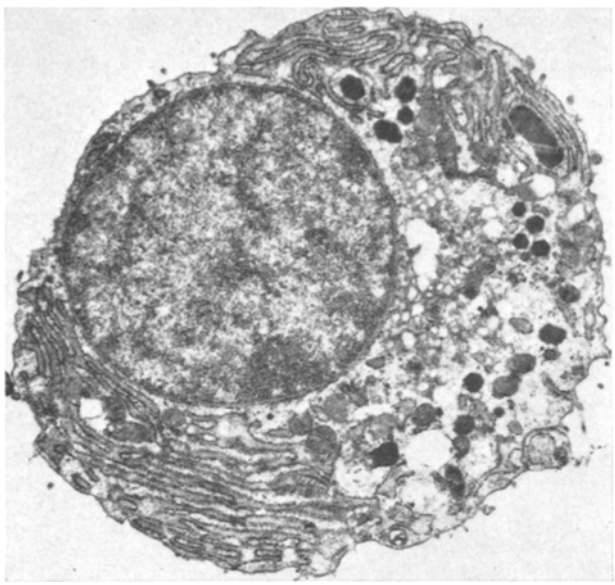

Fig. 1. Electron micrograph of a human myeloma cell treated with peroxidase-conjugated anti-$\varkappa$. Intracellular immunoglobulin is revealed as electron-dense precipitate on ergastoplasm including its membrane and membrane-bound ribosomes and the external layer of nuclear envelope. Lead citrate and uranyl acetate. × 11,600.

increased after the same treatment (Figures 4 and 5). The failure of staining of these crystalline structures with enzyme label of any antibody reactivity was rather unexpected, because they have long been postulated as immunoglobulin molecules produced in ergastoplasm[6]. Stimulated by this unexpected result, we performed close morphological examination of these crystalline structures on electron microscopy. Now, we have the strong impression that these crystalline substances originated from Golgi complex rather than from ergastoplasm by the following reasons: (1) Crystalline structures were usually detected outside the ergastoplasmic cavity. (2) Crystalline structure was always surrounded by a single smooth membrane which closely resembled that of Golgi vacuoles observed in Golgi area. In Golgi area were found numerous vacuoles which contained rounded, slightly electron-dense substance. Some of them contained fine periodic structure very similar to that observed in crystalline structure above described. Those electron-dense inclusions may represent a growing form of the paracrystalline structure (Figure 3). (3) Well-developed Golgi complex encountered in our myeloma cells was definitely negative in the reaction to peroxidase-conjugated anti-$\varkappa$ antibody (Figures 1 and 5).

In the light of the widely accepted hypothesis that Golgi complex plays a role in the elabolation and secretion of proteins[15–18], the unreactiveness of this organelle and

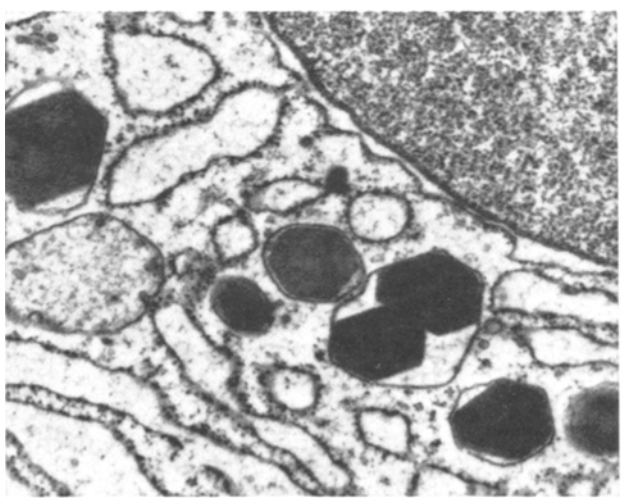

Fig. 2. Electron micrograph of a neoplastic plasma cell showing numerous cytoplasmic inclusions with periodic fine structure very clearly shown. Note that those inclusions are surrounded by a single, smooth membrane resembling that of Golgi vacuole. Lead citrate and uranyl acetate. × 12,000.

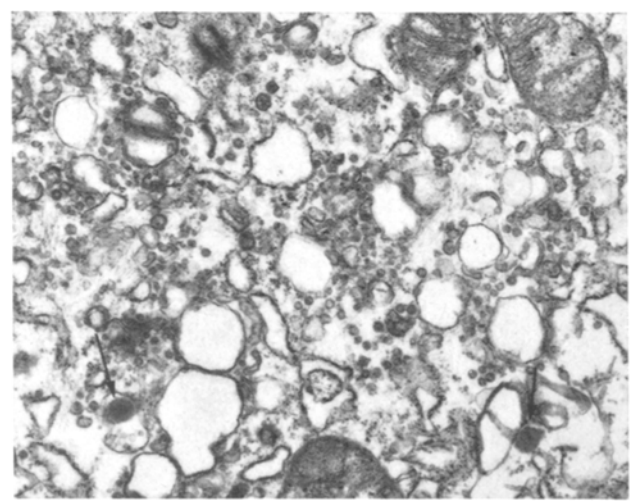

Fig. 3. Golgi area consisting of many vacuoles and vesicles. Golgi lamellae are usually lacking in our myeloma cells. Slightly electron-dense substance presenting crystalline fashion in large magnification was seen (arrow).

1). Enlarged intracisternal cavity of ergastoplasm was often stained with peroxidase-conjugated anti-$\varkappa$ antibody, too. The general pattern of $\varkappa$-chain distribution in the cytoplasm of myeloma resembled that encountered in non-neoplastic immature and mature plasma cells obtained from hyperimmunized rabbits[12–14].

In Figure 2 is shown an electron micrograph of crystalline structures which are electron dense even without treatment with peroxidase label. When reacted with peroxidase-conjugated anti-$\varkappa$ antibody, the electron opacity of crystalline inclusions remained unchanged, whereas the electron density of ergastoplasm significantly

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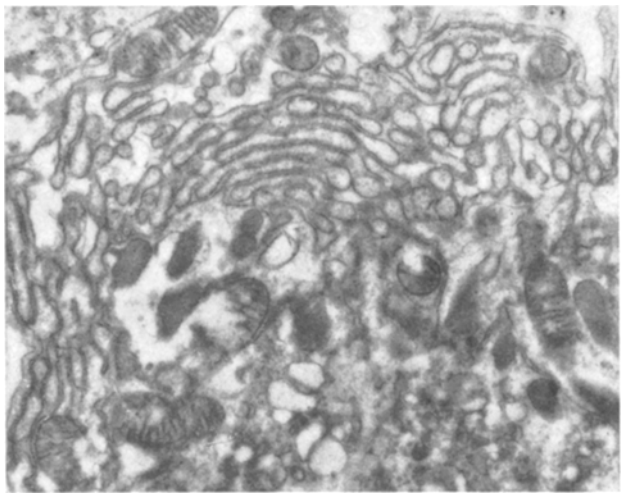

Fig. 4. Electron micrograph of myeloma cell demonstrating a variety of cell organelles including ergastoplasm, mitochondria, Golgi complex and crystalline structures. Note electron lucidness of these organelles. No electron staining. × 10,000.

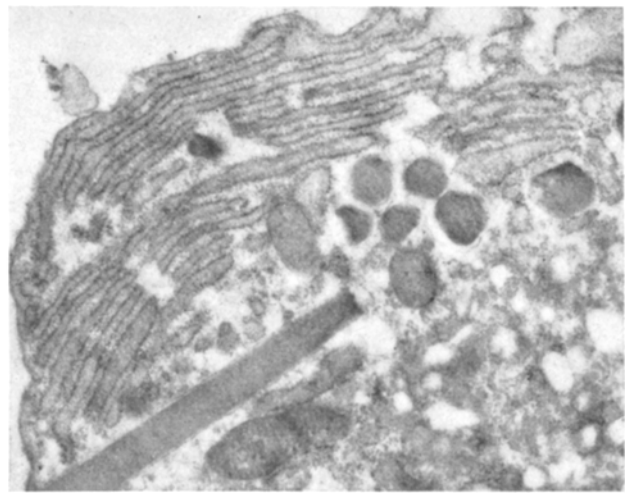

Fig. 5. Myeloma cells reacted with peroxidase-conjugated anti-$\varkappa$ antibody, showing the existence of electron-dense reaction precipitate on ergastoplasm, ergastoplasmic ribosomes and intracisternal cavity. In comparison with Figure 4, it is noted that electron density of other cell organelles remains unchanged by peroxidase reaction. No electron staining. × 15,000.

cytoplasmic crystalline structures which apparently originated from Golgi complex in the reaction with peroxidase label seems of importance. Whether this unresponsiveness reflects the functional abnormality of Golgi complex following malignant transformation in neoplastic plasma cells, or it merely represents a degeneration of proteins resulting in the loss of antigenicity of immunoglobulin molecules during the storage of produced immunoglobulin inside Golgi complex awaits further investigation[19].

Zusammenfassung. Menschliche Myelomazellen wurden immunchemischelektronenoptisch untersucht. Durch die Behandlung mit peroxidasekonjugiertem Antikörper gegen die Immunoglobuline H- oder L-Kette wurde keine H-Kette beobachtet.

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[19] We thank Dr. S. Ito of the Anjo Hospital of Aichi for providing the myeloma specimen.

Ultrastructural Localization of Heavy- and Light-Polypeptide Chains in Human Long-Term Culture Cells Detected by Peroxidase-Conjugated Antibodies

A long-term culture line, R.P.M.I. No. 4666 was originally established from peripheral blood of a patient with chronic myelogeneous leukemia and since has been propagated in suspension culture[1]. Extensive studies revealed that this culture consisted of very uniform cell types and was producing monoclonal immunoglobulin A (IgA) of $\varkappa$-type along with free $\varkappa$-type light chain simultaneously in the same cells[2].

In an attempt to reveal electron microscopic localization of component polypeptide chains of human immunoglobulin in the cytoplasm of the culture cells of human hematopoietic origin, we employed in the present study peroxidase-conjugated antibodies directed to human immunoglobulin component chain. By reacting fixed cells with peroxidase-conjugated antibody and revealing peroxidase activity cytochemically, ultrastructural localization of immunoglobulin was readily demonstrated as distinct electron-dense reaction product.

Antibodies monospecific either for α-heavy chain or for $\varkappa$-light chain were purified specifically by use of immunoadsorbents as described previously[3,4], and purified antibody was coupled to horseradish peroxidase using *p,p'*-difluoro-*m,m'*-dinitrodiphenyl sulfone as a coupling reagent as described by Nakane and Pierce[5]. The anti-

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