A UNIQUE CYTOPLASMIC STRUCTURE IN PAPULAR HISTIOCYTOMA

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Intracytoplasmic granules with a unique and highly complex ultrastructure, have been observed within the cellular infiltrate of the lesions of two patients with papular histiocytosis. Examination of serial sections has enabled construction of a three-dimensional model of these granules, which appear to be composed of an elaborate arrangement of membranes and vesicles. Two hypotheses (endocytosis and exocytosis) are proposed for the mode of formation of these unique cytoplasmic structures.

In benign and malignant histiocytic proliferations of the skin, electron microscopy has revealed various cytoplasmic structures including Langerhans granules [1-5], worm-like bodies [3,6-8], dense bodies [9], and myelinated bodies [8,10] whose nosologic role has been given different interpretations [5,8,10]. In 1974 Rodriguez et al [11] described a case of "nodular cutaneous reactive histiocytosis" exhibiting "pleomorphic cytoplasmic inclusions" never observed before. In 1975 Touraine et al (unpublished data) found the same cytoplasmic structures in a case of unclassified histiocytosis, and Degos et al [12] made similar observations in a case of multicentric reticulohistiocytoma. Recently, we have had the opportunity to observe two cases of papular histiocytosis, clinically difficult to classify and exhibiting differences in infiltrate distribution and evolution of the lesions. Light and electron microscopic analysis of biopsy specimens of the lesions has revealed a large number of cells containing granules identical to those reported by Rodriguez et al [11], Touraine et al (unpublished data), and Degos et al [12]. In this paper, the ultrastructural features of these unique granules are illustrated, a three-dimensional model is proposed, and their possible origin is discussed.

MATERIALS AND METHODS

Case 1. A 13-year-old girl had asymptomatic, dome-shaped papulonodular lesions 3 to 5 mm in diameter, of a red-brownish color on the face, trunk, and upper limbs. There were no oral lesions and no palpable lymph nodes. Hematologic, enzymatic, radiographic, and bacteriologic tests revealed no abnormality. Within approximately 4 months, the lesions regressed spontaneously, leaving no trace. No relapses have been observed during a 4-year follow-up. Histologically, the infiltrate was arranged in nests in the superficial and middermis, at some sites affecting also the dermoeidermal junction. The infiltrate was monomorphous and appeared to consist of histiocytic cells with abundant cytoplasm which was vacuolated, granular, and slightly basophilic in appearance. Nuclei were bulky and irregular. Mitoses were rare. In many cells, portions of the cytoplasm were PAS positive. Thin reticulin fibers were present in abundance, enmeshing individual histiocytes. The vessels were unaffected. Sudan, Giemsa, and Ziehl-Neelsen stains were negative. A diagnosis of generalized eruptive histiocytoma was proposed [13].

Case 2. A 30-year-old woman had an eruption of light pink, papulonodular lesions, 2 to 4 mm in diameter on both forearms. The lesions quickly spread almost symmetrically over the whole skin surface. Lymph nodes were not affected. The eruption spontaneously disappeared after 1 month, leaving no sequelae. After approximately 1 year, red-brownish, firm, elastic nodular elements 5 mm in size appeared on the forearms, lower limbs, face, and trunk. The patient's general health was good and hematologic, enzymatic, radiologic, and bacteriologic tests were noncontributory. Liver scintigraphy, carried out after the appearance of mild hepatomegaly, showed a uniformly decreased uptake of tracer. The patient refused a sternal puncture and liver biopsy and no specific treatment was given. Histologically, the infiltrate was monomorphous and had the same features as in Case 1; however, it was dense and replaced most of the normal structures in the superficial and middermis. The number of cells undergoing mitosis was higher. This second case, initially diagnosed as generalized eruptive histiocytoma, during its evolution has come to show similarities with the case described by Rodriguez et al [11] as "reactive nodular histiocytosis."

Specimen Preparation for Thin and Semithin Sections

In both cases, biopsies were performed from the forearms. Half of each specimen was cut into small pieces and fixed in 1% osmium tetroxide in Millonig buffer [14] for 4 hr at 4°C, dehydrated in acetone, and embedded in Vestopal, according to the technique described by Caputo and Lombardi [15]. The sections, cut on an LKB Ultratome microtome, were stained with uranyl acetate and lead citrate and observed with EM 200 and EM 300 Philips electron microscopes. In order to identify the light microscopically visible granules, 1-μ thick sections from epoxy-embedded tissues were stained.
with aniline blue stain, as suggested by Biagini et al [16].

RESULTS

In 1-μ-thick sections (Fig. 1, inset) the cells of the infiltrate looked like large histiocytes characterized by irregular nuclei with margination of chromatin and 1 or 2 nucleoli, and by abundant cytoplasm containing a large number of strongly basophilic, rounded, oval or irregular granules. Essentially similar ultrastructural findings were observed in all biopsies of both patients. Most "granular cells" had a characteristic appearance. They were large, exhibiting numerous peripheral "villi" (Figs. 1, 4); nuclei were irregular and often polynucleate with nucleoplasm of medium electron density and 1 or 2 nucleoli. The cytoplasm contained one or more Golgi apparatus rich in vesicles (Fig. 2) and a large number of granules which had an extremely polymorphous appearance (Figs. 1, 2). These granules consisted mainly of unit membranes 90-Å thick, occasionally limiting electron-dense areas containing vesicles 300 to 800 Å in diameter (Figs. 2, 3). The thickness of the vesicle membrane contained in these granules was the same as that of the limiting membrane of the granule (Fig. 3b). Some granules appeared to have a plain ringlike structure and were composed of 2 concentric membranes, separated by a light space of approximately 150 Å. Some others were shaped like a signet ring (Fig. 3a) or a saddle (Fig. 3a), and still others comprised a unit membrane which contained vesicles (Fig. 3b). Serial sections revealed these images often to be sections of the same granule at different levels (Fig. 3f). In other words, in serial sections a ring-shaped granule (Fig. 3f) exhibits a widening of the space between 2 limiting membranes in which vesicles are found. Finally, there were also some granules exhibiting a quite complex structure with convoluted and folded membranes (Figs. 3c,d). Concentrically arranged membranes were rarely observed inside these structures (Fig. 3c) and, occasionally, the contents were formed by a dense matrix and membrane fragments rather than by vesicles. The limiting membranes enclosed areas of cytoplasm sometimes containing mitochondria, glycogen granules, and lipid droplets. Even though the limiting membranes have the same thickness as the plasma membrane, only the portion of the granules containing vesicles comes in contact with the plasma membrane (Fig. 4, inset). The plasma membranes of these cells are often surrounded by numerous vesicles, which are of the same size and membrane thickness as those inside the granule (Fig. 4). In spite of the presence of numerous centrioles, we have never found any cells undergoing mitosis. Langerhans granules and wormlike bodies were absent.

DISCUSSION

The granules present in the cytoplasm of the cells of these two cases of histiocytic proliferation of the skin consist of membranes and vesicles. Their structure, though quite complex, is typical. Their marked polymorphism makes it extremely difficult to construct a three-dimensional model (Fig. 5). An analysis of serial sections of the granules seems to indicate that they have a wall consisting of 2 parallel unit membranes separated by a light space of approximately 150 Å and a portion formed as a result of the enlargement of the space between the 2 unit membranes and containing vesicles. Both the wall and the solid portion occasionally surround areas of cytoplasm and cytoplasmic organelles. Rodriguez et al [11] have interpreted these granules as unidentified microorganisms, "in spite of negative cultures and multiple stains for bacteria, fungi, and parasites. Degos et al [12] regard them as a consequence of the cells' macrophagic function, marked by increased membrane activity.

Our findings do not permit any definite statement as to the nature of these granules, but enable us to make some interesting remarks: (1) the thickness of the limiting membranes of the granules is identical to that of plasma membranes; (2) the thickness of the membranes of the vesicles inside the granules is identical to that of the limiting membranes, and is normally greater than that of the numerous Golgi vesicles present in the cytoplasm; (3) the thickness of the membranes of the vesicles outside the plasma membranes is identical to that of the vesicles contained inside the granules; (4) the vesicles are present only around the cells containing these unique granules.

On the basis of our observations, two hypotheses can be proposed on the mode of formation of the granules. The first hypothesis is that they are the consequence of a peculiar kind of endocytosis. In epidermal Langerhans cells, Olmos [17] has succeeded in inducing the formation of dense cytoplasmic bodies, in some respects similar to the cytoplasmic granules found in these histiocytoses, following the application of an alcoholic solution of podophyllin resin at concentrations above 10% for 7 hr. In our patients, however, no topical treatment had ever been given. As claimed by Hashimoto [18] with regard to Langerhans granules, the similarity of the limiting membrane of intracytoplasmic granules to plasma membranes seems to support an endocytic origin of these granules. To prove this possibility, we would have to elucidate the nature of the vesicles surrounding the plasma membrane, which apparently induce the phenomenon.

An alternative hypothesis is that the granules are the expression of an exocytosis phenomenon: the vesicles originate from the Golgi apparatus (Fig. 2), are surrounded by membranes, and are carried outside the cells. The fact that the membrane of the vesicles surrounding the Golgi apparatus is generally thinner than that of the vesicles contained in the granules and found around the plasma membrane argues against the hypothesis that these granules might be the expression of a type of exocytosis.
Fig. 1. Case 1. Typical "granular cell" at low magnification. The nucleus (N) is irregular, the plasma membrane exhibits peripheral villi (arrows), and the cytoplasm contains many granules which have an extremely polymorphous appearance (×11,500). Inset: One-micron-thick section of Case 1 in which granular cells are evident (× 1,250).
FIG. 2. Case 2. Part of the cytoplasm of a "granular cell" containing numerous Golgi complexes (arrows) and many granules which have an extremely polymorphous appearance (× 25,200).
Our ultrastructural findings are not sufficient to support either hypothesis but we believe that the use of tracers and cytochemical investigations will elucidate the nature of these granules. Further ultrastructural studies involving other histiocytoses will enable us to establish whether or not these cytoplasmic structures are typical of some histiocytic proliferations of the skin, or whether
Fig. 4. Case 2. "Granular cell" around which one can see transversally cut villi (star) and many vesicles \((\times 43,400)\). Inset: Peripheral cytoplasmic area of a "granular cell" where a granule is in contact with the plasma membrane. Vesicles outside the cell (arrow) have the same size and membrane thickness as those inside the granules \((\times 65,000)\).
Fig. 5. Three-dimensional model of the granules on the basis of serial sections. The granules have a wall consisting of 2 parallel unit membranes separated by a light space of approximately 150 Å and a portion formed as a result of the enlargement of the space between the 2 unit membranes and containing vesicles.

they merely represent an unusual but nonspecific behavior of the membranes of some histiocytes in response to still undetected stimuli.

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REFERENCES


