Morphological Analysis of Multinucleated Giant Cells Occurred in Experimental Autoimmune Myocarditis

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SAEKI, M., TAKAHASHI-IWANAGA, H., IWANAGA, T., FUJITA, T., KODAMA, M., HANAWA, H., ZHANG, S., IZUMI, T. & SHIBATA, A. *Morphological Analysis of Multinucleated Giant Cells Occurred in Experimental Autoimmune Myocarditis*. Tohoku J. Exp. Med., 1994, **172** (3), 195-204 — Our previous study reported the rich existence of multinucleated giant cells in an autoimmune myocarditis experimentally induced in rats. The present study investigated the histochemical and ultrastructural characteristics of these giant cells. Histochemistry for an acid phosphatase clearly demonstrated multinucleated giant cells dispersed at the inflammatory foci. Ultrastructurally, the giant cells were shown to be single cells, but not clustered cells. Their ultrastructural characteristics were very similar to the basic features of macrophages, except that the giant cells were poor in lysosomes and phagosomes. It was noticeable that some macrophages possessed three or more nuclei, displaying an intermediate form between mononuclear macrophages and multinucleated giant cells. These findings suggest that the giant cell in the experimental autoimmune myocarditis is a single multinucleated cell, and possibly derived from macrophages by cell-to-cell fusion. ——— autoimmune myocarditis; rat; ultrastructure; macrophages; multinucleated giant cell

It is well known that multinucleated giant cells are recognizable in some cases of human myocarditis. Such types of myocarditis are called giant cell myocarditis and have been given special interest for their roles in pathogenesis (Wynne and Braunwald 1992). Origin of the giant cell in the myocarditis still remains controversial. Theaker et al. (1985) have emphasized through their pathological studies on autopsied hearts of humans that the giant cells must be derived from macrophages. Meanwhile, Tubbs et al. (1980) are against this proposal, insisting that the giant cells originate from cardiac muscle cells.

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Recently, we succeeded in establishing a novel model of autoimmune myocarditis by immunizing Lewis rats with human cardiac myosin. This lethal myocarditis was pathologically characterized by the occurrence of multinucleated giant cells in the inflammatory foci (Kodama et al. 1990). Therefore, it is possible to investigate the origin of the giant cells and their involvement in the pathogenesis of myocarditis. Our previous immunohistological study of the experimental myocarditis showed that the giant cell possessed the same cell-surface antigens as macrophages (Kodama et al. 1991). Thus, in the present study, our interests are directed to the ultrastructural characteristics of the multinucleated giant cell, and to making clear whether the giant cell is a cluster of several macrophages or a single multinucleated cell.

MATERIALS AND METHODS

Production of the experimental autoimmune myocarditis has been precisely described in our previous paper (Kodama et al. 1990). Briefly mentioned, cardiac myosin was purified from normal human hearts according to Murakami's method (Murakami et al. 1976). Male Lewis rats (7 weeks in age) were immunized with 0.5 mg of cardiac myosin in an equal volume of complete Freund's adjuvant, and were sacrificed on the 21st day after the immunization.

For the general pathological examination, the hearts were removed and immersed overnight in Bouin fixation. Paraffin sections were cut at 4–5 μm in thickness and stained with hematoxylin-eosin in a conventional way.

For histochemistry, the hearts were removed and fixed for 12 hr with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The tissues were then immersed overnight in 30% sucrose and rapidly frozen in liquid nitrogen. The frozen materials were sequentially cut at 15 μm thickness in cryostat. An acid phosphatase staining was employed according to Burstone's method (1958).

For electron microscopy, the rats were perfused with physiological saline followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The perfused hearts were removed, chopped into 1 $\times$ 1 $\times$ 1 mm cubes, and immersed in the same fixative for an additional 3 hr. The specimens were postfixed in 1% OsO_4 for 2 hr, and after dehydration, embedded into Araldite resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate.

RESULTS

Macroscopically, the hearts obtained from immunized rats displayed obvious enlargement, massive pericardial effusion and gray discoloration of the cardiac muscle. A histological survey demonstrated serious infiltration of cells and extensive necrosis in the cardiac walls as shown in Fig. 1A. The major population of the infiltrates was composed of lymphocytes and mononuclear cells; the former were small spherical cells with an intensely stained nucleus and a thin rim of cytoplasm, whereas the latter were large irregular-shaped cells with a broad, frequently foamy, cytoplasm. The latter cells were thus largely identified macrophages. In addition, multinucleated giant cells were found in groups in massive inflammatory foci, being intermingled with a considerable number of macrophages (Fig. 1B).

Fig. 1. A: An epicardial inflammatory lesion of the left ventricle of a Lewis rat immunized by human cardiac myosin, Hematoxylin-eosin staining. There are huge number of infiltrates among cardiocytes (asterisks). $\times 74$ B: A closer view of Fig. 1A. Two giant cells (G) which possess several nuclei in the peripheral cytoplasm are intermingled with mononuclear cells. $\times 1,100$

Fig. 2. Acid phosphatase activity demonstrated on a cryostat section from the left ventricle. A multinucleated giant cell with five or six nuclei (arrow heads) shows intense activity in the cytoplasm. $\times 1,100$

Acid phosphatase activity was positive both in the macrophages and the giant cells (Fig. 2). The macrophages showed the activity in various intensity, while the giant cells had a constantly strong reaction. The acid phosphatase activity in both cells had a fine granular appearance and spread throughout in the cytoplasm.

Ultrastructurally, the macrophages could be identified as large mononuclear cells possessing developed Golgi apparatus and numerous phagosomes, namely, secondary lysosomes (Fig. 3). These phagosomes were various in size and electron density. Larger ones contained some cellular elements, although structures reminiscent of cardiac muscle cells were not recognizable in them (Fig. 3B). Many lipid droplets were also found in the peripheral cytoplasm of the macrophages (Fig. 3). Some of the macrophages contacted each other, displaying an epit-

Fig. 3. Macrophages (M) in the necrotic portions of the inflammatory foci. They are rich in phagosomes (P) of various size and lipid droplets (LD). Fig. 3A Bar = 3 μ m; Fig. 3B Bar = 1 μ m

Fig. 4. A: Ultrastructure of a multinucleated giant cell which possesses at least twenty nuclei. The Golgi apparatus (G) is extremely developed in the central region, while lipid droplets (LD) are gathered at the periphery. L: lymphocyte Bar = 5 μ m. B: Closer view of Fig. 4A. No demarcating membrane is recognizable among nuclei. The surface of the cell is covered with irregularly shaped microvilli. Bar = 1 μ m

Fig. 4.

Fig. 5. An electron micrograph showing two types of predominant cells present in inflammatory lesion. Cells indicated by arrows possess well-developed Golgi apparatus (G) and many lipid droplets (LD), being very similar to the giant cell as shown in Fig. 4. Right one possesses one or two nuclei, while left one appears to contain more than four nuclei, therefore being classified as multinucleated giant cell. Another type of cell indicated by an asterisk lacks lysosomes, lipid droplets and developed Golgi apparatus. Bar = 3 μ m

elioid arrangement. Occasionally, macrophages were in very close contact with multinucleated giant cells.

The multinucleated giant cells were usually oval in shape, their size ranging from 5 to 30 μm in diameter (Fig. 4A). Their cell surface was irregular due to many villus-like processes, 1 to 3 μm in length. Lymphocytes were frequently found to be in contact with the giant cells or to be invaginated into the cytoplasm of the giant cells (Fig. 4A). When counted in an ultrathin section, the giant cells possessed 5 to 20 nuclei which were often arranged rosette-like in the periphery of the cytoplasm. Since no demarcating membrane was recognizable among nuclei, the multinucleated giant cell is a single cell (Fig. 4B). In each nucleus, chromatin was usually gathered along the nuclear membrane and a nucleolus was located at the center. The cytoplasm of giant cells possessed well-developed organella, especially Golgi apparatus and mitochondria. Primary lysosomes of various sizes were seen though not frequently, and no typical phagosomes were recognizable (Fig. 4B). Abundant lipid droplets tended to be deviated to a peripheral part of the cytoplasm. The above mentioned ultrastructural characteristics of the giant cells were identical to basic features of the macrophages (Fig. 3), except for phagosomes.

Electron microscopic observation also demonstrated cells, which had the same ultrastructural characteristics as giant cells but possessed less than 5 nuclei (Fig. 5). They were twice or more in size than macrophages.

Another type of large mononuclear cells, different from the macrophages, were found in this model. They were lacking in developed Golgi area, lysosomes and lipid droplets, and were characterized by interdigitation of the cell membrane (Fig. 5). The cells seemed to be cardiac interdigitating cells.

DISCUSSION

The present ultrastructural study demonstrated that multinucleated giant cells observed in the experimental autoimmune myocarditis were conspicuously similar to macrophages. This finding agrees with the present histochemical staining for acid phosphatase, an enzyme specific for macrophages, and also with our previous immunohistochemical staining around the surface markers of the giant cells (Kodama et al. 1991). It is worth noting that the giant cells were ultrastructurally shown to be single cells, possibly derived from macrophages. Giant cells of smaller size suggesting an intermediate form from mononuclear macrophages to multinucleated giant cells were recognizable through observation. These findings support the view that the multinucleated giant cells may be formed by cell-to-cell fusion of macrophages.

Occurrence of lysosome- and phagosome-rich macrophages in our model for autoimmune myocarditis suggests their active phagocytosis at the inflammatory lesions. We failed to identify any cellular remnants of the cardiac muscle in their phagosomes. This findings may be accounted for by that the materials examined

correspond to advanced stage in which the phagocytosis of degenerated cardiocytes has finished. Our preliminary observation in earlier stages of this myocarditis model showed that macrophages directly contact with cardiocytes and phagocytose them (Suzuki, unpublished data). The giant cells also occurred in the central portion of the inflammatory lesions where extremely large amounts of necrotic debris are present. It is possible that the giant cells act as scavenger, like macrophages, in the inflammatory foci of the myocarditis. In vitro study by Schlesinger et al. (1984) have reported that multinucleated giant cells, derived from human monocytes after 9-14 days in culture, display much more intense phagocytotic activity than macrophages. However, the giant cells were shown in the present study to be poor in lysosomes and phagosomes as compared with the macrophages, indicating a lower capability of phagocytosis in the giant cells. Therefore, the question arises as to a role of the giant cells.

Although functional significance of the giant cells without scavenger activity remains to be elucidated, the following two explanations are available. First, the appearance of giant cells in our experimental myocarditis may be only an epiphenomenon induced by elevated activity of lymphocytes. According to McInnes and Rennick (1988), when monocytes obtained from the bone marrow and lung were incubated in vitro with mouse interleukin-4 (IL-4), multinucleated giant cells were easily formed via cell fusion. This lymphokine has been designated as a macrophage-fusion factor (MFF). Our preliminary study (Kodama et al. 1992) showed that the experimental myocarditis could be transferred from rats with the myocarditis to normal rats by intravenous injection of spleen cells or lymph node cells. Therefore, the myocarditis was considered to be mediated by lymphocytes, possibly T-lymphocytes (Kodama et al. 1992). The occurrence of numerous lymphocytes adjacent to giant cells in the experimental myocarditis suggests a possibility that the lymphocytes are involved in the formation of the multinucleated giant cells. Secondly, giant cells in this myocarditis may be engaged in a function other than phagocytotic processes. There is a general agreement that multinucleated giant cells in granulomas are formed by the fusion of exudative macrophages, which possess less intense phagocytotic activity than so-called tissue macrophages (van Furth 1992). Papadimitriou and Robertson (1980) ultrastructurally demonstrated an active secretion of lysosomal contents by multinucleated giant cells. Langer and Thoenes (1981), on the other hand, reported that the giant cell in granuloma resembled a secretory gland cell as to cytoplasmic components. The possible secretory function of the giant cells in our experimental myocarditis is supported by their well-developed Golgi apparatus.

The present study also demonstrated the existence of another type of mononuclear cells, which were identifiable under electron microscope. Their ultrastructural characteristics resemble those of interdigitating cells which normally occur in the thymus and lymph nodes, and function as an antigen-presenting cells (Kaiserling et al. 1974; Veerman 1974; Ushiki et al. 1984; Steinman 1991).

Ultrastructurally, there was no similarity between the interdigitating cells and the giant cells. No morphological evidences for their cell-fusion into giant cells were recognizable. From this data, it seems unlikely that this type of cell may give rise to the giant cells. Further studies are required to investigate the morphological characteristics of the interdigitating cells and their roles in the pathogenesis of the experimental autoimmune myocarditis.

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