AN ELECTRON MICROSCOPIC CYTOCHEMICAL STUDY OF MACROPHAGES DURING UTERINE INVOLUTION

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ABSTRACT

Acid phosphatase was localized at the fine structural level in rat endometrial phagocytes during the period of postpartum involution. These cells showed intense phagocytotic and pinocytotic activities, which were accompanied by the development of abundant lysosomes. Phagosomes acquired their enzymatic complement by fusion with lysosomes; the same appeared to be true in the case of pinocytotic vesicles, but, because of the small size of these vesicles, this point could not be established with certainty. Digestion within some phagolysosomes led to the formation of electron-lucent vacuoles containing solubilized products. Other phagolysosomes showed accumulation of lipid residues in the form of droplets and myelin figures, and the structures acquired the appearance of residual bodies. In many macrophages, overfeeding led to the formation of unusually large numbers of phagolysosomes, which occupied almost the entire cytoplasm with exclusion of other cell organelles. In these cells the presence of abundant lead deposits, apparently free in the cytoplasm suggested an intracytoplasmic release of hydrolases.

INTRODUCTION

During pregnancy, the growth of the uterus involves an increase of intracellular and extracellular components, which are removed rapidly during the period of postpartum involution. The marked changes in acid hydrolytic activities observed in the uterus during the postpartum period (1-3) suggested the involvement of lysosomal enzymes in the involution processes that occur during the return of the organ to a nongravid condition. By light microscopic histochemistry (1), these changes were seen to affect the endometrium and myometrium, and were accompanied by a marked infiltration of macrophages.

Electron microscopic-cytochemical studies have served to elucidate some of the mechanisms operating in the involvement of lysosomes in intracellular degradative events both in surface epithelial cells and in smooth muscle fibers (4). The present investigation reveals some of the ultrastructural-cytochemical mechanisms relating to pinocytosis and phagocytosis in the uterine macrophages of rats during postpartum involution.

MATERIALS AND METHODS

Holtzman female albino rats were killed on the 17th day of gestation, at 0 hr, and 1, 2, 3, 4, 6, 10, and 17 days postpartum. The uteri were fixed for 2 hr in glutaraldehyde (6%) in 0.1 M cacodylate buffer, pH

7.4, containing 0.33 M sucrose (5). Tissues were rinsed and stored (at 4°C) in the same buffer. For conventional electron microscopy, portions of the tissues were postfixed in 1% OsO₄ and embedded in Epon 812. Ultrathin sections were cut on an LKB or Porter-Blum microtome, and the grids were stained with lead citrate, uranyl acetate, or uranyl acetate followed by lead citrate. Electron micrographs were taken with the RCA EMU-3F electron microscope.

Histochemistry

Frozen sections were cut at a thickness of 8 µm and 36 µm. The 8-µm sections were mounted on slides and incubated for the demonstration of:

(a) Acid phosphatase. Gomori's lead salt method (6), with β-glycerophosphate, Grade I as substrate (Sigma Chemical Co., St. Louis, Mo.); 45 min at 37°C. Counterstaining was with methyl green.

(b) β-Glucuronidase. Simultaneous coupling azo-dye technique (7), with naphthol-AS-BI-β-glucuronide as substrate (Calbiochem, Los Angeles, Calif.); for 30 min at 37°C. Counterstaining was with methyl green.

(c) E600-resistant esterase. Indoxyl acetate method (8), with 5-bromoindoxyl acetate as substrate (Sigma Chemical Co., St. Louis, Mo.): 1 hr at 37°C counterstaining was with nuclear fast red.

(d) N-acetyl-β-D-glucosaminidase. Simultaneous coupling azo-dye technique (9), with naphthol-AS-BI-N-acetyl-β-D-glucosaminidase as substrate (Pierce Chemical Co., Rockford, Ill.). The 36-µm sections were floated in 0.33 M sucrose, incubated in Gomori's lead salt mixture for acid phosphatase for 20-30 min, postfixed in buffered OsO₄, and embedded in Epon 812. Subsequent preparative procedures were identical with those of the conventional preparation.

RESULTS

Immediately following parturition, minimal acid hydrolytic activity was detected in the stromal cells of the mucosa of the uterus (Fig. 1), but moderate activity was seen in the glandular epithelium. Similar results were obtained with sections stained for the demonstration of β-glucuronidase, E600-resistant esterase, and N-acetyl-glucosaminidase. From the 1st day postpartum onward, an intense positive reaction for several hydrolases became apparent in numerous macrophages in the stroma. Figs. 2-4 illustrate results obtained with the acid phosphatase, β-glucuronidase, and E600-resistant esterase methods, respectively.

The enzymatic activity and the number of macrophages rose during the first few days after parturition, and reached a peak between the 4th and 6th day. From 1 wk postpartum onward, there was a decline in enzymatic activity and in the number of macrophages; by the 17th day postpartum, the various histochemical reactions were similar to those prevailing before the onset of involution.

Sections stained for the demonstration of acid phosphatase at the ultrastructural level (electron-microscopic-cytochemical preparations) revealed some of the subcellular mechanisms related to lysosomal involvement in pinocytic and phagocytic processes.

Phagocytic cells contained lysosomes of the dense body and Golgi vesicle types, and showed morphological evidence of surface activity related to phagocytosis (Fig. 5).

Interaction between such lysosomes and phagocytized material was illustrated in Fig. 6 and its insets A, B, and C. Lysosomes were seen to coalesce with absorption droplets (Inset A, Fig. 6), and sometimes appeared to protrude into the droplets by finger-like projections (Inset B, Fig. 6). In what seemed to represent a more advanced stage of the process of interaction between lysosomes and phagosomes, the latter contained incipient lead deposits indicating that transfer of enzymatic activity from lysosomes to phagosomes had taken place (Inset C, Fig. 6). In accordance with the prevailing nomenclature the absorption droplets will be referred to as phagosomes before they acquire the enzymatic complement from lysosomes, and as phagolysosomes after that event.

The phagocytic activities of macrophages appeared also to be directed toward other cell types; and similarly, fusion of lysosomes with phagosomes containing ingested cells was observed (Fig. 7). Lead deposits, indicative of enzyme transfer, were seen even in the nuclei of ingested cells.

Differences in the stages of digestion of the content of the various phagolysosomes in a cell seemed to indicate the ability of macrophages to participate in successive lytic events. The macrophage in Fig. 7, for example, contains a recently ingested cell, two phagolysosomes with abundant lipid residues, as well as a residual body with numerous arrays of membranes having the characteristic appearance of myelin figures.

Phagocytic types of cells appeared to play a role in phagocytosis, pinocytosis, and degradation of engulfed material to such an extent as to cause an overload of lysosomes, which apparently led to disappearance of vital cytoplasmic organelles and...
FIGURE 1 Noninvolutionary endometrium, 17 days postpartum. Acid phosphatase reaction. The surface epithelium (arrow) and the stromal cells show minimal reaction. A moderate reaction is seen in the glandular epithelium. LU, lumen. × 350.

FIGURE 2 Endometrium, 4 days postpartum. Acid phosphatase stain. Intense reaction in the epithelium (arrow); moderate activity in most stromal cells. Extremely intense reaction in macrophages. LU, lumen. × 350.

FIGURE 3 Endometrium, 4 days postpartum. β-glucuronidase reaction. Moderate reaction in the surface epithelium (arrow); intense reaction in the macrophages. LU, lumen. × 350.

FIGURE 4 Endometrium, 4 days postpartum. E{sup}600{-resistant esterase reaction. Intense reaction in macrophages. × 350.
FIGURES 5–10  Electron microscopic histochemical preparation from postpartum endometrium. Acid phosphatase stain.

FIGURE 5  Endometrial phagocyte, 24 hr postpartum. Moderate number of lysosomes of the Golgi vesicle type (arrowheads) and of the dense body types (LY) are visible. N, nucleus. × 32,300.
FIGURE 6  Endometrial phagocyte, 24 hr postpartum. Interaction between lysosomes and phagosomes are shown in detail in blocked areas, A, B, C. A, Conglomerate of lysosomes (LY) and a phagosome (P). Areas of contact and fusion between a lysosome (LY) and the phagosome (P) are indicated by arrows. N, nucleus. B, A lysosome (LY) is protruding into a phagosome (P). C, A lysosome (LY) appears to have fused (arrow) and transferred some of its enzymic content to a phagosome. The similarity between the matrix of the resulting phagolysosome (PLY) and the matrix of phagosomes in A and B supports this assumption. Fig. 6, X 16,000; Inset A, X 35,800; Inset B, X 35,800; Inset C, X 35,800.
Figure 7  Indications of successive phagocytic events in a macrophage, 2 days postpartum. It contains a polymorphonuclear leukocyte (PC). Lead deposits in the cytoplasm and even in the nucleus of the ingested cell indicate enzyme transfer, presumably through fusion (arrows) of lysosome (LY) with phagocytic vacuoles. Phagocytic vacuoles (PV) with lipid droplet residues and a residual body with abundant myelin figures (MF) indicate different chronological stages in the digestive events. N, nucleus. × 10,700; Inset, × 35,800.
seemed to provoke diffuse lytic changes. In early stages of this process, pinocytotic and phagocytotic activities seemed to be accompanied by the development of lysosomes of the dense-body type and Golgi vesicle type (Fig. 5), and in later stages there was a tendency to the formation of larger lytic structures through fusion of lysosomes and also by the development of less well defined areas of focal cytoplasmic degradation (Fig. 8). In some phagocytes (Fig. 9) these areas became confluent and the abundant lead deposits, apparently free in the cytoplasm, suggested an intracellular release of lysosomal hydrolases.

Early and late stages in the life cycle of lysosomes (10) occurred in these cells as judged from the presence of primary lysosomes of the Golgi vesicle type and myelin figures presumably related to the formation of residual bodies.

Progression of the digestive process within the phagolysosomes (Fig. 10) resulted in the development of electron-lucent vacuoles and in the accumulation of lipid droplets and myelin figures, probably derived from the degradation of the protein component of lipoprotein complexes. Notwithstanding these marked degenerative changes, characterized by the loss of most of the subcellular organelles, by lipid deposition, and by apparent diffuse hydrolytic activity in the cytoplasm (Figs. 9, 10), unequivocal signs of autolysis were not detected in the macrophages present in the endometrium.

**DISCUSSION**

Increased lysosomal activity in sex target organs in the course of physiological involution has also been reported in the prostate glands of castrate rodents (11) and in rat mammary gland upon cessation of lactation (12, 13). Many other examples of physiological involution involving lysosomes have been recently reviewed by de Duve and Wattiaux (14).

In the case of involution of the uterus, changes in lysosomal distribution have been related, at least partly, to the loss of placental hormones, as judged from the similarities of response upon hormone withdrawal in ovariectomized rats treated with estrogen and progesterone (1). By light microscopic histochemistry (1), these changes in lysosomes have been shown to affect all layers of the uterus, including the endometrium, lamina propria, and the smooth muscle fibers. Biochemical studies under similar conditions have shown a rapid increase in total activity for various acid hydrolases (3).

The present results have revealed some of the subcellular mechanisms related to lysosomal participation in digestive activities in endometrial macrophages during postpartum involution.

Pinocytotic and phagocytotic activities in the macrophages were accompanied by the appearance of lysosomes of the Golgi vesicle type and by dense bodies. The latter appeared to fuse with the various types of phagosomes represented by absorption droplets or phagocytized cells, and resulted in the formation of phagolysosomes (15).

The influence of pinocytosis in the development of lysosomes, and acid hydrolytic synthesis in macrophages has been thoroughly investigated during in vitro differentiation of mouse mononuclear phagocytes (16-18). Pinocytosis is believed to be a prime regulator in lysosome formation, involving enzyme synthesis at the endoplasmic reticulum, transfer of hydrolases to Golgi vesicles, and fusion of these vesicles with pinosomes to give rise to secondary lysosomes (19, 20). Therefore, it is suggested that phagocytosis and pinocytosis by uterine macrophages also constitute a regulatory factor in lysosome formation.

The nature of the material ingested by phagocytosis or pinocytosis, and subsequently degraded within phagolysosomes, has not been characterized in this study, but can be inferred for the data furnished by other authors (2, 3, 21, 22). It is known that, during postpartum involution of the uterus, considerable amounts of collagen, elastic fibers, and intercellular amorphous substances that has greatly increased during pregnancy are degraded and reabsorbed within the organ (2, 3, 21, 22). The materials contained in the phagosomes may represent byproducts of collagen catabolism originated from preliminary conversion of fibrous collagen into soluble forms (21), as well as mucopolysaccharides (22). Fusion of these phagosomes with lysosomes might provide the enzymes required for the breakdown of such substances, especially in view of the fact that certain collagenases (23, 24) and acid cathepsins that may act upon solubilized collagen (25), and also various glycosidases (14) have been reported to be present within lysosomes.

Differences observed in the course of digestion of the material within the various types of lysosomes would tend to indicate the heterogeneous
FIGURES 8-10 are intended to illustrate apparent progression of intracellular digestive events within phagocytic cells. Acid phosphatase preparations.

FIGURE 8 3 days postpartum. Phagocyte with increasing number of lysosomes (LY), some of which tend to fuse into large bodies (arrows). One of these bodies shows abundant material undergoing degradation (FD) and apparent accumulation of lipid residues. X 28,600.
FIGURE 9 4 days postpartum. Dissemination of lead deposits within the general cytoplasm, indicative of release of lysosomal enzymes. An uninvolved portion of the cytoplasm shows activity in Golgi elements (G), including Golgi vesicles (arrows). Myelin figures are clearly visible (arrowheads). LY, lysosomes. X 28,600.

nature of these bodies, in relation either to their enzymic complement or to the type of material engulfed. As seen in our preparations, digestion in some of the phagolysosomes resulted in the development of electron-lucent vacuoles containing solubilized material. In other instances, apparent failure to achieve the breakdown of lipid components led to the accumulation of undigested lipid residues, in the form of droplets or myelin figures. Accumulation of lipid residues within autophagic and phagocytic vacuoles and their evolution towards residual bodies have been attributed to the more rapid degradation of the protein component in lipoprotein complexes (26).

The presence of phospholipase (27) and sphingolipid hydrolyses (28) in lysosomes tends to weaken the assumption that such deposition of lipid material results from a poor lipolytic activity in lysosomes (29).

Some of the structures containing myelin figures
Figure 10 4 days postpartum. More advanced stage in intracellular digestion in a macrophage. The areas of complete digestion and solubilization appear as electron-lucent vacuoles (V) which tend to fuse. Lipid residues, in the form of droplets (LD) and myelin figures (arrowhead), are more abundant. X 19,700.
showed a similarity to residual bodies (14, 30) and lipofuscin granules (31). Since such similarities exist in numerous other tissues, de Duve (29) has suggested that inability of many cells in higher organisms to eliminate these digestive residues may result in the gradual accumulation of age pigment. In the present case, however, the overload of the macrophages with lytic bodies containing very high concentration of degradative enzymes, as judged from the unusually heavy lead deposits, seemed to have a deleterious effect on the phagocytic cells. Most of the vital cytoplasmic organelles, such as mitochondria and the endoplasmic reticulum, were replaced by lysosomes, and abundant lead deposits appeared free in the cytoplasm. Such changes in the phagocytic cells could be related to the biochemical concept of selective retention and intracytoplasmic release of hydrolases in relation to tissue regression (30), and also in accordance with the “suicidal bag” theory of de Duve (32).

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