Lysosomal compartment of macrophages: Extending the definition of GERL

(acid phosphatase activity/cholesterol/lipoprotein particles/reticuloendothelial system)

PHYLLIS M. NOVIKOFF, ANA YAM, AND ALEX B. NOVIKOFF

Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York 10461

Contributed by Alex B. Novikoff, June 15, 1981

ABSTRACT “Professional phagocytes” such as Kupffer cells show an extensive network of interconnected residual bodies with cytochemically demonstrable acid phosphatase [orthophosphoric monoester phosphohydrolase (acid optimum), EC 3.1.3.2]. In such cells and in others (hepatocytes, etc.) this compartment may be considered a part of GERL [a hydrolase-rich region of endoplasmic reticulum (ER) situated at the *trans* face of the Golgi apparatus from which various Lysosomes appear to arise].

Cytochemistry has been used in our laboratory for visualizing intracellular organelles at the light and electron microscope levels (1, 2). The use of acid phosphatase [AcPase; orthophosphoric monoester phosphohydrolase (acid optimum), EC 3.1.3.2] to discriminate GERL from the *trans* (3) element of the Golgi apparatus [this displays nucleosidediphosphatase (EC 3.6.1.6) activity] has added insights regarding events in secretory cells and other cells (1). In a 1977 review (2) we wrote,

“Although the status of GERL as a distinct organelle is now more firm, we wish to note that parts of ER other than GERL may show some of its properties. These properties include a delimiting membrane which is straighter and thicker than that of ER generally, an electron-lucent area (‘halo’) beneath the membrane, materials within the cisterna, sometimes consisting of electron-opaque grains, ‘membranous arrays,’ lipid-like substances; and demonstrable acid phosphatase activity. In the beige mouse hepatocytes, this region of ER may or may not extend beyond the Golgi zone; from our observations most of GERL appears to be in the Golgi zone. In the pancreatic exocrine cells of the beige mouse, ER with hallmarks of GERL probably extend considerably beyond the Golgi zone However, the extent to which such ER is removed from the Golgi zone cannot be firmly established without serial sectioning.”

In their 1966 review, de Duve and Wattiaux (4) considered “the manner in which two lysosomal vacuoles merge and share their content. The generally accepted hypothesis involves membrane coalescence: as the membranes of the approaching vacuoles meet, they become reorganized into a single continuous membrane lining a single space made up of the sum total of the two merging spaces The alternative hypothesis that lysosomes may be dilatations of a continuous tract interconnected by canals has sometimes been considered; we have rejected it mainly because such connections have never been seen, even when the lysosomes are highly swollen and closely packed. However, this opinion may need revising in the light of recent experiments in which connections between dense bodies have been found by serial sectioning.” The serial sections and a model derived from it have since been published by us (5, 6). These establish that lysosomes may appear separate in

thin sections although they are in fact interconnected by endoplasmic reticulum (ER).

Evidence has been presented that when the obese Zucker (*ff*) rat (OZ) (7) is fed a cholesterol (Chol)-rich diet, Kupffer cells increase in size and number. These cells endocytose particles that appear, upon, thin-section electron microscopy to be lipoprotein (LP) particles. Four endocytic mechanisms will be summarized below. These endocytosed LP particles gain access to the “lysosomal system, with its cytochemically demonstrable acid phosphatase activity [consisting] largely of a network of interconnected residual bodies” (8). The system shows the two morphological hallmarks referred to in the first paragraph. We now demonstrate that intravenously injected tracer, horseradish peroxidase (HRP), is rapidly transported to this compartment.

MATERIALS AND METHODS

OZ rats were fed pellets containing 2% chol (prepared by Bio-Serv, Frenchtown, NJ) for 2 weeks. Livers and spleens were fixed and processed for electron microscopy by procedures previously described (2). Nonfrozen Vibratome sections, $\approx 30 \mu\text{m}$ thick, were incubated for AcPase activity or for peroxidase activity prior to these electron microscopic procedures.

Type II salt-free HRP (Sigma) was dissolved in saline and injected into the tail vein. The dose was 10 mg of HRP per 100 g of body weight, and the intervals between injection and removal of tissue from the rats were 5 min and 10 min. Peroxidase activity was demonstrated by the Graham and Karnovsky method (9). In these experiments the endogenous peroxidase activity in the ER of the Kupffer cells was inhibited by prolonged fixation (5 hr) in 2.5% glutaraldehyde/2% formaldehyde (10) (see reference to this Karnovsky fixative in ref. 2). Incubations for peroxidase activity were at room temperature for 10 min.

RESULTS

As described in abstract form (8), endogenous (i.e., not injected as LP particles) LP particles ranging in size up to 200 nm are endocytosed from the blood sinusoids in Chol-fed rats by Kupffer cells. There are four morphologic forms of such endocytosis: filopodia (Figs. 1A and 2A), membrane indentations (Fig. 2B, upper right), “vermiform invaginations” of the plasma membrane (Fig. 2C), and coated pits which form coated vesicles (Fig. 2A). For a description of such endocytic forms, see Wisse and Knook (11).

The extensive network of interconnected residual bodies with cytochemically demonstrable AcPase activity is illustrated in Fig. 1A. Fig. 1B illustrates the presence in this lysosomal system of endocytosed LP particles.

Intravenously injected HRP gains access to the lysosomal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: AcPase, acid phosphatase; Chol, cholesterol; ER, endoplasmic reticulum; HRP, horseradish peroxidase; LP, lipoprotein; OZ, obese Zucker (*ff*) rat.

FIG. 1. (A) Portion of a Kupffer cell from a Chol-fed OZ rat; section incubated for AcPase activity 10 min at 37°C. The Golgi apparatus is twisted so that this thin section shows several Golgi stacks (G). No Golgi element demonstrates AcPase activity. In contrast, reaction product is present in the extensive lysosomal compartment, including GERL (GE) and a coated vesicle (C) probably derived from GERL. The coat of this coated vesicle (C) is not evident at this low magnification. F, filopodium; P, portion of platelet; arrow, LP particle in the blood. ($\times 19,500$.) (B) A small portion of another Kupffer cell from a section incubated as in A. Both LP particles (arrows) and reaction product are seen in the lysosomal compartment. ($\times 15,000$.) (C) A portion of a macrophage from the spleen of a Chol-fed OZ rat. Uncrossed arrows, some of the numerous LP particles in the extensive lysosomal compartment; crossed arrows, LP particles which appear to have undergone transformation into larger and more irregular particles; arrowheads, two morphological hallmarks (cf. Fig. 2B). A portion of the ER close to the lysosomal compartment is indicated. A part of the nucleus is seen at N. ($\times 15,000$.)

system of Kupffer cells in the shortest time tested, 5 min (Fig. 3A and B).

In numerous places the ER lies very close to, and is probably continuous with, the lysosomal system (Figs. 1C and 2). The relevant observations from other laboratories as well as our own and the significance of such continuities are discussed in ref. 2.

The endogenous ER peroxidase of Kupffer cells was inhibited by prolonged fixation (10). Thus, the injected HRP is clearly evident in the extensive lysosomal compartment (Fig. 3).

Only one figure is used to show a part of a spleen macrophage in the Chol-fed OZ rat (Fig. 1C); others will be published elsewhere. Many endocytosed LP particles are seen in the lysosomal compartment; and the compartment has the two morphological features described in the Introduction.

DISCUSSION

A number of reports on macrophages are directly relevant to our findings. Essner and Haimes (12) showed that mouse al-

veolar macrophages endocytosed colloidal silver from the nasal passages and transported it directly into an extensive system such as we describe in Kupffer cells and spleen macrophages. We note an early report from our laboratory (13) and a recent one on macrophages of human fetal membranes (14). Phaire-Washington *et al.* (15) have reported effects of phorbol myristate acetate on mouse thioglycolate-stimulated peritoneal macrophages. An extensive lysosomal compartment develops with all features we have described in the Kupffer cells: the two morphological hallmarks; cytochemically demonstrable AcPase activity; and the uptake of HRP into this system. Both unstimulated and proteose peptose-stimulated rat peritoneal macrophages were described by Friend *et al.* in 1969 (16). They showed AcPase-rich "dense bodies" which we can view as constituting an extensive lysosomal compartment.

Also relevant is the recent report by Gonatas *et al.* (17). When cultured neuroblastoma cells are exposed to HRP, "residual bodies" are involved in its "bulk uptake." However, when the HRP is conjugated to ricin, the conjugated tracer is transported to an extensive system considered by the authors to be GERL.

FIG. 2. (A) Portion of a Kupfer cell from a Chol-fed OZ rat. Arrows, LP particles; F, filopodium; CP, coated pit or coated vesicle. The arrow at the right is directed to a LP particle apparently being endocytosed by the filopodium. Not illustrated are coated vesicles containing endocytosed LP particles. The arrow at the left shows a LP particle in the lysosomal compartment. ER, portion of ER close to the compartment. ($\times 38,000$.) (B) The major part of the field shows a Kupfer cell from a Chol-fed OZ rat. At the upper right, LP particles are seen in a forming endocytic vacuole. An arrowhead points toward a region of the lysosomal compartment showing its two morphological hallmarks: a thick delimiting membrane; and a halo between the delimiting membrane and the contents of the system. ER, region of ER close to the compartment. ($\times 27,000$.) (C) Portion of a Kupfer cell from a Chol-fed OZ rat. LP particles (which show circular profiles) are seen within the "vermiform invaginations" and in vacuoles presumably attached to, or separated from, these invaginations (see ref. 11). Arrowheads point to parts of the lysosomal compartment which show the two hallmarks referred to in B. ER, a portion of ER close to the compartment. ($\times 26,000$.)

For earlier descriptions of an extensive lysosomal compartment in the hepatocytes and exocrine pancreas cells of beige mice see ref. 2.

The extensive lysosomal compartment such as found in mac-

rophages are also present in other cells, including rat hepatocytes. The use of lactosaminated ferritin to demarcate this compartment in hepatocytes is in press (18). In an important paper on the catabolism of low density lipoprotein in the livers of es-

FIG. 3. OZ rats fed Chol for 2 weeks and then injected with HRP. (A and B) At 5 min after injection; (C) at 10 min after injection. The endogenous ER peroxidase activity has been inhibited. (A) At this low magnification, a single Kupfer cell is seen surrounded by HRP reaction product in the blood sinusoid around the cell. Only the extensive lysosomal compartment shows reaction product. The other organelles (mitochondria, etc), lacking reaction product, are barely evident (compare B and C). L, large lipid spheres in adjacent hepatocytes. ($\times 5800$.) (B) A portion of another Kupfer cell at higher magnification. Arrows, three of the many electron-lucent LP particles in the lysosomal compartment; arrowhead, thickened membrane and a halo beneath it. ($\times 17,000$.) (C) A portion of another Kupfer cell and parts of the adjacent blood sinusoid. Arrow, one of the numerous LP particles in the lysosomal compartment; arrowhead, thickened membrane and a halo beneath it. Note the absence of endogenous peroxidase activity from the ER. ($\times 32,000$.)

trogen-treated rats (19). The authors failed to appreciate the continuity of the structures labeled "multivesicular bodies" or "secondary lysosomes" in their figure 4; see the model in ref. 5 and figures in ref. 6.

We have not studied receptors that may be involved in the uptake of LP particles by the Kupfer cells; therefore, no attempt is made to review the extensive literature on receptors (20, 21) or the literature on factors that stimulate macrophages (22). Also, we have not studied the origin (hepatic, intestinal, etc.) of the circulating LP particles; cultured Kupfer cells are known to degrade LP particles (23).

We gratefully acknowledge the preparation of the final photographs by Mr. George Dominguez and the typing of successive versions of the manuscript by Ms. Fay Grad. This work was supported by National Institutes of Health Grant AM23078 to P.M.N. from the National Institute of Arthritis, Metabolic and Digestive Diseases, and Grants CA06576 and CA14923 (Research Career Award) from the National Cancer Institute and American Cancer Society Grant PDT-11A to A.B.N.

- Novikoff, A. B. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2781-2787.
- Novikoff, A. B. & Novikoff, P. M. (1977) *Histochem. J.* **9**, 525-551.
- Ehrenreich, J. H., Bergeron, J. J. M., Siekevitz, P. & Palade, G. E. (1973) *J. Cell Biol.* **59**, 45-72.
- de Duve, C. & Wattiaux, R. (1966) *Annu. Rev. Physiol.* **28**, 435-492.
- Novikoff, A. B. & Shin, W.-Y. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5039-5042.
- Novikoff, A. B. (1973) in *Lysosomes and Storage Diseases*, eds. Hers, G. & Van Hoof, F. (Academic, New York), pp. 1-41.
- Novikoff, P. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3550-3554.
- Novikoff, P. M. & Yam, A. (1980) *J. Cell Biol.* **87**, 311a.
- Graham, R. C., Jr., & Karnovsky, M. J. (1956) *J. Histochem. Cytochem.* **14**, 291-302.
- Fahimi, H. D. (1970) *J. Cell Biol.* **47**, 247-262.
- Wisse, E. & Knook, D. L. (1977) *Kupffer Cells and Other Liver Sinusoidal Cells* (Elsevier/North-Holland, Amsterdam).
- Essner, E. & Haimes, H. (1977) *J. Cell Biol.* **75**, 381-387.
- Novikoff, A. B. (1963) in *Rôle du Système Réticulo-endothélial dans l'Immunité Antibactérienne et Anti-tumorale*, ed. Halpern, N. B., pp. 67-84.

14. Nehemiah, J. L., Schnitzer, J. A., Schulman, H. & Novikoff, A. B. (1981) *Am. J. Obstet. Gynecol.* **140**, 261-268.
15. Paire-Washington, L., Silverstein, S. C. & Wang, E. (1980) *J. Cell Biol.* **86**, 641-655.
16. Friend, D. S., Rosenau, W., Winfeld, J. S. & Moon, H. D. (1969) *Lab. Invest.* **20**, 275-282.
17. Gonatas, J., Stieber, A., Olsnes, S. & Gonatas, N. K. (1980) *J. Cell Biol.* **87**, 579-588.
18. Haimes, H. B., Stockert, R. J., Morell, A. G. & Novikoff, A. B. (1981) *Proc. Natl. Acad. Sci. USA*, in press.
19. Chao, Y.-S., Jones, A. L., Hradek, G. T., Windler, E. E. T. & Havel, R. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 597-601.
20. Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1979) *Nature (London)* **279**, 679-685.
21. Neufeld, E. F. (1981) in *Lysosomes and Lysosomal Storage Diseases*, eds. Callahan, J. W. & Lowden, J. A. (Raven, New York), pp. 115-129.
22. Stanley, E. R. (1981) in *The Lymphokines*, eds. Stewart, W. E. & Hadden, J. W. (Humana), pp. 101-132.
23. Van Tol, A. & Van Berkel, T. J. C. (1980) *Biochim. Biophys. Acta* **619**, 156-166.