ULTRASTRUCTURAL STUDY OF GERL IN BEIGE MOUSE ALVEOLAR MACROPHAGES

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ABSTRACT

Alveolar macrophages of the beige mouse mutant have a system of smooth-surfaced elements with the hallmarks of GERL. GERL also appears to produce residual bodies, and both organelles show cytochemically demonstrable acid phosphatase activity. When cells are exposed to colloidal silver, the tracer is endocytosed via pinocytic vacuoles to GERL.

KEY WORDS GERL beige mouse alveolar macrophage lysosomes endocytosis

GERL (Golgi apparatus, endoplasmic reticulum, lysosomes) is a term used to describe a hydrolase-rich structure located near the "trans" (3) Golgi saccule. It is considered to be a specialized area of endoplasmic reticulum that is apparently involved in the formation of various types of lysosomes (16, 18, 19). The structure was first described by Novikoff (16) in the small neurons of rat dorsal root ganglia. Novikoff and his colleagues subsequently described GERL in diverse cell types (see reference 18, for recent review).

In a preliminary study of mouse alveolar macrophages (4), we described a system of smooth-surfaced elements that appeared to be analogous to GERL. This report provides additional morphological and cytochemical evidence for the presence of GERL in the alveolar macrophage. Although GERL has also been identified in alveolar macrophages of normal Swiss mice, it is more highly developed and thus easier to study in the beige mouse, a mutant considered to be homologous to human Chediak-Higashi syndrome (1, 13, 20).

Recently, Gonatas et al. (8) demonstrated that when cultured neurons are exposed to conjugates of ricin or phytohemagglutinin and horseradish peroxidase, the material is endocytosed into GERL. In the present study, we show that when colloidal silver is endocytosed by alveolar macrophages, it first appears in pinocytic vacuoles and then in elements of GERL. Residual bodies (secondary lysosomes; reference 17), some of which contain colloidal silver, appear to originate from GERL.

MATERIALS AND METHODS

A total of 25 adult beige (bg/bg) and control (C57BL/6J) mice (The Jackson Laboratory, Bar Harbor, Me.) were used in these experiments.

For electron microscopy, mice were sacrificed by cervical dislocation. Small pieces of lung were fixed in 3% glutaraldehyde (3 h, 4°C; reference 22) or in Karnovsky's fixative (1-3 h, room temperature; reference 10), diluted with an equal volume of 0.1 M cacodylate buffer, pH 7.4. The tissue was rinsed three times in the same buffer containing 5% sucrose and stored overnight at 4°C. The tissues were fixed in 1% OsO4-5% sucrose (1 h, 4°C), rinsed in sucrose buffer, and soaked (4 h, 4°C) in 0.5% uranyl acetate in Michaelis veronal-acetate buffer, pH 7.4. The tissue was rinsed three times in the same buffer containing 5% sucrose and stored overnight at 4°C. The tissues were fixed in 1% OsO4-5% sucrose (1 h, 4°C), rinsed in sucrose buffer, and soaked (4 h, 4°C) in 0.5% uranyl acetate in Michaelis veronal-acetate buffer, pH 7.4 (6), then dehydrated in graded alcohols and propylene oxide and embedded in Spurr's mixture (25) or in Epon (12). Thin sections, prepared on a diamond knife, were stained with lead citrate (21) and examined in a Siemens 101B electron microscope.

For demonstration of acid phosphatase activity, thin strips of tissue were fixed in glutaraldehyde or in diluted Karnovsky's fixative as described above, rinsed in su-
Electron microscopy of beige mouse alveolar macrophages revealed a system of smooth-surfaced elements distributed throughout the cytoplasm. Although some elements were seen near the Golgi apparatus, no particular spatial relationship between the two structures was noted. When viewed in longitudinal section, some elements appeared as relatively narrow, possibly tubular structures, whereas others were more dilated (Figs. 1 and 2). Continuities were evident between the narrow and dilated regions (Figs. 1 and 2), suggesting that these regions formed a continuous, interconnecting network.

The various elements contained a finely granular matrix together with focal deposits of dense material (Figs. 1 and 2). Some of this material appeared in the form of elongated, crystalline-like bodies that were embedded in the matrix and caused distortion of the element (Fig. 3). Many of the larger accumulations appeared in dilated elements that were morphologically similar to residual bodies (Figs. 1 and 2). They had a "thick" delimiting membrane and subjacent "halo" (Figs. 4 and 5). Continuities were evident between the delimiting membranes of these bodies and the smooth-surfaced elements (Figs. 4-6).

Acid phosphatase reaction product was demonstrable in the various elements as well as in the residual bodies (Fig. 7).

Within 15 min after mice were exposed to colloidal silver, deposits of silver were seen in numerous pinocytic vacuoles (Fig. 8). By 30 min silver deposits were seen in pinocytic vacuoles (Fig. 9), in narrow and dilated smooth-surfaced elements, including those containing accumulations of dense material (Figs. 9-12), and in some residual bodies (Fig. 9). Because the colloidal silver deposits were of very high density and had relatively small dimensions and sharply defined edges, they could be distinguished from the naturally occurring dense material seen in the smooth-surfaced elements and in residual bodies.

DISCUSSION

A system of smooth-surfaced elements is present in alveolar macrophages of mutant beige mice. The system appears to be analogous to GERL because the elements bear the same morphological and cytochemical hallmarks: a delimiting membrane of the thick variety and subjacent halo and demonstrable acid phosphatase activity. In many cell types, GERL is located near the trans face of the Golgi apparatus. However, no such relationship is observed in the alveolar macrophage, and the elements of GERL are distributed throughout the cell. The reasons for these differences are not apparent but are probably related to the specific functions of these cells.

Although elements similar to GERL are also seen in alveolar macrophages of normal Swiss mice, its labyrinthine nature is more apparent in beige mice. It is also of interest to note that GERL is hypertrophied in beige mouse hepatocytes (5).

To our knowledge, GERL has not been described previously in macrophages. However, in mouse alveolar macrophages, Karrer (11) described "elongated inclusions" (ib4 in Fig. 27, reference 11) with a "centrally dense matrix... surrounded by a less dense shell" (ib4 in Fig. 27, reference 11), which closely resemble the elements of GERL described in the present study. Recently, Sorokin (24), in a preliminary report on mouse alveolar macrophages that had been exposed to iron oxide particles, described a "labyrinth of branched and smooth-surfaced tubules and cisterns." The system appeared to produce lysosomes, and its relationship to GERL was suggested. These observations, together with those of the present study, suggest that GERL may be more highly developed and thus more readily de-
Figure 1 Portion of alveolar macrophage showing elongated and dilated elements (GE). Note matrix of moderate density and focal deposits of dense material (arrows). Accumulations of dense materials are also evident in residual bodies (RB). G, Golgi apparatus and related structures. × 21,000.

Figure 2 Higher magnification shows elongated elements (GE). Note "thick" type membrane and subjacent "halo" that delimits the elements (GE) and the residual bodies (RB). × 30,000.
FIGURE 3 Crystalline-like bodies are seen within dilated, distorted smooth-surfaced elements (GE). ≥ 38,000.

FIGURES 4–6 These micrographs illustrate continuities (arrows) between residual bodies (RB) and smooth-surfaced elements (GE). Note thick membrane and halo delimiting both organelles. Fig. 4, ≥ 54,700. Fig. 5, ≥ 54,600. Fig. 6, ≥ 26,000.

FIGURE 7 Alveolar macrophage, incubated for acid phosphatase activity (45 min). Enzyme reaction product is localized in smooth-surfaced elements (GE) and in a residual body (RB). Unreactive endoplasmic reticulum is shown at arrows. N, nucleus. ≥ 27,000.
tected in alveolar macrophages compared with macrophages in other tissues.

As mentioned above, in the alveolar macrophage, acid phosphatase activity is restricted to the elements of GERL (and residual bodies) and is not seen in other organelles. In contrast, Nichols et al. (14) have demonstrated acid phosphatase and aryl sulfatase reaction products in parts of the endoplasmic reticulum (and Golgi apparatus) of macrophages in peritoneal exudates from rabbit, guinea pig, and man. These differences in localization of acid phosphatase may also reflect differences in the structure and function of macrophages, depending on their location in tissues.

In several cell types, Novikoff and his colleagues have shown that GERL produces residual bodies as well as other kinds of lysosomes. In the present study, varying amounts of material were seen in GERL. These accumulations had the morphological features of residual bodies and showed cytochemically demonstrable acid phosphatase activity. Furthermore, continuities were apparent between the delimiting membranes of the larger residual bodies and those of GERL. These observations suggest that, as in other cell types, the residual bodies in the alveolar macrophage arise from GERL.

In macrophages and certain other cells that show endocytic activity, it is generally considered that the endocytic vacuoles merge with either primary or secondary lysosomes (residual bodies), thereby initiating the events that lead to degradation of the endocytosed material (2, 9, 17). The present findings, however, raise the possibility of another pathway for the transport of exogenous materials. Within a relatively short time after exposure of mice to colloidal silver, particles were seen within elements of GERL, suggesting that at least a portion of the material was endocytosed into GERL. The possible interrelations of the two pathways and their relative importance require further study. Silver particles were also seen in some residual bodies. These bodies may have obtained the marker during their formation from GERL, or alternatively, they may represent preexisting residual bodies that had fused with endocytic vacuoles containing silver. Further studies with different markers may help to distinguish between these possibilities.

That exogenous material may be endocytosed into GERL, rather than merge with lysosomes, has recently been demonstrated by Gonatas et al. (8) in cultured neurons derived from mouse dorsal root ganglion. By labeling the plasma membranes
Figure 9  Alveolar macrophage, 30 min after nasal instillation of colloidal silver. Silver deposits are seen in pinocytic vacuoles (arrows) beneath plasma membrane, in residual bodies (RB), and in elongated and dilated, smooth-surfaced elements (GE). × 34,000.

Figure 10  Higher magnification of element marked GE in Fig. 9. Deposits of silver, identifiable by their high density, are present in dilated (S) and elongated (T) elements. Arrow indicates continuity between the two regions. × 67,000.

Figure 11  Alveolar macrophage, 30 min after nasal instillation of colloidal silver. Area is from same cell as that shown in Fig. 9. Dense silver deposits are seen in elongated elements (GE) and in circular profiles that represent transverse and oblique sections through the elements (arrows). RB, residual body. × 34,000.

Figure 12  Higher magnification of elements marked GE in Fig. 11. Silver deposits are seen in elongated elements (GE). × 81,000.
with conjugates of ricin or phytohemagglutinin and horseradish peroxidase, these authors were able to visualize the internalized plasma membrane and demonstrate its transport into GERL. These investigators suggested that agents such as lectins that are bound to the plasma membrane may be handled differently than soluble materials. Further studies of alveolar macrophages with the use of soluble and insoluble substances that have specificity for plasma membrane receptors may help to clarify the functions of GERL in alveolar macrophages.

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