ELECTRON-MICROSCOPIC STUDY OF LYSOSOMES OF MACROPHAGES WITH DIFFERENT PHAGOCYTIC ACTIVITY

V. V. Rogovin

UDC 612.112.9-086 :537.533.35

In the analysis of the digestive power of the phagocytes attention has been concentrated on the lysosomes because they contain enzymes which may carry out the final stage of phagocytosis [6]. However, our views on the lysosomes are still largely hypothetical, and further experimental study is needed.

Cytochemical investigations have shown that after vaccination, stimulation of the digestive activity of the macrophages is combined with an increase in the acid phosphatase activity in these cells [1]. An increase in the acid phosphatase and acid desoxyribonuclease activity in the macrophages has been demonstrated in association with a nonspecific increase in their digestive power under the influence of the bacterial polysaccharide prodigiosan. Cortisone in large doses produced the opposite changes [2].

This paper describes the results of an electron-microscopic study of the lysosomes of macrophages with different levels of digestive power. To increase the latter, the bacterial polysaccharide prodigiosan was used, and to depress it – large doses of cortisone. The lysosomes of the macrophages were also studied electron-cytochemically using a reaction for acid phosphatase.

EXPERIMENTAL METHOD

The macrophages used were taken from the peritoneal cavity of 200 albino mice weighing 16-20 g. The animals were divided into three groups. Group 1 consisted of mice receiving prodigiosan in a dose of 25 μg subcutaneously 24 h before extraction of the macrophages, group 2 contained mice each receiving cortisone in a dose of 5 mg, 3 times at intervals of 48 h between injections (the macrophages were extracted from the peritoneal cavity 24 h after the 3rd injection), and group 3 were the control mice.

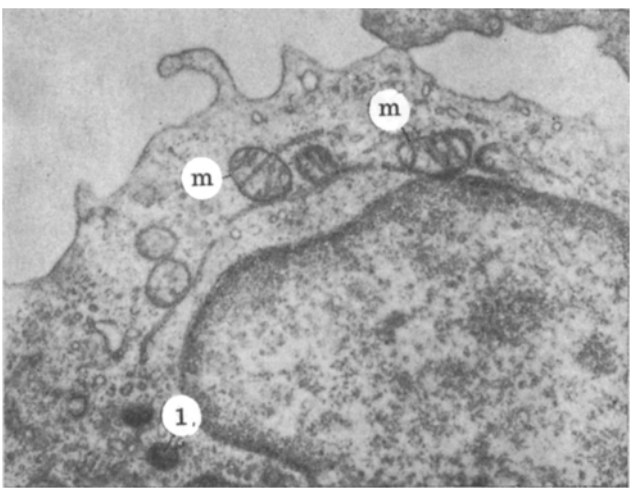

Fig. 1. Area of the young macrophage from a mouse of the control group. Mitochondria (m) and a lysosome (l) can be seen. Fixation with a buffered 1% OsO_4 solution for 30 min, pH 7.4, 16,000×.

Laboratory of Medical Cytology, Department of Microbiology, Central Postgraduate Medical Institute and Polyclinic No. 21, Timiryazev District, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR Z. V. Ermol'eva). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 2, pp. 66-70, February, 1967. Original article submitted May 13, 1965.

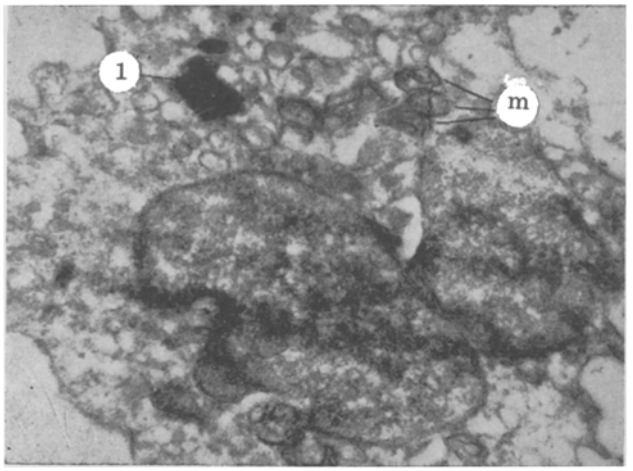

Fig. 2. Adult macrophage of a mouse receiving prodigiosan. Mitochondria and a lysosome showing acid phosphatase activity can be seen. Prefixation with buffered 1% OsO_4 solution with the addition of 7% sucrose by weight. Incubation for 1 h at room temperature in Gomori's medium with addition of 7% sucrose by weight. Subsequent fixation by buffered 1% OsO_4 solution for 1 h, pH 7.4, 13,000×.

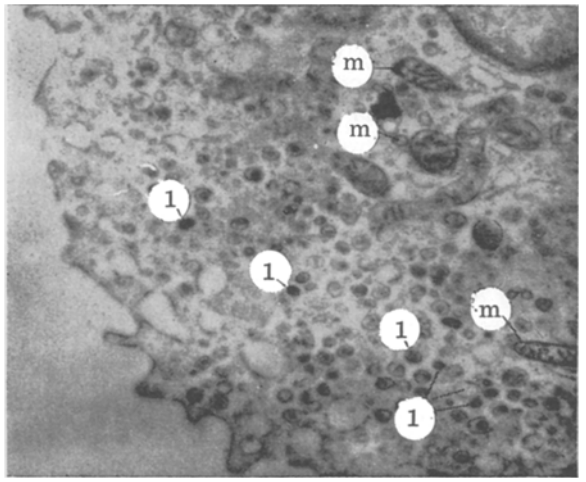

Fig. 3. Area of an adult macrophage of a mouse receiving cortisone. Small lysosomes and mitochondria can be seen. Fixation with buffered 1% OsO_4 solution for 30 min, pH 7.4, 14,000×.

The macrophages were fixed with a 1% OsO_4 solution by Palade's method, dehydrated in alcohols of increasing concentration, and embedded in a mixture of methacrylates. Thermal polymerization continued for 12 h. To detect acid phosphatase activity the macrophages were first fixed for 5 min in 1% OsO_4 solution with the addition of 7% sucrose by weight [5], and then incubated for 1 h at room temperature in Gomori's medium with the same addition of sucrose. The subsequent treatment of the macrophages was as described above. Ultrathin sections were cut on the LKB microtome. The sections were examined with the JEM-5Y electron microscope.

EXPERIMENTAL RESULTS

Because of the well known polymorphism of the macrophages, for convenience of description they were subdivided into two main types, linked by intermediate forms.

The cells of type 1 were small in size, with regular oval outlines of their cell body, and with weak development of the rough endoplasmic reticulum. Many ribosomes were present, lying freely in the cytoplasm. Mitochondria with the typical structure were relatively few. The nucleus was not segmented and occupied a considerable area of the cell section. Isolated, oval, electron-dense structures were found inconstantly in the cytoplasm, surrounded by a single membrane, and corresponding in their morphology to lysosomes (Fig. 1). Cavities were seen only occasionally in the lysosomes.

The cells of type II were large. The cytoplasmic membrane formed processes of different shape, sometimes branching. The lysosome in these cells was much larger than in the macrophage of type I. In their size and shape the lysosomes were similar to those in the cells of the previous type, but several larger examples were found. In the same cell, lysosomes of different electron density could occur. Electron-cytochemically, acid phosphatase activity was demonstrated in the lysosomes, but the intensity of the reaction varied from one macrophage to another.

In the type II macrophages the mitochondria were typical in structure, the endoplasmic reticulum was of the rough type, and the Golgi complex was moderately developed. The cytoplasm occupied a large proportion of the section of the cell. The nucleus was irregular in shape. Besides these structures, numerous tiny more or less oval structures could be seen in the cytoplasm, surrounded by a single membrane without ribosomes on the surface. An osmiophilic area could be seen inside many of these formations. A translucent space was always left between this area and the outer membrane.

The macrophages of the mice receiving prodigiosan could also be divided into two types. However, relatively few type I cells were present. The type II cells were larger than the corresponding macrophages of the control animals. The cells were enlarged mainly because of the abundant cytoplasm, characterized by considerable development of the endoplasmic reticulum, by the numerous free-lying ribosomes, mitochondria, and small structures with osmiophilic contents already described in the cells of the control animals. The Golgi complex was highly developed. The number of lysosomes in the cell was increased, and they were larger than in the controls. The shape of the lysosomes was also modified: many of them were hollow (the electron-dense substance was situated entirely at the periphery by the membrane), the rest, although enlarged, remained entirely filled with electron-dense contents. On electron-cytochemical investigation, acid phosphatase activity was found at the periphery of the hollow lysosomes, where the electron-dense material was situated; the other lysosomes were completely filled with lead phosphate (Fig. 2). Even in the same macrophage, there was considerable variation in the acid phosphatase activity of the different lysosomes.

In experiments in which cortisone was given, as in the previous experiments, two main types of macrophages were observed. In the type I cells the lysosomes were smaller in size following the action of cortisone. The lysosomes were tightly packed with electron-dense contents, and hardly any hollow lysosomes were seen. The structure of the membrane of the lysosomes was unchanged. The nuclei were irregular in shape, and sometimes appeared segmented. The type II cells in the experiments with cortisone were similar to the analogous cells in the control series, but slightly smaller and they contained lysosomes which, as in the type I cells, were small and completely filled with electron-dense material (Fig. 3).

Hence in both the control and the experimental mice, some degree of polymorphism of the macrophages was observed. The type I macrophages, judging by their small content of cytoplasm with a feebly developed endoplasmic reticulum, were young and comparatively undifferentiated cells. The type II macrophages, on the other hand, showed a higher level of differentiation. In the cells of both types examined, as in the intermediate forms, a parallel trend was observed between the development of the endoplasmic reticulum, the Golgi complex, and the number of lysosomes: in the type I macrophages with a feebly developed endoplasmic reticulum there were few lysosomes; significantly more were seen in the type II macrophages with a well developed endoplasmic reticulum and Golgi complex. Under the influence of prodigiosan the number and size of the lysosomes increased, and this was observed during the first 24 h of the experiment. Similar results have been obtained previously in experiments in which mice were given brucellosis vaccine [3]. In the control mice, and especially in the experiments with prodigiosan, variability of the structure of the lysosomes was observed: side by side with typical forms, hollow lysosomes were seen in which the electron-dense material was situated at the periphery, immediately next to the membrane. Finally, attention is directed to the structures present in the type II macrophages resembling the cross-sections of the tubules of the endoplasmic reticulum without ribosomes on the surface, but containing osmiophilic material in the center or eccentrically.

As a whole the observations described above agree well with the view that the lysosomes originate as a distinctive secretion. According to this view the formation of the protein of the lysosomes starts in the cavities of the endoplasmic reticulum of rough type and ends in the Golgi complex [7]. From this point of view the parallel noted above between the degree of development of the endoplasmic reticulum, the Golgi complex, and the lysosomes, and also the presence of intermediate forms between the typical tubules of the endoplasmic reticulum and formation resembling lysosomes can readily be understood. On this basis it must be assumed that the increase in the number and size of the lysosomes in response to administration of prodigiosan or vaccine is connected with the intensified synthesis of the specific lysosome proteins – the hydrolases. This increase in protein synthesis results in increased formation of the lysosomes of the macrophages, and thereby in the principal specific function of these cells – the digestion of phagocytosed objects.

Meanwhile, in the experiments described above cortisone led to a decrease in the size of the lysosomes. Remembering the depressant action of cortisone on protein synthesis it may be postulated that the decrease in the size of the lysosomes is associated with inhibition of the synthesis of their specific proteins under the influence of large doses of cortisone. If this hypothesis is correct, it must be accepted that the action of cortisone on the lysosomes is not limited to its effect on their membrane, but is more widespread [8, 9]. This hypothesis is also in agreement with the results of previous cytochemical investigations showing a reduction in the acid phosphatase activity in the macrophages under the influence of large doses of cortisone.

It was shown above that the changes in the lysosomes reflect changes in certain functional states of the cell, and observations showing the morphological heterogeneity of the lysosomes even in the same macrophage were described. The polymorphism of the lysosomes is a fact which has been known for a long time [4, 6]. The results of the electron-cytochemical investigation of the macrophages also showed that not all structures which from their morphology may be regarded as lysosomes are identical. In some lysosomes the acid phosphatase activity is much higher than in others. Further investigations are needed to reveal the causes of this heterogeneity of the lysosomes.

LITERATURE CITED

1. A. I. Braude, N. N. Braude, and E. A. Zavenyagina, Doklay Akad. Nauk SSSR, 145, No. 3, 677 (1962).
2. A. I. Braude and V. V. Rogovin, Doklady Akad. Nauk SSSR, 153, No. 1, 229 (1963).
3. A. I. Braude and V. V. Rogovin, Byull. Moskovsk. Obshchestva Ispyt. Prirody. Otdel Biol., No. 6, 140 (1964).
4. A. Novikoff, The Functional Morphology of the Cell [Russian translation], Moscow (1963), p. 113.
5. C. T. Ashworth, F. J. Luibel, and S. C. Stewart, J. Cell. Biol., 17 (1963), p. 1.
6. C. de Duve, In the book: Structure and Function of the Cells [Russian translation], Moscow (1964), p. 90.
7. E. Essner and A. B. Novikoff, J. Cell. Biol., 15 (1962), p. 289.
8. W. Jacobson, In the book: Cellular Injury, London (1964), p. 136.
9. R. Llamas and E. Coronas, An. Inst. Biol., Mexico, 34 (1963), p. 11.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
