Response of Peritoneal Cells to Horseradish Peroxidase and Aldehyde-Fixed Erythrocytes in the Mouse: An Electron Microscope Study*

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Summary. In adult dd-mice, response of peritoneal free cells to either horseradish peroxidase (HRP) or glutaraldehyde-fixed erythrocytes was examined by electron microscopy.

As reported previously (ABE et al., 1979), peritoneal free cells are classified into three types: type I, II and III cells. Type I cells are small lymphocytes, type II cells are medium-sized mononuclear cells, and type III cells are macrophages.

The amount of HRP taken up is very large in type III cells. The cells contain numerous vacuoles filled with HRP. Type I and II cells also have vesicles containing HRP. The vesicles are fewer in type I cells than in type II.

Type III cells rapidly enclose erythrocytes by thin cytoplasmic flaps and then ingest several erythrocytes. In type III cells, a labyrinth of ramifying tubes seen in the peripheral cytoplasm may serve as a reserve of the membrane system for allowing a rapid coverage and internalization of large foreign materials. A part of type II cells attach to erythrocytes and take them by a varying depth of invagination. Type I cells do not respond to erythrocytes at all.

In conclusion, the three types of peritoneal cells can be differentiated by their response to foreign materials as well as by their cytologic features.

As generally accepted, the peritoneal cavity contains macrophages and lymphocytes (BALNER, 1963; BENNETT, 1965; FORBES, 1965, 1966; CARR, 1967; ELVES, 1970; DAEMS and BREDEROO, 1973; GALLILY and BEN-ISHAY, 1974). In separate papers (ABE et al., 1978, 1979a, b; HONMA et al., 1978), however, we have reported that free cells in the mouse peritoneal cavity are classified into three types: type I, II and III cells and that type I cells are small lymphocytes, type III cells are macrophages and type II cells are medium-sized mononuclear cells which are intermediate in size between lymphocytes and macrophages. The type II cells seem to have been regarded as lymphocytes or immature macrophages by previous authors (BALNER, 1963; BENNETT,

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1965; FORBES, 1965, 1966; CARR, 1967; ELVES, 1970; DAEMS and BREDEROO, 1973; GALLILY and BEN-ISHAY, 1974), but they are distinguishable by their cytologic features from either lymphocytes or macrophages. Phagocytic activity of peritoneal cells has repeatedly been studied particularly for macrophages (NORTH and MACKANESS, 1963; NORTH, 1966; DUMONT and ROBERT, 1970; DAEMS and BREDEROO, 1973; CATANZARO and GRAHAM, 1974), but information is not sufficient as to how peritoneal free cells other than macrophages respond to foreign materials injected intraperitoneally. The present study was undertaken to examine response of the three types of peritoneal cells to horseradish peroxidase and aldehyde-fixed erythrocytes. It was particularly hoped to clarify whether or not the type II cells can be distinguished from macrophages or lymphocytes functionally as well as morphologically.

MATERIALS AND METHODS

A total of 49 two-month-old dd-mice of both sexes were used.

In 12 mice, 0.12 mg of horseradish peroxidase (HRP) (Sigma, Grade II) in 0.1 ml saline was injected intraperitoneally. The mice were killed with chloroform 5, 15 and 30 min after injection and then immediately given an intraperitoneal injection of 0.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5). After a gentle massage of the abdomen for a while, the fixing fluid in the peritoneal cavity was aspirated. Peritoneal free cells in the aspirate were further fixed in the same fixative for 5 min, washed in the same buffer and then reacted by the DAB method (FAHIMI et al., 1976) for demonstrating peroxidase. Then the cell suspension was post-fixed with 2% OsO_4 for 30 min and centrifuged. The pellet containing the peritoneal cells was solidified in 1% agar solution and embedded in Epon. Ultrathin sections either unstained or stained with uranium acetate and lead nitrate were observed with the electron microscope.

In 24 mice, aldehyde-fixed erythrocytes from the fowl or mouse were injected intraperitoneally. The erythrocytes were prepared as follows: Blood was obtained from a vein of a fowl or from the heart of a dd-mouse with a heparinized syringe and then washed three times with saline. Erythrocytes thus obtained were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.5) and stocked at 4°C. The erythrocytes were washed five times with saline to remove the fixative. Then 0.1 ml of suspension in saline containing about 1×10^8 erythrocytes was injected into the peritoneal cavity. The mice were killed 5, 10, 15, 30, 45 and 60 min after injection. Three mice received a second injection of erythrocytes at 15 min after the first injection, and they were killed 15 min after the second injection.

The peritoneal free cells were fixed in situ with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) in the same way as done after HRP injection. The peritoneal free cells in the fixative were aspirated, centrifuged and then fixed for 2 hrs. Then the pellet was post-fixed in 2% OsO_4 for 30 min, immersed in 0.5% uranyl acetate overnight and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead nitrate and examined with the electron microscope.

As controls, peritoneal free cells from 10 normal mice were observed in the same way.

RESULTS

As described in separate papers (ABE et al., 1978, 1979a, b; HONMA et al., 1978), peritoneal free cells can be divided into three types: type I, II and III (Fig. 1).

Fig. 1. Peritoneal free cells in the normal mouse. **a.** Three types of peritoneal cells. 1 Type I cell, 2 type II cell, 3 type II cell, *T* tubes of the labyrinth. **b.** Type II cell. *NB* nuclear body, *DB* dense bodies, *F* bundle of filaments. Notice a few flat cisterns of rough endoplasmic reticulum. *a:* $\times 7,000$; *b:* $\times 20,000$

Fig. 2. Response of peritoneal free cells to HRP, 15 min after administration (Unstained sections). **a.** Type I cell (1) is not labeled with HRP, whereas type II cell (2) contains vesicles and vacuoles labeled with HRP. **b.** Type III cells (3) have numerous vesicles and vacuoles densely labeled with HRP. **c.** Type II cell (left) contains a few vesicles and vacuoles with HRP. Type III cell (right) possesses numerous pinocytotic vesicles and vacuoles labeled with HRP. Rough endoplasmic reticulum (double arrows) in type III cell contains reaction products, though less dense, for intrinsic peroxidase, whereas rough endoplasmic reticulum (arrows) in type II cell contains no reaction product.
a, b: $\times 8,000$; c: $\times 26,000$

Type I cells are about 20% of the peritoneal cells. They are small lymphocytes which have round nuclei surrounded by a narrow rim of cytoplasm.

Type II cells, about 45% of the peritoneal cells, are medium-sized mononuclear cells. The nucleus is rounded or somewhat irregularly shaped with infoldings of the nuclear membrane. It often contains nuclear bodies (Fig. 1b). The cytoplasm is more abundant than that of small lymphocytes, and contains rough endoplasmic reticulum, which is generally flat and long in profile around the nucleus. Several electron dense granules are scattered, and thick bundles of filaments often appear in the cytoplasm (Fig. 1b).

Type III cells are macrophages, larger than the other two types of cells and occupy about 35% of the peritoneal cells. The nucleus is irregular in shape and eccentric in position. The cytoplasm is abundant. The peripheral zone of the cytoplasm contains an intricate labyrinth of anastomosing tortuous tubes which usually appear as tubes or vacuoles in profile near the cell surface (Fig. 1a). The tubes sometimes open to the extracellular space on the cell surface. In addition to well developed organelles such as rough endoplasmic reticulum and the Golgi apparatus, the cytoplasm contains numerous vesicles and varying amounts of granules of various sizes and appearance.

The cytological features of the three types of cells have been described in detail in a former paper (ABE et al., 1979a).

Response to horseradish peroxidase (HRP):

Type I, II and III cells have taken up HRP 5 min after the intraperitoneal injection, although the amounts of HRP incorporated differ among different cell types (Fig. 2). In general, type III cells (macrophages) contain large amounts of HRP, type II cells relatively small amounts and type I cells (small lymphocytes) very few HRP. In type III and II cells, the HRP taken up tends to increase in amount with the lapse of time.

In type III cells (Fig. 2b, c), HRP appears as electron dense dots on the cell surface and just inside the limiting membrane of labyrinthine tubes in the superficial cytoplasm. HRP is also contained within pinocytotic vesicles varying from 0.1 to 0.2 μm in diameter. Small vesicles with a diameter of about 0.1 μm are often coated with fuzzy material such as is seen in coated vesicles. The vesicles filled with HRP are particularly numerous just close to the cell surface, and they are often fused with the cell membrane or the tubes. In addition to vesicles, the cells of this type contain HRP-labeled vacuoles with a diameter of about 0.5 μm . Some vacuoles are filled densely with HRP, some have a densely labeled rim inside the limiting membrane and some others contain core-like reaction products of HRP. The vacuoles are sometimes fused with pinocytotic vesicles.

Fig. 3. Schematic diagram of uptake of HRP by type I, II and III cells.

Fig. 4. Response of peritoneal free cells to fowl erythrocytes, 15 min after injection. In type III cells (3) thin cytoplasmic flaps (arrows) protruding from the surface enclose the erythrocytes. Some type II cells (2a) do not respond to erythrocytes, some (2b) attach to an erythrocyte and others (2c) take an erythrocyte with a deep invagination. a: $\times 6,000$; b: $\times 12,000$

Fig. 5. Response of type III cells to mouse(a)- or to fowl(b)-erythrocytes, 15 min after injection. The type III cell (3) that has engulfed several erythrocytes has few profiles of labyrinthine tubes. Thin cytoplasmic flaps protruding from type III cells wrap the surface of erythrocytes. Arrows indicate the felt-like cytoplasm. *T* tubes of the labyrinth.
 × 10,000

The HRP-labeled vacuoles are few at 5 min, but increased at 15 and 30 min after HRP injection.

In type III cells, reaction products for peroxidase are also demonstrated, though less strongly, in the nuclear envelope and rough endoplasmic reticulum (Fig. 2c).

Type II cells can take up considerable amounts of HRP by pinocytosis (Fig. 2a). They have small vesicles containing HRP. The reaction products also appear within some larger vacuoles and sometimes inside the bounding membrane of vacuoles. When two type II cells are adjacent to each other, HRP is seen in the intercellular space between their adjoining cell membranes.

Type I cells (small lymphocytes) contain only a few vesicles labeled with HRP (Fig. 2a).

The uptake of HRP by the three types of peritoneal cells is diagrammatically shown in Figure 3.

Fig. 6. a-d. Response of type II cells to mouse-erythrocytes, 15 min after injection. NB nuclear bodies, F bundle of filaments. a: $\times 9,000$; b: $\times 12,000$; c, d: $\times 8,000$

Fig. 7. **a** and **b.** Response of type II cells to fowl-erythrocyte, 15 min after injection. Felt-like cytoplasm with fine filaments (double arrows) is seen beneath the invaginated cell membrane facing the erythrocytes. Type II cells are also seen attaching to another erythrocyte. *F* bundle of filaments. Arrows indicate cytoplasmic flaps of type III cells.
 × 28,000

Response to aldehyde-fixed erythrocytes:

Peritoneal free cells respond to either the fowl or mouse erythrocytes in almost the same manner (Fig. 4-7).

Type III cells (macrophages) first attach to the injected erythrocytes and then engulf them. Five min after injection, several erythrocytes are often seen around a single type III cell. On the contrary, an erythrocyte is sometimes surrounded by two or more type III cells (Fig. 4, 5). Type III cells occasionally have completely engulfed erythrocytes. In any case, type III cells are generally seen in aggregate with erythrocytes. Erythrocytes that have attached to type III cells are usually enclosed by thin cytoplasmic flaps, 0.1 μm in thickness, protruding from the surface of the latter cells (Fig. 4, 5). The intercellular space between erythrocytes and type III cells are 10 to 20 nm in width. The cytoplasmic flaps and the superficial cytoplasm just beneath the cell membrane facing the attached erythrocytes appear felt-like with filaments, being free of cell organelles including ribosomes (Fig. 5). The superficial felt-like cytoplasm contains neither vacuoles nor tubes. Some type III cells have engulfed five or more erythrocytes 15 min and later. The more erythrocytes type III cells have engulfed, the fewer labyrinthine tubes or vacuoles they have (Fig. 5a).

Type II cells, though not all of them, respond to erythrocytes (Fig. 4, 6, 7). Type II cells are often seen attaching to one or a few erythrocytes 5 min after injection. Their cell membrane is invaginated, to a varying depth, by the attached erythrocyte (Fig. 4, 6, 7). The intercellular space between the type II cell and erythro-

Fig. 8. Diagram of the responses of peritoneal free cells to aldehyde-fixed erythrocytes. Shaded circles represent erythrocytes. Dotted areas represent nuclei of peritoneal cells. Type I cells (I) do not respond to erythrocytes. Of type II cells (II), some do not respond, some attach to erythrocytes, and others take up an erythrocyte with invagination of the cell membrane. A few completely engulf an erythrocyte. Type III cells attach to erythrocytes, enclose them with thin cytoplasmic flaps protruding from the cell surface and engulf several erythrocytes. Labyrinthine tubes of type III cells become less apparent with the progress of erythrocyte internalization. In type II and III cells, the superficial cytoplasm (indicated by dotted lines) close to the erythrocytes is felt-like in appearance.

cyte is 10 to 20 nm in width. In type II cells, the superficial cytoplasm just beneath the cell surface confronted with the erythrocyte is felt-like in appearance, and it is generally wider than that in type III cells, being occasionally $1\text{ }\mu\text{m}$ or more in width (Fig. 7). Thick bundles of filaments are also visible in type II cells attaching to erythrocytes (Fig. 6c, 7b). The type II cells responding to erythrocytes appear to be almost unchanged in frequency 10 min or later and even after the second injection of erythrocytes. A few type II cells have completely engulfed erythrocytes even at 60 min. One third to half of type II cells respond to erythrocytes, but the remaining remain intact. Type II cells and type III cells occasionally lie close together around a single erythrocyte.

Type I cells (small lymphocytes) are singly scattered, and they do not respond to erythrocytes.

Response of the three types of peritoneal cells to erythrocytes is schematically summarized in Figure 8.

DISCUSSION

As seen in the Results, the three types of peritoneal free cells differ in response to foreign materials as well as in cytologic features. Type III cells are most avid in phagocytic activity, being distinctly distinguishable from cells of other types. Thus there is no doubt that they are macrophages. It has recently been postulated that macrophages generally originate in the bone marrow (VAN FURTH, 1975). However, it has been suggested that macrophages may also be formed *in situ* where they are populated (DAEMS and KOERTEN, 1978). According to DAEMS and his colleagues (DAEMS and BREDEROO, 1973; DAEMS and KOERTEN, 1978), the peritoneal cavity contains two types of macrophages, resident macrophages and blood-borne ones, the former exhibiting peroxidase activity in the nuclear envelope and rough endoplasmic reticulum, whereas the latter show no peroxidase reaction. In normal unstimulated peritoneal cavity, as reported in another paper of ours (ABE *et al.*, 1979a), type III cells are similar in peroxidase activity to the resident macrophages. In newborn mice, as described separately (ABE *et al.*, 1979b), the majority of free cells in the peritoneal cavity are type III cells, which would have been produced *in situ* rather than migrating from the blood.

Phagocytosis is generally divided into two phases, attachment phase and ingestion phase (RABINOVITCH, 1967; VERNON-ROBERTS, 1972; NELSON, 1976). HRP or exogenous erythrocytes are seen attached to phagocytic cells and then ingested. The present observation shows that type III cells (macrophages) attach to erythrocytes and ingest them quickly. A single cell can usually ingest more than one erythrocyte. In such phagocytosis of large foreign materials, an extensive membrane system seems necessary for prompt expansion of the cell surface, in order that the materials to be phagocytosed can be first enclosed by it. In alveolar macrophages, for instance, about 50% of the surface membrane was estimated to be interiorized by phagocytosis of plastic particles (TSAN and BERLIN, 1971). As seen in the previous and present results, a labyrinth of ramifying tubes which are in community with the extracellular space, is characteristic of type III cells. The finding that the profiles of the tubes become fewer in type III cells as the cells have engulfed more

erythrocytes suggests that the labyrinth of tubes may serve as a reserve of the membrane system for allowing a rapid enclosure and ingestion of large foreign materials. Thus, the labyrinth of tubes in type III cells may resemble the fisiform vesicles which are seen beneath the free surface in the superficial cells of the transitional epithelium and are thought to provide for rapid expansion of the epithelial surface during distension of the organ (PORTER et al., 1965; HICKS, 1966).

In the peritoneal cavity, macrophages are known to increase in number rapidly under stimulated conditions (VAN FURTH, 1975; DAEMS and KOERTEN, 1978). The increase is considered due to migration of bone marrow-derived macrophages which are few in the normal unstimulated peritoneal cavity (DAEMS and KOERTEN, 1978). The immediate precursors of macrophages derived from marrow have been considered as monocytes (VAN FURTH, 1975). However, the possibility that lymphocytes might transform into macrophages has also been suggested from the presence of intermediate forms between lymphocytes and macrophages in inflammatory conditions (BALNER, 1965; FORBES, 1965, 1966; ELVES, 1970). According to these concepts, type II cells that are intermediate in size between lymphocytes and macrophages, may have been regarded as representing either lymphocytes or immature macrophages. As shown in the previous and present results (ABE et al., 1979a), however, type II cells are distinguishable from either macrophages (type III cells) or lymphocytes (type I cells) not only in cell size and fine structure but also in activity in uptake of foreign materials, and they appear neither to transform into macrophages (type III cells) nor to derive from lymphocytes (type I cells).

Type II cells often attach to an erythrocyte and seize it by invagination of the cell surface, whereas type I cells (lymphocytes) do not respond to erythrocytes. Thus, type II cells clearly differ in phagocytic activity from either type III or type I cells. As seen in the Results, essentially every type III cell internalizes exogenous erythrocytes, while a part of the type II responds to erythrocytes. The frequency of type II cells which respond to erythrocytes seems almost definite from the beginning and remains unchanged even after the second administration of erythrocytes. It is likely that some, not all, of the type II cells are naturally endowed with ability to respond to fixed erythrocytes. Thus, the type II cells at least partly, appear different in response to the erythrocytes from type III cells. The difference may presumably be related to that in the so-called immunological receptors on the cell surface (GRIFFIN et al., 1975; MUNTKE-KAAS et al., 1976).

In conclusion, type I, II and III cells in the peritoneal cavity can be differentiated by response to either HRP or fixed erythrocytes, although type I and II cells are similar in response to HRP. In particular, type II cells are distinguishable from either macrophages (type III cells) or lymphocytes (type I cells), and they should be separately dealt with as a population of peritoneal cells functionally as well as morphologically.

マウス腹膜腔細胞の西洋ワサビペルオキシダーゼとアルデヒド固定赤血球に対する反応： 電子顕微鏡的観察

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成熟 dd-マウスを用い，西洋ワサビペルオキシダーゼ（HRP）とグルタルアルデヒド固定赤血球に対する腹膜腔自由細胞の反応を電子顕微鏡で観察した。

腹膜腔自由細胞は形態のうえでⅠ型，Ⅱ型，Ⅲ型の3型に分類され，Ⅰ型は小リンパ球，Ⅱ型は中型単核細胞，Ⅲ型は大食細胞である。

HRPを腹膜腔内に注射すると，Ⅲ型細胞は大量のHRPを摂取する。細胞にはHRPを満たす多数の空胞がみられる。Ⅰ型とⅡ型細胞も，Ⅲ型細胞に比べると少量ではあるが，HRPを摂取する。Ⅰ型とⅡ型細胞ではHRPは小胞によってとりこまれるが，Ⅰ型細胞ではHRP含有小胞はまれにしか認められない。

赤血球を腹膜腔内に注射すると，Ⅲ型細胞は細胞表面から薄膜状の胞体突起を出して赤血球を囲み，これをきわめて速やかに取り込む。Ⅲ型細胞では，周縁部胞体に迷路状に吻合する管状構造がみられるが，この構造は赤血球のような大きな粒子に接着し，直ちにこれを包みこんで取り込むことを可能とする細胞膜の予備と考えられる。Ⅱ型細胞では，その一部が赤血球に接着し，これを種々の深さの細胞膜の陥入によって捕える。Ⅰ型細胞は赤血球に対して反応しない。

以上のように，3型の腹膜腔細胞は細胞形態のみならず，異物に対する反応においても区別できる。

REFERENCES

- Abe, K., S. Honma and T. Ito:** Population and morphology of peritoneal free cells in mice. (Abstr. in Japanese). *Acta anat. nippon.* 53: 97-98 (1978).
- : Peritoneal cells in mice: quantitative and qualitative cell morphology. *Amer. J. Anat.* (1979a, in press).
- : Age-related changes in peritoneal free cells in mice. (Abstr. in Japanese). *Acta anat. nippon.* (1979b, in press).
- Balner, R. H.:** Identification of peritoneal macrophages in mouse radiation chimeras. *Transplantation* 1: 217-223 (1963).
- Bennett, B.:** A method for performing differential counts on peritoneal cell populations from mice. *Transplantation* 3: 254-257 (1965).
- Carr, I.:** The fine structure of cells of the mouse peritoneum. *Z. Zellforsch.* 80: 534-555 (1967).
- Catanzaro, P. J. and R. C. Crahan:** Normal peritoneal lymphocytes. *Amer. J. Pathol.* 77: 23-48 (1974).
- Daems, W. Th. and P. Brederoo:** Electron microscopical studies on the structure, phagocytic properties, and peroxidatic activity of resident and exudate peritoneal macrophages in the guinea pig. *Z. Zellforsch.* 144: 247-297 (1973).

- Daems, W. Th. and H. K. Koerten:** The effects of various stimuli on the cellular composition of peritoneal exudates in the mouse. *Cell Tiss. Res.* 190: 47-60 (1978).
- Dumont, A. and M. D. Robert:** Electron microscopic study of phagocytosis of *Histoplasma capsulatum* by hamster peritoneal macrophages. *Lab. Invest.* 23: 278-286 (1970).
- Elves, M. W.:** Some observations on the life history of the macrophage. *J. Pathol.* 101: 333-338 (1970).
- Fahimi, H. D., B. A. Gray and V. K. Herzog:** Cytochemical localization of catalase and peroxidase in sinusoidal cells of rat liver. *Lav. Invest.* 34: 192-201 (1976).
- Forbes, I. J.:** Induction of mitosis in macrophages by endotoxin. *J. Immunol.* 94: 37-46 (1965).
———: Mitosis in mouse peritoneal macrophages. *J. Immunol.* 96: 734-743 (1966).
- Gallily, R. and Z. Ben-Ishay:** Interaction between normal or irradiated macrophages and lymphocytes in mice. *Cell. Immunol.* 11: 314-324 (1974).
- Griffin, F. M., J. A. Griffin, J. E. Leider and S. C. Silverstein:** Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle-bound ligands to specific receptors on the macrophage plasma membrane. *J. exp. Med.* 142: 1263-1282 (1975).
- Hicks, R. M.:** The function of the Golgi complex in transitional epithelium. Synthesis of the thick cell membrane. *J. Cell Biol.* 30: 623-643 (1966).
- Honma, S., K. Abe and T. Ito:** Sex difference in peritoneal free cells in mice. (Abstr. in Japanese). *Acta anat. nippon.* 53: 98 (1978).
- Munthe-Kaas, A. C., G. Kaplan and R. Seljelid:** On the mechanism of internalization of opsonized particles by rat Kupffer cells *in vitro*. *Exp. Cell Res.* 103: 201-212 (1976).
- Nelson, D. S. (ed.):** Immunology of the macrophages. Academic Press, New York, 1976.
- North, R. J.:** The localization by electron microscopy of acid phosphatase activity in guinea pig macrophages. *J. Ultrastr. Res.* 96-108 (1966).
- Porter, K. R., K. R. Kenyon and S. Badenhausen:** Origin of discoidal vesicles in cells of transitional epithelium. (Abstr.) *Anat. Rec.* 151: 401 (1965).
- Ravinovitch, M.:** The dissociation of the attachment and ingestion phases of phagocytosis by macrophages. *Exp. Cell Res.* 46: 19-28 (1967).
- Tsan, M.-F. and R. D. Berlin:** Effect of phagocytosis on membrane transport of nonelectrolytes. *J. exp. Med.* 134: 1016-1035 (1971).
- van Furth, R. (ed.):** Mononuclear phagocytes in Immunity, infection, and pathology. Blackwell Scientific Publications, Ltd., Oxford, 1975.
- Veron-Roberts, B.:** The macrophage. Cambridge University Press, Cambridge, 1972.

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