Acid Phosphatase Release from Intact Phagocytic Cells Surrounding a Large-Sized Parasite (35275)

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(Introduced by M. Wolman)

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Intraperitoneal injection of the large-sized (>70μ) yeast *Cryptococcus neoformans* into rabbits and guinea pigs is followed by migration of polymorphonuclear (PMN) cells (1, 2); the PMN form rings around the yeast cells and are later replaced by rings of monocytes (MN). This phenomenon can also be reproduced *in vitro* by adding *C. neoformans* to PMN or MN cultures. It is assumed that the ring formation, which takes place when organisms too large to be ingested are involved, is a process analogous to that of phagocytosis of small-sized bodies. Hirsch and Cohn have shown that phagocytosis is followed by release of lysosomal acid hydrolases and antibacterial substances into the cytoplasm (3). Therefore, the possibility of an enzyme release from the ring cells into the encircled yeast was examined in the present system. Preliminary results (4) showed that enzymes of leukocytic origin were indeed present in the surrounded yeast. It seemed important to ascertain whether enzyme release is accompanied by destruction of the leukocytes, or that the cells remain intact and functional. This question was investigated by using the histochemical reaction for acid phosphatase as a marker for enzymic activity in both phagocyte and yeast cell; and phagocytosis of bacteria as an indicator for the functional activity of the PMN and MN.

Materials and Methods. Our experiments were conducted in Leighton tubes containing cover-slips, and employed rabbit or guinea pig phagocytes. PMN or MN were collected after an ip injection of saline, either 18 or 72 hr before harvesting. 5×10^5 of either PMN or MN were mixed with 10^4 yeast cells and were allowed to settle in tubes containing Tyrode's medium and 40% isologous serum. The *C. neoformans* was heated for 10 min at 90°, to abolish its own enzymic activity, prior to its addition to the system.

PMN or MN rings are generally formed within 2 hr of implantation. At this stage, as well as a few hours later, histochemical reactions and the capability of phagocytosis were tested as follows:

a. Some cover slips were fixed in 2% glutaraldehyde for 5 min, then acid phosphatase activity was demonstrated by the procedure of Burstone (5) or by a modification of the Gomori method (6).

b. Phagocytic ability was tested by adding 10^7 *E. coli, S. albus* or *B. subtilis* into the Leighton tubes. The histochemical staining for acid phosphatase was done as described in (a).

In addition, the behavior of ring phagocytes *in vitro* was followed by observation of the cells in a perfusion chamber, under a phase contrast microscope enclosed in a 37° incubator.

Results and Discussion. Observation under phase contrast revealed that the ring cells displayed considerable pseudopodial activity throughout and after the formation of the ring, indicating the viability of the cells during the process (Fig. 1). In Giemsa-stained preparations, the ring cells ingested bacteria as much as did the free phagocytes (Fig. 2).

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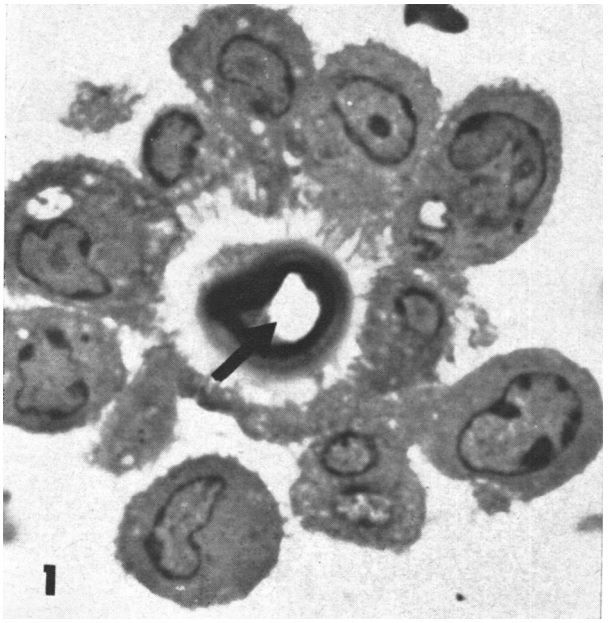

FIG. 1. A "mixed" ring (PMN and MN) enclosing a *C. neoformans* cell (arrow). Note signs of destruction of the encircled yeast; and pseudopodia of the leukocytes penetrating the yeast capsule. Epon embedding, 1μ section; ×1000.

In both PMN and MN rings all the enclosed cryptococci showed considerable acid phosphatase staining, whereas free cryptococci were entirely negative or only slightly positive (Fig. 3). Considering that enzymic activities in the yeast cells were previously abolished by heat, the source of the acid phosphatase observed in the yeast is presumably the surrounding leukocytes. The same results were obtained in the experiment in which ring formation was followed by phagocytosis of bacteria; again there was a release of enzymes from phagocytic cells into the encircled cryptococci.

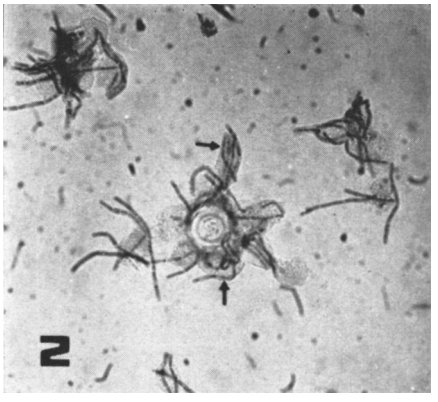

FIG. 2. Phagocytosis of bacteria (arrows) by PMN cells in a ring which was formed earlier around a *C. neoformans* cell. Note for comparison the phagocytosis in free PMN cells. Giemsa stain; ×600.

Additional experiments were conducted to validate the use of this histochemical reaction in the present system.

1. Short incubation times (2–5 min), resulted in the reaction product appearing simultaneously in the surrounding cells as well as in the enclosed cryptococci, thus reducing the possibility of its diffusion from the cells into the encircled yeast.

2. Heat-inactivated cryptococci incubated with acid phosphatase (Sigma) added to the medium, also resulted in the appearance of reaction product in the yeast, which shows that free enzyme may penetrate into the yeast cells.

3. Addition of NaF 0.1 m to the reaction mixture caused a complete inhibition of the phosphatase activity.

The main point which emerges from the present study is that the phagocytic cells in the rings may release hydrolytic enzymes into

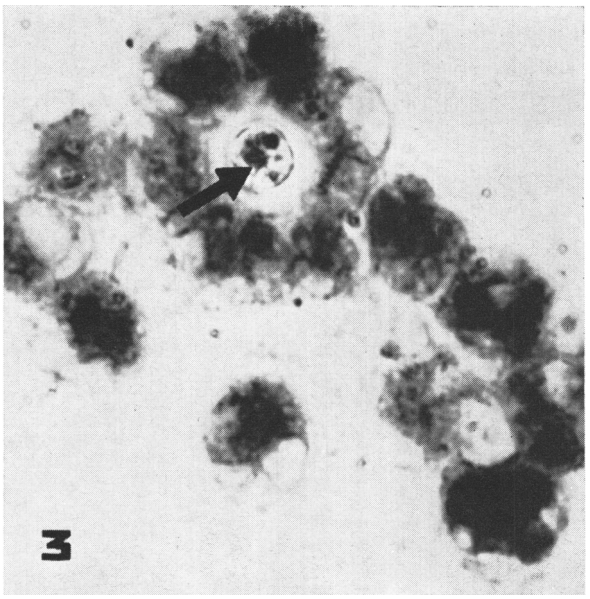

FIG. 3. Acid phosphatase release by ring PMN into the surrounded yeast cell. Note the granular appearance of the reaction product and the dense precipitate in the enclosed cryptococcus (arrow); ×1000.

the encircled cryptococci and yet remain viable and functional. Recent results (unpublished) have shown that the ring cells are able to destroy the encircled yeast, and it is plausible that this effect is mediated by the release of lysosomal enzymes from the phagocytes.

Such extracellular enzyme release associated with phagocytosis has been reported by several investigators (7–10). Crowder *et al.* (9) reported the extracellular release of histamine and lysosomal enzymes by human leukocytes during phagocytosis of staphylococci; trypan blue exclusion being the criterion for intactness of the blood cells. It is interesting that a suicidal cell like the PMN (11) is capable of lysosomal enzyme release without getting destroyed in the process. The significance and the mechanism of the extracellular release of enzymes and other substances from leukocytes is unknown. However, in the case of *C. neoformans*, such a release might be interpreted as constituting part of a defense mechanism of the phagocytic system against a large parasite, and as pointing to the similarity between the ring structure and normal phagocytosis.

Summary. Cryptococcus neoformans is a parasite, too large (>70 μ) to be phagocytosed by leukocytes. Under suitable conditions polymorphonuclear and/or mononuclear cells surround the yeast cells, forming a ring-like structure. It was found that acid phosphatase, originating from the leukocytes within the ring, penetrated the enclosed yeast. The enzyme release was not accompanied by destruction of the leukocytes, which remained active and capable of phagocytosing bacteria.

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