Gentamicin thus has a marked inhibitory action on a strain of *Staph. aureus* sensitive to it, growing in medium with serum. In medium without serum, the inhibitory action of gentamicin is reduced. In medium without serum, the inhibitory action of gentamicin is reduced. The antibiotic does not significantly change the ingestive capacity of neutrophils from healthy blood donors or patients with burns but enhances natural bactericidal (killing) activity of the neutrophils. If the level of the natural bactericidal action of the neutrophils is high, the inhibitory action of gentamicin is manifested only weakly.

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ULTRASTRUCTURAL ANALYSIS OF NEUTROPHIL–MACROPHAGE INTERACTION IN AN INFLAMMATORY FOCUS

B. V. Vtyurin, R. I. Kaem, and N. V. Chervonskaya

UDC 616-002-07:616.155.3-008.13]-076.4

KEY WORDS: leukocyte, macrophage, phagocytosis

Interaction of leukocytes with macrophages (Mph) in an inflammatory process in the course of phagocytosis has not been adequately studied. The generally accepted view that the function of polymorphonuclear leukocytes (polymorphs) is concerned entirely with the control of infection [2] has recently been disputed. Recent investigations [3-5] have shown that many polymorphs, especially in a burn wound, contain wound debris in their phagosomes much more often than bacteria. Meanwhile, phagocytosis of some species of bacteria by Mph has been quite widely reported in the literature [8, 10, 13]. More complex interactions between polymorphs and Mph during phagocytosis evidently arise than was hitherto supposed. After contact with microorganisms and toxins polymorphs quickly perish, but Mph are more resistant. Their role is more varied, for it embraces demarcation of the inflammatory focus, neutralization of toxic breakdown products, and regulation of various cellular systems [2].

The aim of the present investigation was to analyze polymorph–Mph interrelations in an inflammatory focus and the particular features of the morphological picture of phagocytosis in these cells and to examine the process of phagocytosis from the dynamic point of view.

Department of Pathological Anatomy. A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 68-71, January, 1989. Original article submitted April 5, 1988.

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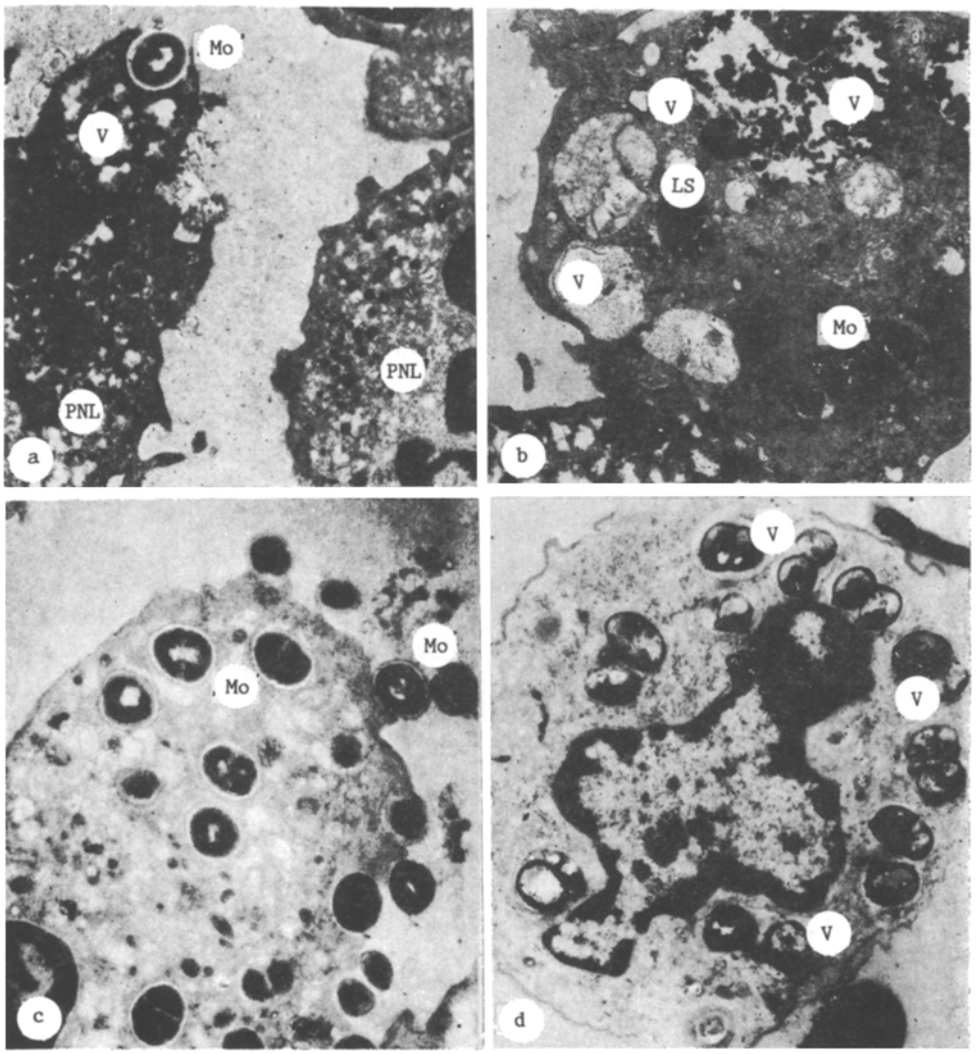

Fig. 1. Interaction of peritoneal exudate cells with microorganisms. a) 30 min after injection of microorganisms into peritoneal cavity, formation of processes can be seen in polymorphs, with uptake of microorganisms (Mo) and an increase in number of digestive vacuoles (V); b) increase in number of digestive vacuoles (V) and lysosomes (LS) and incorporation of microorganisms (Mo) into MPH. 12,000 ×; c) 60 min after injection of staphylococci. Single contacts of microorganisms (Mo) with surface of leukocyte and their penetration by invagination into cell cytoplasm (PNL). Most microorganisms (Mo) are in a state of division; d) after single penetrations of microorganisms into cytoplasm of leukocytes and Mph their number in nearly every vacuole (V) is doubled. 12,000 ×.

EXPERIMENTAL METHOD

Aseptic peritonitis [12], whose morphology has been adequately studied experimentally [2], was chosen as the model of the inflammatory focus. A particular feature of this model is that it is possible to obtain an adequate number of Mph as well as polymorphs in the exudate as early as the end of the 1st or 2nd days of the experiment. Experiments were carried out on 12 noninbred male rats weighing 200-250 g. The animals were given an intraperitoneal injection of 2 ml of 3% peptone solution mixed with 0.2 ml of xylol. After 36 h the peritoneal cavity was flushed out with 6 ml of medium 199. The peritoneal washings, containing a suspension of cells, were mixed with 2 ml of a 1×10^9 suspension of a pathogenic staphylococcus (strain No. 82). Taking into account the data of Cohn [9] showing death of 96% of bacteria takes place 1 h after their contamination with polymorphs, and that mortality is complete after 2 h, and wishing to trace the process of phagocytosis over a period of time, after different periods of contamination of the microorganisms with exudate cells in the experiment (after in-

cubation of the mixture of microorganisms and peritoneal exudate — three series of experiments) at 37°C for 30, 60, and 90 min respectively, the resulting mixture was centrifuged at 1000 rpm for 5 min. The residue was fixed in 2% glutaraldehyde solution and postfixed in 1% OsO_4 solution. After dehydration, the resulting material was embedded in a mixture of Epon-812 and Araldite. Ultrathin sections were examined in the JEM-100cx transmission electron microscope. For quantitative evaluation of the time course of phagocytosis the number of polymorphs and Mph was counted in five sections from each block and the phagocytic number (PN) and phagocytic index (PI) were determined.

EXPERIMENTAL RESULTS

The relative numbers of polymorphs and Mph in the exudate obtained 36 h after the beginning of the experiment were 70 ± 1.6 and 30 ± 1.2% respectively. After contamination of the microorganisms with the exudate for 30 min about half of the polymorphs remained intact. The structure of these cells was characterized by many granules, by a good state of preservation of the intracellular organelles, and by a small number of processes. They were surrounded by a number of unchanged staphylococci. Various phases of phagocytosis could be clearly seen in many cells. Numerous processes and invaginations, with staphylococci adjacent to the cell membrane in contact with them, could be seen in the polymorphs.

The following fact is noteworthy: everywhere in the invaginations of the polymorphs staphylococci are single, i.e., only single cocci are taken up during ingestion. Some cocci are located in the cytoplasm of the leukocyte beneath the cell membrane. They also are single. More than 80% of polymorphs taking part in phagocytosis contain many cocci in their cytoplasm; most of the latter are located in digestive vacuoles. The structure of many of the cocci is preserved, and some of them are dividing. The number of polymorphs in a state of destruction is small, not more than 10% of their total number. PI was 43 ± 1.2 and PN 6.3 ± 0.8 (Fig. 1a).

Among the macrophages most were intact cells with many mitochondria and a well marked Golgi complex. Their cytoplasm contains many large vacuoles, either optically empty or filled with finely granular contents. Signs of phagocytosis are present in only some Mph; adhesion, ingestion, and digestion of the phagocytosed microorganisms. PI was 20 ± 2.1 and PN 3.9 ± 1.8 (Fig. 1b).

After contamination of the microorganisms with the exudate for 60 min, the degree of phagocytosis reached a maximum. Among the polymorphs more than 60% were in various phases of phagocytosis; most cells were in phases of ingestion and digestion. Some polymorphs were literally "stuffed" with microbial particles, located mainly in phagosomes. Different phases of division of cocci can be seen in many of them, with two to four cocci present in each. In some vacuoles capsule formation around the cocci can be seen. PI was 62 ± 2.3 and PN 8 ± 1.9 (Fig. 1c).

At this stage of contamination a marked increase was observed in the number of macrophages involved in phagocytosis (PI 45 ± 1.8, PN 7.2 ± 1.4). Most macrophages show morphological signs of high functional activity: many cytoplasmic processes and an increase in the number of lysosomes and vacuoles. The number of Mph in phases of ingestion and digestion of microorganisms was much greater than at previous periods of contamination. Here also, just as in the polymorphs, invagination and ingestion only of single cocci are in progress. However, the digestive vacuoles contain not only single cocci, but also groups of them as well as dividing microorganisms. Many cocci lie freely in the cytoplasm, but no capsule has formed around them, only thickening of the cell membranes of some of the bacteria. Various phases of capsule formation can be seen in many cocci lying in the vacuoles (Fig. 1d).

After contamination of the microorganisms with exudate for 90 min the number of polymorphs exhibiting phagocytosis in the exudate was reduced a little (PI 38 ± 2.1, PN 6.1 ± 1.1). These same parameters of phagocytosis in Mph remained quite high (PI 40.2 ± 2.0, PN 8.9 ± 1.5). The morphological picture of incomplete phagocytosis could be seen in several polymorphs and some Mph (Fig. 2). Signs of cellular destruction also were seen in many polymorphs and a few Mph.

Thus in the experimental model a clearly defined picture of interaction of polymorphs with Mph in phagocytosis of pathogenic staphylococci can be clearly observed. Whereas in the early stages of development of this process signs of leukocytic phagocytosis predominated, later, Mph were increasingly more involved in the process. This is evidence that the behavior of phagocytic cells in the microphage—macrophage system is more flexible. Considering data [3] showing that polymorphs can ingest destroyed collagen, fibrin, and antigen complexes, this suggests that polymorphs and Mph are not so strictly determined in the process of phagocytosis,

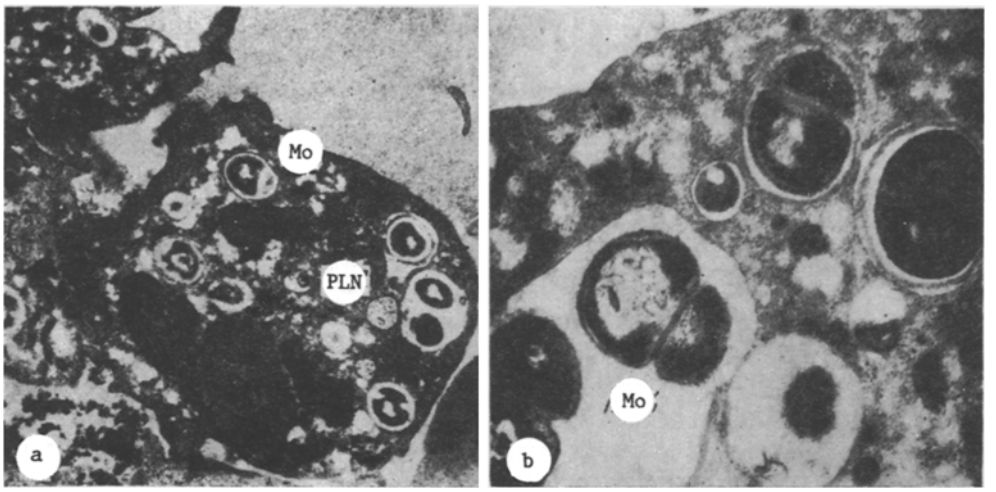

Fig. 2. After microorganisms have been present for 90 min in peritoneal exudate. a) Destruction and lysis of microorganisms (Mo) in cytoplasm of polymorph (PLN). 12,000 ×; b) discovery of unchanged microbial cells with capsules (Mo) in digestive vacuoles of macrophage reflects a picture of incomplete phagocytosis. 32,000 ×.

and that if one of them is "deficient" compensatory "replacement of its functions" takes place by cells involved in that process. The possibility that Mph are extensively involved in phagocytosis is evidently due to the close similarity between the composition of the set of lysosomal enzymes of pH and enzymes of the cytoplasmic granules of the polymorphs [11].

The mechanism of ingestion of staphylococci by phagocytes "single" with the formation of a vacuolar inclusion initially for each microbial cell, also is interesting. Division of microorganisms and an increase in their number are observed later in these single vacuoles. The presence of a capsule and thickening of the cell membrane, found in many bacteria in vacuoles, is observed under these circumstances. The morphological components ultimately guard the microorganisms against the lysosomal enzymes of the cell. Thus during incomplete phagocytosis, microorganisms may accumulate in some macrophages and leukocytes during the first 30 min. These morphological data confirm the hypothesis of Sarkisov and co-workers [5] that polymorphs and Mph with signs of incomplete phagocytosis may play the role of "depots" of infection, and that this factor has an important role in the development of chronic infections and of sepsis. Moreover, as a result of this experiment an original model has been developed, whereby leukocytes and macrophages can be obtained simultaneously from an inflammatory focus, by means of which interaction of leukocytes and Mph with microorganisms and the phases of phagocytosis can be studied qualitatively and quantitatively from the dynamic aspect.

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