CHANGES IN ACTIVITY OF HYDROLYTIC ENZYMES IN TISSUE CULTURES OF BONE-MARROW MACROPHAGES ACCOMPANYING INTRACELLULAR PARASITIZATION BY Salmonella typhosa

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Parasitization by Salmonella typhosa in macrophage cultures leads to an initial increase in activity of the lysosomal enzymes cathepsin C and acid phosphatase, followed by a decrease in this activity and by destruction of the lysosomes. Activity of the cytoplasmic enzymes of the macrophages (aliesterase, lipase, lipoprotein lipase, aminopeptidases, alkaline phosphatase) is reduced in the early periods after infection of the culture.

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Recent findings [1-3, 4, 6] suggest that the typhoid carrier state is based on parasitization of cells of the reticulo-endothelial system by the infecting agent, which is associated, among other factors, with a disturbance of cellular immunity. It is thus important to determine the principles governing the relations between the infecting agent and cells of the reticulo-endothelial system.

The object of the present investigation was to study the activity of some hydrolytic enzymes of macrophages during intracellular parasitization by Salmonella typhosa.

EXPERIMENTAL METHOD

Tissue cultures of macrophages from the bone marrow of guinea pigs were used. A primary explant culture was infected with S. typhosa in doses of 100-200 million cells/ml medium No. 199 with the addition of 20% inactivated and absorbed bovine serum. Periodic studies were made of the activity of the lysosomal enzymes cathepsin C and acid phosphatase, and also of enzymes connected with the endoplasmic reticulum: aliesterase, lipase, leucine-aminopeptidase, and alanine-aminopeptides.

EXPERIMENTAL RESULTS

In uninfected cultures of macrophages the activity of the lysosomal enzymes (acid hydrolases) rose significantly with an increase in the period of cultivation from 1 to 6 days (Fig. 1), whereas the activity of the neutral hydrolases (nonlysosomal enzymes) gradually fell.

Marked changes in enzyme activity of the macrophages took place in cultures of cells infected with S. typhosa (Fig. 2). On the first day after the beginning of contact with the infecting agent, a decrease in activity of the neutral hydrolases distributed throughout the cytoplasm took place. This concerned particularly the amino peptidases, which (in any event their activity in macrophages is low) completely ceased to be detectable in the infected cells. On the other hand, the lysosomal enzymes (cathepsin C and acid phosphatase) became activated on the first or second day after infection in most cells, and this was accompanied by the appearance of juxtanuclear foci of brightly stained, and apparently slightly enlarged lysosomes against the background. The distribution of these particles was unchanged at this period of the experiment.

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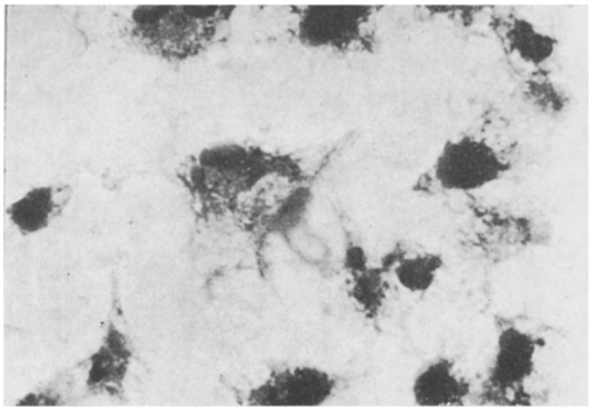

Fig. 1. Moderate activity of cathepsin C in uninfected cells after cultivation for three days: enzyme distributed as poorly distinguishable granules (lysosomes) near the nucleus. Pearse's reaction, 280×.

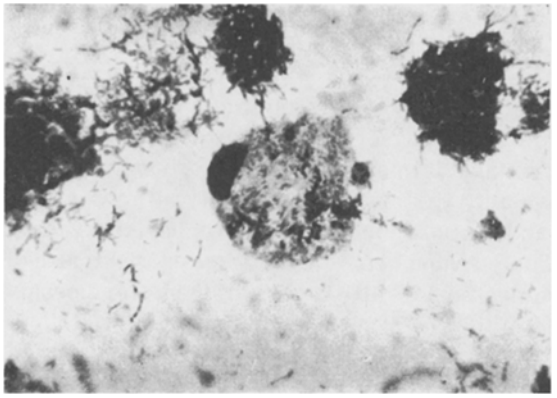

Fig. 2. Multiplication of S. typhosa in macrophages. Giemsa – Romanovsky, 280×.

In the later periods after infection, and in some cells which contained a large number of bacteria as early as the first or second day of the experiment, a further decrease in intensity of the reactions for neutral hydrolases occurred throughout the cytoplasm, and also of reactions for cathepsin C and acid phosphatase in the juxtanuclear focus. Meanwhile a distinctive picture was observed: the penetration of lysosomes, increased in number and size, beyond the boundaries of the juxtanuclear focus into the adjacent cytoplasmic zone. The brightly stained lysosomes stood out clearly against the unstained background of the cytoplasm (Fig. 3).

The number of lysosomes remaining within the juxtanuclear focus was reduced, but their staining properties in reactions for acid hydrolases were unchanged.

When degeneration of the macrophages was far advanced, as shown by the fact that they were packed with bacterial cells, which was usually observed in the late periods after infection (4th-5th days) a further decrease in intensity of the reaction for acid hydrolases could be seen in the juxtanuclear zone, with the almost complete absence of reactions for aliesterase throughout the cytoplasm. A further migration of lysosomes, detected by their reactions for acid phosphatase and cathepsin C, could be observed. They had almost completely left the juxtanuclear zone and were now in the middle and outer zones of the cell where vacuolation was taking place, and they had also penetrated into the vacuolated processes. It is interesting to note that the reaction for acid hydrolases in these parts of the cell, and consequently the intensity of staining of the lysosomes, were not reduced. Neither aliesterase nor acid hydrolases could be found in the dying cells, which were predominant in the latest periods after infection (4th-7th day). Lysosomes with indistinct outlines, weakly stained by the reaction for cathepsin C, could be observed only in the outermost, vacuolated part of the cytoplasm and in the processes; it was as if the lysosomes had melted. The reaction for acid phosphatase in such cells was negative, evidently because of the lower sensitivity of this reaction. Hence, at this period of the experiment, the final stage of activity of the lysosomes could be observed as phagocytosis of the bacteria was carried out by the cells: their transition into an outer zone of cytoplasm packed with bacteria and, evidently, the lysis of these organelles, discharging their enzymes onto the phagocytosed objects [9, 13]. Destruction of the lysosomes was probably caused by the endotoxin of S. typhosa, which is capable of dissolving the lysosome membranes [11]. It is interesting to note that a similar pattern of behavior of lysosomes has been observed by other workers, both during cultivation of uninfected macrophages and after introduction of various bacterial lipopolysaccharides and viruses into the medium [7, 8, 10, 12].

A similar sequence of enzyme changes was also observed in the cells after introduction of an antiserum into the system or when the cells used were taken from immune animals. The only differences observed were quantitative, an increase or decrease in the rate of changes in the lysosomes. In the presence of antiserum, for instance, presumably because of stimulation of phagocytic activity [5], in some cases the microorganisms accumulated more rapidly in the cells, the activity of the neutral hydrolases fell sooner, and pictures of lysosome destruction and massive death of the cells appeared 2-3 days after infection.

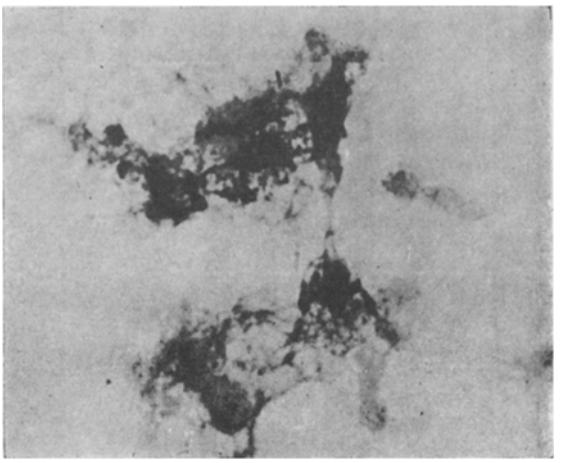

Fig. 3. Macrophages infected with S. typhosa (period of cultivation 3 days): penetration of lysosomal granules can be seen into outer, vacuolated part of cell (upper cell) and progressive decrease in activity of lysosomal enzymes with disappearance of pattern of lysosomes (flower cell). Pearse's reaction for cathepsin C, 280×.

The picture was different when immune cells were parasitized by the microorganisms. Despite the fact that phagocytosis was just as intensive as in the control, the activity of the lysosomal enzymes persisted rather longer than in infected macrophages from nonimmune animals. These differences did not apply to the endoplasmic enzymes (neutral hydrolases), whose activity fell just as rapidly as in the control. Frequently, against the background of marked degeneration and vacuolation of the macrophages, accompanied by intensive multiplication of bacteria in the cells and by complete absence of a reaction for neutral hydrolases, brightly stained (reactions for acid hydrolases) lysosomes could still be seen in the outermost part of the cytoplasm and in the cell processes.

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