NOTES

Surface Morphology of Peritoneal Macrophages During the Attachment of Brucella melitensis

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Received for publication 8 August 1973

When macrophages from immunized rabbits are in the act of phagocytosis of the homologous organism, the macrophages are seen by scanning electron microscopy to possess undulating membranes and microvilli at various stages of formation and spatial relationships to the organisms.

We report here our observations of the attachment of a particle by surface membranes as seen by scanning electron microscopy and extend earlier observations by light and transmission electron microscopes (3-6).

Rabbit peritoneal mononuclear cells, oil-induced, were harvested 3 weeks or more after subcutaneous injection of 1 \times 10^8 living Brucella melitensis, strain Rev. 1, cells. Mononuclear cells (5 \times 10^4 per ml) in 20% homologous antiserum and 80% Tyrode solution were implanted overnight onto small, flying cover slips before infection with heat-killed brucellae in the ratio of cell-bacilli of 1.5 \times 10^9. This large ratio assisted in the microscopy detection of the microorganisms. Phagocytosis proceeded for 2 h before fixation. A Pearse tissue dryer (Edwards High Vacuum Ltd.) was used to remove water (7).

The monocyte surface is covered extensively with undulating membranes which have formed innumerable interstices (Fig. 1). Some of the membranes possess a cup-like appearance. Numerous brucellae attached to the distal or terminal edges of these membranes are readily visible.

A stereoscope is essential to see the structural details presented in Fig. 2-4. The broad expanse of the undulating membrane is especially evident in the upper, right quadrant of the stereophotograph (Fig. 2). One membrane (see arrow number 1) appears to have arisen along a winding, broad front on the cell surface. When viewed on edge, the membranes take on a ridge-like or flattened microvillus-like appearance (arrow number 2). Arrow number 3 points to a clump of brucellae attached to undulating membranes and located within crevices formed by these membranes. The tip of one membrane appears to have attached to the upper, exposed surface of one brucella. Brucellae at various stages of adherence can be seen at the lower, right quadrant of Fig. 2. Arrow number 4 points to an organism which is situated in a network of membranes. One undulating membrane is membranous at its base (arrow number 5), but appears to terminate in a "microvillus" type structure.

Numerous brucellae at various stages of adherence can be seen in Fig. 3; some are located on the surface of membranes while others lie within crevices. These crevices suggest the beginning of the formation of phagosomes or phagocytic vacuoles.

The surface of a small number of peritoneal exudate cells appears to be predominantly composed of microvilli (Fig. 4) as opposed to the undulating membranes (Fig. 1-3) present on the majority of the cell population. It is not known whether the former cell type was engaged in or was capable of phagocytosis.

The basic surface morphology of the macrophage from immunized animals is very similar to that reported earlier for the peritoneal macrophage derived from normal animals (7) and to the monocyte from circulating blood (1). The greater prominence of the microvilli on the cell in Fig. 4 is worthy of comment, and the question remains as to the final identification of this cell. The microvilli (Fig. 4) may be similar to the finger-like processes on macrophages recently reported by Carr and Carr (2).
Fig. 1. *Brucella melitensis* strain Rev. 1 attached to the undulating membranes of a macrophage derived from a homologous, immune rabbit. ×3,900; bar represents 1 μm.

Fig. 2. Stereophotograph showing detailed surface morphology of the macrophage seen in Fig. 1. ×17,000; bar represents 1 μm.
Fig. 3. Stereophotograph of a macrophage in the process of phagocytosing brucellae. ×21,500; bar represents 1 μm.

Fig. 4. Stereophotograph of a peritoneal exudate cell with surface microvilli. ×18,900; bar represents 5 μm.
This investigation was supported by Public Health Service research grant AI-00022 from the National Institute of Allergy and Infectious Diseases and in part by the United States Atomic Energy Commission. We thank T. E. Everhart for the generous use of the scanning electron microscope which was purchased under Public Health Service grant GM-17523 from the National Institute of General Medical Sciences and operated under National Science Foundation grant GB-6428.

The technical assistance of M. Maglio and H. Sampson is gratefully acknowledged.

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