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SCRUB TYPHUS: MECHANISM OF INFECTION AT THE CELLULAR LEVEL,(U)
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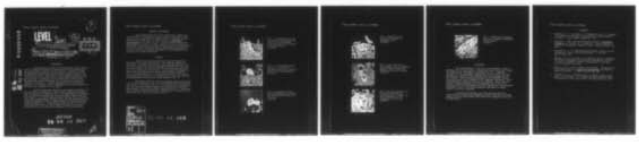

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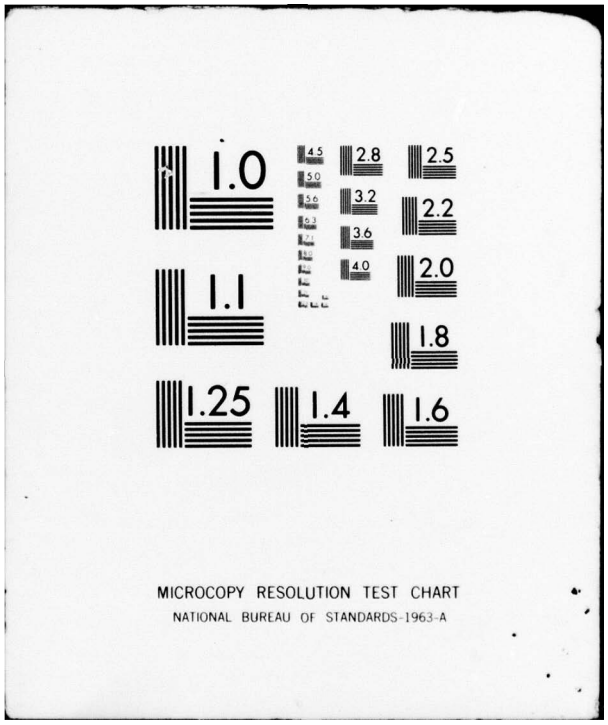
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*EWING, TAKEUCHI, SHIRAI, and OSTERMAN

SCRUB TYPHUS: MECHANISM OF INFECTION AT THE CELLULAR LEVEL

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INTRODUCTION

Scrub typhus is an acute infectious disease of man that has caused significant morbidity in troops operating in Southeast Asia during World War II and the Vietnam conflict. Infection is caused by Rickettsia tsutsugamushi and is acquired through the bite of an infected larval mite. Rickettsiae, living organisms similar to but smaller than most bacteria, require an intracellular environment for multiplication. Rickettsiae must therefore leave infected cells and enter other cells efficiently for an infection to proceed and disease to be produced. A better understanding of the infection mechanism will hopefully lead to improvement of existing measures for prevention and treatment of scrub typhus.

Previous morphologic studies of scrub typhus rickettsiae in various types of cultured cells have revealed organisms protruding from the surfaces of undamaged cells (2,6), suggesting the possibility that rickettsiae can escape from cells by budding, leaving the host cells intact. It is known that susceptible mice as well as cultured cells can be experimentally infected (1,5), and that mesothelial cells lining the abdominal cavity of these animals will vigorously support rickettsial growth (4). In the present study, we focus on infected mouse mesothelial cells to reveal events occurring at the ultrastructural level as an infection progresses in the living animal.

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MATERIALS AND METHODS

Adult female BALB/c mice (Flow Laboratories, Dublin, Va.) were divided into infected and control groups of four animals each. Infection was accomplished by injection into the abdominal cavity of 1000 50% mouse lethal doses of the Karp strain of Rickettsia tsutsugamushi in a volume of 0.2 ml. Control animals received the same volume and dilution of normal, uninfected yolk sac suspension. All animals were killed by cervical fracture on day 10 postinoculation. Portions of spleens bearing peritoneal mesothelium were collected, fixed in chilled half-strength Karnovsky's (3) glutaraldehyde, and processed for electron microscopy.

RESULTS

Rickettsiae were often numerous within the cytoplasm of mesothelial cells from infected mice. The organisms appeared as pleomorphic coccobacilli bounded by a double membrane made up of an outer cell wall and an inner cell membrane separated from each other by a narrow space. The interior had a mottled, granular structure with a loose network of fine fibrils. An occasional rickettsia was found bulging up beneath the host cell plasma membrane slightly (Fig. 1) or markedly (Fig. 2). Rickettsiae were also found free of any apparent cell connection, yet covered by an extra, third membrane indistinguishable from host cell plasma membrane (Fig. 3).

Rickettsiae bearing a third coat appeared within mesothelial cell surface invaginations (Fig. 4) and within membrane-lined vacuoles near the free cell surface (Fig. 5). On occasion, the extra membrane coat and the vacuole membrane appeared as discontinuous, electron-dense fragments encircling an intact rickettsia (Fig. 6). Rickettsiae showing central annular constriction characteristic of binary fission were always free within the cytoplasm (Fig. 7).

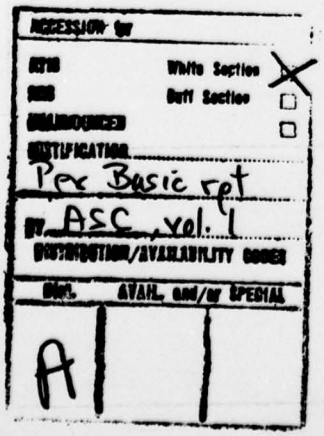

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Fig. 1 A rickettsia has moved to a position just beneath the host cell plasma membrane. Double membrane of the organism is clearly visible. (x33,000)

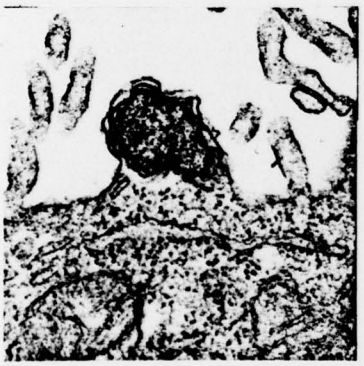

Fig. 2 An organism protrudes from the free surface of a mesothelial cell, still covered by host cell plasma membrane. (x33,000)

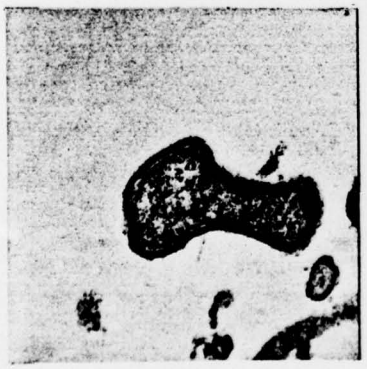

Fig. 3 Rickettsia in extracellular environment, enveloped by third membrane derived from host cell. (x33,000)

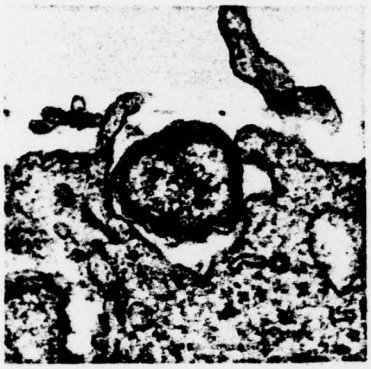

Fig. 4 Phagocytosis of enveloped rickettsia by mesothelial cell. (x33,000)

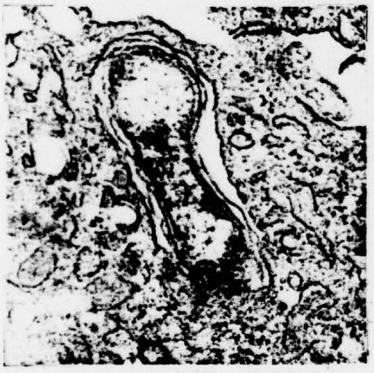

Fig. 5 Still enclosed by a host membrane coat, an organism appears within a phagocytic vacuole near the cell surface. (x33,000)

Fig. 6 The host membrane coat and vacuole membrane are disintegrating to liberate an intact organism into the cytoplasm of a host cell. (x33,000)

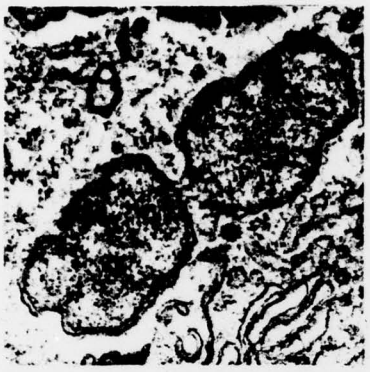

Fig. 7 Rickettsia free within host cell cytoplasm, undergoing binary fission. (x33,000)

DISCUSSION

The plasma membrane of the host cells appeared to play an important role in the spread of rickettsiae from one host cell through the extracellular environment to another host cell. Organisms multiplied in the cytoplasm of mesothelial cells, moved to the cell periphery and acquired a host membrane coat as they budded from the cell surface. Free rickettsiae enveloped by this membrane entered other mesothelial cells, apparently by a phagocytic mechanism. Entry of rickettsiae lacking this coat was not observed. After internalization, organisms escaped from the phagocytic vacuole into the cytoplasm as the vacuole membrane and host membrane coat disintegrated. Rickettsiae devoid of extraneous membranes replicated by binary fission in the cell cytoplasm.

A host-derived membrane coat enveloping extracellular rickettsiae may stabilize the organisms, protect them from the immune system of the host and promote their entry into susceptible host cells by phagocytosis.

REFERENCES

1. Catanzaro, P. J., A. Shirai, P. K. Hildebrandt, and J. V. Osterman. Host defenses in experimental scrub typhus: Histopathological correlates. Infect. Immun. 13:861-875, 1976.

2. Higashi, N. 1962. Electron microscopic studies of Rickettsia orientalis, p. 145-156. In T. Tamiya (ed.), Recent advances in studies of tsutsugamushi disease in Japan. Medical Culture, Inc., Tokyo.

3. Karnovsky, M. J. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27:137A-138A, 1965.

4. Kokorin, I. N., C. Din Kyet, N. G. Kekcheeva, and E. D. Miskarova. Cytological investigation on Rickettsia tsutsugamushi infection of mice with different allotypic susceptibility to the agent. Acta Virol. 20:147-151, 1976.

5. Kundin, W. D., C. Liu, P. Harmon, and P. Rodina. Pathogenesis of scrub typhus infection (Rickettsia tsutsugamushi) as studied by immunofluorescence. J. Immunol. 93:772-781, 1964.

6. Schaechter, M., F. M. Bozeman, and J. E. Smadel. Study on the growth of rickettsiae. II. Morphologic observations of living rickettsiae in tissue culture cells. Virology 3:160-172, 1957.