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Light and Electron Microscopic Studies on the Attachment of *Ureaplasma urealyticum* to Human Leukemia Cell

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ABSTRACT

Interaction between human leukemia cell and *Ureaplasma urealyticum* was investigated light and electron microscopically. The cells adhered with their pseudophodia to the surface of colonies of *Ureaplasma urealyticum*. A large number of pleomorphic *Ureaplasma urealyticum* adhered to the cell surface and were seen in the intracellular vacuoles. *Ureaplasma urealyticum* was captured by the villus-like structure of the cells and actively phagocytized.

Recent studies recognize and accept that the adherence of bacteria to mucosal epitherial surface is the initial stage in the pathogenesis of most bacterial infections[2]. During the last decade, the adherence of *Mycoplasma pneumoniae* and some other mycoplasmas to cells of human and animal origin has been evaluated extensively[1,4,5,8,9]. Mycoplasmas adhere to and colonize on the epitherial cell surfaces of the infected organ, but rarely invade the tissue and bloodstream, so that the pathogenic mycoplasmas are considered to be membrane parasites[7,11].

Little information is available about the interaction between *Ureaplasma urealyticum* and eukaryotic cells. The present paper describes the attachment of *Ureaplasma urealyticum* to human leukemia cells and intracellular localization of the organism.

The strain of *Ureaplasma urealyticum* T 960 was kindly supplied by Dr. J.A. Robertson, Department of Medical Microbiology, University of Alberta, Alberta, Canada. The established cell line of Human Leukemia Cell K 562 was a gift from Dr. S.D. Ihm, Kyung-Hee Medical Center, Immunology Laboratory, Seoul, Korea. *Ureaplasma urealyticum* was cultured in a standard liquid medium 10-B formulated by Shepard and Lunceford[14]. A 0.2 ml of stocked culture of *Ureaplasma urealyticum* was inoculated in 2 ml of the standard liquid medium, and incubated at 37°C for 18 hr. The culture was serially diluted in 2 ml of the medium from 10^{-1} to 10^{-3}, and a 0.01 ml of each dilution was inoculated on a A_7 agar plate[13] and incubated in a gas pak system at 37°C for 48 hr. The plate was observed for the colonial growth of the organism under a 100-fold magnification with a light microscope. K 562 cells were cultured in RPMI 1640 medium (Grand Island Biological Co., U.S.A.) supplemented with 10 % fetal calf serum (Grand Island Biological Co., U.S.A.). After developing into a confluent monolayer, the cells were washed, trypsinized and suspended in phosphate buffered solution (PBS) at a density of $1x10^6$ cells per ml. On the plate with *Ureaplasma urealyticum* growth, several penicillin cups were placed for each to cover five to ten colonies. Three drops of previously prepared cell suspension were poured into each cup and

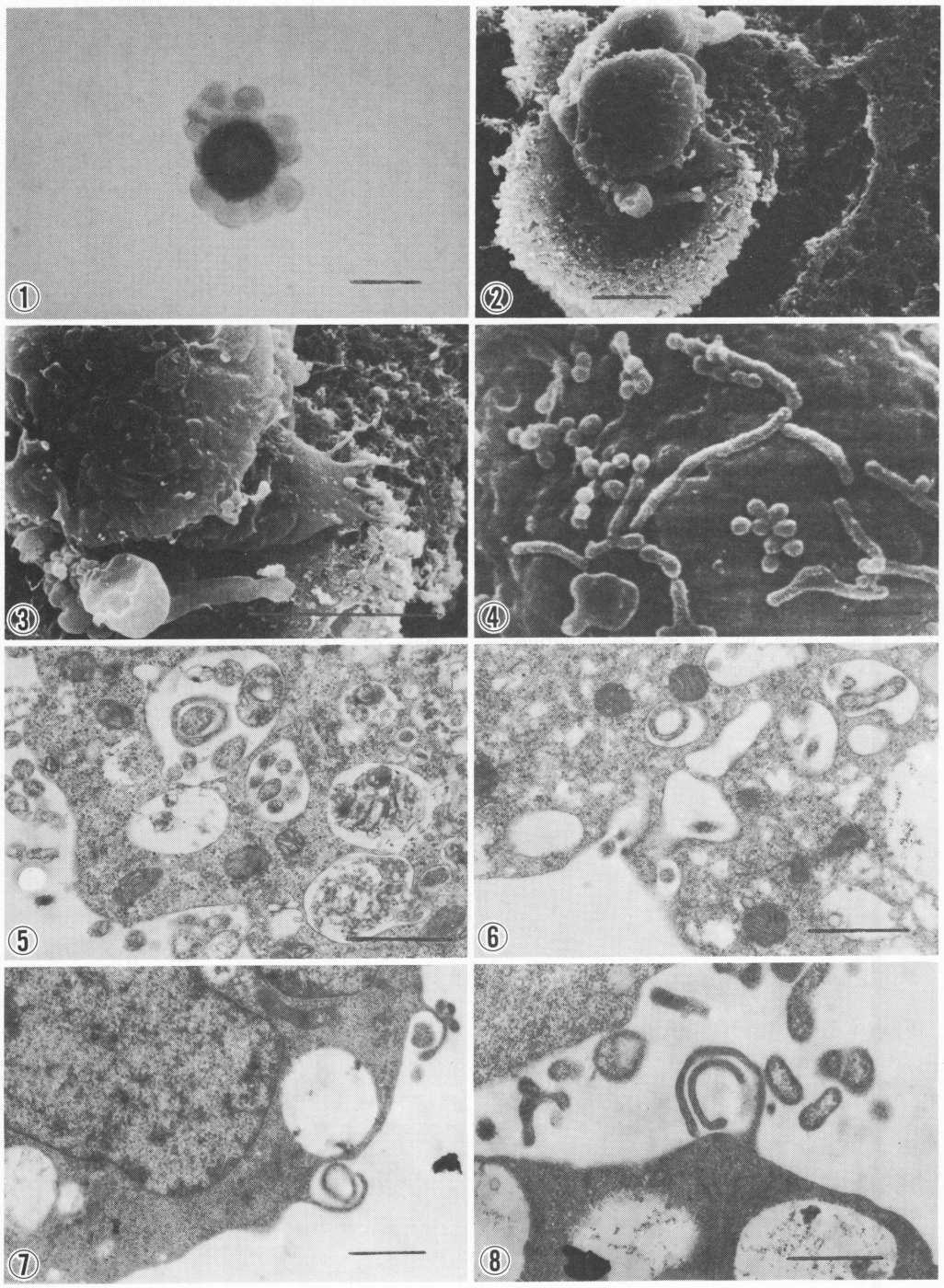
incubated for two hr at 37°C. After the incubation, the penicillin cup was removed and the plate was washed three times with PBS. The adherence of K 562 cells to colonies of *Ureaplasma urealyticum* was examined by direct microscopic observation with a 100-fold magnification. The colonies of *Ureaplasma urealyticum* adhered K 562 cells were fixed with 2 % glutaraldehyde and 1 % osmium tetraoxide at 4°C overnight, washed first with PBS and then with distilled water, dehydrated with acetone and dried by the critical point drying technique. The samples were coated with Au-Pd and examined with a scanning electron microscope (JSM T 3000, JEOLCO, Tokyo, Japan, 15 kv.). On the other hand, a 5.0 ml volume of K 562 cell suspension was mixed with 10 ml of 18 hr culture of *Ureaplasma urealyticum* and incubated for 32 hr at 37°C. After the incubation, the mixture was centrifuged at 1,000 rpm for 10 min and the pellet was washed three times with PBS. The sedimented cells were fixed with 2 % glutaraldehyde and 1 % osmium tetraoxide, and washed three times with PBS and distilled water. The cells were suspended in 5 ml of PBS and divided into two portions. One portion was subjected to scanning electron microscopic observation in the same way as mentioned above. Another portion was embedded in 1 % agar and cut into 3 mm^2 agar blocks. The agar blocks were dehydrated with ethanol and embedded in Epok. The samples were ultrathin-sectioned, stained with UA-LC and observed with a transmission electron microscope (TEM 200 CX, JEOLCO, Tokyo, Japan, 100 kv).

Fig. 1 shows K 562 cells adhered to an ureaplasma colony 2 hr after incubation. Scanning electron micrographs confirmed that the cells adhered with their pseudophodia (tip structure) on the surface of the colony as shown in Figs. 2 and 3. Fig. 4 demonstrates many particles of *Ureaplasma urealyticum* on the surface of the K 562 cell incubated for 32 hr. Spherical forms of the particle were 0.2-0.4μm in diameter and rod forms were 0.5-2.5μm in length. In ultrathin-sections of K 562 cells, *Ureaplasma urealyticum* appeared pleomorphic and was located close to or directly on the cell surface. In addition, the organisms attached to the cells via membrane to membrane as shown in Figs. 5 and 8. Intracellular vacuoles were frequently seen where the pleomorphic *Ureaplasma urealyticum* was present (Figs. 5 and 6). Occasionally the organisms were actively phagocytized by the cells (Fig. 6), and the villus-like structure of the cells captured them directly (Figs. 7 and 8).

Shepard[12] reported that *Ureaplasma urealyticum* was successfully grown in experimentally infected HeLa cells, and that light microscopic observation revealed a progressive and degenerative cytopathogenic infection. Horikawa et al[6] observed the attachment of HeLa cells to colonies of *Ureaplasma urealyticum* by light microscopic observation. According to Nakamura et al[10] *Ureaplasma urealyticum* attached to the surface of MDBK and human leukemia cells or existed intercellularly but the organisms were not seen intracellularly by electron microscopic observation.

The present study demonstrated that human leukemia cells adhered to the surface of colonies of *Ureaplasma urealyticum* by their pseudophodia and that the organisms attached to the cell surface via membrane to membrane. The organisms were actively phagocytized by the cells or captured by the villus-like structure of the cells. The fate of phagocytized organisms is under investigation.

Figures. 1) The human leukemia cells adhered to a colony of *Ureaplasma urealyticum*. Light micrograph (the bar: 35μm). 2) Ibid. Scanning electron micrograph (the bar: 10μm). 3) Scanning electron micrograph (the bar: 10μm). Tip structures of the cell adhered to an ureaplasma colony. 4) Ibid. (the bar: 1 μm). Pleomorphic ureaplasmas adhered to the cell surface. 5) Transmission electron micrograph (the bar: 1 μm). Many ureaplasmas are seen in vacuoles within the cell. 6) Ibid. Ureaplasmas actively phagocytized by the cell. 7) Ibid. Ureaplasmas captured by villus-like structure of the cell. 8) Ibid.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