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Ingestion of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* by Human Peritoneal Mesothelial Cells

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In the present study we examined whether mesothelial cells can ingest and digest bacteria. The results showed that all strains were ingested. Ingested staphylococci proliferated abundantly, and only a few were digested. *Escherichia coli*, however, was digested during the first 8 h, whereafter the mesothelial cells disintegrated and proliferation of bacteria could be observed. The clinical implications of these findings are discussed.

Most studies on phagocytosis emphasize the role of macrophages and neutrophils in this process (10). However, it has been demonstrated that stromal cells are also able to phagocytose bacteria (16). This ability indicates that stromal cells play a role in local host defense mechanisms or, alternatively, that these cells can act as a hiding place for bacteria. Which of these phenomena is most important depends on the capacity of stromal cells to ingest and digest the bacteria. Whether these cells are also capable of killing the ingested bacteria is unknown; most studies have found no proof of any intracellular killing (16).

Some cell types, such as epithelial cells in the gut, are frequently exposed to bacteria. It is evident that many stromal cells are able to produce proinflammatory cytokines, such as interleukin-6 (IL-6), IL-8, huGRO, and monocyte chemotactic protein (MCP-1), after stimulation with IL-1β, tumor necrosis factor alpha, or gamma interferon (3, 6, 14, 19). Therefore, the importance of stromal cells in interactions with other cells has been reemphasized (4), and it is now widely accepted that these cells are important in the local host defense mechanisms.

Mesothelial cells (MC), the cells lining the serosal cavities, are stromal cells which are able to produce several cytokines, such as IL-8, IL-6, and MCP-1 (3, 8, 14). Recently, we have reported that MC are able to produce IL-8 after direct stimulation with viable staphylococci (18). MC are less likely to encounter bacteria than, for instance, epithelial cells under physiological circumstances. However, in peritoneal dialysis, a renal replacement therapy, the MC are the first cells to encounter bacteria because they are the most numerous cells in the peritoneal cavity. There are indications that MC are relevant in the prevention of peritonitis in patients treated by peritoneal dialysis because these cells can produce several cytokines (2, 7, 15). Moreover, MC might also be able to eliminate bacteria. This capability would imply a more important role for these cells.

The aim of this study was to investigate whether MC are able to ingest and subsequently kill clinically relevant bacteria in vitro.

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MC were isolated from samples of human omenta, obtained during elective surgery, as previously described (13). Cells were grown on fibronectin (Sigma, St. Louis, Mo.)-coated culture flasks, and 3T3 supernatant was added to the medium as a growth factor (20). All experiments were performed with confluent monolayers of MC in culture passage 1. The purity of the MC was determined by distinct morphological characteristics, such as the presence of microvilli, and positive staining with antibodies against cytokeratins and cancer antigen 125 (17, 20).

MC (2×10^4) were incubated with different bacteria (inoculum of 10^5/100 μl, opsonized and nonopsonized) for 1, 2, 4, 8, 12, 16, and 24 h. All bacteria were isolated from peritoneal dialysis effluents of patients suffering from peritonitis: one isolate each of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*. MC were also incubated for 16 h with latex particles (10^2 to 10^7/ml).

After stimulation, the MC were harvested, washed three times, and processed for transmission electron microscopy (9) and for quantification of the intracellular CFUs by culture of the lysed MC on broth-agar plates. By light microscopy of the material processed for transmission electron microscopy, it was shown that very few adherent bacteria were present.

Ingestion of bacteria by MC. Both *S. aureus* and *S. epidermidis* were ingested by the MC. There was no difference between opsonized and nonopsonized bacteria. The viability of these MC remained relatively high, with an average of 80% after 24 h of incubation with the bacteria. Intracellular proliferation of the staphylococci was observed after 8 h of incubation (Fig. 1a). By 4 h of incubation some of the staphylococci were being digested by the MC (Fig. 1b).

Escherichia coli could be observed intracellularly after 45 min of incubation (Fig. 2a). After 1 h of incubation, digestion of the bacteria started to take place. By up to 8 h of incubation, all of the ingested bacteria had been digested or digestion was taking place (Fig. 2b). After 8 h of incubation, digestion was less efficient and more intact bacteria were present intracellularly. After 16 h of incubation, intracellular proliferation of *E. coli* could be observed. After 18 h of incubation, the majority of the MC had disintegrated (Fig. 2c) and extracellular proliferation of the bacteria was abundant. At 24 h, only MC debris was left among proliferating *E. coli* cells (Fig. 2d).

Quantitative assessment of the intracellular presence of the staphylococci and *E. coli* was demonstrated by culture of the

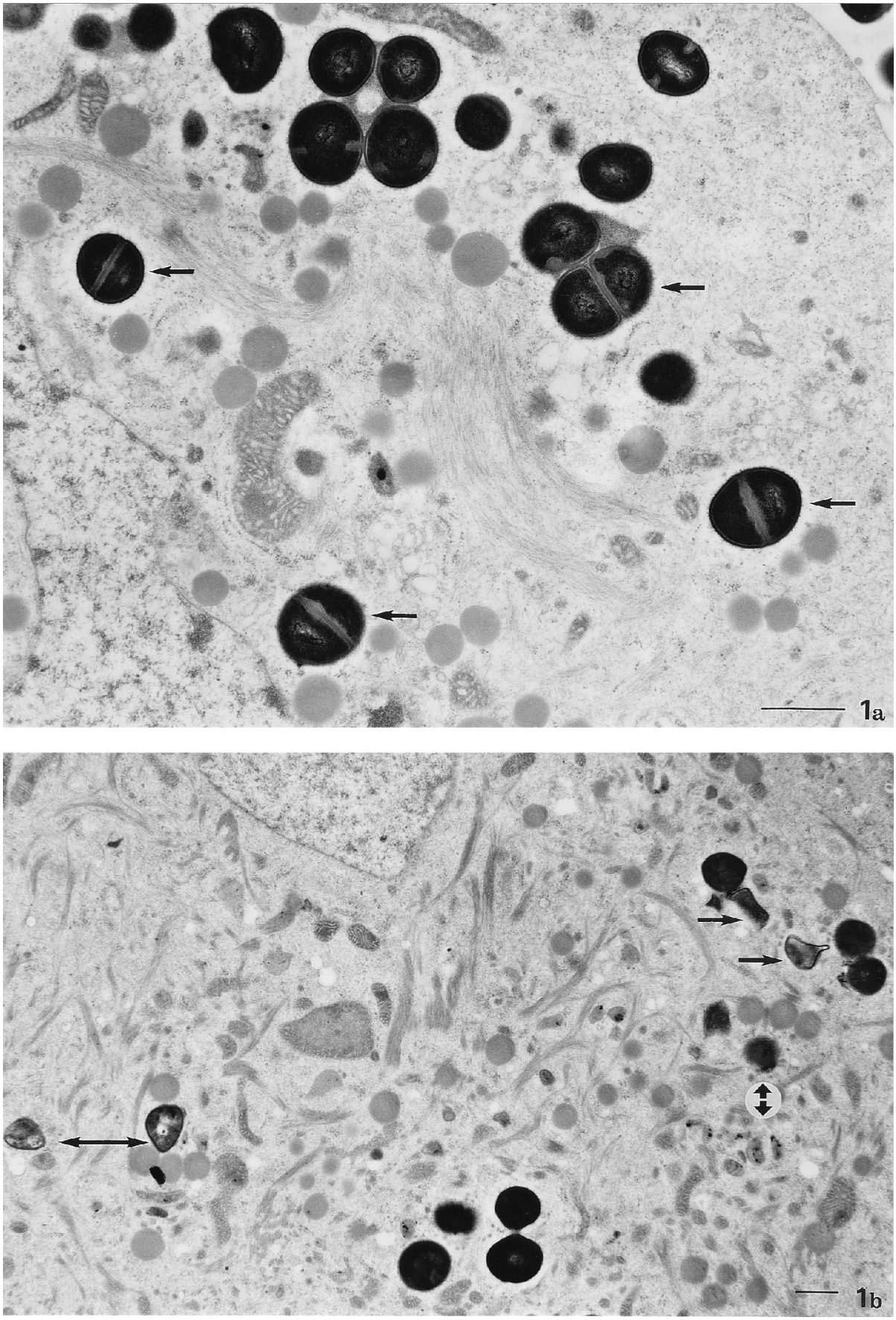

FIG. 1. Electron micrographs (bars, 1.0 μm) of MC after 24 h of incubation with staphylococci. (a) Detail of intracellular proliferation of *S. aureus* (arrows); (b) digestion of *S. epidermidis* by MC (arrows).

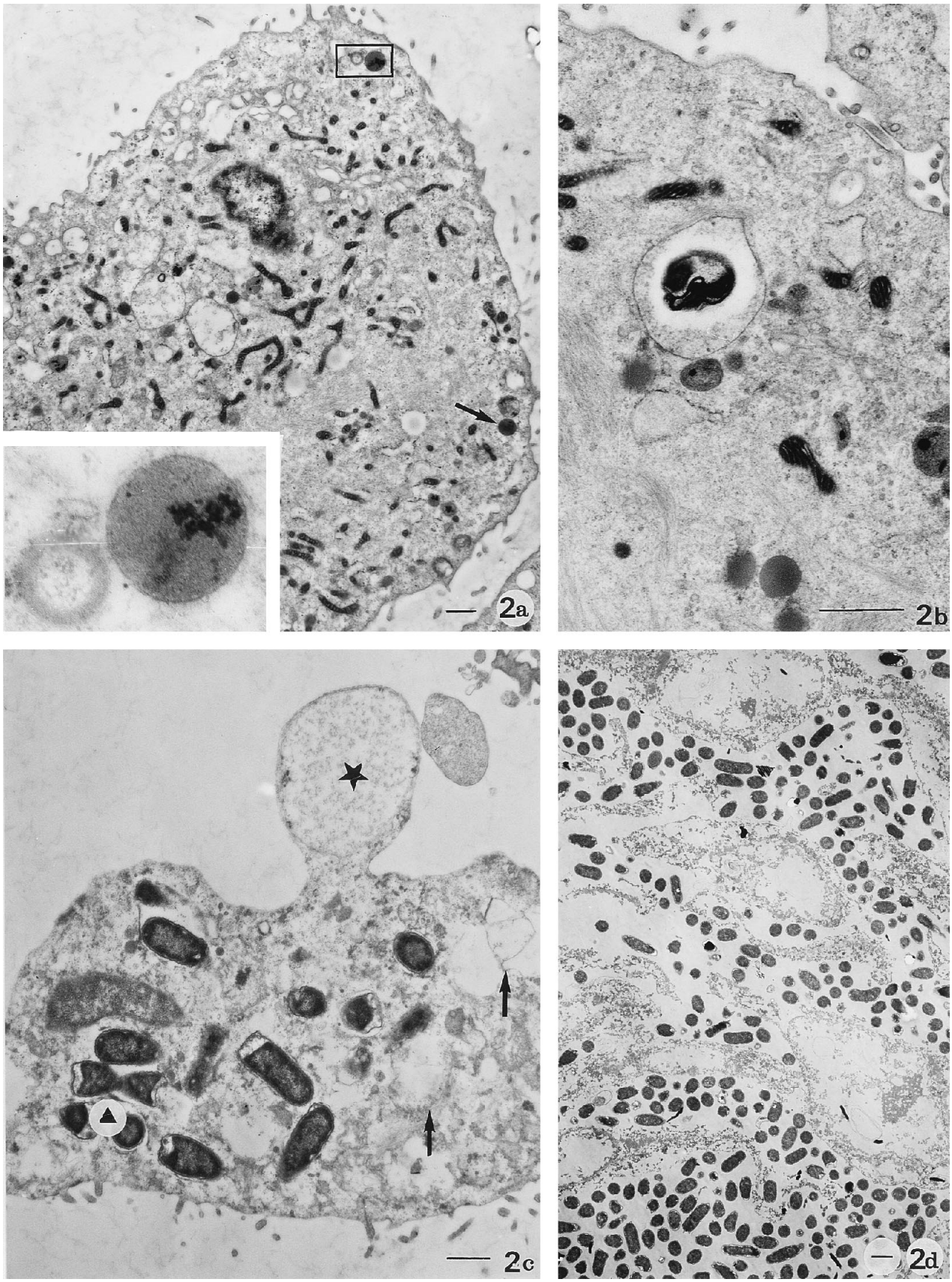

FIG. 2. Electron micrographs (bars, 1.0 μm) after incubation with *E. coli*. (a) Overview of MC after 45 min of incubation with *E. coli*. The intact bacteria are present intracellularly (arrow and inset showing a magnification of the boxed area). (b) Detail of digestion of the bacteria after 8 h of incubation; (c) disintegration of MC, indicated by the presence of blebs (★) and the disruption of lysosomal membranes (arrows) after 16 h of incubation. Intracellular proliferation can also be observed (▲). (d) Massive proliferation of *E. coli* and remaining cell debris after 24 h of incubation.

lysate of MC after 24 and 16 h of incubation, respectively. This was because all MC had disintegrated after 16 h for *E. coli*. The number of intracellular staphylococci reached a maximum of 10^6 CFUs/ml after 24 h of incubation. Shorter incubation periods did not lead to differences in outgrowth between intracellular and extracellular bacteria. The number of intracellular *E. coli* organisms also reached a maximum of 10^6 CFUs/ml after 16 h of incubation. When incubation periods were less than 6 h, no bacteria could be cultured.

Latex particles were also ingested by the MC; however, no cytokine production could be induced and the viability remained about 98%.

MC are the most numerous cells in the peritoneal cavity and are therefore more likely to be exposed to microorganisms than are peritoneal macrophages. With peritoneal dialysis, this chance is markedly enhanced, because the leukocytes present in the effluent are rinsed from the abdominal cavity every 4 to 8 h and the load of invading microorganisms is increased compared with the normal load (7). Therefore, it would be important to elucidate the capacity of MC to eliminate bacteria and to produce cytokines in case of a bacterial invasion. As described above, MC are able to produce cytokines and chemokines after stimulation with proinflammatory cytokines or after direct stimulation with bacteria.

This study demonstrates that MC are able to ingest and kill invading bacteria. However, this elimination is not equally efficient and differs among strains of bacteria. The ingested staphylococci survive and proliferate intracellularly. Nevertheless, the viability of the MC is not dramatically influenced. Moreover, the MC produce IL-8 after direct stimulation with staphylococci (18).

All MC stimulated with *E. coli* died after 24 h of incubation. Although most ingested *E. coli* organisms are digested within the first 12 h, intracellular proliferation can be observed after a 16-h incubation period, and the MC start to disintegrate after 18 h of incubation. Cytokine production induced by the incubation with *E. coli* could not be detected (18).

The ingestion of staphylococci does not lead to abundant digestion but does induce an IL-8 response. This response leads to an influx of neutrophils, which are the most important eliminators of bacteria. The ingestion of *E. coli*, however, induces an active process of digestion of these bacteria. However, this digestion is increasingly less efficient and induces no IL-8 response. The MC subsequently die and are not able to mediate the recruitment of neutrophils. Digestion of *E. coli* within the first few hours might be due to the production of antibacterial substances, such as superoxide, nitric oxide, or cathepsins (1, 11, 12). This difference in the reactivity of the MC might explain why peritonitis caused by gram-negative bacteria, such as *E. coli*, is clinically more severe than peritonitis caused by staphylococci (5). The rapid influx of neutrophils due to the presence of IL-8 might influence the severity of symptoms and the damaging effects of the invading microorganisms. Moreover, the rapid destruction of the MC monolayer may have a worsening effect on the course of the peritonitis. There are indications that a lack of MC in the abdominal cavities of peritoneal dialysis-treated patients predisposes the patients to a higher risk for developing bacterial peritonitis (7). The results of the present study strengthen the hypothesis that MC are involved in the local host defense mechanism of the peritoneal cavity in peritoneal dialysis-treated patients.

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