Epithelial cell phagocytosis of *Listeria monocytogenes* in the conjunctiva.

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Guinea pig conjunctivae inoculated with *Listeria monocytogenes* were studied by light microscopy and transmission electron microscopy. The epithelial cell of the conjunctiva was found to phagocytose L. monocytogenes as early as 15 minutes after inoculation, long before the appearance of polymorphonuclear cells. The epithelial cells of the mucous membranes appear to participate actively in the host response to this pathogenic organism.

In extensive studies of phagocytosis, Metchnikoff\(^1\) described two types of functionally important phagocytic cells. These he called "microphages" and "macrophages," neutrophilic or eosinophilic leukocytes acting as microphages, and large mononuclear cells acting as macrophages, a concept which has been widely accepted.\(^2\)

One aspect of this concept that has never been fully explained is the role of the epithelial cell in the phagocytosis of parasitic microorganisms on mucous membranes. In 1921, Lindner\(^3\) observed epithelial phagocytosis in the conjunctiva and his observations were extended by Howard.\(^4\) A number of bacteria have been shown to be taken into epithelial cells, both in experimental and naturally occurring infections.\(^5\) The importance of epithelial cell phagocytosis in the host defense against infection has not yet been determined.

In rabbits and guinea pigs *Listeria monocytogenes* produces a severe keratoconjunctivitis.\(^7\) Typically, *L. monocytogenes* induces a mononuclear cell response in the involved host and bacteria can be identified in some of these monocytes.\(^8\) Recent studies by Racz and co-workers\(^9\) have shown that *L. monocytogenes* penetrates into epithelial cells of the cornea, intestine, and bladder. In order to study the role of conjunctival epithelial cell phagocytosis in *Listeria* infection, we have made sequential observations by light and electron microscopy following inoculation of the guinea pig conjunctiva with this organism.

Colonies from 48-hour cultures of an *L. monocytogenes* strain\(^*\) were inoculated onto the superior and inferior palpebral conjunctivae of albino guinea pigs with a sterile cotton-tipped applicator. After intervals of 15, 30, 60, 90, and 120 minutes, and 4 hours the animals were killed and specimens were obtained.

For light and transmission electron microscopy the superior and inferior palpebral conjunctivae were removed in toto and immersed immediately in cold, buffered, 3 per cent glutaraldehyde at a pH of 7.4. A 2 mm. central strip of conjunctiva was postfixed for two hours with 2 per cent osmic tetroxide in veronal acetate buffer, and embedded in araldite epoxy resin. Sections 1 to 2 \(\mu\)m thick were cut for light microscopy and stained with basic fuchsin and methylene blue. Thicker sections stained with uranyl acetate and lead citrate were examined with an RCA electron microscope.

Adherence of bacteria to epithelial cells. In electron photomicrographs, bacteria were found at the exposed surface of conjunctival epithelial cells at 15, 30, and 90 minutes after inoculation even though the tissue had been thoroughly washed and rinsed during processing (Figs. 1 and 2). In control (noninoculated) conjunctivae no bacteria were found at the cell surface or within...
Fig. 2. Electron photomicrograph of bacteria at the external surface of a conjunctiva epithelial cell 30 minutes after inoculation with *L. monocytogenes*. One bacteria is partially surrounded by the conjunctival cell. Another bacteria is in a vacuole, but does not appear to be adherent to the cell membrane of the vacuole. The clear area within the ingested bacterium is due to the failure of the embedding media to penetrate completely. Inset: A bacteria closely adherent to the cell membrane at the surface of a conjunctival epithelial cell 90 minutes after inoculation. (Both x44,835.)

cells which suggests that most of the bacteria in inoculated specimens were indeed *Listeria*. In some inoculated specimens the bacterial and cellular membranes are in close apposition, but in others there appeared to be a space between the bacteria and cell membrane (Fig. 2).

**Phagocytosis by epithelial cells.** Microorganisms appeared within epithelial cells in 30 minutes indicating that phagocytosis occurs rapidly in this system (Fig. 2). Most intracellular bacteria were within cellular vacuoles with clearly defined limiting membranes (Figs. 2 and 3). With some organisms (Fig. 4) it is possible that a complete limiting membrane is not seen separating the bacteria from the cytoplasm because of fixation or handling artifacts or due to the plane of the section.

In some vacuoles the bacteria are separated from the vacuole membrane by a clear space (Figs. 2 and 3). While this separation may be due to an attachment by capillary strands or flagella not apparent in this section, it is also possible that a closer attachment between the bacteria and cell occurs in only one region (Fig. 2), so it would be out of the plane of section.

At two hours after inoculation, bacteria were seen only within cells, so all bacteria attached to the surface had either been phagocytosed or had lost their attachment. Microorganisms were also found within polymorphonuclear leukocytes (PMN's) which appeared among the conjunctival epithelial cells in the specimens at two hours and four hours (Fig. 4). There is no indication that these bacteria within PMN's had first been ingested by epithelial cells.

**Inflammatory response in the conjunctiva.** As in uninoculated specimens, only a few PMN's were noted in infected conjunctivas at 15, 30, 60, and 90 minutes. In the specimens at two hours, PMN's were first noted in the subepithelial and epithelial tissues, but the capillaries were not dilated. At four hours even more PMN's had appeared and the small subepithelial capillaries were distended with red blood cells (Fig. 4). At all these times numerous bacteria were found in the epithelial cells.

*L. monocytogenes* is one of the few bacteria capable of consistently producing conjunctivitis in rabbits and guinea pigs. In the early stages this infection is associated with a marked polymorphonuclear response with occurrence of mono-
cytes two days later. It has been shown that macrophages from susceptible mice and rabbits rapidly engulf and may support the growth of L. monocytogenes. In the intact animal, however, the monocytes become resistant to infection about four days after the initial challenge.

In the ocular model there appeared to be a rapid mobilization of polymorphonuclear leukocytes which appeared in large numbers by two hours after inoculation. It was also apparent that these leukocytes had ingested some bacteria (Fig. 4). It is characteristic of L. monocytogenes to proliferate within infected cells, but leukocytes probably destroy the organisms, while the epithelial cells may remain infected for some days.

In the model of L. monocytogenes keratoconjunctivitis, the conjunctival epithelial cells participate actively in the ingestion of bacteria as do PMN's and monocytes. Since monocytes show solid immunity to infection four days after inoculation, it would be of interest if the epithelial cells showed a similar immune response. Since the destruction or survival of intracellular bacteria seems to be mediated by exposure to intracellular enzymes, the role of these enzymes in L. monocytogenes-infected conjunctival epithelial cells should also be explored.

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Fig. 4. Guinea pig conjunctiva four hours after inoculation. Polymorphonuclear leukocytes (PMN's) are just beneath the superficial epithelial cells. Bacteria are present both in the epithelial cell and in the PMN's. (x9,000.)

REFERENCES