Phagocytosis of E. coli by Renal Tubular Epithelia

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Despite significant advances in our understanding of renal tubular cell function, the in vivo handling of E. coli by renal tubules has not been previously investigated. The present studies were, therefore, designed to study this aspect of nephron function. Live and dead E. coli and vehicle alone were microinjected into the proximal tubular lumen of a single nephron of rats, and the microinjected tubules were morphologically studied at one-half, two, four, and six hours after. The bacteria initially contacted the luminal cell membrane. The luminal cell membrane adjacent to the bacteria subsequently invaginated, and both live and dead E. coli eventually became internalized into the tubular epithelial cytoplasm. Since dead E. coli are unlikely to invade the cells, their intracytoplasmic localization is a result of tubular epithelial phagocytosis. Similar microinjections of dead E. coli together with rat erythrocytes revealed a preferential phagocytosis of dead E. coli. Examination of the microinjected nephron with dead E. coli 48 hours after also demonstrated a development of microscopic interstitial nephritis surrounding the microinjected tubule. In conclusion, the renal tubular epithelia of the proximal and distal segments of rat nephron have phagocytic potential for E. coli which are further capable of inducing an inflammatory reaction around the microinjected tubule.

INTRODUCTION

The complex mechanisms by which renal tubules transport various solutes, proteins, and water have been extensively studied and characterized. The tubular uptake of macromolecular complexes, however, has received little attention. Madsen et al. [1] observed uptake of erythrocytes by renal proximal tubular epithelia. Based on this observation, they concluded that the proximal tubular epithelia were capable of phagocytizing erythrocytes. Additional reports on the renal tubular handling of macromolecular complexes have not been found. In order to study the tubular handling of macromolecular complexes, a suspension of E. coli or a mixture of E. coli and erythrocytes was microinjected into early proximal tubular sites of rat kidneys by micropuncture techniques, and the tubular handling of these macromolecular complexes was investigated by morphologic methods. These studies demonstrate that proximal and distal tubular epithelial cells possess phagocytic potential for E. coli and that E. coli can initiate a peritubular inflammatory reaction when introduced into the tubular lumen.

METHODS

Holtzman male rats with an average weight of about 300 grams were anesthetized by intraperitoneal injections of Inactin (100 mg/kg of body weight). The left kidney
with prior mechanical ureteral obstruction for 24 hours [2] was surgically exposed and live E. coli (strain IMRU-54), suspended in Ringer's solution, or Ringer's solution alone, and tinted with 1 percent lissamine green, were microinjected into an early proximal tubular site of a single nephron at a rate of 15 to 20 nl/minute over ten minutes by methods previously reported [3]. The number of E. coli in the suspension was approximately $9.85 \times 10^8$ per milliliter as assessed by colony count. At the end of the microinjection, the area of the microinjected nephron was marked by placing the tips of two micropipettes into the superficial renal cortical tissue several tubules away from the puncture site on both sides of the microinjected tubule. The pipette tips were then cut at the level of renal cortical surface and left in place as a marker to identify the microinjected single nephron [4]. Control rats were similarly injected with Ringer's solution without E. coli. A group of three experimental rats and corresponding controls were sacrificed at one-half, two, four, and six hours. At the time of sacrifice, the left kidney was perfusion-fixed with buffered 4 percent formalin and 1 percent glutaraldehyde [5], postfixed with 1 percent osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) for one hour, dehydrated, and embedded in Epon-Araldite mixture No. 1 of Mollenhauer [6]. One-micron-thick sections were cut with LKB Ultrrotome III, stained with alkaline toluidine blue or Ladd multiple stain solution, and examined by light microscopy for identification of the microinjected nephron. Thin sections were doubly stained with uranyl acetate and lead citrate for ultrastructural examination.

In a separate experiment, rats without ureteral obstruction were similarly microinjected with live E. coli into the proximal tubular lumen of a single nephron. A single nephron of control rats was microinjected with Ringer's solution alone. Three control and three experimental rats were sacrificed at two and four hours, respectively, and the microinjected nephron studied as described above.

In addition, E. coli were killed by exposure to ultraviolet light, suspended in Ringer's solution, and these dead E. coli were similarly microinjected into the proximal tubular lumen of nonobstructed kidneys. The rats were sacrificed in a group of three at two and four hours, and the microinjected nephron studied as previously described.

Furthermore, fresh rat erythrocytes were washed three times with 0.01 M phosphate-buffered saline (pH 7.2). Dead E. coli were mixed with these washed erythrocytes. The suspension containing dead E. coli and rat erythrocytes was similarly microinjected into the proximal tubular luminal site. A single nephron of control rats was similarly microinjected with the phosphate-buffered saline alone. Three experimental rats and the corresponding controls were sacrificed at four hours, and the nephron studied as described.

As long-term studies, dead E. coli (4 $\times$ $10^{12}$ ml) were similarly microinjected into a single nephron of four rats. The ureter of half of these was obstructed after the microinjection. Controls were microinjected with Ringer's solution. At 48 hours, all rats were sacrificed together with their corresponding controls. The tissue containing the microinjected nephron was processed and embedded in the Epon-Araldite mixture. One-micron-thick sections were stained with Ladd multiple stain solution and examined by light microscopy.

RESULTS

Microinjection of Live E. coli into a Single Nephron of an Obstructed Kidney

Proximal Tubular Segment At 30 minutes, many bacteria were closely opposed to the surface of the brush border membrane. On some occasions, filamentous
pilar structures extending from the bacteria were contacting the brush border membrane. In places, the microvilli adjacent to a bacterium became effaced, creating a small space which was just large enough to accommodate the bacterium. The bacteria occupying these tiny spaces formed by effacement of microvilli were frequently present. After complete effacement of microvilli that occurred adjacent to a bacterium, the luminal cell membrane, now devoid of microvilli, invaginated further to carry the bacterium deeper into the cell, eventually leading to a complete internalization of the bacterium into the cell. The bacteria within the cytoplasm were sometimes in a vacuole lined by a unit membrane, but more often the cytoplasmic space containing bacteria had no visible lining of unit membrane. At four and six hours, the process observed at the prior time intervals continued. The bacteria in the cytoplasm revealed little tendency to be degraded. The cells with bacteria showed little ultrastructural changes beside those due to prior ureteral obstruction [2]. No *E. coli* were observed in the control nephrons.

**Distal Tubular Segment** Uptake of *E. coli* in the thick ascending limb of the loop of Henle and in the distal convoluted tubules was noted to be similar to that of the proximal tubular segments. Upon contact of *E. coli* or pili with the luminal membrane, the latter invaginated at the area of contact. Some bacteria were already internalized within the cytoplasm of distal tubular cells as early as two hours after microinjection. This process of internalization of *E. coli* was also observed at four and six hours (Figs. 1A, 1B, 2). The tubular epithelia exhibited little evidence of significant structural alterations as late as six hours after microinjection. No *E. coli* were observed in any of the control nephrons.

**FIG. 1.** A (*top*). A thick ascending limb of loop of Henle of an obstructed kidney four hours after microinjection of live *E. coli*. Many *E. coli* are in the lumen. Two *E. coli* have been internalized into the cytoplasm of the epithelial cells.  × 10,000. **B (bottom).** A higher magnification of a part shown in A, demonstrating attachment of *E. coli* pili to the luminal cell membrane. Invagination of the luminal cell membrane is seen at the right upper corner.  × 36,300.
Microinjection of Live E. coli into a Single Nephron of Nonobstructed Kidney

As in the previous experiments, entrance of E. coli into the epithelia of the proximal (Fig. 3) and distal segments of the nephron was noted at two and four hours. The mode of tubular uptake of the bacteria was identical to that of the obstructed kidneys. No E. coli were present in the control nephrons.

Microinjection of Dead E. coli into a Single Nephron of Nonobstructed Kidney

As in the previous two sets of experiments, E. coli were taken up by the epithelia of the proximal and distal tubular segments. No bacterial colonies grew on three MacKonkey agar plates when aliquots of ultraviolet-treated E. coli were incubated for 24 hours.

Microinjection of Mixture of Dead E. coli and Rat Erythrocytes into a Single Nephron of Nonobstructed Kidney

Dead E. coli were taken up preferentially in comparison to erythrocytes by the epithelia of proximal and distal nephron segments. There was little evidence that the erythrocytes were phagocytized and internalized by the tubular cells as late as four hours after microinjection. The cytoplasm of one epithelium was found to be loaded with phagocytized E. coli (Fig. 4), while other cells had a few to several or none in a given plane of section. Yet there was no evidence of cellular uptake of erythrocytes. The tubular lumen remained filled with erythrocytes as late as four hours, and there was considerable loss of brush border membrane throughout the proximal tubular segment.

Forty-Eight Hours after Microinjection of Dead E. coli into a Single Nephron of Obstructed and Nonobstructed Kidneys

Interstitial edema and interstitial infiltration of mononuclear cells mixed with polymorphonuclear leukocytes were noted around the microinjected nephrons of
FIG. 3. A proximal tubule of a non-obstructed kidney four hours after microinjection of live *E. coli*. Several *E. coli* are seen deep in the brush border where these bacteria occupy the former site of microvilli. A loss or effacement of microvilli is pronounced in the central area where several *E. coli* are in the process of being internalized. Two *E. coli* are within the lining cell that has electron-lucent cytoplasm. ×12,000.

FIG. 4. A proximal tubule four hours after microinjection of dead *E. coli* and erythrocytes into a single nephron of a nonobstructed kidney. The cell on the left is avidly phagocytic while the other on the right with large cytoplasmic vacuoles shows no evidence of phagocytosis. The *E. coli* are avidly phagocytized while the erythrocytes remain in the lumen. ×13,400.
Forty-eight hours after microinjection of dead *E. coli* into a proximal tubular lumen of an obstructed kidney, there is interstitial nephritis exhibiting interstitial edema and infiltration of mononuclear cells that are mixed with polymorphonuclear leukocytes. The microinjected nephron in the center of the inflamed area is hardly recognizable. ×165.

Forty-eight hours after microinjection of dead *E. coli* into a proximal tubular lumen of nonobstructed kidney, the lumen and the peritubular space of the microinjected nephron exhibit inflammatory infiltrate of mixed cell types. The interstitium is edematous. ×165.

FIG. 5. Forty-eight hours after microinjection of dead *E. coli* into a proximal tubular lumen of an obstructed kidney, there is interstitial nephritis exhibiting interstitial edema and infiltration of mononuclear cells that are mixed with polymorphonuclear leukocytes. The microinjected nephron in the center of the inflamed area is hardly recognizable. ×165.

FIG. 6. Forty-eight hours after microinjection of dead *E. coli* into a proximal tubular lumen of nonobstructed kidney, the lumen and the peritubular space of the microinjected nephron exhibit inflammatory infiltrate of mixed cell types. The interstitium is edematous. ×165.

obstructed and nonobstructed kidneys. The microinjected tubule of the obstructed kidney was hardly recognizable due to replacement of the necrosed tubule by inflammatory cells (Fig. 5). In contrast to the obstructed kidney, the microinjected tubule of the nonobstructed kidney was better preserved, exhibiting swollen lining epithelia and inflammatory cells in its lumen. The interstitium was edematous with mixed cellular infiltration (Fig. 6). *E. coli* were no longer identifiable in the inflamed areas of the obstructed and nonobstructed kidneys. The control kidneys showed no inflammation in either obstructed or nonobstructed kidneys.

**DISCUSSION**

The microinjection of live *E. coli* into the lumen of a single nephron of obstructed rat kidneys resulted in entrance of the bacteria into the tubular epithelial cytoplasm from the luminal side of the proximal and distal nephron segments. Since the experiment was initially carried out in obstructed kidneys, live *E. coli* were similarly microinjected into a single nephron of nonobstructed kidneys to determine whether or not tubular uptake of *E. coli* occurred in the nonobstructed kidney as well. The results clearly indicate that *E. coli* also became internalized within tubular epithelia of nonobstructed kidneys, suggesting that obstruction was not necessary for this phenomenon to occur. A question further arose as to whether or not this intracytoplasmic localization of *E. coli* was a phenomenon which occurred only with live *E. coli*. Dead *E. coli* were, therefore, similarly microinjected into the lumen of a single nephron of nonobstructed kidneys. The results indicate that dead *E. coli* were similarly taken up by the tubular cells of the proximal and the distal nephron segments. Since dead *E. coli* are unlikely to invade, their entrance into the cytoplasm of tubular cells was clearly the result of tubular epithelial phagocytosis and not due to invasion of the cells by *E. coli*. Our data, however, do not completely exclude the possibility that, in
addition to tubular epithelial phagocytosis, live *E. coli* might also enter the cells by active invasion.

The question of selective or specific uptake of *E. coli* by tubular epithelial phagocytosis was further investigated by microinjection of dead *E. coli* that were mixed with fresh erythrocytes. The results demonstrate that *E. coli* were preferentially phagocytized by the tubular cells up to four hours after microinjection. There was no evidence of phagocytosis of erythrocytes by tubular cells during this time interval. It is possible that differences in size, charge selectivity, or even receptor-mediated tubular epithelial phagocytosis might account for the preferential tubular uptake of *E. coli* over that of erythrocytes [7]. Madsen et al. [1] demonstrated that the proximal tubular epithelia needed much longer than four hours for phagocytosis of erythrocytes to occur. Accordingly, the preferential phagocytosis of *E. coli* within four hours after microinjection may be due to size selectivity and not due to a phenomenon that is specific to *E. coli*, since *E. coli* are much smaller in size than erythrocytes.

Forty-eight hours after the microinjection of dead *E. coli* into single nephrons of obstructed and nonobstructed kidneys, there was focal interstitial nephritis which was more intense in the obstructed kidney as compared to the nonobstructed kidney. Since the single nephrons of obstructed and nonobstructed kidneys were microinjected with comparable concentrations of dead *E. coli*, which would not replicate in the tubular lumen, the more intense interstitial nephritis noted in the obstructed kidneys could be interpreted as enhancement of the inflammatory reaction by obstruction. Moreover, it is possible that the dead *E. coli* in the nonobstructed kidney were subjected to a constant tubule urine flow and thus decreased the concentration and contact time with the luminal membrane. On the other hand, in the obstructed kidney the concentration of *E. coli* might have been higher and the urine flow sufficiently decreased to increase contact time even though the obstruction was induced following completion of the microinjection. The severe inflammation in the obstructed kidney, therefore, could have resulted from a higher concentration of *E. coli* within the tubular lumen and increased contact time of the obstructed kidney. The reason for the enhanced inflammatory reaction in the obstructed kidney and the precise mechanism(s) by which *E. coli* initiates the inflammatory response, however, remain unresolved.

Erythrophagocytosis by epithelial cells has been reported in placenta [8], epidermal cells or keratinocytes [9,10], transitional epithelium [11], and proximal tubular cells [1,12]. Lande et al. demonstrated phagocytosis of polystyrene latex beads by keratinocyte [10]. Râcz et al. reported an apparent endocytosis of listeria by vesical epithelia [13]. Fukushi et al. described endocytosis of *E. coli* by transitional cells of the urinary bladder [14]. These previous reports indicate that some epithelial cells have phagocytic capabilities. Our findings are consistent with this view and suggest the presence of a phagocytic potential in both the proximal and distal segments of the nephron for macromolecules such as *E. coli*.

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