The Effects of Cobra Venom Factor, an Inhibitor of the Complement System, on the Sequence of Morphological Events in the Rat Kidney in Experimental Pyelonephritis

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Received November 29, 1976

Acute experimental pyelonephritis has been produced by a combination of mechanical ureteral obstruction and intravenous injection of *E. coli* (strain IMRU-54). The effects of administration of cobra venom factor, an inhibitor of the complement system, on the sequence of morphologic events in the kidneys have been studied by light and electron microscopy.

Pronounced bacterial colonization and suppression of the infiltration of acute inflammatory cells into the kidney were present in the cobra venom factor treated rats on day 2. In these rats, in which the infiltration of polymorphonuclear leukocytes was inhibited, renal structural damage was significantly reduced. The findings appear to indicate that the polymorphonuclear leukocytes infiltrating into the kidney play some role in damaging the renal parenchymal tissue in the early phase of *E. coli* induced acute pyelonephritis in rats.

INTRODUCTION

The complement system plays a vital role in production of mediators of the acute inflammatory response [1,2]. The role of chemotactic factors generated through activation of complement system in the production of glomerular structural damage (as seen, for example, in immunologically induced glomerulonephritis) has been well established [3,4].

A tissue protease released from injured myocardial fibers is capable of cleaving the third component of the complement system (C3) into chemotactically active fragments. Depletion of C3 has inhibited infiltration of acute inflammatory cells into the injured site of myocardium; it has, however, no obvious effects on the ultimate sequence of morphologic events of the experimentally-induced myocardial infarction [2]. This is probably due to the fact that the necrosis of the myocardial fibers was initiated by ischemia, not by leukocytic lysosomal enzymes.

Acute pyelonephritis is one of the most common diseases in humans. Little is known, however, about the mechanism of renal tissue damage due to bacterial acute pyelonephritis. It occurred to us that polymorphonuclear leukocytes infiltrating into the kidney may play some role in inflicting renal structural damage in the early stage of the *E. coli* induced acute pyelonephritis. The present investigation was undertaken to determine the effects of cobra venom factor, an inhibitor of complement systems, on the sequence of morphologic events in the kidney infected with *E. coli*.

MATERIALS AND METHODS

Forty-two rats (Holzman strain, Wisconsin), weight averaging 90 gms, were anesthetized by inhalation of ether, and their left ureters were surgically ligated to...
their abdominal muscle by a thick nylon thread at the ureteropelvic junction [5].
Inactivators of the third component of complement, cobra venom factor (CVF), were prepared from venom of Naja naja (obtained from Ross Allen Reptile Inst., Silver Spring, Fla.) according to the directions of Ballow and Cochrane [6]. Intraperitoneal injection of the purified CVF into one month old rats indicated an absence of lethal action of neurotoxin. Immediately following the ureteral ligation, one milliliter of solution containing 18 units of C3 inactivator [6] was injected intraperitoneally into 22 rats. The injection was repeated every 5 hours for 4 consecutive days [7]. The remaining 20 rats were similarly injected with one milliliter of physiological saline. Immediately following the ureteral ligation, both CVF-treated and untreated rats were injected through the tail vein with one-half milliliter of Ringer's solution containing 2.5 x 10⁸ E. coli (IMRU-54, obtained from the Institute of Microbiology, Rutgers University). Two days after the injection the ureteral obstruction was released. Four, three, three, and three of the CVF-treated and the corresponding number of CVF-untreated rats were killed at the end of days 2, 4, 7, and 14, respectively, after ureteral ligation. Nine of the CVF-administered rats died within the first 2 days, two from intraperitoneal hemorrhage apparently caused by the needle puncture, the others from an unknown cause possibly gram negative shock. These 9 rats were excluded from the present study. Also excluded were three rats that failed to recover from hydronephrosis following the release of the ureteral obstruction. The left kidneys were fixed in ice-cold 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) for 24 hours, and then washed overnight with the phosphate buffer. A part of the kidney was post-fixed in 1%OsO₄ buffered with 0.1M phosphate, dehydrated, and embedded in Epon-Araldite mixture No. 1 of Mollenhauer [8]. One-micron-thick sections made from these tissue blocks were stained with Paragon Epoxy tissue stain for light microscopy. Thin sections were cut by LKB Ultrotome III, and were doubly stained with uranyl acetate and lead citrate for ultrastructural examination. The remaining portion of the left kidney was embedded in paraffin, sectioned, and stained with hematoxylin eosin and Brown-Brenn bacterial stain for light microscopy.

At the time of sacrifice, 0.2 ml of blood obtained via aortic puncture was placed on a MacConkey agar plate.

RESULTS

Gross Findings

On day 2 after injection of E. coli and ureteral ligation, the kidneys of the CVF-treated rats had slight hydronephrotic change and only one of four had barely recognizable pale cortical and cut surface. The kidneys of the complement non-depleted rats had pale cortical and cut surface, slightly swollen and edematous parenchyma, and minor dilation of the pelvic cavity.

Following the release of the ureteral obstruction, recovery of the kidney was rapid. No demonstrable difference was observed between the kidneys of the CVF-treated and the CVF-untreated rats on days 4 through 14. A minor dilation of the pelvic cavity was noticeable on day 4. By days 7 and 14, the kidneys appeared normal.

Light Microscopic Findings

On day 2, three of the four CVF-treated rats had no demonstrable infiltration of acute inflammatory cells (Fig. 1) and the other one had a virtual absence of the infiltration. Colonization of the bacteria in the kidney, however, was quite prominent, and it was seen in the tubular lumina (Fig. 2) and rarely in Bowman's space.
FIG. 1. The kidney of a cobra venom factor (CVF) treated rat killed on day 2. No evidence of infiltration of acute inflammatory cells is present. The renal parenchyma shows good preservation of structural integrity. Most of the tubular casts are colonies of bacteria as illustrated in Fig. 2. (H & E, × 140).

FIG. 2. The kidney of a CVF-treated rat killed on day 2. Prominent bacterial colonization of the tubular lumina is illustrated. Acute inflammatory cells are hard to see despite the presence of numerous bacteria in the tubular lumina. (Paragon Epoxy tissue stain, × 360).

These bacteria were gram negative rods as assessed by Brown-Brenn stain. Despite the presence of bacteria, these areas typically lacked infiltration of inflammatory cells (Fig. 2) and little evidence of cellular degeneration was seen in the renal tubular epithelia. In no instance could we see any bacteria within the renal interstitium. In contrast, the CVF-untreated rats had overt acute inflammatory reaction characteris-
tic of acute pyelonephritis (Fig. 3). Cellular degeneration was observed both in cortex and medulla.

Following the release of the ureteral obstruction, recovery from hydronephrosis and pyelonephritic changes was rapid, and infiltration of acute inflammatory cells in the renal parenchyma was not detectable on day 4 in either CVF-treated or untreated rats. On days 7 and 14, both groups of rats had essentially normal kidneys.

_Ultrastructural Findings_

Ultrastructural examinations were done only on those rats killed at the end of day 2. In the kidneys of the CVF-treated rats, structures believed to be _E. coli_ were numerous within tubular lumina. Despite the presence of so many bacteria, ultrastructural alterations of the tubules (Fig. 4) were minor. Leukocytes were rare in the areas where bacteria were found. Of interest was the phagocytosis of these bacteria by the proximal convoluted tubular epithelia (Fig. 4). There was evidence that the phagocytized bacteria degenerated within the phagolysosomes of the tubular epithelia indicating that the tubular epithelia are capable of destroying the bacteria within their cytoplasm. In contrast, the kidneys of the CVF-untreated rats showed prominent infiltration of acute inflammatory cells in the interstitium as well as into the tubular lumina. Degeneration of some of the tubular epithelia was evident as indicated by increased cell sap, dilated endoplasmic reticula, and swollen mitochondria and nuclei (Fig. 5). The basement membrane of proximal convoluted tubules was perforated in some areas.

_Blood Cultures_

The blood cultures of the CVF-treated rats were positive in all cases on day 2, while all of the corresponding CVF-untreated rats had negative results. The cultures on days 4, 7, and 14 were all negative in both CVF-treated and untreated rats except one rat killed on day 4.
DISCUSSION

The infiltration of acute inflammatory cells was substantially reduced when the rats were treated with CVF. The suppression of the leukocytic infiltration was associated with better structural preservation of the cellular elements of the kidney. This indicates that the release of the leukocytic enzymes in the inflamed areas may at least in part be responsible for renal tissue damage seen in E. coli induced acute pyelonephritis [9].

Although better structural preservation of the kidney was observed on day 2 of our present experiment, the administration of CVF resulted in more prominent forma-
Fig. 5. Tubular epithelium of a CVF-untreated rat killed on day 2. Bacterial bodies are present in the necrotic tubular epithelium which shows swollen mitochondria, dilated endoplasmic reticulum, and a large vacuole. The tubular basement membrane is perforated (larger arrow). A polymorphonuclear leukocyte is present at left lower corner. Smaller arrow: tubular basement membrane. (X 12,600).

tion of bacterial colonies within the kidneys. The presence of numerous bacteria in the kidneys of CVF-treated rats enabled us to observe the apparent route of passage of the intravenously administered bacteria from the tubular lumina toward the interstitium. The fact that we were able to detect bacteria in the tubular lumina and not in the interstitium suggests that the initial site of colonization in the kidney of intravenously administered E. coli may be the tubular lumina. Urinary stasis is associated with a higher incidence of acute pyelonephritis. It may be that stasis of urine in the tubular lumina favors initial colonization of bacteria thus leading to development of acute pyelonephritis.

Numerous E. coli were observed within the cytoplasm of the proximal convoluted tubular epithelia. The overt degeneration of some of the bacterial bodies within the structures believed to be phagolysosomes indicates that the tubular epithelia have phagocytic capability as well as bactericidal action.
Positive blood cultures from day 2 of CVF-treated rats, but not from the untreated animals, appears to be related to the prominent bacterial colonization seen in CVF-treated rats. This prominent bacterial colonization in CVF-treated rats leads us to believe that these rats will ultimately develop severe infection and damage to the kidney if the ureter is left obstructed and CVF continuously administered.

Once the ureteral obstruction was released on day 2, the recovery of the damaged kidneys was rapid. This is in part due to the strong inherent resistance of the rat kidneys to bacterial infection. This rapid recovery made it difficult to assess the differences in intensity and extent of damage to the kidneys between the CVF-treated and the untreated rats on days 4 through 14.

Although a great deal remains to be elucidated, we believe that there is evidence indicating the existence of a role played by polymorphonuclear leukocytes in causing structural damage to the kidneys in the early phase of the E. coli induced experimental pyelonephritis in rats.

Technical assistance of Mrs. J.C.W. Wang, Miss J. Cotton, and Mrs. Anne Barry is gratefully acknowledged.

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