Apoptosis of Renal Cortical Cells in the Hemolytic-Uremic Syndrome: In Vivo and In Vitro Studies

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This study examined apoptotic cell death associated with Shiga-like toxin (Stx)-producing Escherichia coli. Renal cortices from three children with postenteropathic hemolytic-uremic syndrome (HUS) and from mice infected with E. coli O157:H7 and pediatric renal tubular epithelial cells stimulated with Stx and E. coli O157:H7 extracts were examined for apoptotic changes. Apoptotic cells were detected by terminal dUTP nick end labeling of tubuli and glomeruli from HUS patients and from mice inoculated with Stx-2-positive and Stx-negative strains. Apoptosis was more extensive and severe ultramorphologically and cytoplasmically than in the Stx-2-negative group. Stx caused DNA fragmentation and ultramorphological changes indicating apoptosis in cultured pediatric tubular cells. DNA fragmentiation increased when cells were prestimulated with tumor necrosis factor alpha. Polymyxin extracts from Stx-2-positive and Stx-negative strains induced DNA fragmentation, but only extracts from Stx-2-positive strains caused ultramorphological changes and extensive DNA fragmentation. The results indicate that HUS is accompanied by increased apoptosis of kidney cells and that bacterial factors, possibly together with host cytokines in vivo, may activate apoptotic tissue injury.

Hemolytic-uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. The syndrome has been divided into two forms: typical (or postenteropathic) and atypical (28). The postenteropathic form of HUS occurs due to infection with Shiga-like toxin (Stx)-producing Escherichia coli, which causes a prodrome of diarrhea or hemorrhagic colitis followed by HUS (3, 22). Stx-producing E. coli O157:H7 has been associated with outbreaks of these conditions (15). The atypical form of HUS is not caused by Stx-producing E. coli and is usually not preceded by a diarrheal prodrome (10).

The most extensive tissue damage in HUS occurs in the kidneys. The injury is most prominent in the renal cortex, with pathologic changes occurring in the glomerular endothelial cells and also in the tubular epithelial cells (16). The mechanism by which Stx-producing E. coli causes tissue damage is not clear. E. coli O157:H7 has not been found to be invasive (33), and it has therefore been assumed that tissue damage occurs as a result of the spread of bacterial products and/or inflammatory mediators from the intestine to target organs (14). Previous studies found that Stx-producing strains cause systemic symptoms in animals (5, 11, 39, 48, 51, 52) and that these symptoms could be reproduced when the animals were injected with purified Stx (2, 18, 47). In a recent study carried out with mice, we found that E. coli O157:H7 that produce Stx caused glomerular and tubular pathology and a higher frequency of systemic symptoms than E. coli O157:H7 that did not produce Stx, suggesting that this toxin may be important for the HUS-related virulence of the strain (24). Furthermore, in vitro studies have found that Stx is cytotoxic for human endothelial cells (27, 36, 49) and that renal endothelial cells are more susceptible to the cytotoxic effect than umbilical vein endothelial cells (37). After binding to cells, the toxin is endocyttosed (44), binds to 60S ribosomes, and inhibits peptide chain elongation and protein synthesis (38, 43), thereby leading to cell death.

Programmed cell death, or apoptosis, is defined by the cell’s ultrastructural morphology (26) and is characterized by cell shrinkage, membrane blebbing, and condensation of nuclear chromatin. The morphological changes are accompanied by DNA fragmentation. This form of cell death is a naturally occurring process by which an organism removes damaged or unnecessary cells and may also be triggered by external stimuli (32). Shigella flexneri (56) has been found to induce apoptosis in host macrophages. This activity was related to the invasive properties of the strain (8, 56) and not the production of toxin. Purified toxins such as diphtheria toxin (6), ricin, and Stx (45) have previously been found to activate apoptosis. Stx induced apoptosis in Burkitt lymphoma cells and Vero cells in vitro (19, 31) and in rabbit intestinal epithelial cells in vivo (25). In addition, plasma samples from patients with atypical HUS, but not from a patient with postenteropathic HUS, were found to induce apoptosis of human microvascular endothelial cells (34).

The aims of this study were to examine kidney tissue from patients with postenteropathic HUS and mice with experimental E. coli O157:H7 infection for signs of apoptosis and to study apoptosis induction in pediatric kidney cultures in vitro.

MATERIALS AND METHODS

Renal tissue specimens from renal biopsies and autopsies of patients and a pediatric control. Renal cortical tissues from four children were studied. A renal cortical biopsy (n = 1) and postmortem tissues (n = 2) were available from three children with postenteropathic HUS. A renal cortical biopsy specimen from one child with nephrotic syndrome was studied as a control.

A renal biopsy was obtained from a 22-month-old Swedish girl hospitalized in 1990 due to bloody diarrhea and anuria. HUS was suspected due to anemia (hemoglobin, 55 g/liter; normal value, 120 to 160 g/liter), thrombocytopenia (platelet count 65 × 10⁹/liter; normal value, 140 × 10⁹ to 400 × 10⁹/liter), and renal failure (creatinine, 438 µmol/liter; normal value, 27 to 62 µmol/liter).
TUNEL assay. The TUNEL method was based on a modified protocol from Gavrieli et al. (12, 41). TUNEL labeling was defined as positive when cells were highly fluorescent against a background of lightly stained cells. Assay controls were performed by preincubation of sections with 1 μg of DNase (Boehringer, Mannheim, Germany) per ml in partial restriction of the TdT reaction of all visible cells. Omission of the biotin-labeled nucleotide resulted in no detectable signal.

HRTEC. Human renal tubular epithelial cells (HRTEC) were isolated from the kidneys of a 3-year-old boy whose kidney was removed due to hydrocephrosis and reduced function. The removal of part of the renal tissue for research purposes was approved by the ethics committee of the University of Lund. The child was not taking medications prior to or at the time of the operation and had a slight elevation of blood pressure (90/70 mm Hg), which was considered by the cardiovascular team as being only slightly thinner than normal. The cortex was dissected from the renal medulla. The cortical cells were isolated according to the method described for human renal microvascular endothelial cells (HRMEC) (37, 54). Briefly, the cortical fragments were incubated overnight in a buffer containing 0.1% collagenase type I (Sigma, Stockholm, Sweden) and 0.04% DNase IV (Sigma). The tissue was applied to the top of a two-step Percoll gradient (Pharmacia Biotech, Stockholm, Sweden), and the interface between the two steps was collected, centrifuged, and resuspended in Primaria flasks (Falconn, Stockholm, Sweden) in Dulbecco’s modified Eagle’s medium (GIBCO, Taby, Sweden) supplemented with 15% fetal calf serum (GIBCO), 2 mM L-glutamine (GIBCO), 20 U of heparin (Pharmacia) per ml, 100 μg of penicillin per ml, and 100 μg of streptomycin per ml (both from GIBCO). The cells were incubated in an atmosphere of 95% air and 5% CO2 and kept in culture. After trypsinization, the cell suspension was filtered through a 35-μm-pore-size nylon mesh and cells passed through the net were replated in Primaria flasks and grown to confluence. The cell isolates were typified, concentrated in EDTA-PBS-A, and stored in liquid nitrogen for subsequent use. Cells used in experiments were passed three to five times and cultured in Primaria flasks or in 24-well Primaria plates in medium with supplements and 1% endothelial cell growth factor (Sigma). Ten days before cell culture, 2.5 μg of RNA was added to the medium. The cells grew to confluence in 12 days.

By light microscopy, more than 90% of the cells had similar morphologies. These cells were characterized and confirmed as epithelial cells by positive staining for cytokeratins (35) with MNF116 (Dako) and CAM 5.2 (Becton Dickinson, San Jose, Calif.) as well as for glomerular, tubular, interstitial, and vascular changes. The biosynthesis of von Willebrand factor was demonstrated with anti-von Willebrand factor antibody (Dako). With anti-endoglin antibody (Dako), approximately 5% of cells stained weakly. Less than 10% of cells contained Weibel-Palade bodies, as determined by TEM (53).

Cell stimulants. (i) Stxs. Purified Stxs was kindly provided by A Lindberg (Department of Microbiology, Karolinska Institute, Huddinge, Sweden). The LPS content of the Stx preparation was 2 endpoint units (EU)/ml at a Stx concentration of 1 μg/ml. Endotoxin was measured with the Limulus amebocyte lysate assay (Coast; Chromogenix, Gothenburg, Sweden), which has a detection level of 0.04 EU/ml.

(ii) Polymyxin extracts. E. coli O157:H7 86-24 and 87-23 and the control E. coli O157:H7 86-24 were used as stimulants. To 1 ml of the cell suspension in endothelial serum-free medium (GIBCO) containing 100 μg of penicillin/ml, 100 μg of streptomycin/ml, 100 μg of heparin/ml, and 200 U/ml of penicillin, polymyxin B sulfate (2 mg/ml; Duomen, Copenhagen, Denmark) was added. The resulting mixture was incubated overnight on tryptic soy agar plates, scraped off the plates, and harvested into PBS-A (0.12 NaCl, 0.03 M phosphate, 0.02% NaN3; pH 7.2) with polymyxin B sulfate (2 mg/ml). The cell suspension was filtered through a 0.2-μm-pore-size filter and centrifuged at 2,000 × g for 10 min at 4°C. The pellets were resuspended in endothelial serum-free medium (GIBCO) containing 100 μg of penicillin/ml and 100 μg of streptomycin/ml (both from GIBCO), and stored in liquid nitrogen for subsequent use. The LPS content of the bacterial extracts was determined by the Limulus amebocyte lysate assay technique. The LPS content of polymyxin B sulfate in PBS-A was 4 to 7 EU/ml.

Cell stimulation assays. Epithelial cells were grown to confluency in 24-well Primaria plates or in Primaria flasks. At time zero, cells were washed in PBS and the culture medium was replaced and replaced with endothelial serum-free medium (GIBCO) supplemented with 2 mM L-glutamine, 20 U of heparin (Pharmacia) per ml, 100 U of penicillin per ml, 10 μg of streptomycin per ml (both from GIBCO), and 1% endothelial cell growth factor (Sigma) with or without 40 ng of tumor necrosis factor alpha (TNF-α; Knoll; BASF, Ludwigshafen, Germany) per ml. At 24 h 20 μg of polymyxin B sulfate per ml were added to the medium, and 10 μg of polymyxin B sulfate per ml to the medium 48 h after the addition of stimulants. The cell suspension was collected, washed, and resuspended in endothelial serum-free medium (GIBCO) containing 100 μg of penicillin/ml and 100 μg of streptomycin/ml (both from GIBCO), and stored in liquid nitrogen for subsequent use. The LPS content of the bacterial extracts was 10,000 EU/ml as tested by the Limulus amebocyte lysate assay. The LPS content of polymyxin B sulfate in PBS-A was 4 to 7 EU/ml.
agiar 100 (Link, Stockholm, Sweden), poststained with 4% uranyl acetate and 2% lead citrate (both from Kebo, Stockholm, Sweden), sectioned, and examined by TEM.

DNA fragmentation. High-molecular-weight DNA fragments were detected by field-inversion gel electrophoresis (FIGE) as described previously (55). DNA size calibration was performed with two sets of pulse markers: chromosomes from Saccharomyces cerevisiae (225 to 2,200 kbp) and a mixture of λ DNA HindIII fragments, λ DNA, and λ DNA concatemers (0.1 to 200 kbp) purchased from Sigma. DNA was visualized under UV light (305 nm) after being stained with ethidium bromide (6 μg/ml) and was photographed with Polaroid 55 positive-film.

Detection of apoptosis. Tissue apoptosis in vivo was detected by the TUNEL assay and TEM. Apoptosis of cultured renal cells in vitro was detected by TEM and FIGE.

Statistics. Differences in the number of TUNEL-positive cells between mice inoculated with Stx-2-positive strains and mice inoculated with Stx-2-negative strains were evaluated by the Student t test. A P of ≤0.05 was considered significant.

RESULTS

Histopathology of human renal cortical tissue. Histopathology of the biopsy from the HUS patient showed extensive renal cortical necrosis (pathological definition). The 10 glomeruli visualized in the biopsy had capillaries filled with thrombi. Cell proliferation was noted in one glomerulus. Blood vessels were dilated and filled with thrombi. Extensive damage to tubular epithelial cells, including pyknotic cell nuclei and fragments of nuclei, was noted. Tubular luminae were filled with these fragments as well as fibrin and erythrocytes (Fig. 1A and B). The interstitium was widened. Immunofluorescence for fibrinogen was strongly positive in glomerular capillaries, blood vessels, and tubuli. Renal cortical necrosis secondary to HUS was diagnosed by a renal pathologist.

The autopsy material from the boy with HUS showed extensive renal cortical necrosis. Only a few areas of the renal cortex had viable cells with nuclei. These areas contained glomeruli with capillary thrombi and pyknotic tubular cells sloughing into the luminae. The autopsy material from the girl with HUS showed a majority of glomeruli with capillary fibrin thrombi and pyknotic nuclei. Focal tubular damage with many pyknotic nuclei were seen.

Light microscopy of the control biopsy showed 10 glomeruli with slight proliferation of mesangial cells. Changes were not noted in the blood vessels, tubuli, or interstitium (Fig. 1C). Immunofluorescence was negative for all examined antisera. Minimal-change nephropathy was diagnosed by a renal pathologist.

TUNEL of human renal cortical tissue. Numerous TUNEL-positive nuclei were identified in the renal cortices from the children with HUS. Labeling was noted in the nuclei of tubular cells (Fig. 1D), and small labeled structures were present in the tubular luminae. Nuclei also stained positively in glomeruli. The renal cortices from mice inoculated with Stx-negative strains (n = 5) from mice inoculated with Stx-2-negative strains (n = 9). One mouse exhibited single fluorescence in multiple cells with various morphologies. Most of these cells appeared to be nonepithelial in size and may have been inflammatory. Patchy fluorescence was also seen in the tubular lumina. Patchy fluorescence was identified in three kidneys, and diffuse cortical fluorescence was observed in five kidneys. Kidney samples from mice inoculated with E. coli 86BL and pretreated with anti-Stx-2 antibody were TUNEL negative regardless of the antibody dose.

Single tubular fluorescence was detected in eight kidneys and single glomerular fluorescence was detected in one kidney from mice inoculated with Stx-negative strains (n = 9). One mouse exhibited single fluorescence in multiple cells with various morphologies. Most of these cells appeared to be nonepithelial in size and may have been inflammatory. Patchy fluorescence was observed in four kidneys.

A comparison of the degrees of single-cell fluorescence between mice inoculated with Stx-2-positive (n = 11) versus Stx-2-negative (n = 9) E. coli O157:H7 strains provided the following results. Mice inoculated with Stx-2-positive strains showed a range of TUNEL-positive single cells of 0 to >1,000 (median, 29); mice inoculated with Stx-2-negative strains showed a range of 0 to 40 (median, 15) (P < 0.0001). The cells in one kidney from a mouse inoculated with a Stx-negative strain that had 40 fluorescent nuclei were nonepithelial in size and appeared to be inflammatory; all other kidneys from mice inoculated with Stx-negative strains had up to 24 fluorescent nuclei.

The renal cortices from mice inoculated with E. coli FN414 were TUNEL negative. Single fluorescent nuclei were found in the renal cortices of two of the PBS control mice, one of which had five and the other of which had eight positive nuclei per section of renal cortex. The renal cortices of the other two control mice were TUNEL negative (Fig. 2D).

TEM of mouse kidneys. Ultramorphological changes indicating apoptosis or necrosis were evaluated by TEM. Marked nuclear and cytoplasmic changes in tubular epithelial cells were noted for all renal cortices from symptomatic mice inoculated with Stx-2-positive strains (n = 5). Cells (5 of 10 cells) exhibited convoluted nuclei with invaginations in their nuclear membranes (Fig. 3A), chromatin condensation (2 of 10 cells) (Fig. 3B), or separation of the nuclear envelopes (1 of 20 cells) (Fig. 3C). Considerable swelling and disruption of the inner mitochondrial cristae were noted for most cells (Fig. 3B). Cells with severely damaged mitochondriae had a total lack of the brush border, and their luminal spaces were filled with granular deposits. A few endothelial cells exhibited convoluted nuclei with disruption of the nuclear membranes, but intracytoplasmic changes were not noted. A few mesangial cells exhibited convoluted nuclei, but most appeared normal. These
FIG. 1. Light microscopy of renal biopsies from a child with D+ HUS (A and B) and a child with nephrotic syndrome (C). (A) Glomerular thrombi are seen in capillaries (arrow), erythrocytes are seen in the urinary space, and tubular luminae are filled with pyknotic nuclei, fragments of nuclei, and erythrocytes. (B) Larger cells, which may be macrophages, were noted between tubular structures (arrow). (C) Glomerular and tubular structures without histopathological changes. Shown are the results of fluorescent microscopy of renal biopsies from the child with D+ HUS (D) and the child with nephrotic syndrome (E) labeled by the TUNEL assay in sections near those seen in panels A and C, respectively. (D) Numerous fluorescent cells in glomerular and tubular structures. (E) Lack of fluorescent labeling. Magnification, x392 (all panels).
changes in tubular epithelial, endothelial, and mesangial cells were noted for all kidneys regardless of when mice were sacrificed.

In contrast, asymptomatic mice inoculated with Stx-2-positive strains \((n = 3)\) did not show ultrastructural changes except for one mouse, which had a few tubular cells (1 of 20) with swollen mitochondriae and disrupted cristae.

Nuclear changes in tubular, endothelial, or mesangial cells from symptomatic mice inoculated with Stx-negative strains \((n = 5)\) were not noted (Fig. 3D). One of 20 tubular cells exhibited slight swelling of mitochondriae with few disrupted cristae. One of two asymptomatic mice exhibited a few tubular cells (1 of 30 cells) with swollen mitochondriae. No ultramorphological changes were noticed in the control mice \((n = 3)\) (Fig. 3E).

The effects of Stx and polymyxin extracts on HRTEC. (i) Stx. Stx had a marked effect on the confluence, adherence, and morphology of HRTEC. At Stx concentrations of 1 ng/ml to 10 pg/ml, considerable dose-related cell detachment was noted by light microscopy after 24 h of stimulation. Cells that did not detach became elongated, with multiple vacuoles and prominent and less centralized nuclei. Stx at concentrations above 1 ng/ml caused detachment of almost all cells. Toxin concentrations below 10 pg/ml did not have a visible effect on the cells. The effect of Stx was enhanced by prestimulation with TNF-α.

Cell changes indicative of apoptosis or necrosis were examined by TEM. HRTEC stimulated with 100 pg of Stx per ml for 24 h exhibited marked nuclear and cytoplasmic changes, with chromatin condensation and disruption of their nuclear envelopes (Fig. 4A), sparsity of rough endoplasmic reticula, slight mitochondrial swelling, blebbing of their cytoplasmic membranes, and disappearance of microvilli.

<table>
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<tr>
<th>Pattern of labeling by TUNEL analysis</th>
<th>No. of mice inoculated with E. coli strain type:</th>
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<tbody>
<tr>
<td></td>
<td>Stx(^+) ((n = 11))</td>
<td>Stx(^-) ((n = 9))</td>
</tr>
<tr>
<td>Patchy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Single cells</td>
<td>9</td>
<td>8</td>
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<tr>
<td>Cortical diffuse</td>
<td>5</td>
<td>4</td>
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<td>None</td>
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The capacity of Stx to induce DNA fragmentation was examined by FIGE. Stx at concentrations of 1 ng/ml and 100 pg/ml and 10 pg/ml induced the formation of high-molecular-weight DNA fragments, seen as weak bands in the 50-kbp range on agarose gels after 24 h. Prestimulation of cells with TNF-α (40 ng/ml) for 24 h increased the degree of fragmentation, which was reflected in stronger high-molecular-weight bands after 24 h of toxin stimulation (Fig. 5). TNF-α alone did not induce DNA fragmentation.

(ii) Polymyxin extracts. E. coli 86-24 extract (Stx-2 positive) caused massive cell detachment after 24 h. TEM revealed convolution of the nuclear envelope (Fig. 4B) and redistribution of chromatin. Mitochondrial swelling and sparsity of microvilli were also apparent. High-molecular-weight DNA fragments (294 and 50 kbp) were formed (Fig. 5). Prestimulation of cells with TNF-α (40 ng/ml) for 24 h increased DNA fragmentation in the 50-kbp range after 24 h of stimulation with the bacterial supernatant. In contrast, E. coli 87-23 extract (Stx negative) did not cause nuclear or intracytoplasmic changes (Fig. 4C) but high-molecular-weight DNA fragments (294 kbp) were formed (Fig. 5) and did not increase after TNF-α prestimulation. E. coli FN414 extract caused the formation of high-molecular-weight DNA fragments in the 294-kbp range. Cells stimulated with this strain were not prestimulated with TNF-α or examined by TEM. Neither morphological changes nor DNA fragmentation was noted in cells exposed to polymyxin B sulfate in PBS-A or in unstimulated cells (Fig. 4D).

DISCUSSION

This study examined apoptosis of renal cortical cells in the HUS. The renal cortical biopsy taken from a child with postenteropathic HUS showed extensive cortical necrosis and thrombotic microangiopathy as defined by pathology. Nuclear fragments were visualized by light microscopy, which indicated that apoptosis was taking place. TUNEL analysis revealed positive staining in the glomerular and tubular structures and in lumina. Labeling was also positive in autopsy material taken from two children with HUS. A recent study showed that processing of tissue postmortem does not, in itself, increase the degree of TUNEL-positive cells (in comparison to the degree of TUNEL-positive cells in biopsy material) (1). Taken together, these results suggest that apoptosis of renal cortical cells takes place in HUS.

Apoptotic cells were also detected in kidneys of mice with experimentally induced E. coli O157:H7 infection. Renal cor-
tices from mice inoculated with Stx-2-positive *E. coli* O157:H7 exhibited high numbers of TUNEL-positive cells. The presence of patches of fluorescence and high numbers of labeled single cells indicated extensive apoptosis. Ultrastructural changes consisted of severe nuclear and cytoplasmic tubular changes. TUNEL-positive cells were also observed, albeit to a lesser degree, in mice inoculated with toxin-negative strains, and a low frequency of TUNEL-positive cells occurred in normal kidneys. TEM did not show tissue damage in mice inoculated with toxin-negative strains, but the sections represent a small portion of the renal cortex and damaged areas may have been missed. Kidneys from mice inoculated with Stx-2-positive strains showed changes in both epithelial and endothelial cells, whereas kidneys from mice infected with Stx-2-negative strains showed fewer changes and these occurred only in epithelial cells. The kidney apoptosis pattern confirmed the observations regarding human HUS and suggested that infection with Stx-2-producing *E. coli* was the cause of these changes.

Further studies showed a direct apoptosis-inducing effect of the bacteria and of Stx on pediatric renal cells in culture. Primary kidney cell cultures were susceptible to the cytotoxic effect of Stx-2-positive *E. coli* O157:H7 and purified Stx. Ultramorphology indicated that the cells were undergoing apoptosis. Furthermore, cells exposed to Stx and polymyxin extracts of Stx-2-positive *E. coli* O157:H7 were shown to undergo DNA fragmentation. Epithelial cells are less likely to produce typical nucleosome ladders (9), but fragments of 50 kbp represent more extensive DNA digestion. HRTEC stimulated with the polymyxin extract of the toxin-negative strain did not show morphological changes indicative of apoptosis but underwent DNA fragmentation in the higher-molecular-weight range (294 kbp). The same pattern of fragmentation was also
noted for cells stimulated with the control strain. These results show that bacteria induced apoptosis in kidney cells and indicate that more than one bacterial virulence factor was involved but that when Stx was present, the damage became more pronounced.

The effect of Stxs has been studied in vitro with various types of endothelial cells. A dose-dependent cytotoxic effect was found when human umbilical vein endothelial cells were stimulated with purified Stx (36). These results have since then been confirmed in several studies which found that Stx-1 and Stx-2 had similar effects on endothelial cells (27, 30). The susceptibility of pediatric renal cortical tubular cells to Stx has not been previously studied. We chose to use pediatric renal cortical cells because children are more susceptible to post-teratogenic death occurs in the renal cortex during HUS. Stx and other E. coli O157:H7 factors were found to induce this process in vitro. Thus, induction of apoptosis may contribute to the renal damage in HUS. A better understanding of the factors involved in this form of tissue injury may lead to new perspectives for therapy.

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REFERENCES
