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Oxalate stimulates IL-6 production in HK-2 cells, a line of human renal proximal tubular epithelial cells

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Oxalate stimulates IL-6 production in HK-2 cells, a line of human renal proximal tubular epithelial cells.

Background. Oxalate is a metabolic end product excreted primarily by the kidney and associated with several pathologic conditions. The most common pathologic condition involving oxalate is the formation of calcium oxalate stones in the kidney. Several stimuli have been implicated in the development of glomerular and tubular injury in various forms of immune-mediated renal diseases. The elevated level of interleukin-6 (IL-6) has been reported in the urine of kidney stone–forming patients. In the present study, we investigated the role of oxalate, a major constituent of calcium oxalate kidney stone disease, in the production of IL-6 in normal human HK-2 kidney cells.

Methods. Confluent cultures of HK-2 cells (a renal epithelial cell line of human origin) were exposed to various concentrations of oxalate (0.2 to 2.0 mmol/L) and lipopolysaccharide (LPS) (0.1 and 10 μg/mL) for various time points (4–24 h) under serum-free conditions. The conditioned mediums were collected, and an IL-6 protein level was measured by enzyme-linked immunosorbent assay (ELISA). The total cellular RNA was isolated from the cells and subjected to relative quantitative reverse transcription-polymerase chain reaction (RT-PCR) to determine the expression of IL-6 mRNA. The statistical analysis of the results was carried out using the Student *t* test.

Results. HK-2 cells express IL-6 mRNA and protein. Oxalate increased the secretion of IL-6 protein in HK-2 cells in a concentration-dependent fashion. Oxalate exposure to HK-2 cells also induced transcriptional up-regulation of the IL-6 gene, as determined by the increased level of IL-6 mRNA expression following treatment with oxalate. Moreover, the effects of oxalate on IL-6 expression were time- and concentration-dependent. This is the first report demonstrating the regulation of IL-6 by oxalate.

Conclusion. This study provides the first direct evidence that oxalate up-regulates the expression and secretion of IL-6 in renal epithelial cells. The increased IL-6 expression and secretion by renal epithelial cells may play a critical role in the progression of urolithiasis in hyperoxaluric conditions.

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Oxalate, a metabolic end product, is excreted primarily by the kidney and is associated with several pathologic conditions. This organic dicarboxylate is freely filtered at the glomerulus and undergoes bidirectional transport in the renal tubules [1–3]. The most common pathologic condition involving oxalate is the formation of calcium oxalate stones in the kidney [4]. Besides renal stone formation, oxalate deposits are also associated with hyperplasic thyroid glands, benign neoplasm of the breast, renal cysts in acquired renal cystic disease, and proliferating cells in the kidney [5–8]. Many of these conditions are associated with aberrant cell proliferation and cell death. Previous studies from our laboratory and those of others have demonstrated that oxalate interaction with renal epithelial cells results in a program of events, selective activation of signaling pathways, reinitiation of DNA synthesis, cell growth, and apoptosis [9–17]. These considerations suggest that oxalate toxicity could result in tissue damage and inflammation.

Several chemotactic factors and adhesion molecules play key roles in the proinflammatory/inflammatory reaction at various sites throughout the body, including the kidney [18]. Cytokines perform ever-increasing roles in both the regulation of general homeostasis, and in orchestrating the immune response during disease. Various cascades of mediator systems can be activated following initial injury or stimulation of the proximal tubular cells, to enhanced local production of complement, chemokines, cytokines, and matrix components. Renal tubular cells have been shown to express various cytokines and other mediators of inflammatory response [19]. Interleukin-6 (IL-6), which was originally identified as a B-cell differentiation factor, is now known

to be a multifunctional cytokine that regulates the immune response, hematopoiesis, the acute-phase response, and inflammation [20]. An increased production of IL-6 from mesangial and proximal tubular epithelial cells has been shown in vitro in response to many diverse stimuli, including interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-α), and lipopolysaccharide (LPS) [21, 22]. Elevation in urinary IL-6 has been noted in disease states such as interstitial cystitis, mesangial glomerulonephritis, and urinary tract infections [23–25]. IL-6 may also function indirectly as a mediator of inflammation, which may act along with other mediators such as IL-1, TNF-α, and LPS in the acute inflammatory response [26]. The elevated levels of IL-6 have been reported in the urine of kidney stone–forming patients [27]. The inflammation following oxalate-induced injury plays a significant role in the pathogenesis of nephrolithiasis, but the molecular mechanism/s and mediators of this proinflammatory/inflammatory response in the interstitium are not delineated.

In the present study, we investigated the role of oxalate, a major constituent of calcium oxalate kidney stone disease, on IL-6 gene expression and protein production in HK-2 cells, a line of proximal tubular epithelial cells from normal human kidney. Our findings provide evidence that IL-6 gene expression and protein production increases in oxalate-exposed HK-2 cells, which may have a critical role in the progression of the hyperoxaluric condition of urolithiasis disease.

METHODS

Materials

HK-2 cells (CRL-2190), a line of renal proximal tubular epithelial cells of human origin, were obtained from the American Type Culture Collection (Manassas, VA, USA). Penicillin and streptomycin antibiotic mixture, Dulbecco's modified Eagle's medium (DMEM), and heat inactivated fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA, USA). LPS was obtained from EMD Biosciences, Inc. (San Diego, CA, USA). Human IL-6 ELISA kit was purchased from ALPCO Diagnostics (Windham, NH, USA). Human IL-6 gene specific relative quantitative reverse transcription-polymerase chain reaction (RT-PCR) kit was purchased from Ambion (Austin, TX, USA). All other chemicals were of analytical grade, and were purchased from Sigma (St. Louis, MO, USA).

Cell culture

HK-2 cells (American Type Culture Collection) were grown on polystyrene T-75 flasks. The cells were serially passaged and maintained in high glucose DMEM, 4.5 g/L glucose supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL) antibiotic mixture at 37°C under 5% CO_2/95% air. For experiments, cells were grown in 6-well plates. Stock solution of sodium oxalate (20 mmol/L) was prepared in phosphate-buffered saline (PBS), pH adjusted to 7.4 and used within 2 to 3 days. Where indicated, sodium oxalate was added at a final concentration of 0.2, 0.8, 1.0, or 2.0 mmol/L (total), which increased the free oxalate levels to 0.07, 0.28, 0.35, or 0.75 mmol/L, respectively. Estimates of free oxalate were obtained using EQUIL program.

Assay for interleukin-6

The human IL-6 assay was performed by using solid-phase enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's recommended protocol (ALPCO Diagnostics). Briefly, cells were grown to confluence in 6-well plates. Serum-starved (12–18 h) cells were exposed for various concentrations of oxalate (0.2–2.0 mmol/L) or LPS (0.1 and 10 μg/mL) for 24 hours. At the end of experimental period, the conditioned medium were collected and centrifuged for 5 minutes at 5000 rpm to remove cell debris. One hundred mL of cultured media from treated cells with oxalate, LPS, or IL-6 standard were added in duplicates to the precoated wells with monoclonal antibodies specific for IL-6. Then, 150 mL of IL-6 calibrator/sample diluents was added to every well and the plate was rotated for 2 hours at room temperature on a plate mixer (350–400 rpm). The nonreactive sample components were removed by washing 4 times with 300 mL wash buffer. Afterward, a second polyclonal horseradish peroxidase-labeled anti-IL-6 antibody (200 mL/well) was added, and the plate was rotated for an additional 2 hours, as mentioned above. An excess of enzyme conjugate was washed 4 times with 300 mL washing buffer. Following washing, 200 mL of chromogenic substrate solution, 3,3,5,5-tetra-methyl-benzidine (TMB), was added to all wells for 30 minutes at room temperature, and the reaction was stopped by the dispensing of 50 mL of 2 mol/L hydrochloric acid. The optical density of the color solution was measured at 450 nm using EL340 Microplate Biotek™ biokinetics reader (Biotek, Winooski, VT, USA). Prior to ELISA, the total protein content of the cultured media from treated and untreated groups was determined using BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

RNA preparation and first-strand cDNA synthesis

Total RNA was isolated according to the guanidium thiocyanate-phenol-chloroform extraction method [28]. HK-2 cells were grown to confluence in 6-well plates. Serum-starved (12–18 h) cells were exposed for various concentrations of oxalate (0.1, 1.5, and 2.0 mmol/L) for 4 hours, or 1 mmol/L oxalate for various time points (4–24 h). The RNA concentration was measured by spectrophotometric absorbance at 260 nm. The total RNA was treated with RQ1 RNase-Free DNase (Promega,

Madison, WI, USA) to maintain the integrity of the RNA, and first-strand cDNA was synthesized by using Moloney Murine Leukemia Virus Reverse Transcriptase kit (M-MLV RT), according to the manufacturer's recommended protocol (Gibco BRL, Life Technologies, Gaithersburg, MD, USA). Briefly, 5 μg total RNA treated with RNase-Free DNase was reverse-transcribed for 60 minutes at 37°C using 1 μL (200 U) M-MLVRT in the presence of 250 ng random hexamer (Promega), 1 μL of 10 mmol/L dNTP mix, 4 μL of 5X first-strand buffer [250 mmol/L Tris-HCl (pH 8.3), 375 mmol/L KCl, 15 mmol/L $MgCl_2$], 2 μL of 0.1 mol/L dithiothreitol (DTT), 1 μL of RNaseOUT recombinant ribonuclease inhibitor (40 U/μL), and 1 μL of 10 mmol/L dNTP mix in 20 μL final volume.

IL-6 relative quantitative RT-PCR

IL-6 PCR reactions were carried out with a human IL-6 gene-specific relative quantitative RT-PCR (Ambion). PCR amplification was carried out by using specific oligonucleotide primers selected within the coding regions of the genes. IL-6 primers were designed to produce a 242-bp product, and 18s primers were designed to produce a 495-bp product. PCRs were composed of 5 μL of cDNA template, 100 ng each of sense and antisense oligonucleotide primers, 2.5 μL of optimized *Taq* PCR buffer (Promega), 0.4 mmol/L dNTP mixture, and 2 U of *Taq* polymerase in a total reaction volume of 25 μL. Following an initial 5-minute incubation at 94°C, PCRs were performed using a 45-second denaturation step at 94°C, followed by a 1-minute annealing step at 57°C, and a 2-minute elongation step at 72.0°C. A total number of 30 PCR cycles were carried out for the detection of IL-6 and 18s RNA, followed by a final elongation reaction for 10 minute at 72.0°C. PCR products were separated by electrophoresis at 80 V for 60 minutes through a 2% agarose gel, and were detected by ethidium bromide staining. Expected sizes of specific PCR products (495 and 242 bp for 18s and IL-6, respectively) were verified by reference to a 100-bp DNA ladder.

Measurement of free radical production

Oxalate-induced changes in the reduction of Nitro blue tetrazolium (NBT), a dye that reacts with superoxide, were used to evaluate oxidant stress as described previously [17]. For these experiments, confluent HK-2 cells grown in 12-well plates were exposed, for varying periods (0 to 180 min), to DMEM containing 25 μg/mL NBT. Where indicated, oxalate was also added alone or in combination with SOD or catalase. At the predetermined intervals (0, 60, 120, or 180 min), the medium was removed and the reaction was halted by the addition of 70% methanol. The monolayers were washed with 4 changes of 100% methanol to remove the unreduced NBT, air dried, and solubilized with a mixture of 2M KOH and dimethyl sulfoxide (DMSO) in a ratio of 1:1.167 (v/v). Samples were then centrifuged for 4 minutes at 15,000 rpm, and supernatants were read at 700 nm (density OD_{700}) using a Beckman DU-650 spectrophotometer (Beckman-Coulter, Fullerton, CA, USA) against a blank containing KOH and DMSO.

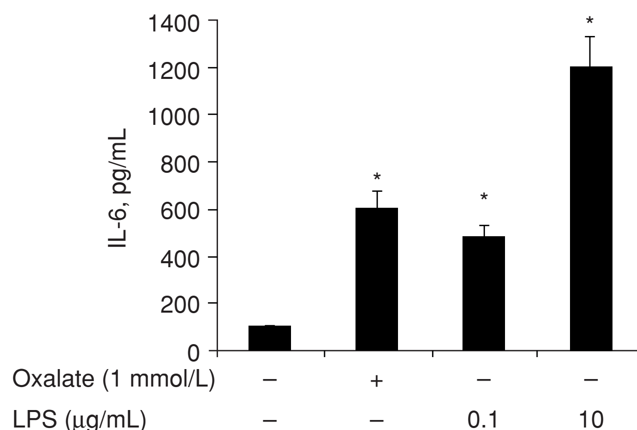

Fig. 1. Oxalate and lipopolysaccharide (LPS) stimulate interleukin-6 (IL-6) production from HK-2 cells. Confluent, growth-arrested, and serum-starved HK-2 cells were exposed to oxalate (1 mmol/L) or LPS (0.1, and 10 μg/mL) for 24 hours. The conditioned mediums were collected and assayed for IL-6 by ELISA, as described in **Methods**. The IL-6 levels were expressed as pg/mL, and each value represents the mean ± SD of at least 3 to 5 experiments, each performed in duplicate. The star indicates a significant difference ($P < 0.001$, Student t test).

Statistical analysis

For analysis of the data, an unpaired Student t test was used for statistical significance. Results are expressed as the mean ± SD, and $P < 0.001$ was deemed significant.

RESULTS

Oxalate and LPS stimulate IL-6 secretion from HK-2 cells

For these studies, confluent, growth-arrested, and serum-starved HK-2 cells were exposed to DMEM alone, or in combination with oxalate (1 mmol/L) or LPS (0.1 and 10 μg/mL) for 24 hours. At the end of experimental periods, the cultured conditioned mediums were collected, and their IL-6 levels were measured by ELISA. As can be seen from Figure 1, the secretion level of IL-6 in oxalate-exposed HK-2 cells, as compared to untreated control cells, was significantly elevated (602 ± 70 vs. 97 ± 12 pg/mL, $P < 0.001$). LPS is known to induce expression of a number of genes, including IL-6; we observed that even a low dose of LPS (0.1 μg/mL) exposed HK-2 cells significantly increases the level of secretion of IL-6 compared to untreated control (467 ± 50 vs. 97 ± 12 pg/mL, $P < 0.001$, Fig. 1). The higher dose of LPS (10 μg/mL) secreted a very high level of IL-6 (1204 ± 130 pg/mL). These results demonstrate that oxalate exposure results

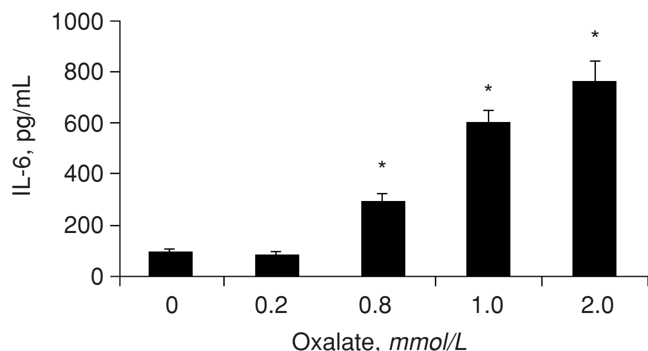

Fig. 2. Oxalate stimulates dose-dependent increases in interleukin-6 (IL-6) production from HK-2 cells. Confluent, growth-arrested, and serum-starved HK-2 cells were exposed to oxalate (0.2 to 2.0) for 24 hours. The conditioned mediums were collected and assayed for IL-6 by ELISA, as described in **Methods**. The IL-6 levels were expressed as pg/mL, and each value represents the mean ± SD of at least 3 to 5 experiments, each performed in duplicate. The star indicates a significant difference ($P < 0.001$, Student *t* test).

in elevated secretion of IL-6. Moreover, oxalate-stimulated IL-6 secretion was comparable to that of LPS, a known inducer of IL-6.

Oxalate stimulates IL-6 secretion in a dose-dependent manner from HK-2 cells

For these studies, confluent, growth-arrested, and serum-starved HK-2 cells were exposed to DMEM alone, or in combination with varying concentrations of oxalate (0.2–2.0 mmol/L) for 24 hours. At the end of experimental periods, the conditioned mediums were collected, and their IL-6 levels were measured by ELISA. Results presented in Figure 2 show that oxalate stimulated secretion of IL-6 in a dose-dependent fashion at all the concentrations tested (0.2–2.0 mmol/L).

Oxalate increases IL-6 mRNA expression in a time-dependent manner

For these studies, confluent, growth-arrested, and serum-starved HK-2 cells were exposed to DMEM alone, or in combination with oxalate (1.0 mmol/L) for 4 to 24 hours. At the end of experimental periods, total cellular RNA was isolated from the cells and subjected to relative quantitative RT-PCR to determine the expression of IL-6 and 18s mRNA. As can be seen from Figure 3A, the expression of IL-6 mRNA was significantly elevated in oxalate-exposed HK-2 cells compared to untreated control cells in a time-dependent manner. Maximum stimulation of IL-6 mRNA expression was observed at about 4 to 6 hours of oxalate exposure, with an average 1.8-fold increase (Fig. 3B). However, there was no significant change in the expression level of 18s mRNA (internal control) following HK-2 cells exposed to oxalate (Fig. 3A). These results demonstrate that oxalate exposure increases expression of IL-6 in a time-dependent fashion.

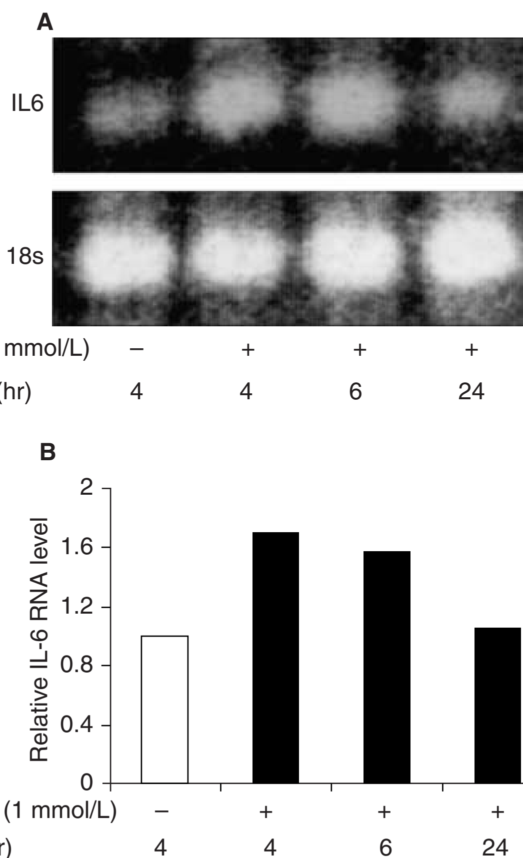

Fig. 3. Oxalate exposure increases time-dependent increase in interleukin-6 (IL-6) mRNA in HK-2 cells. Confluent, growth-arrested, and serum-starved HK-2 cells were exposed to oxalate (1.0 mmol/L) for various time points (4, 6, and 24 h). Total RNA was isolated according to the guanidium thiocyanate-phenol-chloroform extraction method, and the human IL-6 gene-specific relative quantitative RT-PCR was carried out as described in **Methods**. (*A*) Representative relative quantitative RT-PCR results for IL-6 and 18s ribosomal mRNA. (*B*) Relative IL-6 mRNA levels of HK-2 cells treated with oxalate (1 mmol/L) for various time points. The values were obtained by dividing the densitometric reading of the IL-6 band by that of the 18s band.

Oxalate increases IL-6 mRNA expression in a dose-dependent manner

For these studies, confluent, growth-arrested, and serum-starved HK-2 cells were exposed to DMEM alone, or in combination with oxalate (1.0–2.0 mmol/L) for 4 hours. At the end of experimental periods, total cellular RNA was isolated from the cells and subjected to relative quantitative RT-PCR to determine the expression of IL-6 and 18s mRNA. As can be seen from the Figure 4A, the expression of IL-6 mRNA in oxalate-exposed HK-2 cells, compared to untreated control cells, was significantly elevated, in a dose-dependent manner. Maximum expression of IL-6 mRNA can be seen at 2.0 mmol/L oxalate (Fig. 4B). However, there was no significant change in the

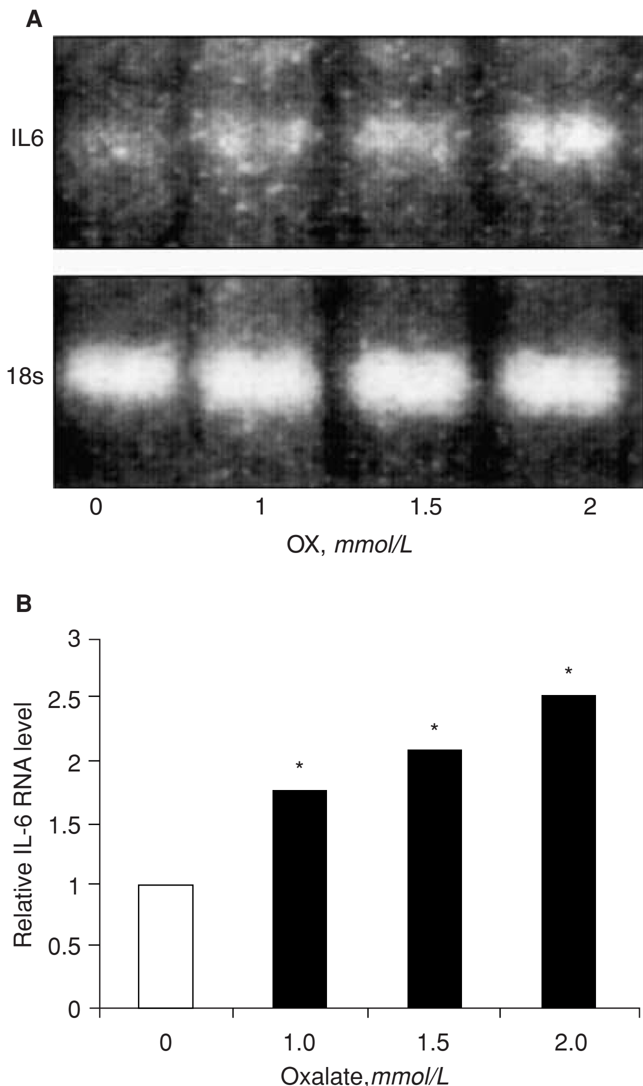

Fig. 4. Oxalate exposure increases dose-dependent increase in interleukin-6 (IL-6) RNA in HK-2 cells. Confluent, growth-arrested, and serum-starved HK-2 cells were exposed to various concentrations of oxalate (1.0 to 2.0 mmol/L) for 4 hours. Total RNA was isolated according to the guanidium thiocyanate-phenol-chloroform extraction method, and human IL-6 gene specific relative quantitative RT-PCR was carried out as described in **Methods**. (*A*) Representative relative quantitative RT-PCR results for IL-6 and 18s ribosomal mRNA. (*B*) Relative IL-6 mRNA levels of HK-2 cells treated with various concentrations of oxalate (1.0 to 2.0 mmol/L) for 4 hours. The values were obtained by dividing the densitometric reading of the IL-6 band by that of the 18s band. *Indicates a significant difference compared to control ($P < 0.001$, Student t test).

expression level of 18s mRNA (internal control) following HK-2 cells exposed to oxalate (Fig. 4A). These results demonstrate that IL-6 mRNA expression is increased by oxalate in a dose-dependent manner.

Oxalate-induced superoxide production is necessary for IL-6 expression

Previous studies from our laboratory have shown that oxalate exposure to HK-2 cells resulted in superoxide

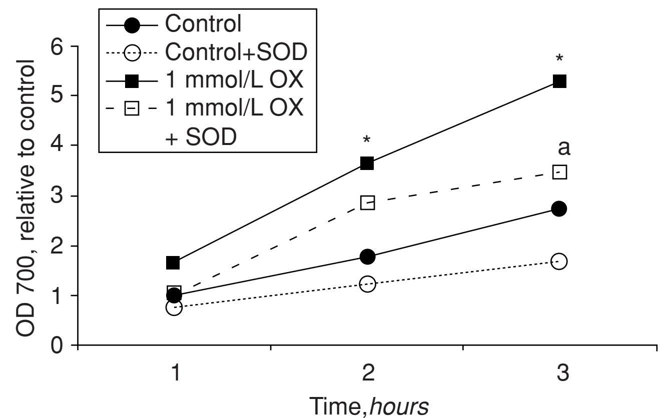

Fig. 5. Oxalate-induced IL-6 expression is mediated in part by free radical production. Effect of pretreatment with SOD on free-radical production: For these experiments, confluent monolayers of HK-2 cells were exposed to DMEM containing 25 μg/mL NBT plus varying oxalate (0 or 1 mmol/L total), alone or in the presence of SOD. *Indicates a significant difference compared to control ($P < 0.005$, Student t test). **a,** Indicates a significant difference compared to oxalate treated group ($P < 0.005$, Student t test).

production [17]. These data presented in Figure 5 indicate that oxalate exposure produces a time-dependent increase in the accumulation of reduced NBT dye, demonstrating increased superoxide production. The addition of superoxide dismutase (SOD) suppressed oxalate-stimulated free radical production. We also observed that the pretreatment with SOD of HK-2 cells resulted in the inhibition of IL-6 expression (Fig. 5B). Taken together, these data demonstrate that oxalate-stimulated IL-6 expression is mediated, at least in part, by free-radical production.

DISCUSSION

The present study provides evidence that human renal epithelial cells (HK-2 cells) express IL-6 mRNA and protein, and the oxalate exposure of these cells resulted in a significant alteration at the transcription and translation levels (Figs. 1–4). These findings are significant because elevation of IL-6 has been shown in the urine samples of kidney stone–forming patients [27]. The increased production of IL-6 may have a crucial role in the pathogenesis and physiopathology of urolithiasis. IL-6 is produced by a wide variety of cell types in response to many different stimuli, including TNF-α, LPS, and IL-1 [29]. We also observed that LPS-exposed HK-2 cells produce a very high level of IL-6 (Fig. 1). It is a typical cytokine, exhibiting functional pleiotropy, and involved in the immune response, inflammation, and hematopoiesis [18].

In the past few decades, accumulating evidence has been generated on the central role of the proximal tubular cell in renal injury and dysfunction, such as can be found in some patients with glomerular proteinuria, in chronic

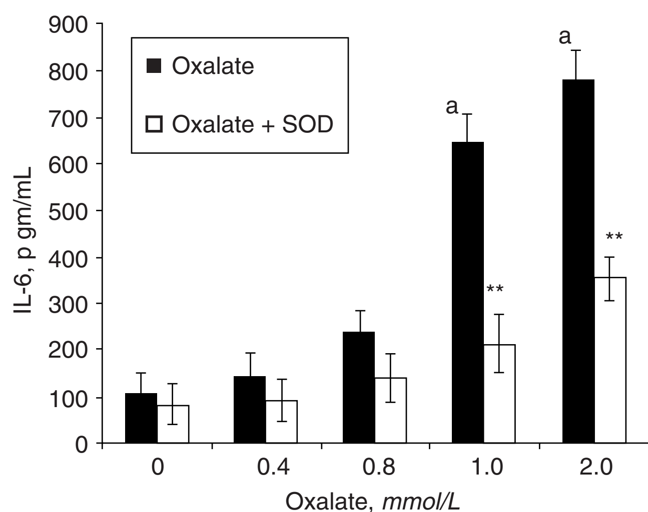

Fig. 6. Effect of SOD on IL-6 expression. Confluent, growth-arrested, and serum-starved HK-2 cells were exposed to oxalate (0 to 2.0) alone or in combination with SOD for 24 hours. The conditioned mediums were collected and assayed for IL-6 by ELISA, as described in **Methods**. The IL-6 levels were expressed as pg/mL, and each value represents the mean ± SD of at least 3 experiments, each performed in duplicate. **a,** Indicates a significant difference compared to control ($P < 0.001$, Student *t* test). **Indicates a significant difference compared to oxalate treated group ($P < 0.001$, Student *t* test).

renal rejection, or following ischemic insult. Following initial injury or stimulation of the proximal tubular cells, various cascades of mediator systems can be activated, leading, for example, to enhanced local production of complement, chemokines, cytokines, and matrix components. The locally produced mediators can subsequently lead to the amplification of injury—either directly or indirectly—by the enhancement of an influx of proinflammatory cells, such as macrophages, polymorphonuclear leukocytes, and T cells. The concerted production of cytokines and chemokines may tip the balance to a more proinflammatory pathway, leading to irreversible interstitial injury of the kidney and loss of renal function [18].

Within the kidney, elevated levels of IL-6 have been demonstrated in both resident and infiltrating cells in various forms of glomerulonephritis and tubulointerstitial nephritis, and, indeed, have been suggested to contribute to the pathogenesis or progression of the disease [30]. An elevated level of IL-6 has been reported in urolithiasis patients. IL-6 is also elevated in bacterial cystitis. The pattern of cytokine elevation in bacterial cystitis is quite different from the pattern observed in urolithiasis. In urolithiasis, only IL-6 was found to be elevated in the urine sample of the patients, while in bacterial cystitis, marked elevations in IL-1 beta, IL-1 alpha, and IL-6 were observed [27]. Bladder epithelium has been shown to produce IL-6 in response to various agents, and IL-6 has been proposed as a potential marker for interstitial cystitis. In the kidney, IL-6 elevation is produced by mesangial cells [31, 32]. A potential involvement of IL-6 in epithelial cell growth and differentiation has been proposed [33]. It was suggested that IL-6 in stone disease is unlikely to be derived from leukocytes; epithelial cells or other cells may possibly contribute to the production of IL-6. Our findings directly demonstrate that oxalate, the major constituent of calcium oxalate kidney stones, stimulates IL-6 secretion, which suggests that inflamed renal epithelial cells directly secrete IL-6 during calcium oxalate kidney stone pathogenesis. It is important to point out here that the results of our study may be relevant only to stone patients showing elevated urinary oxalate excretion. Indeed, other studies have suggested lack of inflammation and or tubular damage in idiopathic stone formers. Nonetheless, two subgroups of patients that have been shown to have elevated hyperoxaluria and proximal tubular damage and or inflammation are the patients with primary hyperoxaluria, and patients suffering from stone disease following intestinal bypass surgery [34].

Our studies presented herein demonstrate that oxalate-stimulated IL-6 expression and secretion could be inhibited by the free-radical scavenging agent (SOD). The results indicate that oxalate-associated free-radical production was at least in part necessary for IL-6 secretion and synthesis. These data suggest a role for oxidative stress in oxalate-stimulated IL-6 expression.

The mechanism/s that elicit the IL-6 release in renal cells are unclear, and the biologic role for IL-6—after its release from renal cells—is uncertain. The pathogenesis which gives rise to IL-6 elevation in urolithiasis is uncertain. There are two alternate scenarios to explain the elevation of IL-6. IL-6 may be released after stone formation, upon the mechanical stimulation (irritation) of epithelial cells [35], or it may be a contributor to the formation of stones. The possibility of a complex interaction between epithelial cells, the stone itself, and other cells may be needed for IL-6 production to occur. Alternately, IL-6 is known to cause bone resorption, and thus, elevated serum calcium levels [36]. Stone formers with idiopathic hypercalciuria have decreased bone mineral density [37, 38]. Our studies strongly suggest increased urinary oxalate as a contributor to increased IL-6 in idiopathic stone formers. However, it is not clear that in vivo IL-6 secreted by renal cells is capable of entering circulation. Whether IL-6 derived from nascent stone disease may contribute to bone resorption and stone formation remains unclear, and requires further study.

CONCLUSION

IL-6 is elevated in oxalate-exposed human renal epithelial cells at the transcriptional and translational

levels. These data suggest that oxalate-induced IL-6 production by the renal epithelium may play a critical role in the initiation/progression of urolithiasis. With idiopathic calcium oxalate urolithiasis likely being a multifactorial disease, it is unlikely any one cause of this disease can be found. Additionally, the biologic role of physiologic processes can be difficult to discern from in vitro studies because local cell death could be beneficial or detrimental in regards to crystalluria and stone formation. The threshold for oxalate-induced IL-6 expression in HK-2 cells is much higher than these cells commonly experience under normal physiologic conditions. The IL-6 production may reflect urothelium changes following oxalate injury and, as such, this may be appropriate only in subgroups of patients suffering from higher levels of urinary oxalate (i.e., PH-1 and intestinal bypass patients, and maybe some idiopathic hyperoxaluric patients).

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