View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Kidney International, Vol. 66 (2004), pp. 655–662

Proximal tubule cells stimulated by lipopolysaccharide inhibit macrophage activation

YIPING WANG, YUET-CHING TAY, and DAVID C.H. HARRIS

Center for Transplantation and Renal Research, The University of Sydney at Westmead Millenium Institute, Westmead, Sydney, Australia

Proximal tubule cells stimulated by lipopolysaccharide inhibit macrophage activation.

Background. Tubule cells can produce a variety of cytokines, extracellular matrix (ECM) components, and adhesion molecules in vitro and in vivo. It is generally assumed that stimulated tubule cells are proinflammatory and at least partially responsible for interstitial inflammation. However, the overall effect of tubular cells on interstitial cells is unknown. In this study, pro- and anti-inflammatory cytokine production and net effects on macrophages of tubule cells activated by lipopolysaccharide (LPS) were examined.

Methods. Tubule cells stimulated with LPS expressed tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-12, monocyte chemoattractant protein-1 (MCP-1), IL-10, and transforming growth factor-β (TGF-β). Conditioned media were collected from confluent monolayers of rat tubule cells stimulated, or not, by LPS for 4 and 18 hours, respectively. Macrophages were cultured with conditioned media and/or LPS (0.5 μg/mL) for 18 hours.

Results. TNF-α and IL-lβ mRNA of macrophages stimulated by LPS increased more than fivefold when cultured with control conditioned media from unstimulated tubule cells. Surprisingly, TNF-α and IL-lβ levels of macrophages stimulated by LPS were not increased when cultured with conditioned media from activated tubule cells. Neutralizing antibodies to IL-10 and TGF-β were used to define the inhibitory component(s) in conditioned medium. Anti-IL-10, but not anti-TGF-β, abolished partially the inhibitory effects of conditioned media on macrophages.

Conclusion. Tubule cells produce both pro- and anti-inflammatory cytokines and the net effect, partially explained by IL-10, of tubule cells activated with LPS is to inhibit activity of macrophages. Thus, the net effect of activated tubule cells on interstitial pathology may in certain circumstances, be anti- rather than pro-inflammatory.

In chronic renal diseases, the severity of tubulointerstitial damage correlates well with impairment of renal function and prognosis. The mechanisms underlying tubulointerstitial injury in chronic renal diseases are not clear, but include interstitial infiltration of macrophages and other cells. Depletion of macrophages has been shown to reduce renal injury in various models of glomerulonephritis [1–3], suggesting that macrophages play an important role in progression of chronic renal diseases. The damaging effects of macrophages involve interactions with intrinsic renal cells; for example, macrophages have been shown to alter the phenotype of tubule cells via the release of soluble mediators or cell to cell surface contact [4].

In response to various stimuli such as lipopolysaccharide (LPS), cytokines, urinary proteins, and hypoxia, tubule cells are capable of producing a large range of mediators, including chemoattractants, cytokines, and matrix proteins [5, 6]. It is generally believed that soluble mediators released from tubule cells worsen interstitial disease by recruiting and activating inflammatory cells such as macrophages or lymphocytes [7]. However, there is no direct proof of this hypothesis. Tubule cells produce a redundant network of mediators, including both proinflammatory and anti-inflammatory cytokines. Thus, the net effect of pro- and anti-inflammatory signals from tubule cells could be to either worsen or limit interstitial inflammation. The effects of single mediators released from tubule cells, including interleukin (IL)-1, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and regulated upon activation, normal T-cell expressed and secreted (RANTES), have been studied intensively [8, 9], yet the overall effects of activated tubule cells on inflammatory cells has been studied less. This study explored the net paracrine effects on macrophages of tubule cells activated by LPS.

Key words: proximal tubule cells, lipopolysaccharide, macrophage.

Received for publication December 2, 2002
and in revised form July 8, 2003, and February 2, 2004
Accepted for publication March 10, 2004

© 2004 by the International Society of Nephrology

METHODS

Animals

Inbred male Wistar rats (250 to 300 g) were supplied by the Animal Care Facility, Westmead Hospital, Sydney, Australia.

Cells and cell culture

For primary culture, tubule cells were isolated and cultured from normal male Wistar rats using isopycnic centrifugation. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with epidermal growth factor (EGF) (10 ng/mL) and insulin (5 mg/mL) in a 5% CO2/95% O2 environment[10]. Experiments were commenced after cells had reached confluence, which was usually between 4 to 5 days after the isolation procedure. The cultured tubule cells were 95% to 99% proximal tubule cells, with the remainder being distal tubule cells. A rat macrophage cell line (CRL-2192) [American Type Culture Collection (ATCC), Manassas, VA, USA] was propagated in F-12K medium with 2 mmol/L L-glutamine and supplemented with 15% fetal calf serum (FCS) in a 5% CO_2 and 95% O_2 air atmosphere. Tubule cells were stimulated with LPS (2.5 μg/mL) for 18 hours and their cytokine expression examined. Rat cytokines (TNF-α, IL-1β, MCP-1, and IL-10) were obtained from Endogen (Cambridge, MA, USA), and antibodies [anti-IL-10 and anti-transforming growth factor-β (TGF-β)] were obtained from A&D System (Oxon, UK).

Insert culture

To examine polarized secretion from tubule cells, bilayer insert culture was used. Confluent cells in primary culture were detached with trypsin treatment and washed with 10% FCS medium. Four hundred microliters of cells (5×10^4/mL) were transferred to 12 mm inserts (0.4 μm pore diameter) (Millipore-CM, Bedford, MA, USA) previously coated with rat tail collagen. These inserts allow tubule cells to grow on a permeable membrane and secrete cytokines into apical and basolateral compartments. After 24 to 36 hours of incubation, cells were confluent on the insert membrane. The permeability of confluent monolayer was assessed by adding albumin I^{125} at 0.2 μCi/mL. Approximately 5% leakage was detected during 24 hours of incubation. LPS (2.5 μg/mL) was added onto inserts (apical surface) or into wells (basolateral surface) and incubated for 8 hours. Aliquots of 100 μL were removed from both apical and basolateral sides for enzyme-linked immunosorbent assay (ELISA).

Preparation of conditional media

Confluent tubule cells in 100 mm culture plates were washed twice with DMEM-F12 Ham without any supplements and then incubated in 4 mL of DMEM-F12 Ham alone or 4 mL of DMEM-F12 Ham containing 2.5 μg/mL LPS for 4 and 18 hours at 37°C. At the end of 4 or18 hours, the media were removed, washed twice with DMEM-F12 Ham, replaced with 4 mL DMEM-F12 Ham with 2% FCS, and incubated for a further 24 hours. Media were then collected, filtered through a 0.2 μm filter and stored at −80°C until use. The amount of residual LPS in conditioned media was equivalent to that of standard commercial media (<0.001 EU), and insufficient to stimulate macrophages. Media conditioned by tubule cells in the absence of LPS served as control-conditioned media. Media conditioned by tubule cells in the presence of LPS were designated as conditioned media.

Dose-dependent and time-dependent stimulation of macrophage cytokines

Macrophages grown in culture plates were harvested on the day of treatment and seeded onto 35 mm culture plates (5 to 10×10^5 cells/plate). LPS (0 to 2.0 μg/mL) was added to the plates, and after 18 hours incubation at 37°C, the cells were harvested for RNA extraction. To follow the time course of cytokine expression in macrophages in response to LPS stimulation, the cells were harvested at 6, 12, and 24 hours after addition of LPS (0.5 μg/mL). Cells grown without LPS were harvested after 24 hours and used as unstimulated control. The combined effects of LPS (0.5 μg/mL) were examined with TNF-α (10 ng/mL), IL-1β (10 ng/mL), MCP-1 (100 ng/mL), and IL-10 (10 ng/mL).

Effect of tubule cells–derived conditioned media on cytokine expression in macrophages

To examine whether tubule cells have the ability to produce modulators of macrophage function, macrophages in F-12K macrophage growth medium were seeded onto 35 mm culture plates (5 to 10×10^5 cells/plate). Conditioned control media (from unstimulated tubule cells) and conditioned media (from LPS-stimulated tubule cells) were diluted serially from 2 to 40 times and added to the culture plates. The plates were incubated at 37°C for 6 hours. After 6 hours, LPS (0.5 μg/mL) was added and the plates were incubated for a further 18 hours. LPS was omitted from some plates to serve as a negative control for unstimulated macrophages. The cells were harvested for RNA extraction. In some experiments macrophages were pretreated with diluted (1:2) conditioned media to which anti-IL-10 antibody (tested at 0.5, 1.0, and 2.5 μg/mL) had been added, and diluted (1:2) conditioned media to which anti-TGF-β had been added. Normal IgG, in amounts equivalent to those of the antibodies, was added to control plates.

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using RNAzol B (Teltest, Frienswood, TX, USA). Transcription of mRNA in tubule cells or macrophages was determined using semiquantitative RT-PCR. The design of primers and conditions for RT-PCR have been described previously [11, 12].

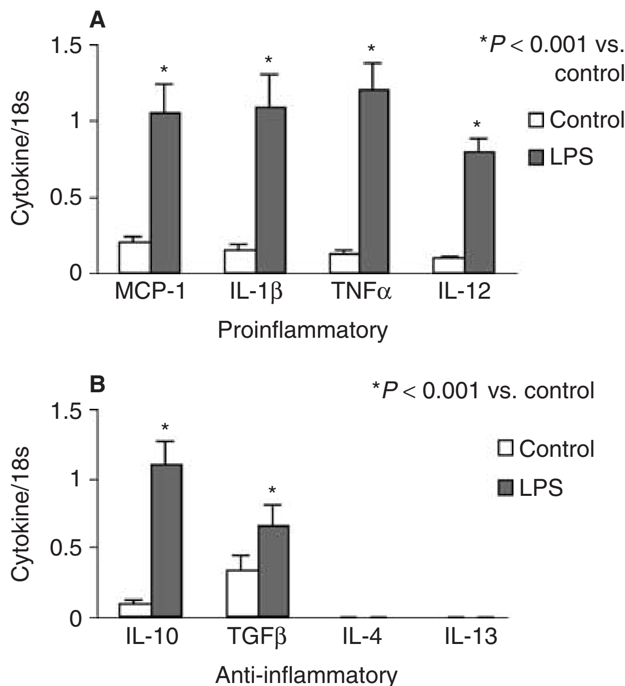

Fig. 1. Expression of pro-inflammatory (*A*) and anti-inflammatory (*B*) cytokines relative to 18 S by reverse transcription-polymerase chain reaction (RT-PCR) in tubule cells exposed to lipopolysaccharide (LPS) (2.5 μg/mL). Expression of proinflammatory cytokines interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), IL-12, and monocyte chemoattractant protein-1 (MCP-1) and anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGF-β) mRNA were significantly increased in tubule cells exposed to LPS. However, IL-4 and IL-13 were undetectable. The results are from five separate experiments and each performed in triplicate. Data are mean ± SD.

PCR product was loaded into a 1.2% agarose gel stained by ethidium bromide (0.5 μg/mL). The gel was viewed and scanned using a Fluo-S MultiImager system from Bio-Rad (Hercules, CA, USA) (170 to 7700). The expression of all cytokines of interest was normalized against 18S expression (Internal Standards, Ambion Inc., Austin TX, USA).

ELISA

TNFα, IL-1β, and IL-10 in conditioned media and the media from apical and basolateral sides of insert culture were assessed by ELISA. The procedures were undertaken according to manufacturer's protocol for individual ELISA kits (BioSource International, Inc., Camarillo, CA USA).

Statistics

The results are expressed as the mean ± SD, and statistical significance of differences within the groups and among groups was determined by one way analysis of variance (ANOVA). The post-test of ANOVA was the post hoc least significant difference (LSD) test (SPSS, Chicago, IL, USA). A value less than 0.05 was considered to be significant.

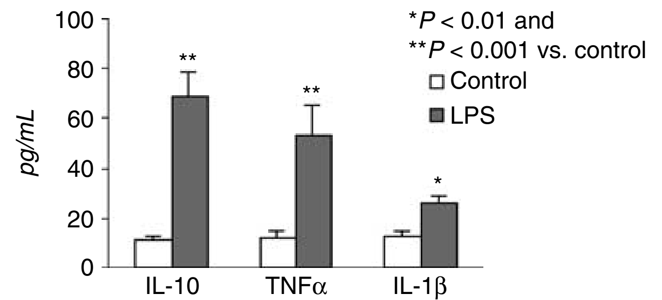

Fig. 2. Concentration of interleukin (IL)-10, IL-1β and tumor necrosis factor-α (TNF-α) protein in conditioned media. IL-10, IL-1β, and TNF-α were examined by enzyme-linked immunosorbent assay (ELISA) in conditioned media from unstimulated tubule cells and tubule cells exposed to lipopolysaccharide (LPS) (2.5 μg/mL). Data are mean ± SD.

RESULTS

Induction of cytokines in tubule cells

By semiquantitative RT-PCR, the proinflammatory cytokines-IL-1β, TNF-α, IL-12, and MCP-1, and anti-inflammatory cytokines-IL-4, IL-10, TGF-β, and IL-13 were examined in tubule cells stimulated with LPS (2.5 μg/mL) for 18 hours. mRNA of proinflammatory cytokines IL-1β, TNF-α, IL-12, and MCP-1 and anti-inflammatory cytokines IL-10 and TGF-β were increased in tubule cells stimulated with LPS, but IL-4 and IL-13 mRNA was not detected in tubule cells either with or without LPS stimulation (Fig. 1). As a positive control, IL-4 and IL-13 mRNA were detected in spleen and renal cortex of rats with adriamycin nephropathy at week 4. mRNA of both proinflammatory cytokines-IL-1β, TNF-α, IL-12, and MCP-1 and anti-inflammatory cytokines IL-10 and TGF-β increased with a similar time frame in tubule cells stimulated with LPS, each starting to increase after 2 hours, reaching a peak at 6 to 12 hours and lasting for at least 24 hours.

To examine whether the induced transcripts of cytokines were translated to proteins and secreted into medium, we assessed IL-1β, TNF-α, and IL-10 protein levels in conditioned media, after tubule cells were stimulated with LPS. IL-10 and TNF-α increased dramatically, about four- to sevenfold, whereas the increase in IL-1β was less marked, about one- to twofold (Fig. 2).

If cytokines are to act as mediators in interaction between tubule cells and interstitial cells, then tubule cells should secrete cytokines across their basolateral surface toward the interstitial space. To explore this important issue, polarized secretion from tubule cells was examined. Tubule cells stimulated by LPS from either the apical or basolateral side secreted IL-1β, TNF-α and IL-10. The concentration of TNF-α and IL-10 proteins was greater in basolateral than in apical media, whereas IL-1β was the same in media of the two sides (Fig. 3). Moreover, apical stimulation of tubule cells with LPS resulted in a

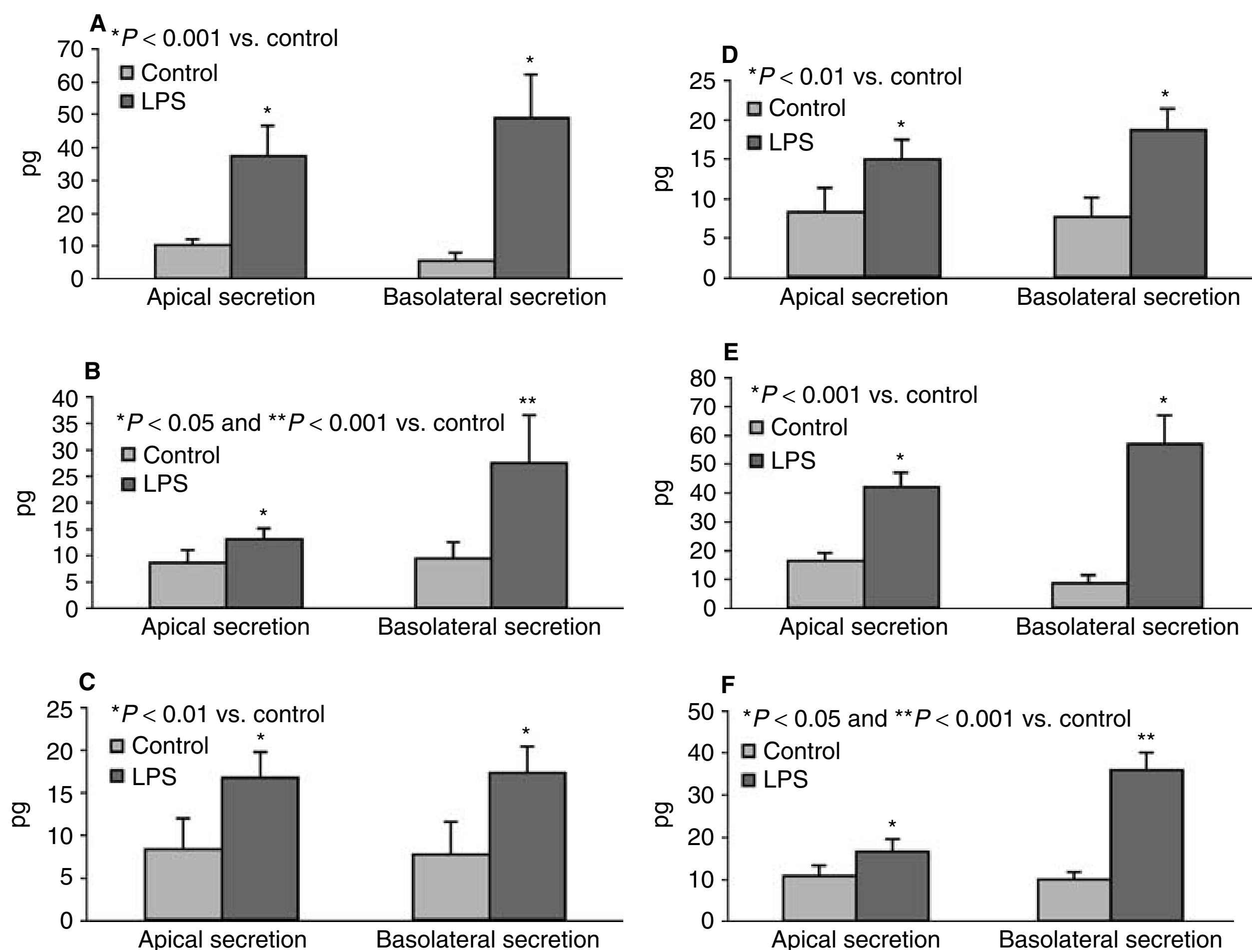

Fig. 3. Polarized secretion of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-10 from tubule cells. The total amount of TNF-α (*A* and *B*), IL-1β (*C* and *D*), and IL-10 (*E* and *F*) secreted into apical and basolateral media were examined after apical lipopolysaccharide (LPS) stimulation (A, C, and E) and basolateral stimulation (B, D, and F). Both apical and basolateral secretion of IL-10 and TNF-α were greater following apical LPS stimulation versus basolateral stimulation. Basolateral secretion was greater than apical secretion with either apical or basolateral stimulation. However, apical and basolateral secretion of IL-1β were no different, nor was there a differential effect of apical versus basolateral stimulation. Data are mean ± SD.

greater release of TNF-α and IL-10 both apically and basolaterally, than did basolateral stimulation with LPS.

Effects of conditioned media on cytokine expression of macrophages

The kinetics of expression of TNF-α and IL-1β were examined in macrophages. Unstimulated macrophages expressed TNF-α and IL-1β weakly. Following exposure to LPS from 0.1 to 2 μg/mL for 18 hours, macrophages produced TNF-α in a dose-dependent manner (Fig. 4). After 2 hours of LPS (0.5 μg/mL) stimulation, an increase in expression of TNF-α was detectable at 3 hours, reached a peak at 6 hours, and was sustained for at least 24 hours (Fig. 5). Macrophages expressed IL-1β mRNA in a dose-dependent and time-dependent manner similar to TNF-α, except there was less increase in IL-1β mRNA (fourfold) than TNF-α (sevenfold) in response to LPS (0.5 μg/mL), indicating TNF-α is a more sensitive marker of activated macrophages (Figs. 5 and 6). Therefore, TNF-α was used as a marker for macrophage activation in most of the following experiments. LPS (0.5 μg/mL) was chosen to activate macrophages in further experiments, as this dose activated macrophages moderately, permitting assessment of both inhibition and stimulation of macrophage TNF-α and IL-1β production. The effect of LPS (0.5 μg/mL) was augmented (TNF-α, IL-1β, and MCP-1) or inhibited (IL-10) by addition of recombinant cytokines (Fig. 7).

Macrophages without any LPS stimulation were cultured in conditioned media. mRNA expression of TNF-α and IL-1β of macrophages was not significantly different whether grown in media obtained from tubule cells stimulated with LPS or from unstimulated tubule cells. However, mRNA expression of TNF-α and IL-1β of macrophages stimulated with LPS (0.5 μg/mL) was reduced significantly by media from tubule cells stimulated

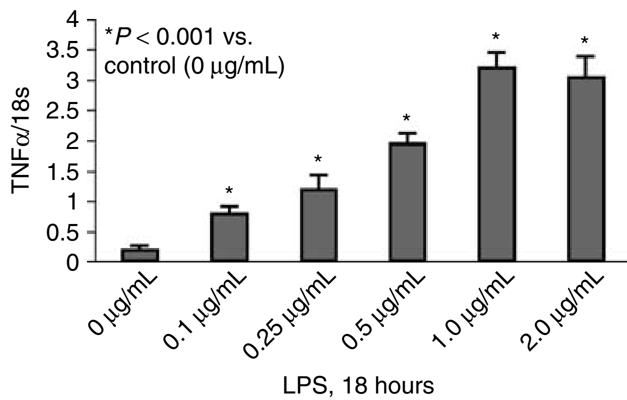

Fig. 4. Dose-dependent effect of lipopolysaccharide (LPS) on tumor necrosis factor-α (TNF-α) mRNA expression in macrophages. Macrophages were stimulated with increasing concentrations of LPS (0.1 to 2 μg/mL). The results are from five separate experiments and each performed in triplicate. Data are mean ± SD.

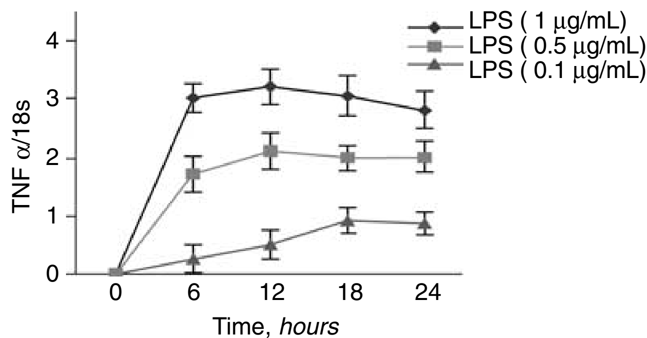

Fig. 5. Time-dependent effect of lipopolysaccharide (LPS) on tumor necrosis factor-α (TNF-α) mRNA expression in macrophages. TNF-α mRNA expression reached its peak in approximately 6 hours and was sustained for at least 24 hours with LPS. The results are from five separate experiments and each performed in triplicate. Data are mean ± SD.

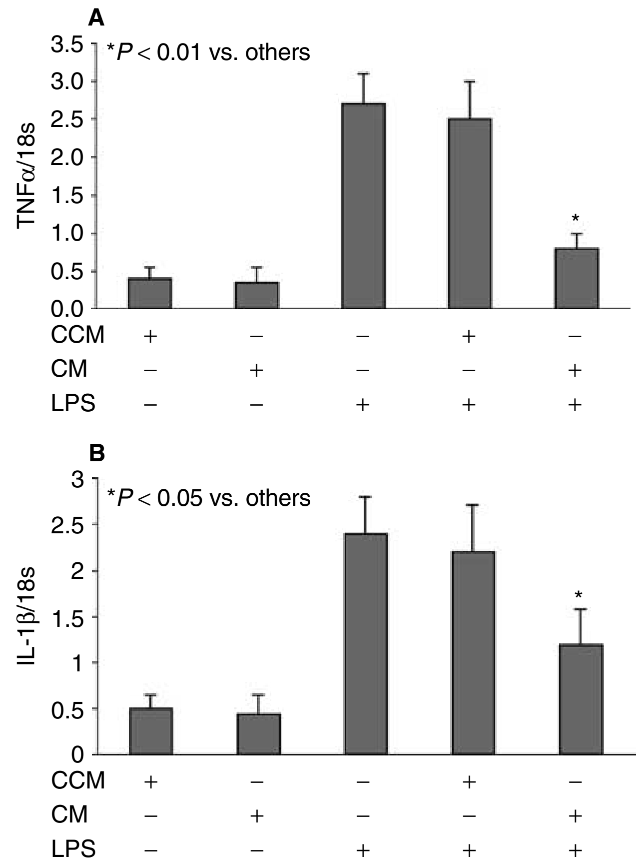

Fig. 6. The effect of tubule cell–conditioned media on tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β mRNA expression of macrophages activated by lipopolysaccharide (LPS) (0.5 μg/mL). TNF-α mRNA expression (*A*) and IL-1β mRNA expression (*B*) of macrophages were increased in control conditioned media (CCM), but increased TNF-α and IL-1β expression were inhibited in conditioned media (CM) from tubule cells exposed to LPS for 18 hours. Very similar results were obtained with conditioned media exposed to LPS for 4 hours. The results are from five separate experiments and each performed in triplicate. Data are mean ± SD.

for 18 hours with LPS (Fig. 6). Similarly, an inhibitory effect on macrophages was also shown with the media from tubule cells stimulated with LPS from an early time point of stimulation (4 hours). This inhibitory effect of conditioned media was concentration-dependent (Fig. 8). Moreover, the inhibitory effect of conditioned media was also exhibited in macrophages activated with TNF-α (Fig. 9).

Identification of the active inhibitor in tubule cell–conditioned media

To define the inhibitors in conditioned media, the effects of inhibiting inflammatory cytokines were examined using neutralizing antibodies. Anti-IL-10 specific antibody abolished partially the inhibitory effect of conditioned media from activated tubule cells. Expression of TNF-α of macrophages in conditioned media increased by 40% with anti-IL-10 (Fig. 10). The inhibitory effect was not increased by higher doses of anti-IL-10. However, anti-TGF-β had no effect on the inhibitory effect of conditioned media (Fig. 10). The combination of both antibodies had a similar effect on TNF-α expression of macrophages to that of anti-IL-10 alone.

DISCUSSION

To examine the influence of stimulated tubule cells on macrophages, we assessed cytokine production by tubule cells and the net effect of conditioned media from tubule cells on macrophages. Tubule cells produced both proinflammatory cytokines IL-1β, TNF-α, IL-12, and MCP-1 and anti-inflammatory cytokines IL-10 and TGF-β. Both proinflammatory and anti-inflammatory cytokines were secreted by tubule cells across their apical or basolateral membranes, but preferentially across their basolateral surface. Surprisingly, conditioned media from

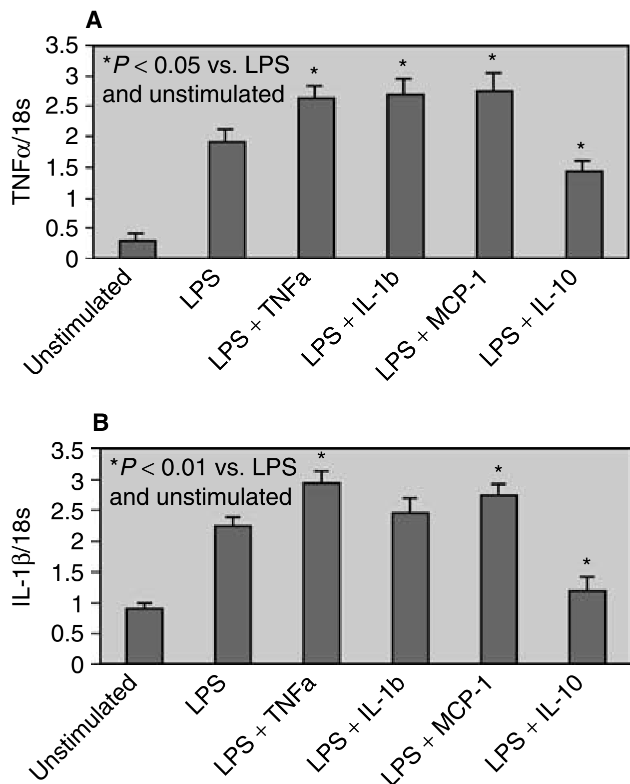

Fig. 7. Combined effects of recombinant cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, monocyte chemoattractant protein-1 (MCP-1) and IL-10] and lipopolysaccharide (LPS). TNF-α, IL-1β, or MCP-1 increased macrophage TNF-α mRNA expression (*A*), and TNF-α and MCP-1 increased macrophage IL-1β mRNA expression (*B*). Recombinant IL-10 inhibited both TNF-α, and IL-1β expression. The results are from three separate experiments and each performed in triplicate. Data are mean ± SD.

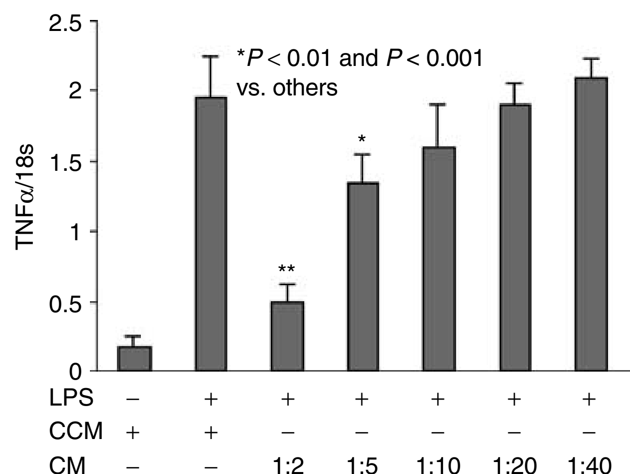

Fig. 8. The effect of tubule cell–conditioned media (CM) in different concentrations on tumor necrosis factor-α (TNF-α) mRNA expression of macrophages. The inhibitory effect of conditioned media in dilutions from 1:2 to 1:40 was dose-dependent. The results are from three separate experiments and each performed in triplicate. Data are mean ± SD. Abbreviations are: LPS, lipopolysaccharide; CCM, control conditioned media.

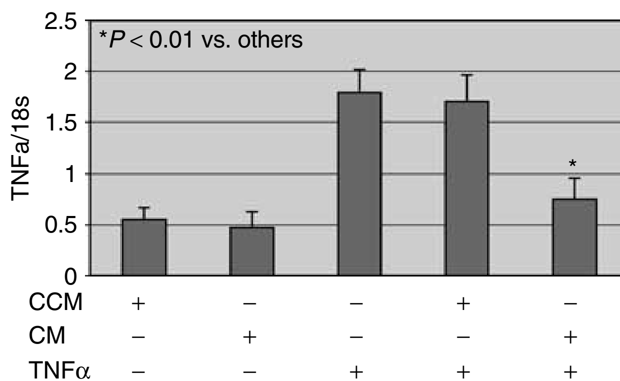

Fig. 9. The effect of tubule cell–conditioned media (CM) on tumor necrosis factor-α (TNF-α) mRNA expression of macrophages activated by TNF-α. TNF-α mRNA expression of macrophages was increased in control conditioned media (CCM), but increased TNF-α expression was inhibited in conditioned media from tubule cells exposed to lipopolysaccharide (LPS). The results are from three separate experiments and each performed in triplicate. Data are mean ± SD.

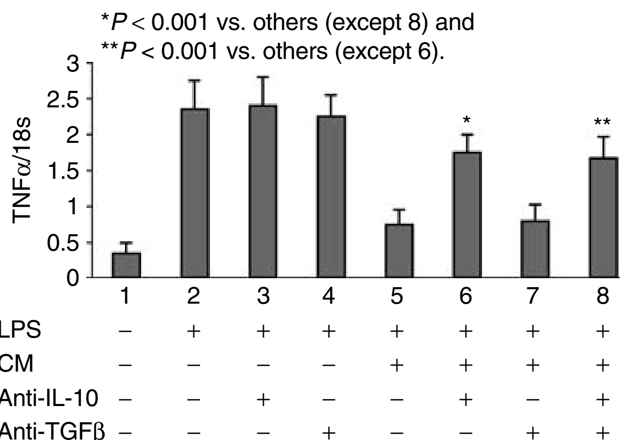

Fig. 10. Effect of neutralizing antibodies against interleukin (IL)-10 and transforming growth factor-β (TGF-β) on tumor necrosis factor-α (TNF-α) mRNA expression of stimulated macrophages in response to tubule cell–conditioned media (CM). Anti- IL-10 partially abolished the inhibitory effect in conditioned media. However, there was no effect of anti-TGF-β on the inhibitory effect of conditioned media. The results are from three separate experiments and each performed in triplicate. Data are mean ± SD.

activated tubule cells suppressed TNF-α and IL-1β mRNA expression of macrophages. To define the inhibitory components involved, the effects of IL-10 and TGF-β were examined with neutralizing antibodies. Anti-IL-10, but not anti-TGF-β, partially abolished the inhibitory effects of conditioned media on macrophages.

Renal interstitial inflammation is an integral pathologic component of chronic proteinuric renal disease, and its presence predicts the progression and prognosis of chronic renal disease [13]. Tubule cells play an important role in development and evolution of interstitial inflammation, due in part to the elaboration of a network of pro- and anti-inflammatory cytokines. Tubule cell cytokines

are of overriding importance in the interaction between tubule cells and the interstitium, but act in concert with other factors, including adhesion molecules, complement, and major histocompatibility complex (MHC). It has been established that expression of a number of individual proinflammatory cytokines such as TNF-α, IL-1β MCP-1, and RANTES are increased in tubule cells in various renal diseases, suggesting that tubular cell cytokines could contribute to the development and progression of interstitial inflammation [14]. Experiments in vitro have also showed that the expression of those cytokines was increased by stimulated tubule cells in response to various phenomena such as proteinuria, hypoxia, and endotoxin, indicating that stimulated tubule cells could be a major source of cytokines involved in pathogenesis of chronic renal diseases [15]. However, the true role of various tubule cell cytokines in modulating interstitial inflammation is unclear, as administration or inhibition of individual cytokines is ineffective or only partially effective in influencing the inflammatory process. This lack of efficacy could be due to a complex network of tubule cell cytokines with overlapping or opposing effects on interstitial target cells. To determine the importance of the balance between pro- and anti-inflammatory cytokines, we surveyed the profile of tubule cell cytokines, and found increased mRNA expression of both proinflammatory cytokines (IL-1β, TNF-α, IL-12, and MCP-1) and anti-inflammatory cytokines (IL-10 and TGF-β). Correspondingly, increased protein levels of IL-1β, TNF-α and IL-10 were also detected in media cultured from tubule cells after stimulation by LPS. Furthermore, tubule cell secretion of TNF-α and IL-10, but not IL-1β, was polarized across the basolateral membrane, consistent with their proposed modulatory effect on the renal interstitium in vivo.

Tubule cells are widely considered to be activators of interstitial inflammation. Yet, as both pro- and anti-inflammatory cytokines are released by tubule cells, it is not possible to predict their net effect on interstitial target cells. To assess the true proinflammatory potential of tubular cells, it was necessary to observe the net effect of conditioned media harvested from tubule cells on an interstitial target. Against expectations, the net signal of conditioned media from stimulated tubule cells on TNF-α and IL-1β production by activated macrophages was inhibitory. The relative importance of potential inhibitory components of conditioned media was defined by neutralizing antibodies against IL-10 and TGF-β. The data demonstrated that IL-10, but not TGF-β was partially responsible for the inhibitory effects of conditioned media. Increased IL-10 expression has been demonstrated in various glomerulonephritides, and interferon-γ (INF-γ)/IL-10 ratio has been shown to correlate with the severity of renal diseases such as IgA nephropathy [16, 17]. In vitro, IL-10 has been defined as a deactivator of macrophages by its inhibition of generation of macrophage products such as IL-1β, TNF-α, reactive oxygen species, and matrix metalloproteinases [18]. IL-10 also inhibits the differentiation of monocytes into macrophages and promotes apoptosis of macrophages in concert with granulocyte-macrophage-colony-stimulating factor (GM-CSF) [19]. In vivo, IL-10 has been reported to attenuate macrophage-induced glomerular injury [20]. However, others have reported an inability of IL-10 to prevent renal injury in anti-glomerular basement membrane (GBM) glomerulonephritis, possibly due to its failure to inhibit macrophages in this situation [21]. The pathophysiologic significance of IL-10 produced by tubule cells in vivo is uncertain and likely to be contextural, and dependent on the nature and phase of the specific renal disease. For example, IL-10 could act as a deactivator of macrophages and also serve as a cofactor for promoting the growth of T cells, B cells, and mast cells and up-regulating the cytolytic activity of natural killer (NK) cells [22]. Moreover, it has been shown that IL-10 mediates mesangial cell proliferation and induces expression of E-selectin on endothelial cells, an effect crucial to the accumulation of neutrophils at the site of inflammation [23, 24].

Although the expression of TGF-β by tubule cells was increased in response to LPS, TGF-β was not one of the inhibitory components of the conditioned media in this in vitro experiment. However, an anti-inflammatory role for TGF-β produced by tubule cells is not excluded in vivo, as TGF-β is secreted in an inactive form and requires enzymatic activation involving the participation of other cells [22]. In vivo, TGF-β converted to its active moiety in extracellular space could stimulate proliferation and matrix production of fibroblasts and mesangial cells, respectively, leading to interstitial and glomerular sclerosis [26].

In present study, anti-IL-10 did not completely block the inhibitory effect of conditioned media on TNF-α production by activated macrophages. One possible reason for this could be related to effects of inhibitory autocrine loop of macrophages. Function of macrophages stimulated by LPS was shown to be controlled by stimulatory autocrine loops involving IL-12 and inhibitory autocrine loops involving IL-10 [27]. Anti-IL-10 not only neutralized IL-10 in conditioned media, but also blocked the IL-10–dependent inhibitory autocrine loop of macrophages, resulting in increase of TNF-α [28]. Another possible reason for the partial blocking effect of anti-IL-10 could be that IL-10 is not the only inhibitory component in conditioned media. There are several other potential inhibitory components, including IL-1 receptor antagonist, insulin-like growth factor-1, macrophage inhibitory factor, and leukemia inhibitory factor. Further studies are necessary to determine whether and to what extent other inhibitory factors are involved.

LPS was chosen as the stimulator of tubule cells in these experiments as it is an important pathogenic factor in both acute and chronic renal disease. Other potential stimulators of tubule cells such as cytokines TNF-α and IL-1β, proteinuria, and hypoxia also have important roles in the development and progression of interstitial inflammation; however, none of the these factors individually was able to stimulate tubule cells sufficiently to produce detectable cytokines under these experimental conditions. Various combinations of those factors might be able to stimulate tubule cells, and it is possible that certain combinations could produce unique macrophage responses to tubule cell conditioned media.

CONCLUSION

Tubule cells produce both pro- and anti-inflammatory cytokines. In vivo these cytokines are likely to gain access to interstitium across tubule cell basolateral membrane, allowing their participation in pathophysiologic processes underlying interstitial inflammation and fibrosis. Surprisingly, the net effect of media from tubule cells activated with LPS was to inhibit activity of macrophages, an inhibitory effect partially dependent on IL-10. These results emphasize that the overall role of tubule cells in progressive chronic renal disease is likely to be complex and contextual, and may in some circumstances be to protect against interstitial injury.

ACKNOWLEDGMENTS

This study was supported by a grant from the National Health and Medical Research Council of Australia (No. 970721) and a grant from the Australia Kidney Foundation.

Reprint requests to Dr. Yiping Wang, Center for Transplantation and Renal Research, The University of Sydney at Westmead Millenium Institute, Westmead, Sydney, Australia 2145.
E-mail: Yiping_Wang@wsahs.nsw.gov.au

REFERENCES

1. D'souza MJ, Oettinger CW, Shah A, *et al*: Macrophage depletion by albumin microencapsulated clodronate: Attenuation of cytokine release in macrophage-dependent glomerulonephritis. *Drug Dev Ind Pharm* 25:591–596, 1999
2. Feith GW, Bogman MJ, Assmann KJ, *et al*: Decreased PMN accumulation and glomerular damage by clodronate liposome treatment in PMN-dependent anti-GBM nephritis in mice. *Exp Nephrol* 5:301–304, 1997
3. Westerhuis R, Van Straaten SC, Van Dixhoorn MG, *et al*: Distinctive roles of neutrophils and monocytes in anti-thy-1 nephritis. *Am J Pathol* 156:303–310, 2000
4. Ong AC, Fine LG: Tubular-derived growth factors and cytokines in the pathogenesis of tubulointerstitial fibrosis: Implications for human renal disease progression. *Am J Kidney Dis* 23:205–209, 1994
5. Bohle A, Mackensen-Haen S, Von Gise H, *et al*: The consequences of tubulointerstitial changes for renal function in glomerulopathies. A morphometric and cytological analysis. *Pathol Res Pract* 186:135–144, 1990
6. Frank J, Engler-Blum G, Rodemann HP, Muller GA: Human renal tubular cells as a cytokine source: PDGF-B, GM-CSF and IL-6 mRNA expression in vitro. *Exp Nephrol* 1:26–35, 1993
7. Jevnikar AM, Wuthrich RP, Takei F, *et al*: Differing regulation and function of ICAM-1 and class II antigens on renal tubular cells. *Kidney Int* 38:417–425, 1990
8. Chen WP, Lin CY: Augmented expression of interleukin-6 and interleukin-1 genes in the mesangium of IgM mesangial nephropathy. *Nephron* 68:10–19, 1994
9. Schmouder RL, Strieter RM, Kunkel SL: Interferon-gamma regulation of human renal cortical epithelial cell-derived monocyte chemotactic peptide-1. *Kidney Int* 44:43–49, 1993
10. Baer PC, Scherberich JE, Bereiter-Hahn J, Geiger H: Induction of RANTES, HLA-DR, and intercellular adhesion molecule-1 on highly purified distal tubular cells from human kidney. *Transplantation* 15:2456–2459, 2000
11. Vinay P, Gougoux A, Lemieux G: Isolation of a pure suspension of rat proximal tubules. *Am J Physiol* 241:F403–F411, 1981
12. Rangan GK, Wang Y, Tay YC, Harris DC: Inhibition of NFkappaB activation with antioxidants is correlated with reduced cytokine transcription in PTC. *Am J Physiol* 277(5 Pt 2):F779–F789, 1999
13. Rangan GK, Wang Y, Tay YC, Harris DC: Cytokine gene expression in adriamycin nephropathy: Effects of antioxidant nuclear factor kappaB inhibitors in established disease. *Nephron* 86:482–490, 2000
14. Schainuck LI, Striker GE, Cutler RE, Benditt EP: Structural-functional correlations in renal disease. II. The correlations. *Hum Pathol* 1:631–641, 1970
15. Chen WP, Lin CY: Augmented expression of interleukin-6 and interleukin-1 genes in the mesangium of IgM mesangial nephropathy. *Nephron* 68:10–19, 1994
16. Healy E, Brady HR: Role of tubule epithelial cells in the pathogenesis of tubulointerstitial fibrosis induced by glomerular disease. *Curr Opin Nephrol Hypertens* 7:525–530, 1998
17. Niemir ZI, Ondracek M, Dworacki G, *et al*: In situ upregulation of IL-10 reflects the activity of human glomerulonephritides. *Am J Kidney Dis* 32:80–92, 1998
18. Lim CS, Zheng S, Kim YS, *et al*: Th1/Th2 predominance and proinflammatory cytokines determine the clinicopathological severity of IgA nephropathy. *Nephrol Dial Transplant* 16:269–275, 2001
19. Fiorentino DF, Zlotnik A, Mosmann TR, *et al*: IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 147:3815–3822, 1991
20. Hashimoto SI, Komuro I, Yamada M, Akagawa KS: Il-10 inhibits granulocyte-macrophage colony-stimulating factor-dependent human monocyte survival at the early stage of the culture and inhibits the generation of macrophages. *J Immunol* 167:3619–3625, 2001
21. Huang XR, Kitching AR, Tipping PG, Holdsworth SR: Interleukin-10 inhibits macrophage-induced glomerular injury. *J Am Soc Nephrol* 11:262–269, 2000
22. Chadban SJ, Tesch GH, Lan HY, *et al*: Effect of interleukin-10 treatment on crescentic glomerulonephritis in rats. *Kidney Int* 51:1809–1817, 1997
23. Moore KW, O'Garra A, De Waal Malefyt R, *et al*: Interleukin-10. *Annu Rev Immunol* 11:165–190, 1993
24. Chadban SJ, Tesch GH, Foti R, *et al*: Interleukin-10 is a mesangial cell growth factor in vitro and in vivo. *Lab Invest* 76:619–627, 1997
25. Vora M, Romero LI, Karasek MA: Interleukin-10 induces E-selectin on small and large blood vessel endothelial cells. *J Exp Med* 184:821–829, 1996
26. Massague J: The transforming growth factor-beta family. *Annu Rev Cell Biol* 6:597–641, 1990
27. Taniguchi Y, Yorioka N, Masaki T, *et al*: Role of transforming growth factor-beta 1 in glomerulonephritis. *J Int Med Res* 252:71–80, 1997
28. Grohmann U, Belladonna ML, Vacca C, *et al*: Positive regulatory role of IL-12 in macrophages and modulation by IFN-gamma. *J Immunol* 167:221–227, 2001
29. Donnelly RP, Freeman SL, Hayes MP: Inhibition of IL-10 expression by IFN-gamma up-regulates transcription of TNF-alpha in human monocytes. *J Immunol* 155:1420–1427, 1995