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Renal cell cytokine production stimulates HIV-1 expression in chronically HIV-1-infected monocytes

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Renal cell cytokine production stimulates HIV-1 expression in chronically HIV-1-infected monocytes. Renal infiltration of human immunodeficiency virus type 1 (HIV-1)-infected monocytes might play an important role in the development of HIV-associated nephropathy (HIVAN). In the present study, we investigated the effects of cytokines produced by cultured human mesangial cells (HMC) and proximal tubular epithelial cells (PTEC) on HIV-1 expression in chronically HIV-1-infected promonocytes (U1 cells). Human mesangial cells constitutively secreted interleukin-6 (IL-6) but not tumor necrosis factor-alpha (TNF-α) into the culture medium, whereas PTEC constitutively secreted both IL-6 and TNF-α. Coculture of U1 cells with HMC or PTEC for 72 hours markedly stimulated HIV-1 expression, with the p24 antigen concentration in the coculture supernatants ranging from approximately 200 to 1850 pg/ml. The presence of anti-IL-6 antibody in the coculture medium nearly completely blocked HIV-1 expression in the HMC/U1 cell cocultures ($P < 0.05$). Anti-IL-6 antibody and anti-TNF-α antibody blocked HIV-1 expression in the PTEC/U1 cell cocultures by 40% and 53%, respectively ($P < 0.05$). Moreover, the combination of anti-IL-6 and anti-TNF-α antibodies additively reduced coculture HIV-1 expression by 87% ($P < 0.05$). We conclude that renal cell production of IL-6 and TNF-α might provide a potent stimulus for HIV-1 expression in HIV-1-infected monocytes that infiltrate the kidney, and that this may play an important role in the pathogenesis of HIVAN.

Infection with human immunodeficiency virus type-1 (HIV-1) can be complicated by renal disease of diverse pathology. Most commonly, human immunodeficiency-associated nephropathy (HIVAN) is characterized by proteinuria, focal segmental glomerular sclerosis (FSGS), marked tubulointerstitial injury, and rapid progression to end-stage renal disease [1–4]. Although the pathogenesis of HIVAN is unknown, recent preliminary studies have suggested that abnormal renal cytokine and chemokine production may be important in the pathogenesis of HIVAN. Kimmel and coworkers reported elevated levels of transforming growth factor-beta (TGF-β), interleukin (IL)-8, and monocyte chemoattractant protein-1 (MCP-I) in glomeruli and interstitium microdissected from renal tissue of HIV-infected patients with FGS [5]. Transforming growth factor-β has also been detected by immunohistochemistry in renal tissue from HIVAN patients [6, 7]. The source of elevated cytokines in renal tissue of HIVAN patients is unknown, and could involve intrinsic renal mesangial cells and tubular epithelial cells, as well as infiltrating mononuclear cells.

Glomerular and interstitial infiltration of monocytes/macrophages has been identified in renal biopsy tissue obtained from HIV-infected patients [8]. Intrarenal replication of HIV-1 in infiltrating HIV-1-infected mononuclear cells might provide a continuous reservoir of HIV-1 that could infect renal cells or stimulate renal cell cytokine production, and contribute to the development of HIVAN. Certain cytokines, including IL-6, TGF-β, and tumor necrosis factor-alpha (TNF-α), which can be produced by intrinsic renal cells, have been shown to up-regulate HIV-1 replication in chronically infected mononuclear cells [9–13]. We hypothesized, therefore, that mesangial and tubular cells produce cytokines that stimulate HIV-1 replication in infected mononuclear cells, and that this is an important mechanism of disease development in HIVAN. To begin to test this hypothesis in the present study, HIV-1 replication was measured in chronically HIV-1-infected promonocytes (U1 cells) that were cocultured with human mesangial or proximal tubular epithelial cells.

Key words: HIV in renal cytokines; cytokines and HIV, mesangial cells, proximal tubular cells, interleukin.

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METHODS

Cell culture

Human fetal mesangial cells were kindly provided by Dr. Youngki Kim (Department of Pediatrics, University of Minnesota Medical School). The mesangial cells were characterized, maintained in culture, and passaged using techniques that we have previously described [14]. For maintenance of the mesangial cell cultures, the culture medium consisted of RPMI-1640 supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml). Because bacterial lipopolysaccharide (LPS) may stimulate cytokine production in cultured cells, the cell culture medium was routinely demonstrated to be free from LPS contamination, using a commercial Limulus Amebocyte Lysate Assay (Biowhitaker Inc., Walkersville, MD, USA). Mesangial cells between the 6th and 10th passages were used for these experiments.

Proximal tubular epithelial cells from normal, adult human kidney were obtained from Clonetics Corp. (San Diego, CA,

USA) and grown in a culture medium consisting of RPMI 1640 supplemented with 1% FBS, epidermal growth factor (EGF; 10 ng/ml), and penicillin (100 U/ml) and streptomycin (100 μg/ml). Routine testing of the culture medium indicated no detectable levels of LPS. Proximal tubular cells in the fifth passage were used for these experiments.

Chronically HIV-1-infected U1 cells were kindly provided by the National Institute of Allergy and Infectious Diseases, and were maintained in RPMI-1640 containing 10% FBS, 2 mM glutamine, and penicillin (100 U/ml) and streptomycin (100 μg/ml). U1 cells cultured in this medium alone demonstrate little or no HIV-1 replication.

Cytokine production

Mesangial cells were seeded in 24-well plates at a concentration of 2×10^4 cells per well, grown to approximately 80% confluence, and then synchronized to quiescence by a 72 hour incubation in culture medium containing 0.5% FBS. To measure constitutive cytokine production, the mesangial cells were then exposed for 24 hours to culture medium containing 10% FBS, and aliquots of the cell supernatants were obtained at 6 and 24 hours and stored at −70°C until assayed for cytokine levels. To measure induced cytokine production, some mesangial cells were exposed for 24 hours to culture medium containing LPS (10 to 100 ng/ml), and aliquots of the cell supernatants were obtained at 6 and 24 hours and stored at −70°C until assayed.

Proximal tubular epithelial cells were seeded in 24-well plates at a concentration of 2×10^4 cells per well, grown to subconfluence, and synchronized by 72 hour incubation in culture medium without EGF. To measure constitutive cytokine production, the tubular cells were then exposed for 24 hours to culture medium containing EGF (10 ng/ml), and aliquots of the cell supernatants were obtained at 6 and 24 hours and stored at −70°C until assayed for cytokine levels. To measure induced cytokine production, some tubular cells were exposed for 24 hours to culture medium containing LPS (10 to 100 ng/ml), and aliquots of the cell supernatants were obtained at 6 and 24 hours and stored at −70°C until assayed.

Cocultures

Mesangial cells were seeded in 24-well plates at a concentration of 2×10^4 cells per well, grown to approximately 80% confluence, and then synchronized to quiescence by a 72 hour incubation in culture medium containing 0.5% FBS. Cocultures of mesangial cells and chronically HIV-1-infected U1 cells were prepared by removing the culture medium from the quiescent mesangial cells, and adding to each well 1 ml of culture medium containing 10% FBS and U1 cells at a concentration of 2×10^3 cells per well. In some coculture wells, anti-IL-6 antibody (20 μg/ml; murine monoclonal IgG_1; R&D Systems, Minneapolis, MN, USA) was added 30 minutes prior to the addition of the U1 cells. Control wells containing U1 cells exposed to an irrelevant, control IgG_1 antibody or U1 cells only were also included. All samples were run in duplicate. After 72 hours of incubation, the cell supernatants were removed, centrifuged, and stored at −70°C until assayed for HIV-1 p24 antigen. As we have previously described [12], measurement of supernatant p24 antigen is used as an index of HIV-1 expression in U1 cells.

Proximal tubular epithelial cells were seeded in 24-well plates at a concentration of 2×10^4 cells per well, grown to subconfluence, and synchronized by a 72 hour incubation in culture medium without EGF. Cocultures of proximal tubular cells and chronically HIV-1-infected U1 cells were prepared by removing the culture medium from the quiescent tubular cells, and adding to each well 1 ml of culture medium containing 10 ng/ml EGF and U1 cells at a concentration of 2×10^3 cells per well. To some of the coculture wells anti-TNF-α antibody (10 μg/ml; murine monoclonal IgG_1; R&D Systems), anti-IL-6 antibody (20 μg/ml), or both was added 30 minutes prior to the addition of the U1 cells. Control wells containing U1 cells exposed to an irrelevant, control IgG_1 antibody or U1 cells only were also included. All samples were run in duplicate. After 72 hours incubation, the cell supernatants were removed, centrifuged, and stored at −70°C until assayed for HIV-1 p24 antigen.

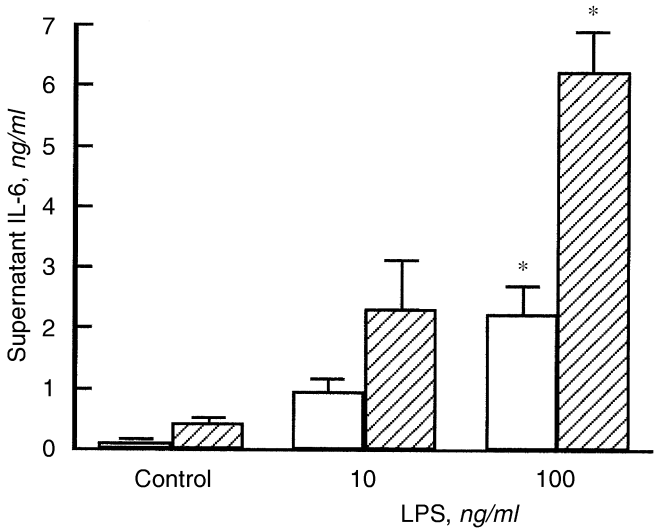

Fig. 1. Mesangial cell supernatant concentrations of interleukin-6 (IL-6) at six hours (□) and 24 hours (▨) of incubation. Control cells incubated in culture medium containing 10% fetal bovine serum (FBS) demonstrated constitutive IL-6 secretion. Addition of lipopolysaccharide (LPS; 10 to 100 ng/ml) to the culture medium induced a dose-related increase in IL-6 secretion. Data are mean ± SEM of three experiments. $^*P < 0.05$ versus control at the same time point.

Assays of cell supernatants

Interleukin-6 and TNF-α concentrations in mesangial cell and proximal tubule cell supernatants were measured using commercial ELISA kits (R&D Systems, Inc.). The sensitivity of the assay for IL-6 was 0.7 pg/ml, and for TNF-α was 4.4 pg/ml. Coculture supernatant concentrations of HIV-1 p24 antigen were measured using a commercial EIA kit (Abbott Laboratories, North Chicago, IL, USA), as we have previously described [12]. The sensitivity of this assay is approximately 50 pg/ml, and both inter- and intra-assay variability are less than 10%.

RESULTS

Mesangial cells incubated in culture medium containing 10% FBS constitutively secreted IL-6 into the cell supernatant (Fig. 1). Moreover, the supernatant concentration of IL-6 increased between 6 and 24 hours of incubation. Interleukin-6 secretion was not maximal in the serum-stimulated cells, as LPS (10 to 100 ng/ml) induced a further dose-dependent stimulation of IL-6

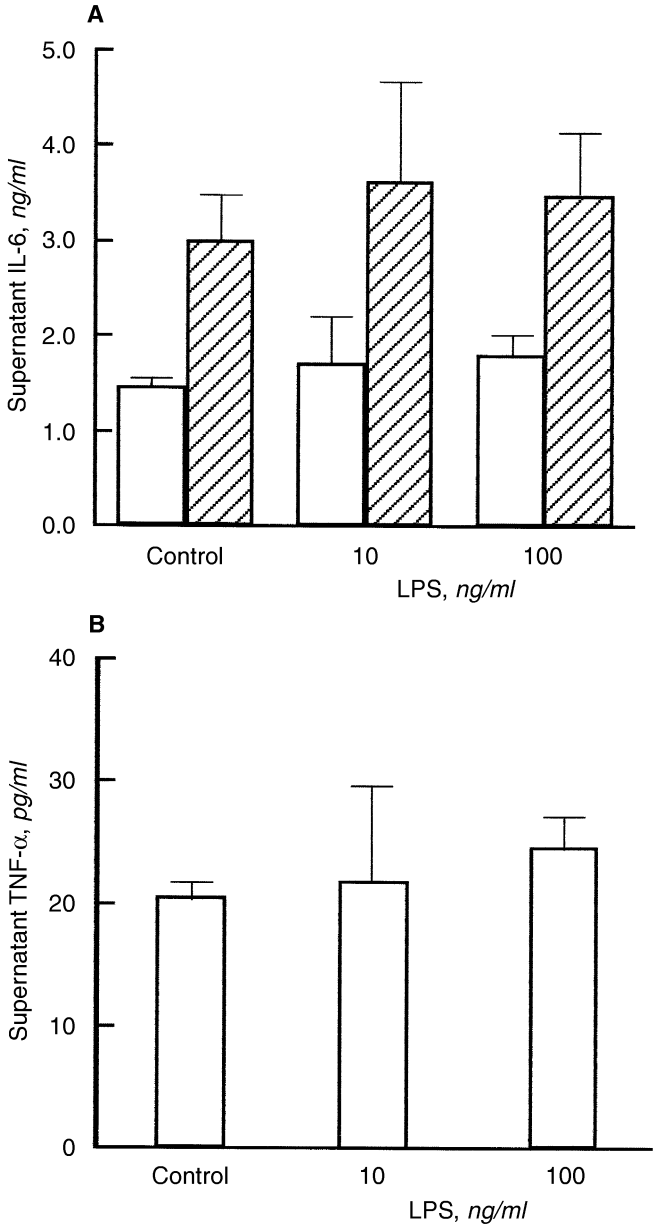

Fig. 2. Proximal tubule epithelial cell supernatant concentrations of interleukin-6 (IL-6; *A*) and tumor necrosis factor alpha (TNF-α; *B*) at six hours (□) and 24 hours (▨) of incubation. Control cells incubated in culture medium containing EGF demonstrated constitutive secretion of both IL-6 and TNF-α. Addition of LPS (10 to 100 ng/ml) to the culture medium did not further increase tubular cell secretion of either IL-6 or TNF-α. Data are mean ± SEM of two experiments.

Table 1. Human immunodeficiency virus-type 1 (HIV-1) expression in U1 cells cocultured with either human mesangial cells (HMC) or human proximal tubule epithelial cells (PTEC)

Sample	Supernatant p24 concentration *pg/ml*		
	Experiment 1	Experiment 2	Experiment 3
U1 cells alone	-0-	-0-	-0-
U1 cells + HMC	1064 ± 154	199 ± 27	471 ± 25
U1 cells + PTEC	1176 ± 95	1781 ± 43	1857 ± 94

Data indicate mean ± SEM of duplicate samples.

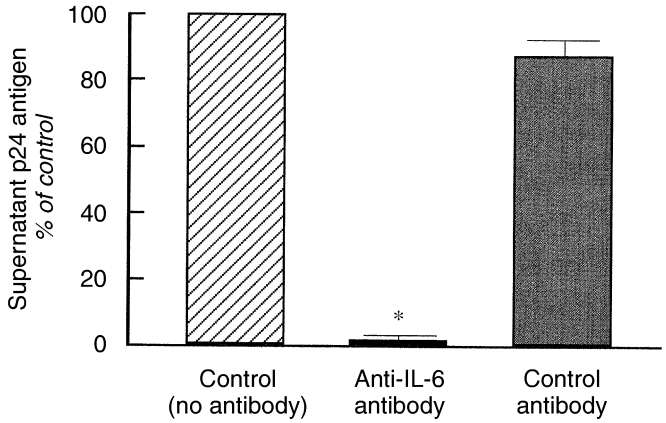

Fig. 3. p24 antigen concentrations in supernatants of human mesangial cell/U1 cell cocultures. Anti-interleukin-6 (anti-IL-6) antibody (20 μg/ml) almost completely inhibited HIV-1 expression. Control antibody (anti-IL-1β; 10 μg/ml) did not affect HIV-1 expression. Data (mean ± SEM) are a summary of three separate experiments. $^*P < 0.05$ vs. control.

secretion (Fig. 1). By contrast, the mesangial cells did not produce a detectable constitutive secretion of TNF-α when incubated in medium containing 10% FBS (data not shown). Moreover, LPS did not induce mesangial cell production of TNF-α.

Proximal tubular epithelial cells incubated in culture medium containing EGF constitutively secreted high levels of IL-6 and TNF-α into the cell supernatant (Fig. 2). Lipopolysaccharide (10 to 100 ng/ml) did not further increase tubular cell secretion of either IL-6 or TNF-α (Fig. 2), possibly due to limited amounts of LPS-binding protein in the relatively low serum (1%) culture medium in which the tubular cells were grown.

Human immunodeficiency virus type-1-infected U1 cells incubated alone in culture medium containing 10% FBS demonstrated no detectable HIV-1 expression, as indicated by lack of measurable supernatant p24 antigen (Table 1). Coculture of U1 cells with mesangial cells markedly up-regulated HIV-1 expression. In three separate experiments, the p24 antigen concentration in the mesangial cell/U1 cell coculture supernatants ranged from approximately 200 to 1000 pg/ml (Table 1). Moreover, in each experiment, the presence of anti-IL-6 antibody in the coculture medium blocked nearly completely HIV-1 expression in the mesangial cell/U1 cell cocultures (Fig. 3). By contrast, a control IgG antibody (anti-IL-1β antibody; 10 μg/ml) did not affect HIV-1 expression in the cocultures.

Coculture of U1 cells with proximal tubular epithelial cells also markedly up-regulated HIV-1 expression. In three separate experiments, the p24 antigen concentration in the tubular cell/U1 cell coculture supernatants ranged from approximately 1200 to 1850 pg/ml (Table 1). Anti-IL-6 antibody and anti-TNF-α antibody blocked HIV-1 expression in the tubular cell/U1 cell cocultures by 40% and 53%, respectively (Fig. 4). The combination of anti-IL-6 antibody and anti-TNF-α antibody reduced coculture HIV-1 expression by 87% (Fig. 4). By contrast, a control IgG antibody (anti-IL-1β antibody) had no effect on HIV-1 expression in the cocultures.

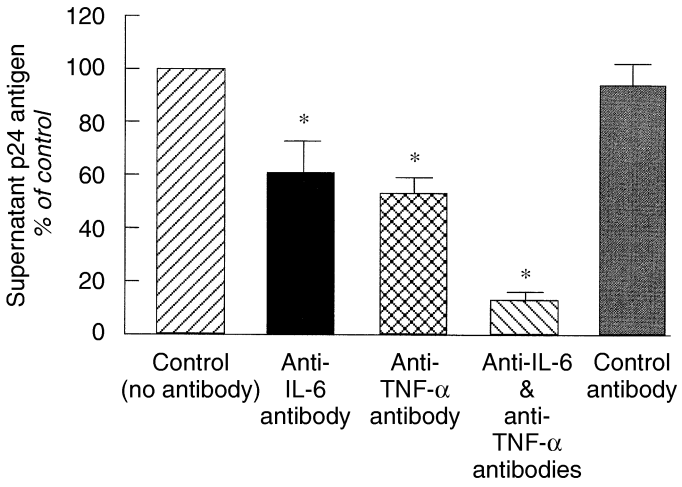

Fig. 4. p24 antigen concentrations in supernatants of proximal tubule epithelial cell/U1 cell cocultures. Anti-IL-6 antibody (20 μg/ml) and anti-TNF-α antibody (10 μg/ml) inhibited HIV-1 expression by 40% and 53%, respectively. The combination of anti-IL-6 antibody and anti-TNF-α antibody reduced HIV-1 expression by 87%. Control antibody (anti-IL-1β; 10 μg/ml) did not affect HIV-1 expression. Data (mean ± SEM) are a summary of three separate experiments. $^*P < 0.05$ vs. control.

DISCUSSION

Recent preliminary reports have indicated increased levels of certain cytokines and chemokines in renal biopsy tissue obtained from HIV-1-infected patients with renal disease, suggesting that abnormal renal cytokine and chemokine production may play a role in the development of HIVAN [5–7]. Moreover, monocytes/macrophages have been identified in glomeruli and renal interstitium of biopsy tissue from HIV-1-infected individuals, suggesting that renal infiltration of these immune cells may participate in the pathogenesis of HIVAN [8]. The mechanisms by which increased cytokine production and macrophage infiltration, alone or in combination, might injure the kidney and lead to the development of HIVAN are not known. Although it has not been specifically demonstrated, it is conceivable that some of the monocytes infiltrating the kidneys in HIV-1-infected patients are infected with HIV-1. If so, then the results of the present study suggest that cytokine production by intrinsic renal cells might provide a potent stimulus for intrarenal HIV-1 expression in these infected monocytes. Importantly, it has been suggested that intrarenal HIV-1 expression might cause, or at least contribute to, the development of HIVAN [4, 15–17].

In the present study we used a coculture system to model a possible *in vivo* interaction between intrinsic renal cells and infiltating HIV-1-infected monocytes. Chronically HIV-1-infected promonocytes (U1 cells) were used to represent HIV-1-infected monocytes that might infiltrate the kidney in HIV-1-infected patients. Human mesangial cells and proximal tubular epithelial cells were used to represent intrinsic renal cells whose cytokine production might up-regulate virus expression in HIV-1-infected immune cells. Human mesangial cells and proximal tubular epithelial cells, at least in culture, produce several substances that might stimulate HIV-1 expression in U1 cells [18–20]. Because previous studies have demonstrated that HIV-1 expression in U1 cells is markedly stimulated by IL-6 and TNF-α [11, 12], we chose to focus on these two cytokines.

The present results confirm observations by others that human mesangial cells can produce and secrete IL-6 [21], and that they do not produce TNF-α [22]. Importantly, the present study also suggests that IL-6 is the principal mesangial cell product that can up-regulate HIV-1 expression in infected monocytes. HIV-1 expression in mesangial cell/U1 cell cocultures was completely blocked by anti-IL-6 antibody. *In vivo*, therefore, mesangial cell IL-6 production might stimulate intraglomerular HIV-1 expression in infiltrating infected monocytes. Whether proliferating HIV-1 in the glomerulus is important in the development of mesangial expansion and FGS characteristic of HIVAN remains to be determined. It should be noted, though, that HIV-1-encoded proteins, which could accumulate in the glomerulus as a consequence of HIV-1 expression, have been reported to facilitate mesangial cell proliferation and matrix synthesis [23].

The present results also confirm previous findings that tubular epithelial cells produce both IL-6 and TNF-α [19, 20]. Moreover, both cytokines appeared to be important mediators of HIV-1 expression in the tubular cell/U1 cell cocultures. Anti-IL-6 antibody and anti-TNF-α antibody each reduced HIV-1 expression in the cocultures by approximately 40 to 50%, and the combination of the two antibodies reduced HIV-1 expression by nearly 90%. The additive effect of the two antibodies is consistent with a previous report indicating that IL-6 and TNF-α act via different mechanisms to stimulate monocyte HIV-1 expression [24].

Marked tubulointerstitial injury is a primary characteristic of HIVAN, and may play a significant role in the deterioration of renal function in HIVAN. Although the mechanism by which tubulointerstitial injury develops in HIVAN is not known, the results of this study suggest that enhanced tubular cell cytokine production, particularly of IL-6 and TNF-α, might provide a potent stimulus for HIV-1 expression in monocytes infiltrating the interstitium and the development of interstitial disease.

In this study we assumed that HIV-1 expression in the renal cell/U1 cell cocultures occurred selectively in the U1 cells. It is conceivable, though, that HIV-1 released from U1 cells might have infected mesangial cells or proximal tubular cells, and that at least some of the observed HIV-1 expression may have occurred in the renal cells. While this is a possibility, previous studies have demonstrated that mesangial cells do not become infected with HIV-1 when exposed to the virus [25], or become infected only to a small extent [15]. Moreover, we have found in preliminary studies that human fetal mesangial cells do not become infected with HIV-1 when cultured in the presence of the virus for up to one week (unpublished data). While the HIV-1 infectivity of proximal tubular epithelial cells has not been reported, one previous study has demonstrated that glomerular epithelial cells do not become infected with HIV-1 when cultured in the presence of the virus [15].

In the present study, constitutive secretion of IL-6 by mesangial cells, and IL-6 and TNF-α by proximal tubular epithelial cells, was likely sufficient to cause the observed stimulation of HIV-1 expression in U1 cells cocultured with the renal cells. Chao et al have previously exposed U1 cells to exogenous IL-6 or TNF-α at concentrations (0.2 to 20 ng/ml and 2 to 20 pg/ml, respectively) similar to those measured in the renal cell culture supernatants in the present study [26]. After three days of exposure to either IL-6 or TNF-α, the U1 cell supernatant p24 antigen levels were approximately 300 to 2000 pg/ml, comparable to the p24 antigen

levels measured in the supernatants of the renal cell/U1 cell cocultures in the present study.

In summary, mesangial cell IL-6 production and proximal tubular cell epithelial production of IL-6 and TNF-α were found to markedly stimulate HIV-1 expression in chronically HIV-1-infected promonocytes *in vitro*. Renal cell cytokine production *in vivo*, therefore, might provide a potent stimulus for intrarenal HIV-1 expression in HIV-1-infected monocytes that infiltrate the kidney, and this may play an important role in the pathogenesis of HIVAN.

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APPENDIX

Abbreviations used in this article are: EGF, epidermal growth factor; FBS, fetal bovine serum; FSGS, focal segmental glomerulosclerosis; HIV-1, human immunodeficiency virus type-1; HIVAN, human immunodeficiency virus-associated nephropathy; HMC, human mesangial cells; IL, interleukin; LPS, lipopolysaccharide; PTEC, proximal tubular epithelial cells; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; U1 cells, chronically HIV-1-infected promonocytes.

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