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Dexamethasone Prevents Monocyte-Induced Tubular Epithelial–Mesenchymal Transition in HK-2 Cells

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ABSTRACT

Epithelial–mesenchymal transition (EMT) is a key cellular event in the early stage of tubulointerstitial fibrosis (TIF). Monocyte infiltration plays an important role in the progression of TIF. We have previously demonstrated that monocytes can directly induce HK-2 cell transition by direct contact. Dexamethasone, an important anti-inflammatory and immunosuppressant agent, has been widely used in renal disease for decades. Whether it could influence the monocyte and HK-2 cell interaction and prevent EMT is still uncertain. In this study, we found that the typical epithelial cell morphology of HK-2 cells disappeared 24 h after co-culture with monocytes, and dexamethasone significantly prevented this change in a dose-dependent manner. In addition, we found that dexamethasone prevented monocytes from binding to HK-2 cells by inhibiting ICAM-1 expression on HK-2 cells. Further analysis demonstrated that there was increased E-cadherin expression and decreased α-SMA and fibronectin expression after co-culture with dexamethasone, suggesting that dexamethasone prevents monocyte-induced HK-2 cell transition. The nuclear transcription factor κB (NF-κB) pathway played an important role in this process. These findings suggest a novel mechanism by which corticosteroids may delay the progression of TIF via preventing EMT. J. Cell. Biochem. 114: 632–638, 2013.

KEY WORDS: MONOCYTES; DEXAMETHASONE; EMT; ICAM-1; NF-κB

Epithelial–mesenchymal transition (EMT) is a process through which epithelial cells lose their epithelial-specific markers, undergo cytoskeletal remodeling and gain a mesenchymal phenotype. EMT plays a prominent role in the development of tubulointerstitial fibrosis (TIF) [Rastaldi et al., 2002; Liu, 2004; Vitalone et al., 2008; Acloque et al., 2009]. Inflammatory cell infiltration, particularly monocyte/macrophage infiltration, has been implicated in the pathogenesis of TIF [Lavaud et al., 1996; Ko et al., 2008; Wang et al., 2008]. The correlation between inflammation and the extent of fibrosis has long been established. Once monocytes from circulating blood are recruited to the injured site in response to cytokine cues, they produce a variety of chemokines and therefore have pathogenic functions that lead to tissue damage and fibrosis. At the same time, our results have recently shown that monocytes can induce HK-2 cell transdifferentiation by direct contact with HK-2 cells [Liu, 2011]. All of these results show that monocyte infiltration is a key event in TIF.

Corticosteroids have been widely used in renal disease as an anti-inflammatory, immunosuppressive agent [Tsao et al., 2004; Johannes et al., 2009]. They inhibit the synthesis of a variety of chemokines and cytokines and therefore have a protective effect against inflammation. At the same time, high doses of dexamethasone inhibit the immune response [Chang and Schumer, 1997; Crinelli et al., 2000]. In addition to inhibiting cytokine and chemokine expression, a study in glioma cell lines demonstrated that dexamethasone inhibited the proliferation, migration and invasion of glioma cell lines [Piette et al., 2009]. Furthermore, dexamethasone could modulate the differentiation of multipotent stromal cells from human adipose tissue [Arutyunyan et al., 2009]. However, whether dexamethasone can modify EMT has not yet been clarified.

In the present study, we applied a monocyte-tubular cell co-culture model and explored the role of dexamethasone on monocyte-induced tubular EMT. Our results provide a novel insight that corticosteroids may influence the progression of chronic renal disease by inhibiting the interaction of monocyte and tubular epithelial cells and subsequent EMT.

MATERIALS AND METHODS

MATERIALS

All of the tissue culture plastics were obtained from Corning Incorporation (Corning, USA). Media and additives for cell culture were purchased from Invitrogen (Gibco, UK) and Sigma (Sigma–Aldrich, UK). Other reagents and sources were as follows: anti-human E-cadherin mAb (Cell Signaling, USA), anti-human alpha

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smooth muscle actin (α-SMA) mAb (Abcam, UK), anti-human fibronectin mAb and anti-β-actin antibody (Santa Cruz, USA), and RT^2 Profiler^{TM} PCR Array Human Signal Transduction Pathway Finder (PAHS-014A; Kang Cheng, China).

CELL CULTURE

All of the experiments were performed in HK-2 cells, which are human proximal tubular epithelial cells immortalized by transduction with human papilloma virus 16 E6/E7 genes. Cells were cultured in DMEM/Ham's F12 (Gibco) supplemented with 10% heat-inactivated fetal calf serum (FCS, Sijiqing, China).

U937 cells, originally derived from a human histiocytic lymphoma, were grown in suspension culture in RPMI medium supplemented with L-glutamine, penicillin/streptomycin and 5% FBS.

CO-CULTURE EXPERIMENTS

All of the co-culture experiments were performed in 6-well plates. U937 cells were washed once with serum-free RPMI 1640 medium, different numbers of U937 cells were added to the HK-2 cell monolayers in 1 ml serum-free DMEM medium, and the cells were co-cultured.

ASSAY FOR LEUKOCYTE ADHESION

U937 cell adhesion was measured as previously described [Cai et al., 2004]. Briefly, HK-2 cells were grown in 6-well culture plates until confluent. On the day of the assay, approximately 70×10^6 cells were resuspended followed by the addition of 5 μg/ml 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM) at 37°C for 30 min in foil-covered tubes. Fluorescently labeled cells were washed twice to remove unincorporated dye and resuspended in DMEM containing 0.2% BSA. Loaded monocyte cells (4×10^6) were added to HK-2 monolayers and incubated at 37°C. After 30 min, unbound monocytes were removed, and HK-2 layers with attached monocytes were gently washed twice with DMEM and lysed with 1.6 ml of 0.1% Triton X-100 in 0.1 M Tris per well. Fluorescence (excitation 485 nm, emission 535 nm) was measured using an fMax microplate reader (Molecular Devices, Sunnyvale, CA). Data were analyzed with SoftMax Pro. The number of adherent cells per well was expressed as the percentage fluorescence of control.

CONFOCAL MICROSCOPY

HK-2 cells were seeded on bacteria-free cover glasses in 12-well plates. When they reached 80% confluence, the cells were cultured in serum-free medium for 24 h. Then, HK-2 cells were separately treated with serum-free medium, U937 cells (1×10^6/ml), or U937 cells (1×10^6/ml) plus dexamethasone (0.5, 5, or 50 μg/ml) for 24 h. The cells on the cover glasses were then fixed, permeabilized, and blocked in blocking buffer. The primary antibodies rabbit anti-human α-SMA and mouse anti-human E-cadherin were incubated with the cells overnight at 4°C in a humidified chamber, followed by another incubation with rhodamine-labeled goat anti-rabbit IgG and FITC-labeled goat anti-mouse IgG (1:100, Boster, China) secondary antibodies, respectively, for 1 h at 37°C. Cells were analyzed under a confocal microscope (Zeiss LSM510, Germany). Imagepro Plus (IPP) was used to analysis the data.

REAL-TIME PCR

Total RNA was extracted by an RNAiso plus reagent, and cDNA was synthesized with a reverse transcription (RT) system kit (Takara, Japan). RT-PCR was performed with an ABI PRISM 7300 real-time PCR System (Applied Biosystems, USA). Primers were based on human sequences, and the amplification efficiencies for all of the genes were similar. The primers used for real-time PCR are shown in Table I.

WESTERN BLOT ANALYSIS

Total proteins extracted from HK-2 cells were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The membrane was blocked with 5% nonfat milk in Tris-buffered saline with 0.5% Tween 20 (TBST) overnight at 4°C. The membrane was subsequently incubated with the primary antibodies rabbit anti-human α-SMA, mouse anti-human fibronectin, mouse anti-human E-cadherin, or mouse anti-human β-actin for 1 h at room temperature, followed by incubation with HRP-conjugated goat anti-rabbit and goat anti-mouse secondary antibodies for an additional 1 h. Finally, the signals were detected using an ECL advanced system (GE Healthcare, UK).

GENE MICROARRAY

We used human signal transduction PCR array profiles, which consist of 84 key genes representative of 18 different signal transduction pathways. Using real-time PCR, we can easily and reliably analyze the expression of a focused panel of genes related to any of the 18 signal transduction pathways in the monocyte and PTC contact process.

STATISTICAL ANALYSIS

All of the data are expressed as the mean ± SD and were analyzed by one-way ANOVA using SPSS 13.0 statistical software. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

DEXAMETHASONE PREVENTS MONOCYTE-INDUCED HK-2 CELL MORPHOLOGICAL CHANGES

After co-incubation with monocytes (100×10^4/ml) for 24 h, HK-2 cells developed a series of phenotypic changes compared with the control, including elongation, branching, and loss of a cobblestone appearance under phase-contrast microscopy. Dexamethasone significantly prevents these phenotypic changes in a dose-dependent manner, with an obvious effect at high dose (Fig. 1).

TABLE I. The Primers Used for Real-Time PCR

Gene	Primer sequence
α-SMA	5′-GACAATGGCTCTGGGCTCTGTAA-3′
	5′-ATGCCATGTTCTATCGGGTACTTCA-3′
Fibrinectin	5′-GAGCTGCACATGTCTTGGGAAC-3′
	5′-GGAGCAAATGGCACCGAGATA-3′
E-cadherin	5′-GGATTGCAAATTCCTGCCATTC-3′
	5′-AACGTTGTCCCGGGTGTCAAG-3′
GAPDH	5′-GCACCGTCAAGGCTGAGAAC-3′
	5′-ATGGTGGTGAAGACGCCAGT-3′

THE INFLUENCE OF DEXAMETHASONE ON IMMUNOSTAINING FOR E-CADHERIN AND α-SMA IN HK-2 CELLS CO-CULTURED WITH MONOCYTES

The expression of E-cadherin and α-SMA protein in HK-2 cells following monocyte stimulation and dexamethasone treatment was measured by immunofluorescence staining. After 24 h of co-culture, there was a significant decrease in E-cadherin (Fig. 2A) and a marked increase in α-SMA compared with the control (Fig. 2B). The mean density of E-cadherin and α-SMA is the mean value of integrated optical density of total pixel optical density in three sections of HK-2 cell layer (Fig. 2C). In parallel experiments, dexamethasone significantly attenuated the changes initiated by monocytes, limiting the down-regulation of E-cadherin and abrogating α-SMA induction (Fig. 2).

DEXAMETHASONE PREVENTS MONOCYTE-INDUCED HK-2 CELL TRANSDIFFERENTION

To determine whether dexamethasone affected the EMT process, we investigated the effects of dexamethasone on the process of monocyte-induced HK-2 cell transdifferentiation. Dexamethasone (0.5, 5, or 50 μg) was added to the co-culture system. The mRNA (Fig. 3A) and protein (Fig. 3B) expression levels of E-cadherin were upregulated by dexamethasone in a dose-dependent manner, and the expression levels of α-SMA and fibronectin mRNA (Fig. 3A) and protein (Fig. 3B) were inhibited by dexamethasone in a dose-dependent manner, with an obvious abrogating effect at 50 μg/ml.

DEXAMETHASONE PREVENTS MONOCYTE BINDING TO HK-2 CELLS

The effect of dexamethasone on monocyte and HK-2 cell binding was assessed with the addition of fluorescently labeled U937 cells to HK-2 cells in the presence of dexamethasone, as described in materials and methods. HK-2 cells were treated with dexamethasone for 24 h previously, and then fluorescently labeled U937 cells were added to HK-2 cells in the presence of dexamethasone for 30 min. The results of this experiment (Fig. 4) demonstrated a significant decrease in monocyte binding to HK-2 cells in dexamethasone group (Fig. 4).

DEXAMETHASONE INHIBITS THE EXPRESSION OF ICAM-1 ON HK-2 CELLS

ICAM-1 is the principal adhesion molecule expressed on resident interstitial cells, and it is recognized by monocytes. Our previous results showed that monocytes induced HK-2 cell transition through CD18-ICAM1 interaction. We determined whether dexamethasone could influence ICAM-1 expression on HK-2 cells. Flow cytometry was used to assay ICAM-1 expression on HK-2 cells. The results showed that dexamethasone significantly inhibited ICAM-1 expression (Fig. 5).

DEXAMETHASONE INHIBITS NF-κB SIGNALING IN HK-2 CELLS

NF-κB signaling plays a key role in monocyte-induced HK-2 cell transition, and inhibition of this signaling pathway in HK-2 cells could inhibit ICAM-1 expression and EMT phenotypes. In this article, we found that dexamethasone inhibited monocyte-induced

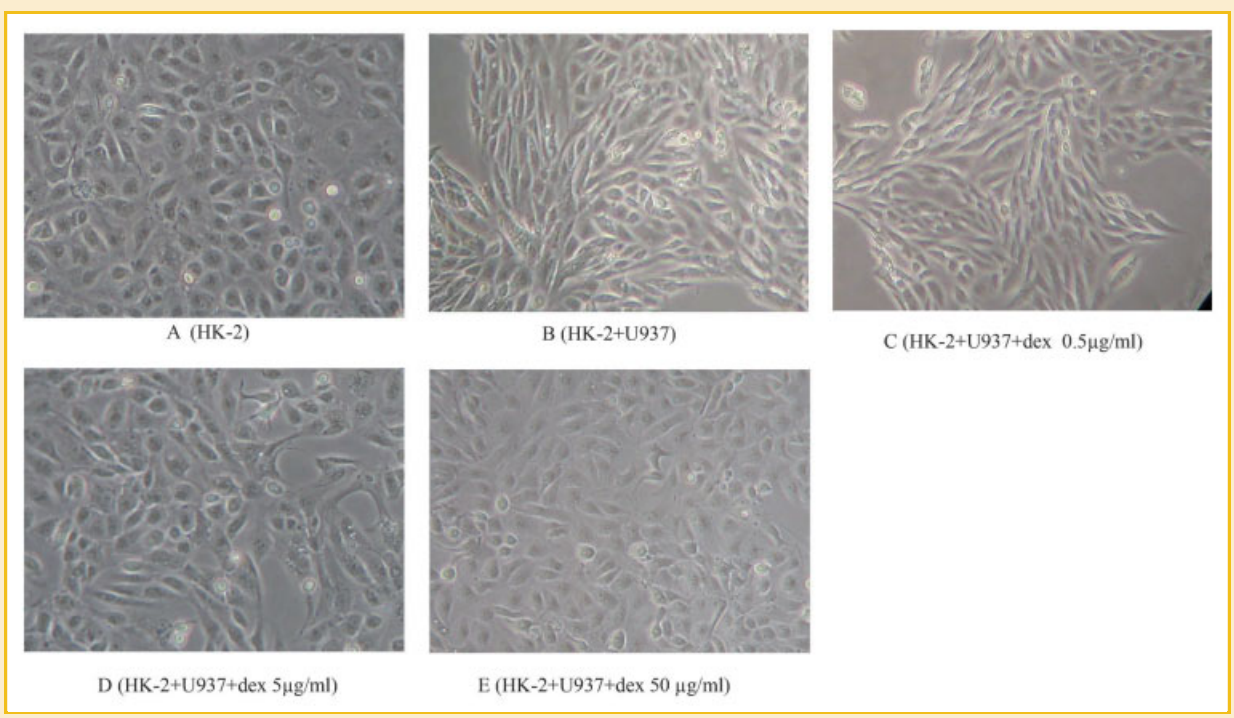

Fig. 1. Dexamethasone prevents monocyte-induced HK-2 cell morphological changes. After co-incubation with monocytes (100 × 104/ml) for 24 h, HK-2 cells became elongated, branched, and lost their cobblestone feature (B) compared with control cells (A). A low dose of dexamethasone had no effect (C), but a high dose of dexamethasone significantly prevents these phenotypic changes, especially at 50 μg/ml (D,E).

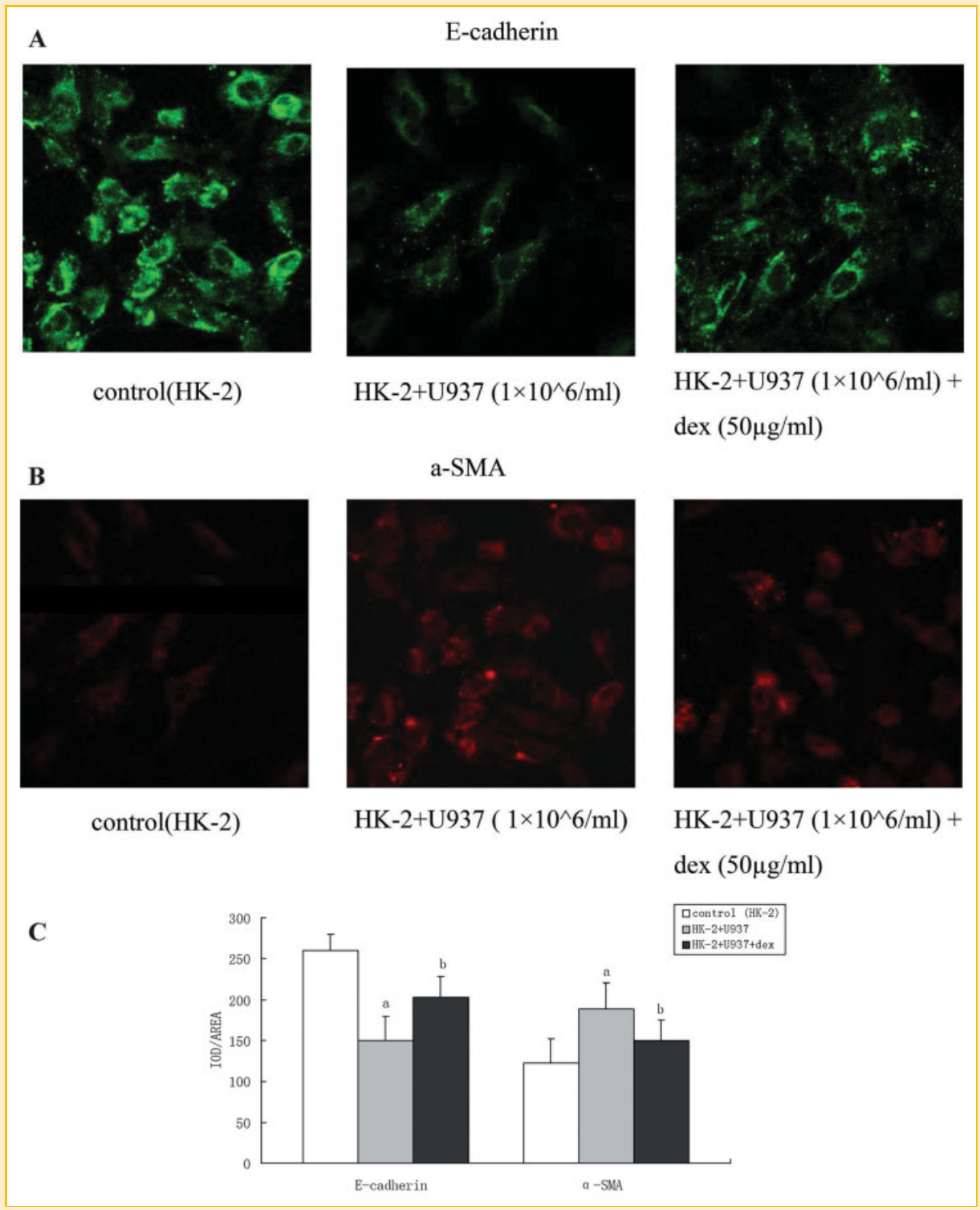

Fig. 2. The influence of dexamethasone on immunostaining of E-cadherin and α-SMA in HK-2 cells co-cultured with monocytes. There was decreased E-cadherin (A, green) staining and increased α-SMA (B, red) staining in HK-2 cell membranes and cytoplasm when they were co-cultured with monocytes. Treatment with dexamethasone could significantly reverse these effects (Leica, TCS-SP5). Imagepro Plus (IPP) was used to analysis the data. E-cadherin and α-SMA mean density is the mean value of integrated optical density of total pixel optical density in three sections of HK-2 cell layer (ap < 0.05 vs. control, bp < 0.05 vs. U937).

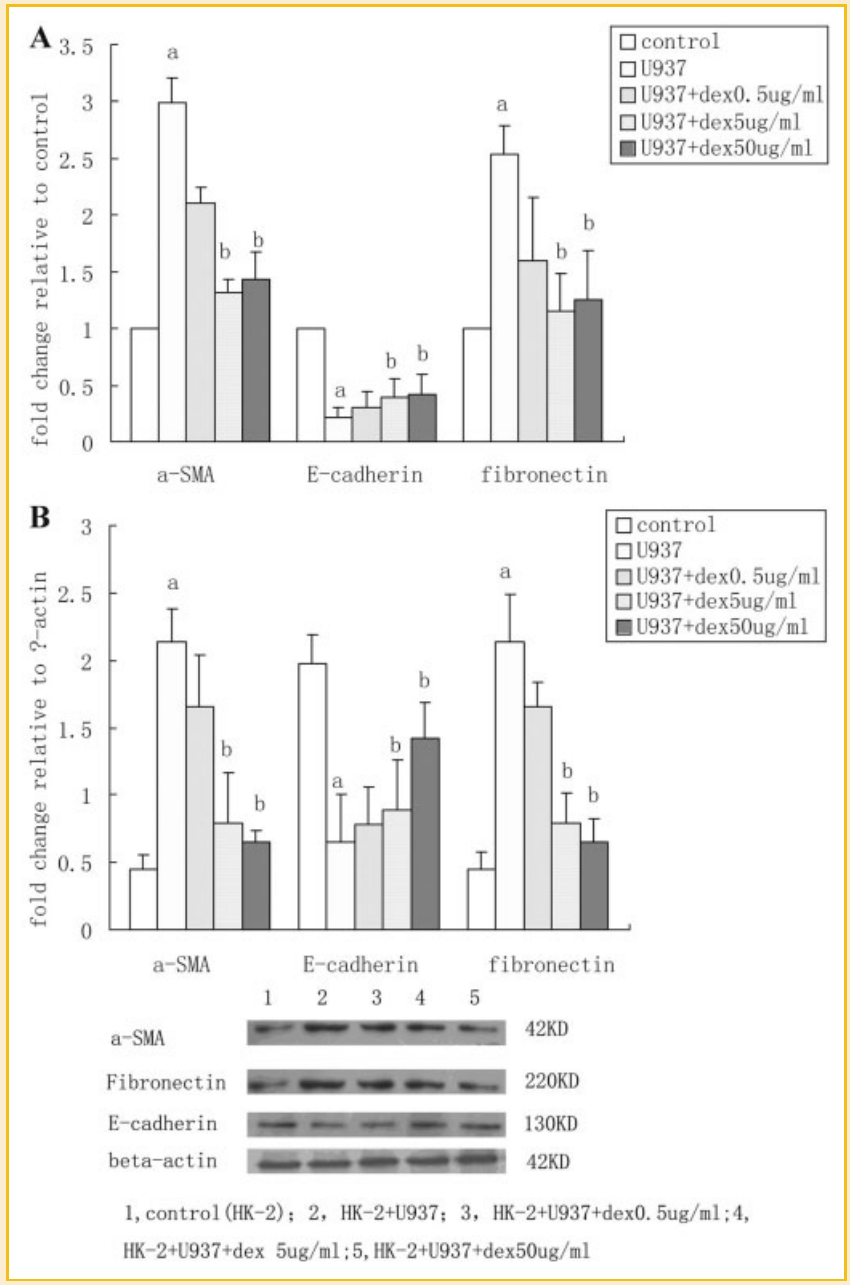

Fig. 3. Dexamethasone prevents monocyte-induced HK-2 cell transition. As shown with real-time RT-PCR (A) and Western blotting (B), dexamethasone significantly upregulated the E-cadherin expression that was inhibited by monocytes ($ap < 0.05$ vs. control, $bp < 0.05$ vs. U937). The α-SMA and fibronectin mRNA and protein expression levels, which were stimulated by monocytes, were significantly downregulated by dexamethasone in a dose-dependent manner ($ap < 0.05$ vs. control, $bp < 0.05$ vs. U937).

HK-2 cell transition through the NF-κB pathway. Using a gene microarray, which conclude 84 keys representative of 18 different signal transduction pathways we found that dexamethasone significantly inhibited the expression of CCL20, IL2, IL8, LTA, and PECAM1, which were involved in NF-κB signaling (Fig. 6A). Using real-time PCR, we analyze the expression of the focused panel of genes related to NF-κB signal transduction pathways, the mRNA expression levels of CL20, IL2, IL8, LTA, and PECAM1 were decreased in the dexamethasone-treated group (Fig. 6B).

DISCUSSION

Tubulointerstitial fibrosis is the common final outcome of almost all progressive chronic kidney diseases, regardless of their etiology, and represents the major lesion of end-stage renal diseases. Inflammation plays a crucial role in the initiation of renal fibrogenesis. Renal fibrosis is almost always preceded by the infiltration of inflammatory cells, including lymphocytes, monocytes/macrophages, dendritic cells and mast cells. Tissue injury induces inflammation by producing proinflammatory cytokines, which provide a directional

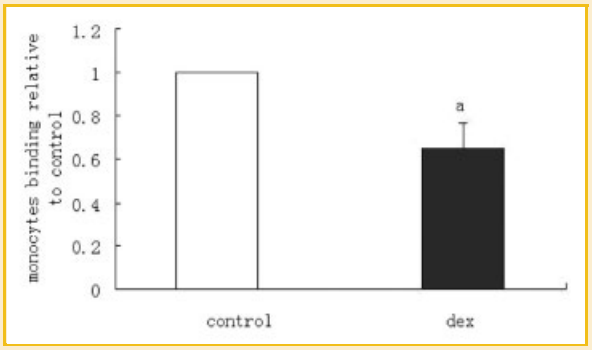

Fig. 4. Dexamethasone prevents monocytes from binding to HK-2 cells. HK-2 cells were treated with dexamethasone for 24 h previously, and then fluorescently labeled U937 cells were added to HK-2 cells in the presence of dexamethasone for 30 min. Dexamethasone significantly decreased monocyte binding to HK-2 cells ($ap < 0.05$ vs. control).

signal for guiding the infiltration of inflammatory cells from the blood to the injured sites [Chung and Lan, 2011]. Inflammation is a host defense mechanism in response to injury. In normal wound healing, inflammation is an early beneficial response to injury. However, when the injury is non-resolving, inflammation is a relentless driver of fibrogenesis because it creates a vicious cycle of inflammation, tissue damage and fibrosis [Nathan and Ding, 2010; Vielhauer et al., 2010]. This cycle builds up sustained profibrotic cytokines and primes fibroblasts and tubular epithelial cells to undergo phenotypic activation or transition and produce a large amount of ECM components [Liu, 2011]. Inflammatory cell infiltration, particularly monocyte/macrophage infiltration, is a feature of tubulointerstitial damage in many chronic renal diseases and has been implicated in the progression of tubulointerstitial

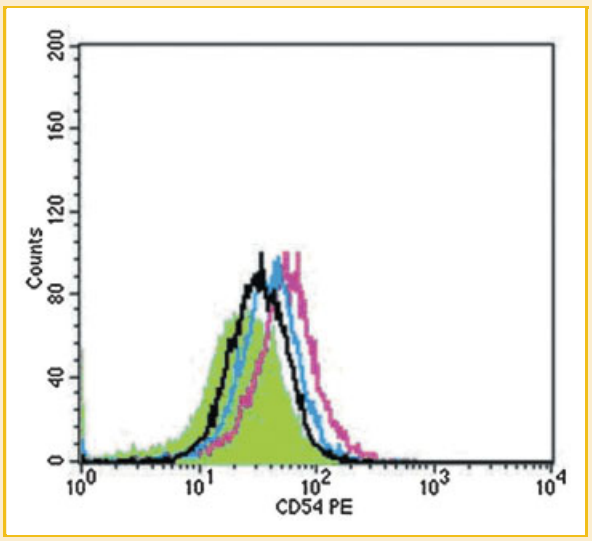

Fig. 5. Dexamethasone inhibits the expression of ICAM-1 on HK-2 cells. Flow cytometry was used to assay ICAM-1 expression on HK-2 cells. Monocytes could upregulate ICAM-1 expression on HK-2 cells (red), whereas dexamethasone significantly inhibited ICAM-1 expression (blue).

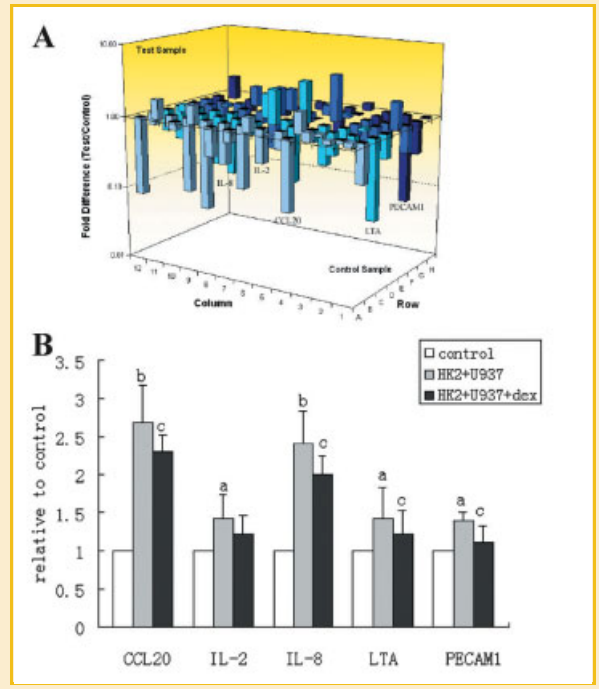

Fig. 6. Dexamethasone inhibits NF-κB signaling in HK-2 cells. Using a gene microarray, which conclude 84 keys representative of 18 different signal transduction pathways we found that dexamethasone significantly inhibited the expression of CCL20, IL2, IL8, LTA and PECAM1, which were involved in NF-κB signaling (A). Using real-time PCR, we analyze the expression of the focused panel of genes related to NF-κB signal transduction pathways, the mRNA expression levels of CL20, IL2, IL8, LTA and PECAM1 were decreased in the dexamethasone-treated group (B; $ap < 0.05$ vs. control, $bp < 0.01$ vs. control, $cp < 0.05$ vs. U937).

fibrosis. However, how these infiltrating inflammatory cells interact with resident cells and how this interaction leads to subsequent fibrotic events is still unclear. Recently, most studies have been focused on the profibrotic cytokines produced by inflammatory cells in a paracrine fashion, which act on resident fibroblasts and tubular cells to promote fibrogenesis [Nightingale et al., 2004; Ricardo et al., 2008; Wang and Harris, 2011]. However, in our present work, we found that in addition to soluble mediators, the direct contact of monocytes and tubular cells might be required to induce tubular EMT by monocytes via an NF-κB-dependent pathway [Li et al., 2011]. These results are consistent with our previous study, in which the interaction between monocytes and PTCs could upregulate TGF-β1 synthesis [Zhang et al., 2005]. The activation resulting from monocyte-resident renal cell interaction is characterized by the sequential activation of adhesion molecules and their ligands on both monocytes and endothelial cells following a coordinated response to inflammatory mediators [Lawrence and Springer, 1991]. PTCs express the adhesion molecule ICAM-1, through which infiltrating monocytes can adhere through CD11a/CD18. The up-regulation of these adhesion molecules predicts outcome in inflammatory renal disease [Daniel et al., 2001].

Dexamethasone, an important anti-inflammatory and immunosuppressant agent, has been widely used in renal disease for decades. Johannes et al. [2009] showed that a low dose of dexamethasone

reverses endotoxin-induced acute renal failure and prevents cortical microvascular hypoxia. Another study demonstrated that dexamethasone combined with antibiotics diminished urinary IL-6 and IL-8 concentrations during the acute phase of pyelonephritis [Sharifian et al., 2008]. All of these data indicated that corticosteroids might prevent renal damage by inhibiting cytokine secretion. In this study, we demonstrated that dexamethasone could significantly inhibit monocyte-induced HK-2 cell transition through the NF-κB pathway. Dexamethasone can double IκBα protein levels within 1 h and reduce complex formation of nuclear NF-κB proteins with DNA [Goppelt-Struebe et al., 2000]. Newly synthesized IκBα may thus bind to NF-κB and interfere with gene activation, which is linked to survival, proliferation, inflammation, and immunoregulation. In our study, we found that dexamethasone inhibited ICAM-1 expression on HK-2 cells through the NF-κB pathway and subsequent monocyte binding. Because the infiltration of monocytes is common in chronic renal disease and they exert a critical role in the development of EMT, our findings suggest that the application of dexamethasone might play a unique role in preventing the chronic progression of renal disease.

In conclusion, in this study, we have identified a novel mechanism that regulates monocyte-dependent PTC activation. Specifically, we have demonstrated that dexamethasone can significantly attenuate monocyte-induced tubular cell transdifferentiation, which provides a novel understanding for the clinical application of corticosteroids to prevent tubulointerstitial fibrosis.

REFERENCES

Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA. 2009. Epithelial–mesenchymal transitions: The importance of changing cell state in development and disease. J Clin Invest 119:1438–1449.

Arutyunyan IVRA, Volkov AV, Goldstein DV. 2009. Effect of dexamethasone on differentiation of multipotent stromal cells from human adipose tissue. Bull Exp Biol Med 147:503–508.

Cai Q, Lanting L, Natarajan R. 2004. Growth factors induce monocyte binding to vascular smooth muscle cells: Implications for monocyte retention in atherosclerosis. Am J Physiol Cell Physiol 287:C707–C714.

Chang CKLS, Schumer W. 1997. Effect of dexamethasone on NF-κB activation, tumor necrosis factor formation, and glucose dyshomeostasis in septic rats. J Surg Res 72:141–145.

Chung AC, Lan HY. 2011. Chemokines in renal injury. J Am Soc Nephrol 22:802–809.

Crinelli RAA, Bianchi M, Gentilini L, Scaramucci S, Magnani M. 2000. Selective inhibition of NF-κB activation and TNF-alpha production in macrophages by red blood cell-mediated delivery of dexamethasone. Blood Cells Mol Dis 26:211–222.

Daniel L, Sichez H, Giorgi R, Dussol B, Figarella-Branger D, Pellissier JF, Berland Y. 2001. Tubular lesions and tubular cell adhesion molecules for the prognosis of lupus nephritis. Kidney Int 60:2215–2221.

Goppelt-Struebe M, Rehm M, Schaefers HJ. 2000. Induction of cyclooxygenase-2 by platelet-derived growth factor (PDGF) and its inhibition by dexamethasone are independent of NF-kappaB/IkappaB transcription factors. Naunyn Schmiedebergs Arch Pharmacol 361:636–645.

Johannes TME, Klingel K, Dieterich HJ, Unertl KE, Ince C. 2009. Low-dose dexamethasone-supplemented fluid resuscitation reverses endotoxin-induced acute renal failure and prevents cortical microvascular hypoxia. Shock 31:521–528.

Ko GJ, Boo CS, Jo SK, Cho WY, Kim HK. 2008. Macrophages contribute to the development of renal fibrosis following ischaemia/reperfusion-induced acute kidney injury. Nephrol Dial Transplant 23:842–852.

Lavaud SMO, Sassy-Prigent C, Heudes D, Bazin R, Bariety J, Chevalier J. 1996. Early influx of glomerular macrophages precedes glomerulosclerosis in the obese Zucker rat model. J Am Soc Nephrol 7:2604–2615.

Lawrence MB, Springer TA. 1991. Leukocytes roll on a selectin at physiologic flow rates: Distinction from and prerequisite for adhesion through integrins. Cell 65:859–873.

Li Q, Liu BC, Lv LL, Ma KL, Zhang XL, Phillips AO. 2011. Monocytes induce proximal tubular epithelial–mesenchymal transition through NF-kappa B dependent upregulation of ICAM-1. J Cell Biochem 112:1585–1592.

Liu YH. 2004. Epithelial to mesenchymal transition in renal fibrogenesis: Pathologic significance, molecular mechanism, and therapeutic intervention. J Am Soc Nephrol 15:1–12.

Liu YH. 2011. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol 7:684–696.

Nathan C, Ding A. 2010. Nonresolving inflammation. Cell 140:871–882.

Nightingale J, Patel S, Suzuki N, Buxton R, Takagi KI, Suzuki J, Sumi Y, Imaizumi A, Mason RM, Zhang Z. 2004. Oncostatin M, a cytokine released by activated mononuclear cells, induces epithelial cell-myofibroblast transdifferentiation via Jak/Stat pathway activation. J Am Soc Nephrol 15:21–32.

Piette C, Roger T, Noel A, Foidart JM, Munaut C. 2009. The dexamethasone-induced inhibition of proliferation, migration and invasion in glioma cell lines is antagonized by macrophage migration inhibitory factor (MIF) and can be enhanced by specific MIF inhibitors. J Biol Chem 284:32483–32492.

Rastaldi MP, Ferrario F, Giardino L, Dell'Antonio G, Grillo C, Grillo P, Strutz F, Muller GA, Colasanti G, D'Amico G. 2002. Epithelial–mesenchymal transition of tubular epithelial cells in human renal biopsies. Kidney Int 62:137–146.

Ricardo SD, van Goor H, Eddy AA. 2008. Macrophage diversity in renal injury and repair. J Clin Invest 118:3522–3530.

Sharifian M, Anvaripour N, Karimi A, Fahimzad A, Mohkam M, Dalirani R, Gholikhani F, Rafiee MA. 2008. The role of dexamethasone on decreasing urinary cytokines in children with acute pyelonephritis. Pediatr Nephrol 23:1511–1516.

Tsao CM, Chen A, Wang JJ, Li CY, Tsai SK, Wu CC. 2004. Low-dose dexamethasone ameliorates circulatory failure and renal dysfunction in conscious rats with endotoxemia. Shock 21:484–491.

Vielhauer V, Kulkarni O, Reichel CA, Anders HJ. 2010. Targeting the recruitment of monocytes and macrophages in renal disease. Semin Nephrol 30:318–333.

Vitalone MJ, O'Connell PJ, Jimenez-Vera E, Yuksel A, Wavamunno M, Fung CL, Chapman JR, Nankivell BJ. 2008. Epithelial-to-mesenchymal transition in early transplant tubulointerstitial damage. J Am Soc Nephrol 19:1571–1583.

Wang Y, Harris DC. 2011. Macrophages in renal disease. J Am Soc Nephrol 22:21–27.

Wang Y, Wang Y, Cao Q, Zheng G, Lee VW, Zheng D, Li X, Tan TK, Harris DC. 2008. By homing to the kidney, activated macrophages potently exacerbate renal injury. Am J Pathol 172:1491–1499.

Zhang XL, Selbi W, de la Motte C, Hascall V, Phillips AO. 2005. Bone morphogenic protein-7 inhibits monocyte-stimulated TGF-beta1 generation in renal proximal tubular epithelial cells. J Am Soc Nephrol 16:79–89.