

Production of inflammatory mediators by renal epithelial cells is insensitive to glucocorticoids

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1 In the present study we investigated the effect of glucocorticoids on the activation of renal tubular epithelial cells, which are thought to play an important role in inflammatory processes in the kidney.

2 Activation of renal epithelial cells by IL-1, TNF- α or CD40L resulted in increased production of cytokines and chemokines. Both in the renal epithelial cell line HK-2 and in primary cultures of human proximal tubular epithelial cells (PTEC) production of IL-6, IL-8 and monocyte chemotactic protein 1 (MCP-1) was not inhibited by glucocorticoids, independent of the stimulus.

3 In contrast, dexamethasone strongly inhibited cytokine production by immortalized renal fibroblasts and an airway epithelial cell line (A549).

4 Stimulation of renal epithelial cells resulted in activation of NF- κ B, a pivotal transcription factor in the regulation of cytokine genes, as was shown by I κ B- α degradation and increased DNA-binding activity. In contrast to dexamethasone, addition of the NF- κ B inhibitors pyrrolidine dithiocarbamate (PDTC) and n-tosyl-l-phenylalanine chloromethyl ketone (TPCK) completely abolished cytokine and chemokine production.

5 Renal epithelial cells express abundant levels of the functional glucocorticoid receptor alpha (GR α) isoform and low levels of the inhibitory beta isoform (GR β).

6 In conclusion, cytokine production by renal epithelial cells is insensitive to the inhibitory effects of glucocorticoids. The lack of dexamethasone-mediated inhibition was specific for renal epithelial cells and could not be explained by an increased expression of the glucocorticoid receptor beta isoform.

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Abbreviations: GR α , β , glucocorticoid receptor alpha, beta; MCP-1, monocyte chemotactic protein 1; PDTC, pyrrolidine dithiocarbamate; PTC, PBS/Tween/2% casein; PTEC, proximal tubular epithelial cells; RANTES, regulated upon activation, normal T-cell expressed and secreted; TPCK, n-tosyl-l-phenylalanine chloromethyl ketone

Introduction

Influx of monocytes and T-cells into the tubulo-interstitial area is a hallmark of many renal inflammatory diseases. Rather than being a passive target, resident proximal tubular epithelial cells (PTEC) are thought to play an active role in the inflammatory process *via* production of inflammatory mediators (van Kooten & Daha, 2001). It was shown before that the cytokines IL-1 and TNF- α and the cell surface molecule CD40L play an important role in T-cell-mediated activation of PTEC (Kuroiwa *et al.*, 2000). Stimulation of PTEC *in vitro* with these cytokines or CD40L results in increased cytokine and chemokine production, including IL-6, IL-8, MCP-1 and RANTES (Deckers *et al.*, 1998; Pattison *et al.*, 1994; van Kooten *et al.*, 1997; 1999). Furthermore, expression of adhesion molecules and MHC class II is increased (Kirby *et al.*, 1993).

Glucocorticoids are potent suppressors of the immune system and are therefore used as therapeutic treatment in a

broad range of autoimmune and inflammatory diseases. One of the major anti-inflammatory effects of glucocorticoids is suppression of cytokine production (Almawi *et al.*, 1998). Glucocorticoids can inhibit gene transcription *via* negative glucocorticoid responsive elements (Tsai & O'Malley, 1994). However, several genes that are negatively regulated by glucocorticoids, including cytokine genes, do not contain a negative response element in their promoter. Expression of these genes is inhibited *via* glucocorticoid-induced repression of transcription factors, including AP-1 and NF- κ B (Cato & Wade, 1996). Many anti-inflammatory effects of glucocorticoids have been linked to their ability to inhibit the activation of NF- κ B (Lee & Burckart, 1998; McKay & Cidlowski, 1999).

NF- κ B is a pro-inflammatory transcription factor that regulates the expression of many proteins involved in inflammation, including cytokines (TNF- α , IL-6), chemokines (IL-8, MCP-1, RANTES) and adhesion molecules (ICAM-1, VCAM-1) (Barnes & Karin, 1997; Lee & Burckart, 1998). The classical form of NF- κ B is a heterodimer of p50/p65 (Barnes & Karin, 1997; Ghosh *et al.*, 1998). In the cytoplasm, NF- κ B is kept inactive through binding to its inhibitor,

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I κ B- α . Stimulation of cells with cytokines, like IL-1 and TNF- α results in phosphorylation and subsequent degradation of I κ B- α . The released NF- κ B translocates to the nucleus to activate transcription of target genes (Ghosh *et al.*, 1998; Karin & Ben-Neriah, 2000).

In the present study we investigated the effect of dexamethasone on cytokine and chemokine production by activated human renal epithelial cells and found that these cells were insensitive to the inhibitory action of dexamethasone. We also investigated the role of NF- κ B in cytokine production by PTEC and the expression of the glucocorticoid receptor to obtain more insight in the mechanism of the observed steroid-insensitivity.

Methods

Cell culture

PTEC Primary human PTEC were isolated from kidneys not suitable for transplantation and from pre-transplant biopsies (van Kooten *et al.*, 1997). Cell monolayers were cultured on a matrix of heat-inactivated foetal calf serum (Δ FCS; Gibco BRL/Life Technologies Inc., Paisley, U.K.) in DMEM/HAMF12 (Bio-Whittaker, Walkersville, MD, U.S.A.) supplemented with insulin ($5 \mu\text{g ml}^{-1}$), transferrin ($5 \mu\text{g ml}^{-1}$), selenium (5 ng ml^{-1}), hydrocortisone (36 ng ml^{-1}), tri-iodothyronine (40 pg ml^{-1}), epidermal growth factor (10 ng ml^{-1} , all from Sigma Chemical Co., St. Louis, MO, U.S.A.) and 100 U ml^{-1} penicillin/ $100 \mu\text{g ml}^{-1}$ streptomycin (Gibco) (Detrisac *et al.*, 1984). Specific outgrowth of PTEC was confirmed by morphological appearance and immunofluorescence staining as described (van Kooten *et al.*, 1997). Cells were used between passage 2–6 of culture.

HK-2 The immortalized PTEC-derived cell line HK-2 (Human Kidney-2) was kindly provided by M.P. Ryan, University College Dublin, Ireland (Ryan *et al.*, 1994). Cells were cultured in RPMI 1640 (Gibco) supplemented with 10% Δ FCS and penicillin/streptomycin.

L-cells Mouse fibroblast L-cells, non-transfected (L-orient) or stably transfected with CD40L (L-CD40L) (Garrone *et al.*, 1995) were grown in IMDM (Bio-Whittaker), 10% Δ FCS and penicillin/streptomycin. For co-culture experiments, L-cells were irradiated (80 Gy) before addition to the target cells.

TK173 The human renal fibroblast cell line TK173 was established from normal human kidney and was kindly provided by F. Strutz, (Dept. of Nephrology and Rheumatology, Georg-August-University, Göttingen, Germany). Cells were cultured in IMDM/10% Δ FCS and penicillin/streptomycin (Muller *et al.*, 1995).

Synovial fibroblasts Primary synovial fibroblasts were cultured from the synovial tissue of patients undergoing total or partial knee or elbow surgery (Goossens *et al.*, 1999). Synovial fibroblasts were cultured in IMDM/20% FCS and penicillin/streptomycin and used between passage 2–6 of culture.

A549 The human airway epithelial cell line A549 was cultured in RPMI 1640/10% Δ FCS and penicillin/streptomycin.

For passage of the cultures, cells were harvested by trypsinization with 0.02% ($w v^{-1}$) EDTA/0.05% ($w v^{-1}$) trypsin (Sigma).

Cytokine production

Production of IL-6, IL-8 and MCP-1 was measured in supernatants of stimulated cells after 48 h unless indicated otherwise. Prior to stimulation, cells were transferred to 48-well plates (Costar, Corning, NY, U.S.A.) at a density of 0.5×10^5 cells per well and serum-starved o/n. Cells were stimulated with IL-1 α , TNF- α or CD40L-transfected L-cells in a 1:1 ratio. For inhibition experiments, cells were pre-incubated for 2 h with pyrrolidine dithiocarbamate (PDTC), dexamethasone and *n*-tosyl-1-phenylalanine chloromethyl ketone (TPCK). Cytokines or L-cells were added in the continuous presence of these inhibitors. Cell viability was checked with Trypan blue staining. The concentration of IL-6, IL-8 and MCP-1 in culture supernatants was measured by specific ELISA as described previously (van Kooten *et al.*, 1997).

Preparation of cell lysates

For preparation of whole cell-lysates, 1×10^6 cells were harvested and incubated in lysis buffer containing 20 mM Tris pH 7.4, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA and a protease inhibitor mix consisting of 5 u ml^{-1} Trasylol (Bayer, Leverkusen, Germany), 1 mM PMSF, $2 \mu\text{g ml}^{-1}$ antipain, $2 \mu\text{g ml}^{-1}$ chymostatin and $2 \mu\text{g ml}^{-1}$ leupeptin (all from Boehringer Mannheim, Germany) for 10 min on ice. After centrifugation for 15 min at 13,000 r.p.m., supernatants were collected and protein concentration was determined with a BCA protein assay reagent (Pierce, Rockford, IL, U.S.A.).

Western blot analysis

Whole cell extracts ($20 \mu\text{g}$) were separated on a 10% SDS-PAGE-gel and blotted to a PVDF-membrane (Millipore, Bedford, MA, U.S.A.). Membranes were blocked o/n in PBS/Tween/2% casein (PTC) at 4°C , incubated with antibodies to I κ B- α or human glucocorticoid receptor for 1.5 h at room temperature and detected with horseradish peroxidase (HRP)-conjugated secondary antibodies. Antibodies were diluted in PTC. Blots were developed with a chemiluminescence substrate (Pierce) and detected with Hyperfilm ECL (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Densitometry was performed using a Stratagene Eagle-sight analysis programme. Efficiency of transfer was verified by staining with coomassie brilliant blue (Sigma).

Detection of NF- κ B DNA-binding activity

Cells were incubated with $1 \mu\text{M}$ dexamethasone for 30 min and stimulated with 5 ng ml^{-1} IL-1 for 30 min. NF- κ B DNA binding activity in whole cell lysates was measured with Trans-AM NF- κ B p65 and p50 transcription factor assay kits (Active Motif Europe, Rixensart, Belgium) according to

manufacturer's instructions. Briefly, 2 μg extract was added to 96-well plates coated with an oligonucleotide containing the NF- κB consensus site. Binding of NF- κB to the DNA was visualized by anti-p50 and anti-p65 antibodies that specifically recognize activated DNA-bound NF- κB . Antibody binding was detected with a secondary HRP-conjugated antibody and developed with TMB substrate. The reaction was stopped with 0.5 M H_2SO_4 and measured at 450 nm. Specificity of NF- κB activation was determined by competition experiments using NF- κB wild-type and mutant consensus oligonucleotides that were provided with the kit.

Glucocorticoid receptor mRNA expression

Total RNA was isolated using RNeasy (Qiagen, Venendaal, The Netherlands) according to manufacturer's instructions. OD260/280 ratios were measured to determine the quantity and purity of RNA preparations. Fixed amounts of total cellular RNA (1 μg) were reverse transcribed into cDNA by oligo-dT priming using M-MLV reverse transcriptase (Gibco). Expression of GR α and GR β was determined by RT-PCR with a common upstream primer (5'-CCT AAG GAC GGT CTG AAG AGC-3') and two specific downstream primers: GR α , 5'-GCC AAG TCT TGG CCC TCT AT-3' (450 bp) and GR β : 5'-CCA CGT ATC CTA AAA GGG CAC-3' (377 bp) (Honda *et al.*, 2000). Expression of β -actin was used as a control. β -actin forward primer, 5'-CTA CAA TGA GCT GCG TGT GG-3'; β -actin reverse primer, 5'-AAG GAA GGC TGG AAG AGT GC-3' (527 bp) (van Kooten *et al.*, 1999). PCR was performed under standard conditions (50 mM KCl, 10 mM Tris-HCl, pH 8.4, 0.06 mg ml⁻¹ bovine serum albumin (BSA), 0.25 mM dNTPs, 25 pmol of each primer, 1 u of Taq polymerase; Perkin Elmer, Norwalk, CT, U.S.A.) with MgCl₂ 1.5 mM for GR α and β -actin and 1 mM for GR β . The following scheme was used: 5 min 95°C, GR α/β : 40 cycli of 30 s 94°C, 30 s 60°C, 1 min 72°C; β -actin: 35 cycli of 1 min 95°C, 1 min 55°C and 1 min 72°C. PCR-products were analysed on a 1% agarose gel containing ethidium bromide.

Materials

Recombinant human IL-1 α and TNF- α were purchased from Preprotech, Rocky Hill, NJ, U.S.A. PDTC and TPCK were supplied by Sigma Chemical Co., St. Louis, MO, U.S.A. whereas dexamethasone was obtained from the Pharmacy of the Leiden University Medical Center, The Netherlands. Primary antibodies used for Western blot were rabbit anti- κB - α antibody (sc-371; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and mouse anti-human glucocorticoid receptor (Transduction Laboratories, Lexington, KY, U.S.A.). The secondary antibodies were purchased from DAKO, Glostrup, Denmark.

Statistical analysis

Cytokine and chemokine production is presented as mean concentration \pm s.d. from representative experiments. Experiments were repeated at least three times. Cytokine production in primary cultures was analysed with a paired student's *t*-test. Data are shown as mean \pm s.e. means. Results were considered significant if $P < 0.05$.

Results

IL-6 production by PTEC is not inhibited by dexamethasone

IL-6 production in primary cultures of PTEC from different isolations ranged from 2.8–14.1 ng ml⁻¹ for baseline levels and was increased to 39.7–121.0 ng ml⁻¹ upon stimulation with 5 ng ml⁻¹ IL-1. Incubation of PTEC with increasing doses of dexamethasone (10⁻⁹–10⁻⁵ M) showed no significant effect on constitutive or IL-1-induced IL-6 production after 48 h (Figure 1A,B). Similar results were obtained after stimulation for 24 and 72 h (data not shown). Increasing doses of IL-1 induced a dose-dependent increase in IL-6 production. No effect of dexamethasone (1 μM) was observed

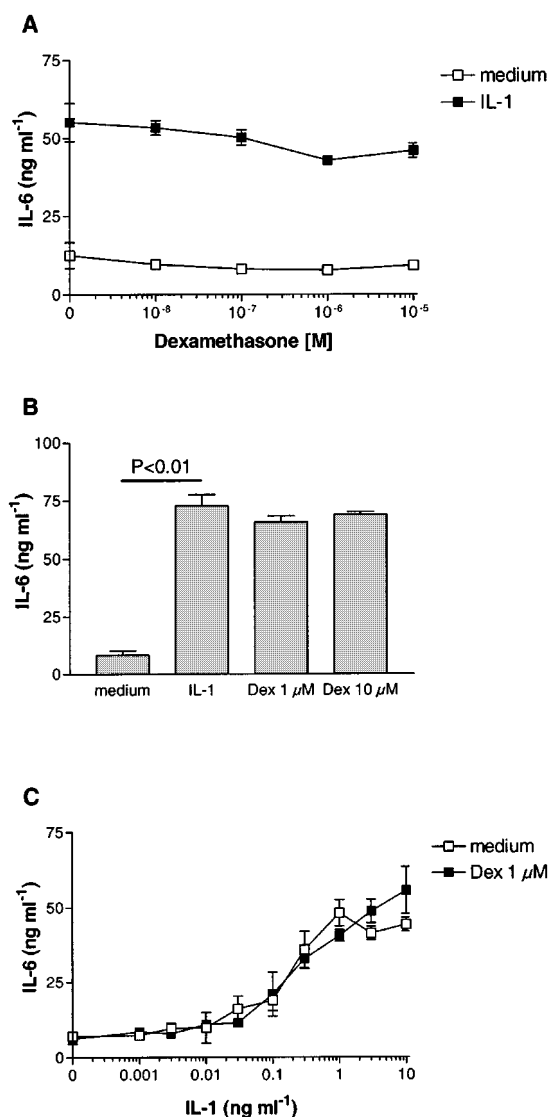


Figure 1 Effect of dexamethasone on IL-1-induced IL-6 production by PTEC. IL-6 production by primary human PTEC stimulated with 5 ng ml⁻¹ IL-1 was not inhibited by increasing doses of dexamethasone. Data shown are mean \pm s.d. of one representative experiment (A) and mean \pm s.e. means of seven independent experiments (B). No effect of 1 μM dexamethasone was found on different doses of IL-1 (C). IL-6 production in culture supernatants was measured after 48 h by specific ELISA.

on either low-dose or high-dose stimulation with IL-1 (Figure 1C), showing that the strength of the IL-1 signal is not masking the inhibitory capacity of dexamethasone. Routinely cells were incubated with dexamethasone for 2 h followed by 48 h stimulation with IL-1 in the continuous presence of dexamethasone. Even incubation with dexamethasone for up to 24 h prior to stimulation with IL-1 showed no effect on IL-1-induced IL-6 production (data not shown). In parallel experiments, the same dexamethasone showed a strong inhibitory action on monocytes as published before (Woltman *et al.*, 2000a).

The absence of dexamethasone-mediated inhibition is specific for renal epithelial cells

As observed with primary cells, IL-1-induced IL-6 production by the PTEC-derived cell line HK-2 was not inhibited by dexamethasone (Figure 2A). In contrast, stimulation of the renal fibroblast cell line TK173 or primary synovial fibroblasts with IL-1 resulted in increased production of IL-6, which was completely inhibited by dexamethasone (Figure 2B,C). Similarly, IL-1-induced production of IL-8 by the airway epithelial cell line A549 was strongly inhibited by low doses of dexamethasone (Figure 2D).

Production of chemokines is insensitive to dexamethasone independent of the stimulus

As shown in Figure 3A, IL-1-induced production of the chemokines IL-8 and MCP-1 by primary PTEC was not

inhibited by dexamethasone. Also, dexamethasone was unable to inhibit IL-6 production by PTEC that were activated with TNF- α (500 u ml⁻¹) and CD40L (Figure 3B). Similar results were obtained for the production of IL-8 and MCP-1 in both primary cells and HK-2.

Activation of PTEC induces NF- κ B activation

Activation of primary PTEC with IL-1 induced degradation of I κ B- α 15–60 min after stimulation (Figure 4A). Maximal degradation was observed after 30 min and I κ B- α levels returned back to normal after 120 min. TNF- α and CD40L also induced degradation of I κ B- α with similar kinetics, although less potent than IL-1 (respectively 48, 75 and 93.6% degradation as determined by densitometry). The lower band in the CD40L-stimulated cells is caused by the murine L-cells used for stimulation. Staining with the human and mouse reactive anti-I κ B- α antibody showed that murine I κ B- α migrated at a slightly smaller size than human I κ B- α and remained stable over time.

We also analysed DNA-binding activity of NF- κ B in the HK-2 cell line. In control cells, a basal level of NF- κ B activity was observed, as shown by DNA-binding of the p65 and p50 subunits (Figure 4B). Stimulation of cells with IL-1 resulted in increased DNA-binding of p65 and p50. Pre-incubation of the plates with a wild-type NF- κ B consensus oligonucleotide, but not a mutant oligonucleotide, completely prevented binding of p65 and p50 to the DNA.

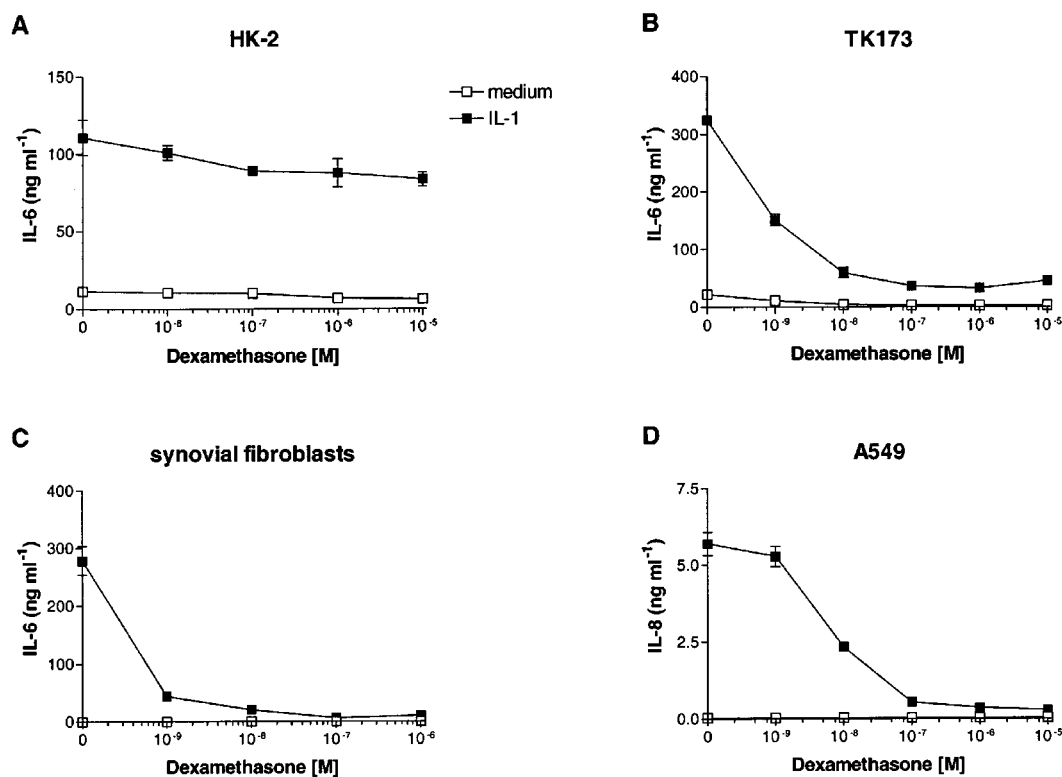


Figure 2 Effect of dexamethasone on different cell types. Dexamethasone was unable to inhibit cytokine production by the renal epithelial cell line HK-2, but strongly abolished cytokine production by a renal fibroblast cell line (TK173), primary human synovial fibroblasts and an airway epithelial cell line (A549). $n=4$.

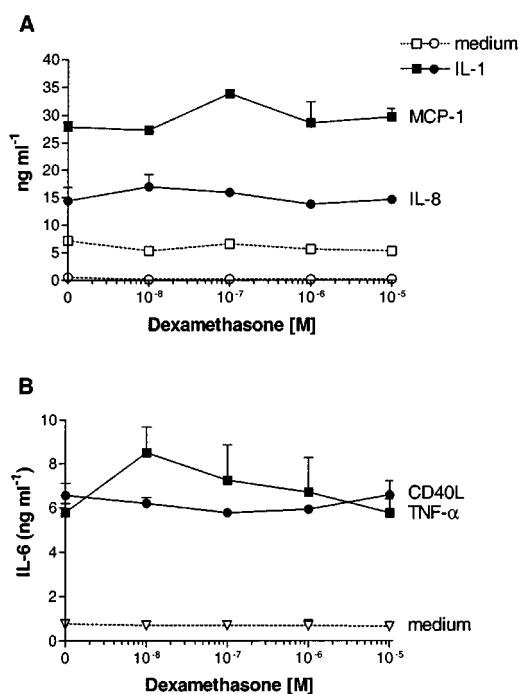


Figure 3 (A) In the same supernatants as described in Figure 1 the effect of dexamethasone on chemokine production by PTEC was determined. IL-1-induced production of IL-8 and MCP-1 was not inhibited by dexamethasone. (B) Representative experiment showing the effect of dexamethasone on PTEC that were activated by TNF- α (500 u ml⁻¹) and CD40L. No inhibition of TNF- α - or CD40L-induced IL-6 production was found in five independent experiments.

Role for NF- κ B in IL-6 production by PTEC

Different NF- κ B-inhibitors were used to study the role of NF- κ B in cytokine production. Stimulation of PTEC in the presence of the anti-oxidant PDTC (3.75–30 μ M) resulted in a strong dose-dependent inhibition of IL-6 production (Figure 5A). Similar results were found for production of IL-8 and MCP-1 (data not shown). This inhibition was not caused by toxicity, since PDTC showed no effect on viable cell counts as measured by Trypan blue staining. The proteasome inhibitor TPCK (2.5–10 μ M) also inhibited IL-1-induced IL-6 production in a dose-dependent manner. Only incubation with the highest concentration of TPCK (10 μ M) resulted in the induction of some cell death (Figure 5B).

Renal epithelial cells express the glucocorticoid receptor

Specific RT-PCR showed that mRNA for the functional glucocorticoid receptor (GR α) was strongly expressed by non-stimulated PTEC. Expression levels of GR α and the inhibitory counterpart GR β were compared semiquantitatively, showing that although GR β was expressed, mRNA for GR α was much more abundant (Figure 6A). Furthermore, Western blot analysis confirmed that GR protein was expressed in PTEC (Figure 6B). As a control for GR expression the steroid-sensitive cell line A549 was used.

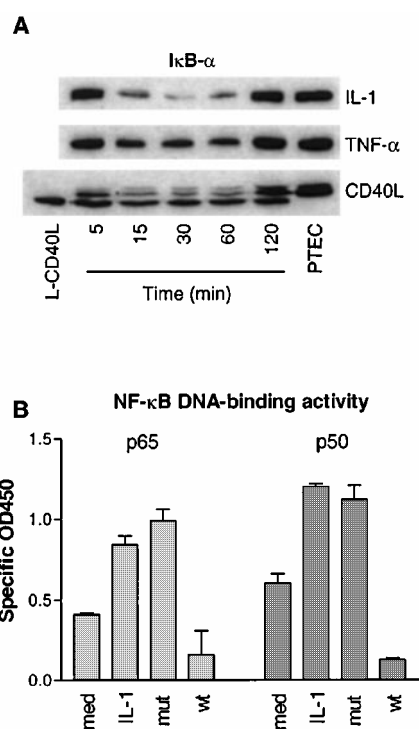


Figure 4 Assessment of NF- κ B activation in activated PTEC. (A) Western blot analysis of I κ B- α degradation in PTEC that were stimulated with IL-1 (5 ng ml⁻¹), TNF- α (500 u ml⁻¹) or CD40L for 5–120 min. Maximal I κ B- α degradation occurred after 30 min. (B) Assessment of NF- κ B DNA-binding activity using TransAm transcription factor assay kits as described in Methods. OD450 values were corrected for background levels. med, medium; mut, mutant and wt, wild-type consensus oligonucleotide. DNA-binding activity of p50 and p65 subunits was increased in HK-2 cells stimulated with 5 ng ml⁻¹ IL-1 for 30 min. $n=3$.

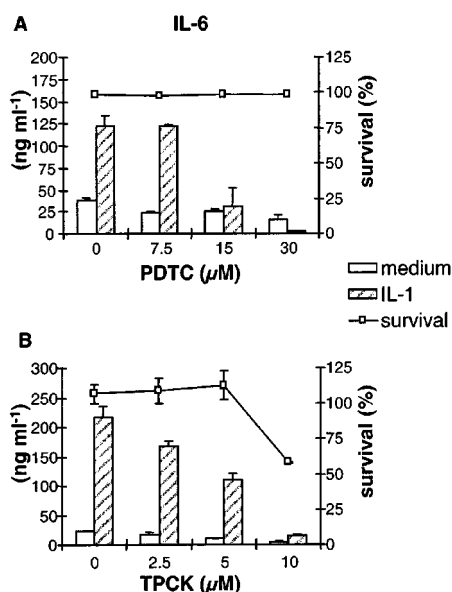


Figure 5 Role for NF- κ B in cytokine production by PTEC. Stimulation of PTEC with 5 ng ml⁻¹ IL-1 in the presence of increasing doses of PDTC (A) or TPCK (B) resulted in strong inhibition of IL-6 production. Data shown are mean \pm s.d. of one representative experiment out of four independent experiments. For TPCK, no inhibition was found when cells were treated with the dissolvent DMSO (data not shown). Cell viability was checked with Trypan blue staining.

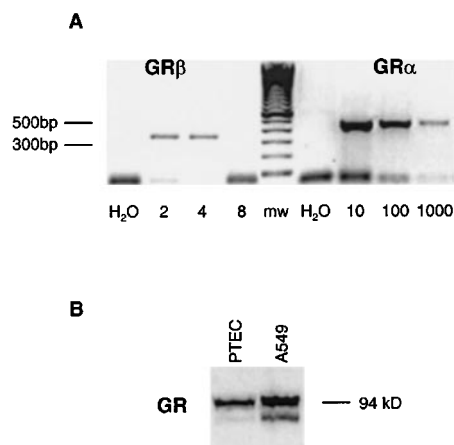


Figure 6 Expression of the glucocorticoid receptor by PTEC. (A) GR α and GR β expression was determined by RT-PCR as described in Methods. For GR β , cDNA was diluted 2–8 times; for GR α , cDNA was diluted 10–1000 times. mw, molecular weight marker. GR α mRNA was abundantly expressed in PTEC. (B) Representative Western blot analysis showing glucocorticoid receptor protein expression in PTEC and A549.

Discussion

In the present study we show that dexamethasone is unable to inhibit production of inflammatory mediators by renal tubular epithelial cells. Production of cytokines and chemokines by proximal tubular epithelial cells (PTEC) is thought to be critical for inflammatory processes in the kidney, including allograft rejection. Although immunosuppressive regimens in transplantation effectively inhibit acute allograft responses, the impact on chronic allograft rejection seems to be limited. Immunosuppression used to prevent allograft rejection usually includes glucocorticoids, which are known to have a profound effect on cytokine production by a variety of cell-types (Auwardt *et al.*, 1998; John *et al.*, 1997; Miyazawa *et al.*, 1998). Unexpectedly, dexamethasone was unable to inhibit IL-6 production by activated primary human PTEC and the PTEC-derived cell line HK-2. Similarly, dexamethasone was unable to inhibit IL-1-induced production of the chemokines IL-8 and MCP-1. In contrast, dexamethasone strongly inhibited cytokine production in renal fibroblasts and lung epithelial cells, emphasising the cell-type specificity of glucocorticoid action.

To investigate the effect of dexamethasone on other stimuli than IL-1, PTEC were stimulated with TNF- α and CD40L, which are known to induce cytokine and chemokine production. As was observed for IL-1, TNF- α - and CD40L-stimulated PTEC were insensitive to the inhibitory action of glucocorticoids. In contrast to our findings, dexamethasone has been shown to partially inhibit MCP-1 production in rat PTEC (Wang *et al.*, 1999) or RANTES production by distal and proximal human tubular epithelial cells stimulated with a combination of IL-1 β , TNF- α and IFN- γ (Baer *et al.*, 2000). Both the origin of the renal epithelial cell or the nature of the stimulus (protein overload or IFN- γ) might contribute to this discrepancy. Using CD40L as a stimulus (van Kooten *et al.*, 1999), we found that RANTES production was not inhibited by dexamethasone (data not shown).

The transcription factor NF- κ B is a key regulator of the expression of cytokine genes (Barnes & Karin, 1997; Lee & Burckart, 1998). In the present study we used degradation of I κ B- α as a marker to show that CD40L, as well as IL-1 and TNF- α induced activation of the NF- κ B signalling pathway. Furthermore, IL-1 induced specific binding of p65 and p50 NF- κ B subunits to DNA, as was previously shown for CD40L (Woltman *et al.*, 2000b). In line with these results, the NF- κ B inhibitors PDTC and TPCK markedly reduced IL-6, IL-8 and MCP-1 production by PTEC, confirming a role for NF- κ B. Glucocorticoid-mediated suppression of cytokine production is mainly attributed to inhibition of NF- κ B (Lee & Burckart, 1998; McKay & Cidlowski, 1999). Initially it was postulated that corticosteroids inhibit NF- κ B activation by increasing the transcription of I κ B- α (Auphan *et al.*, 1995; Scheinman *et al.*, 1995). Subsequent studies have suggested that glucocorticoids can prevent binding of NF- κ B to DNA or interfere with the transactivating potential of NF- κ B *via* protein–protein interaction with the p65 subunit (De Bosscher *et al.*, 2000; Ray & Prefontaine, 1994; Reichardt *et al.*, 2001).

The lack of inhibition by dexamethasone suggests that in PTEC NF- κ B signalling is insensitive to glucocorticoids, as has been shown in other cell systems (Amrani *et al.*, 1999; Auwardt *et al.*, 1998; Bourke & Moynagh, 1999). However, in these studies expression of adhesion molecules and cytokines is reduced by dexamethasone, while in activated human PTEC, all inflammatory mediators studied so far, including IL-6, RANTES, MCP-1, IL-8 and ICAM-1 seem to be insensitive to dexamethasone. In addition to the p50/p65 pathway of NF- κ B activation, alternative pathways have been described (Bourke *et al.*, 2000; Bours *et al.*, 1993; Dechend *et al.*, 1999; Thompson *et al.*, 1995), which are regulated *via* different I κ B-family members (I κ B- β , I κ B- ϵ , I κ B- γ , p105, p100, Bcl-3) and lead to the activation of different NF- κ B complexes (p50/p50 homodimers) or different subunits (c-rel, relB, p52). Although the functional role of these alternative pathways of NF- κ B activation is not fully understood, they might play a role in dexamethasone-insensitive activation of NF- κ B.

Alternatively, the lack of inhibition by dexamethasone could be explained by aberrant glucocorticoid receptor signalling. We have shown that glucocorticoid receptor mRNA and protein is expressed in renal epithelial cells. Furthermore we have excluded that lack of inhibition by dexamethasone was caused by over-expression of the glucocorticoid receptor beta isoform (GR β) that could competitively inhibit the functional glucocorticoid receptor (GR α) as was suggested in other studies (Bamberger *et al.*, 1995; Oakley *et al.*, 1999). Unravelling the mechanism of the steroid-insensitivity of PTEC will be the subject of further studies.

In conclusion, we show that production of cytokines and chemokines by activated renal epithelial cells is insensitive to treatment with dexamethasone. These results emphasise the cell-type specific characteristics of glucocorticoid action. Although the mechanism of steroid-insensitivity of PTEC is not yet known, this finding might have important implications for use of glucocorticoids in treatment of acute inflammatory responses in the kidney. While glucocorticoid treatment might efficiently inhibit T-cell-mediated immune responses, the effect on PTEC activation might be limited.

Considering the important role of PTEC in the amplification of the local immune response, this might allow for an ongoing low-grade immune response ultimately leading to chronic inflammation and fibrosis. The inability of dexamethasone to down-modulate the active state of PTEC therefore necessitates the development of novel immunosuppressive agents that are able to inhibit the pro-inflammatory role of PTEC during renal inflammation.

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