



Glucocorticoids potently block tumour necrosis factor- α - and lipopolysaccharide-induced apoptotic cell death in bovine glomerular endothelial cells upstream of caspase 3 activation

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1 Endothelial cell damage in glomeruli and kidney arterioles appears to play a pivotal role in glomerular inflammatory diseases. Glomerular endothelial cells, a specialized microvascular cell type involved in the regulation of glomerular ultrafiltration, die by apoptosis in response to tumour necrosis factor- α (TNF- α), TNF- α /basic fibroblast growth factor (bFGF), TNF- α /cycloheximide, and bacterial lipopolysaccharide (LPS). Apoptotic cell death is characterized by extensive DNA cleavage, DNA ladder formation, and characteristic morphological alterations.

2 In search for apoptosis-preventing signals, we identified glucocorticoids as potent death preventing factors. Co-treatment of cells with 10 nM dexamethasone and TNF- α , TNF- α /bFGF, TNF- α /cycloheximide, or LPS blocked roughly 90% of apoptotic cell death in glomerular endothelial cells.

3 Similarly to dexamethasone (TNF- α - and LPS-induced apoptosis are prevented with IC₅₀ values of 0.8 and 0.9 nM, respectively), other synthetic and natural forms of glucocorticoids, such as flucinolone, prednisolone, hydrocortisone, and corticosterone potently inhibited cell death with IC₅₀ values of 0.2, 6, 50 and 1000 nM, for TNF- α and 0.7, 8, 100 and 500 nM for LPS, respectively.

4 Apart from glucocorticoids, mineralocorticoids such as aldosterone also blocked TNF- α /LPS-induced apoptosis (IC₅₀ ~ 500 nM for TNF- α and ~ 500 nM for LPS), whereas sex hormones, i.e. β -estradiol and testosterone remained without effect.

5 The protective effect of glucocorticoids (and mineralocorticoids) required glucocorticoid receptor binding as it could be antagonized by the glucocorticoid receptor antagonist RU-486. Concerning TNF- α and LPS signal transduction, we found that dexamethasone efficiently prevented TNF- α - and LPS-induced activation of caspase-3-like proteases. Therefore, we postulate inhibitory mechanisms upstream of terminal death pathways.

Keywords: Glomerular endothelial cells; TNF- α ; LPS; apoptosis; glucocorticoids; dexamethasone; caspase-3

Abbreviations: aFGF, acidic fibroblast growth factor; AP-1, activator protein 1; bFGF, basic fibroblast growth factor; CHAPS, 3-[(3-chloramidopropyl)-dimethylammonio] propanesulphonate; DEVD-AMC, N-acetyl-aspartyl-glutamyl-valinyl-aspartyl-7-amino-4-coumarin; Dex, dexamethasone; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HUVEC, human umbilical vein endothelial cells; IAP, inhibitor of apoptosis; IFN- γ , interferon- γ ; I- κ B α , inhibitor κ B α ; I- κ B β , inhibitor κ B β ; LPS, lipopolysaccharide; MnSOD, manganese superoxide dismutase; NF- κ B, nuclear factor κ B; TE, Tris-EDTA; TNF- α , tumour necrosis factor α ; TNF-RI, tumour necrosis factor receptor I; TNF-RII, tumour necrosis factor receptor II; XIAP, X-chromosome-linked inhibitor of apoptosis

Introduction

Glomerular inflammatory diseases and acute or end-stage renal failure are leading problems in critical health care. Mediators from both immune and non-immune cells play major roles in these glomerular disease processes. Mesangial cells, which occupy a central anatomical position within the glomerulus, are thought to play a major role in the orchestration of the progression of glomerular injury (Pfeilschifter, 1994). Apart from their function as smooth muscle-like cells and their role in extracellular matrix production, mesangial cells release different cytokines which has been related to renal disease severity (Ortiz *et al.*, 1994). Mesangial cells are a major intraglomerular source of tumour necrosis factor- α (TNF- α) which is both expressed in the cell membrane and released following lipopolysaccharide (LPS) challenge (Baud *et al.*, 1989; 1992). Besides mesangial cells also glomerular epithelial and endothelial cells themselves are

potential sources of TNF production and release (Egido *et al.*, 1993). TNF- α in concert with other cytokines and lipid mediators is discussed to participate in tissue injury, i.e. glomerular diseases such as nephrotoxic nephritis, acute and chronic proliferative nephritis, and in diabetic nephropathy (Egido *et al.*, 1993). Apart from its potential to recruit inflammatory cells to the glomerulus, TNF- α was postulated to directly attack glomerular endothelial cells as continuous intravenous infusion of TNF- α produced endothelial cell damage and accumulation of neutrophils in the glomerular capillary lumen in rabbits (Bertani *et al.*, 1989). In experimental models of glomerulonephritis, i.e. a progressive model of anti-glomerular basement membrane glomerulonephritis glomerular endothelial cell apoptosis was identified in damaged glomeruli along with the destruction of the glomerular capillary network (Shimizu *et al.*, 1997). Moreover, also in glomerular capillary repair significant apoptosis of glomerular endothelial cells was demonstrated (Shimizu *et al.*, 1998) and conclusively, in glomerular capillary repair

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apoptosis seems necessary in regulating the number of intrinsic endothelial cells.

Apoptosis of vascular endothelial cells i.e. human umbilical vein endothelial cells (HUVEC) and several microvascular endothelial cell types in response to growth factor deprivation or TNF- α and cycloheximide was clearly established several years ago (Polunovsky *et al.*, 1994). The pleiotropic cytokine TNF- α is one of the most intensively investigated endothelial cell death inducers whose effect was transmitted *via* cross-linking of the membrane bound receptor molecules TNF-RI and TNF-RII. TNF-RI-mediated apoptosis signal transduction in HUVECs involves a coordinated network of intermediate signalling molecules leading to the activation of the caspase protease family including caspase-3 activation.

Recently, we successfully established an *in vitro* system of bovine glomerular endothelial cells (Briner & Kern, 1994) and clearly demonstrated that these cells die by apoptosis in response to TNF- α or bacterial LPS (Meßmer *et al.*, 1999). The apoptotic cell death of glomerular endothelial cells could be characterized by an increase or activation of several proapoptotic signalling pathways terminating in the activation of caspase-3-like proteases. In our current work we intended to investigate a potential pharmacological modulation of glomerular endothelial cell death by glucocorticoids.

Glucocorticoids are well known anti-inflammatory and immunosuppressive drugs that have been used successfully for many years as repressors of immune response and inflammatory processes. Some properties of glucocorticoids are attributed to its effects on transcription factors and gene expression. One important mechanism is inhibition of the nuclear factor kappa B (NF- κ B) activation which may be either achieved by protein-protein interactions between NF- κ B and the glucocorticoid receptor (Wissink *et al.*, 1998) and/or induction of the inhibitor- κ B α (I- κ B α) inhibitory protein (Auphan *et al.*, 1995; Xie *et al.*, 1997) which, however, was not demonstrable in every cell type (De Bosscher *et al.*, 1997; Newton *et al.*, 1998). Because NF- κ B activates many immunoregulatory genes in response to pro-inflammatory stimuli, the inhibition of its activity would be one major component of the anti-inflammatory activity of glucocorticoids (Dumont *et al.*, 1998; Wiegers & Reul, 1998). In addition to their anti-inflammatory activity, glucocorticoids such as dexamethasone are also used as immunosuppressive drugs and in this context are known as classical inducers of apoptotic cell death in lymphocytes (Osborne *et al.*, 1996). However, apart from lymphocytes non-lymphoid cells are resistant to dexamethasone-induced apoptosis and moreover, dexamethasone has been shown to prevent drug-induced apoptosis in malignant glioma cells (Naumann *et al.*, 1998) and in actinomycin D-induced cell death of the human gastric cancer cell line TMK-1 (Chang *et al.*, 1997).

In our previous work we investigated the response of bovine glomerular endothelial cells to TNF- α or LPS. We now report that dexamethasone as well as structurally related glucocorticoid analogues potently prevented apoptotic cell death induced either by TNF- α or LPS. Mechanistically, dexamethasone required glucocorticoid receptor binding and blocked apoptotic signalling upstream of caspase-3-like protease activation.

Methods

Cell culture and cell treatment

Bovine glomerular endothelial cells were cultivated as described previously (Briner & Kern, 1994). In brief,

approximately 10 g of renal cortex tissue were minced, passed through a sterile 240 μ m stainless steel sieve, and suspended in HBSS. This suspension was then poured through a 180 μ m stainless sieve followed by a 100 μ m mesh. The glomeruli retained by the 100 μ m sieve were washed three times in HBSS and were then incubated for 10–15 min at 37°C in HBSS containing 1 mg ml⁻¹ collagenase (type V, Sigma, Deisenhofen, Germany). After digestion, glomerular remnants were sedimented at 500 g for 5 min. The supernatant was centrifuged at 1000 \times g for 5 min, and the pellet was suspended in RPMI 1640 medium containing 20% FCS, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin, 50 μ g ml⁻¹ heparin sodium, and 5 ng ml⁻¹ of acidic fibroblast growth factor. Cells were plated on 0.2% gelatin-coated tissue culture plates. Primary cultures of endothelial cell clones were isolated with cloning cylinders, detached with trypsin-EDTA, and passaged at cloning density onto gelatin-coated 35-mm diameter plates. Individual clones of endothelial cells were characterized by positive staining for Factor VIII-related antigen and uniform uptake of fluorescent acetylated low-density lipoproteins (Ballermann, 1989). Negative staining for smooth muscle actin and cytokeratin excluded mesangial cell and epithelial cell contaminations, respectively. For the experiments, passages 9 to 19 of endothelial cells were used.

For experiments, endothelial cells were grown to confluency in 60-mm or 100-mm petri-dishes with RPMI 1640 medium containing 15% FCS, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin, 50 μ g ml⁻¹ heparin sodium, and 5 ng ml⁻¹ of acidic fibroblast growth factor and then incubated in RPMI 1640 containing 2% FCS, 100 U ml⁻¹ penicillin, and 100 μ g ml⁻¹ streptomycin.

Quantitation of DNA fragmentation

DNA fragmentation was essentially assayed as reported previously (Meßmer *et al.*, 1998). Briefly, after incubation cells were scraped off the culture plates, resuspended in 250 μ l TE-buffer, (Tris, 10 mM, EDTA, 1 mM, pH 8.0) and incubated with an additional volume lysis-buffer (Tris 5 mM, EDTA, 20 mM, pH 8.0, 0.5%, Triton X-100) for 30 min at 4°C. After lysis, the intact chromatin (pellet) was separated from DNA fragments (supernatant) by centrifugation for 15 min at 13,000 \times g. Pellets were resuspended in 500 μ l TE-buffer and samples were precipitated by adding 500 μ l 10% trichloroacetic acid at 4°C. Samples were pelleted at 4000 r.p.m. for 10 min and the supernatant was removed. After addition of 300 μ l 5% trichloroacetic acid, samples were boiled for 15 min. DNA contents were quantitated using the diphenylamine reagent (Burton, 1956). The percentage of DNA fragmented was calculated as the ratio of the DNA content in the supernatant to the amount in the pellet.

DNA agarose gel electrophoresis

For the preparation of DNA for agarose gel electrophoresis, cells were cultured, harvested, lysed, and centrifuged as described above to separate DNA fragments from intact chromatin. Supernatants were precipitated overnight with two volumes ice-cold ethanol and 50 μ l 5 M NaCl at -20°C, centrifuged at 13,000 \times g for 15 min followed by an incubation of the pellet in 500 μ l TE-buffer supplemented with 100 μ g ml⁻¹ RNase A at 37°C for 30 min. Samples were extracted with phenol:chloroform:isoamylalcohol (25:24:1) and once again with chloroform:isoamylalcohol (24:1). DNA was precipitated and pellets were recovered by centrifugation (13,000 \times g, 15 min), air dried, resuspended in 10 μ l TE-buffer,

supplemented with 2 μ l sample buffer (0.25% bromophenol blue, 30% glyceric acid), and electrophoretically separated on a 1% agarose gel containing 1 μ g ml⁻¹ ethidium bromide for 2.5 h at 100 V. Pictures were taken by UV transillumination.

Morphological investigations

Glomerular endothelial cells were grown in 60-mm culture plates to nearly confluency. Cells were stimulated, followed by fixation with 3% paraformaldehyd for 5 min onto glass slides. Samples were washed with phosphate-buffered saline, stained with Hoechst dye H33258 (8 μ g ml⁻¹) for 5 min, washed with distilled water, and mounted in KAISER'S glycerol gelatin. Nuclei were visualized using a Zeiss Axiovert fluorescence microscope.

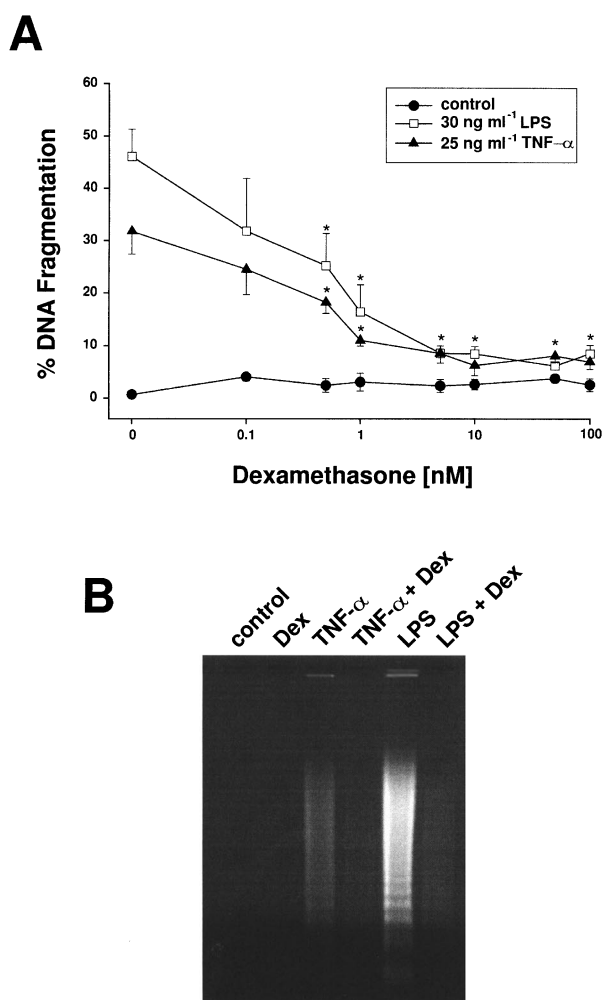


Figure 1 Dexamethasone suppressed apoptotic DNA fragmentation induced by TNF- α and bacterial LPS. (A) Bovine glomerular endothelial cells were incubated for 24 h with 25 ng ml⁻¹ TNF- α and 30 ng ml⁻¹ LPS in the absence or presence of increasing concentrations of dexamethasone. DNA fragmentation was quantitated by the diphenylamine reaction. Data are means \pm s.e. mean of four separate experiments. * P < 0.05 (ANOVA and for multiple comparison the data were corrected by Dunn's Method). (B) The formation of oligonucleosomal DNA fragments was assessed by conventional agarose gel electrophoresis. Cells were incubated with vehicle (control), 10 nM dexamethasone (Dex), 25 ng ml⁻¹ TNF- α (TNF- α), 25 ng ml⁻¹ TNF- α /10 nM dexamethasone (TNF- α +Dex), 30 ng ml⁻¹ LPS (LPS), and 30 ng ml⁻¹ LPS/10 nM dexamethasone (LPS+Dex) for 24 h as indicated. The formation of a DNA ladder is a marker of apoptosis rather than necrosis. The results are representative of three independent experiments.

Caspase-3 enzyme activity

For detection of caspase-3 activity, glomerular endothelial cells were incubated as indicated and lysed in lysis buffer (Tris/HCl 10 mM, sucrose 0.32 M, EDTA 5 mM, 1% Triton X-100, phenylmethanesulphonyl fluoride 1 mM, 1 μ g ml⁻¹ aprotinin, 10 μ g ml⁻¹ leupeptin, DTT, pH 8.0, 2 mM) for 30 min. Following sonication (10 s, output control 1) lysates were centrifuged (10,000 $\times g$, 5 min, 4°C) and stored at -80°C. Protein determinations were performed with the Bradford method (Bradford, 1976). Caspase-3 activity was detected by measuring the proteolytic cleavage of the fluorogenic substrate N-acetyl-aspartyl-glutamyl-valinyl-aspartyl-7-amino-4-coumarin (Ac-DEVD-AMC). Cell lysates (50 μ g protein) were incubated in HEPES 100 mM, 10% sucrose, 0.1% CHAPS, pH 7.5, phenylmethanesulphonyl fluoride 1 mM, 1 μ g ml⁻¹ aprotinin, 10 μ g ml⁻¹ leupeptin, DTT 2 mM at 37°C with 12 μ M DEVD-AMC in a total volume of 700 μ l. Substrate cleavage and AMC accumulation was followed fluorometrically with excitation at 380 nm and emission at 460 nm.

Materials

Diphenylamine, lipopolysaccharide (LPS, *E. coli* serotype 0127:B8), heparin sodium, cycloheximide, Hoechst dye 33258, dexamethasone, fluocinolone acetonide (fluocinolone), prednisolone, 17-hydroxycorticosterone (hydrocortisone), corticosterone-21-acetate (corticosterone), 4-androsterone-17 β -ol-3-one (testosterone), 17 β -estradiol (estradiol), aldosterone, and aldosterone-21-acetate were purchased from Sigma (Deisenhofen, Germany). DEVD-AMC was provided by Bachem (Heidelberg, Germany). RU-486 was from Roussel-UCLAF. Bovine acidic fibroblast growth factor (aFGF) and human basic fibroblast growth factor (bFGF) were purchased from R&D Systems (Wiesbaden, Germany), and recombinant human TNF- α (specific activity: 6.6 $\times 10^6$ units mg⁻¹) was a

Table 1 Comparison between different glucocorticoids of its suppressive effect on apoptotic DNA fragmentation induced by TNF- α and bacterial LPS

A	TNF- α		LPS
	Control	(25 ng ml ⁻¹)	
DNA fragmentation (%)	Control	(25 ng ml ⁻¹)	(30 ng ml ⁻¹)
Control	0.7 \pm 0.6	25.3 \pm 4.2	46.1 \pm 5.2
Dexamethasone 10 nM	2.6 \pm 1.0	6.2 \pm 1.9	8.5 \pm 1.4
Fluocinolone 1 nM	0.5 \pm 0.5	7.5 \pm 1.1	8.0 \pm 1.4
Prednisolone 10 nM	0.7 \pm 0.6	3.6 \pm 1.7	8.4 \pm 2.5
Hydrocortisone 100 nM	3.0 \pm 0.5	5.8 \pm 2.9	20.6 \pm 3.9
Corticosterone 1 μ M	0.4 \pm 0.4	11.2 \pm 2.3	17.5 \pm 6.6
B			
IC ₅₀ values (nM)	TNF- α	LPS	
Dexamethasone	0.8	0.9	
Fluocinolone	0.2	0.7	
Prednisolone	6	8	
Hydrocortisone	50	100	
Corticosterone	1000	500	

(A) Bovine glomerular endothelial cells were incubated for 24 h with 25 ng ml⁻¹ TNF- α and 30 ng ml⁻¹ LPS in the absence (control) or presence of different concentrations of glucocorticoids. DNA fragmentation was quantitated by the diphenylamine reaction. Values represent per cent DNA fragmentation (means \pm s.e. mean) out of four to five separate experiments. (B) Cells were incubated for 24 h with 25 ng ml⁻¹ TNF- α or 30 ng ml⁻¹ LPS in the presence of increasing concentrations of different glucocorticoids as indicated. IC₅₀ values were determined graphically.

generous gift from Knoll AG, Germany. RPMI 1640, cell culture supplements and foetal calf serum were from Gibco (Eggenstein, Germany). All other chemicals were of the highest grade of purity commercially available.

Statistical analyses

Each experiment was performed at least three times and statistical analysis were performed using the two tailed Student's *t*-test or ANOVA and for multiple comparison the data were corrected by Dunn's Method.

Results

Effect of glucocorticoids on glomerular endothelial cell apoptosis

As shown in Figure 1A, a 24-h exposure to 25 ng ml⁻¹ TNF- α or 30 ng ml⁻¹ LPS caused 31.8 \pm 4.3 and 46.1 \pm 5.2% DNA fragmentation (means \pm s.e.mean, *n* = 4), respectively. In contrast, co-incubation of bovine glomerular endothelial cells with 25 ng ml⁻¹ TNF- α or 30 ng ml⁻¹ LPS and increasing

concentrations of the synthetic glucocorticoid analogue dexamethasone revealed that dexamethasone concentration-dependently suppressed DNA fragmentation measured by the diphenylamine reaction with IC₅₀ values of 0.8 and 0.9 nM, respectively. In addition, the pattern of DNA fragmentation elicited by TNF- α and LPS showed the characteristic DNA ladder which is nearly absent when the apoptosis inducers were combined with dexamethasone (Figure 1B) and morphological alterations, i.e. chromatin condensation induced by TNF- α and LPS were prevented by dexamethasone (data not shown). We also questioned whether dexamethasone shared its anti-apoptotic effect with other synthetic and natural glucocorticoids. Remarkably, no significant differences in cell viability and total cell number were observed in cells treated for 24 h with different glucocorticoid analogues alone. In addition to dexamethasone, flucinolone, prednisolone, hydrocortisone, and corticosterone were able to decrease the susceptibility of glomerular endothelial cells towards apoptosis induced either by TNF- α or LPS (Table 1A). Comparing the inhibitory potency, flucinolone and dexamethasone appeared to be very potent exhibiting IC₅₀ values < 1 nM (Table 1B). Prednisolone showed an intermediary potency (IC₅₀ around 6–8 nM) whereas hydrocortisone and corticosterone proved to be less efficient (IC₅₀ up to 1000 nM).

As it would be relevant for kidney function, in addition to glucocorticoids we also tested mineralocorticoids. Coincubation of glomerular endothelial cells with 10 ng ml⁻¹ TNF- α or 10 ng ml⁻¹ LPS and either increasing concentrations of aldosterone or aldosterone-21-acetate revealed that mineralocorticoids blocked apoptosis induction at high concentrations exhibiting IC₅₀ values of ~500 nM for TNF- α and

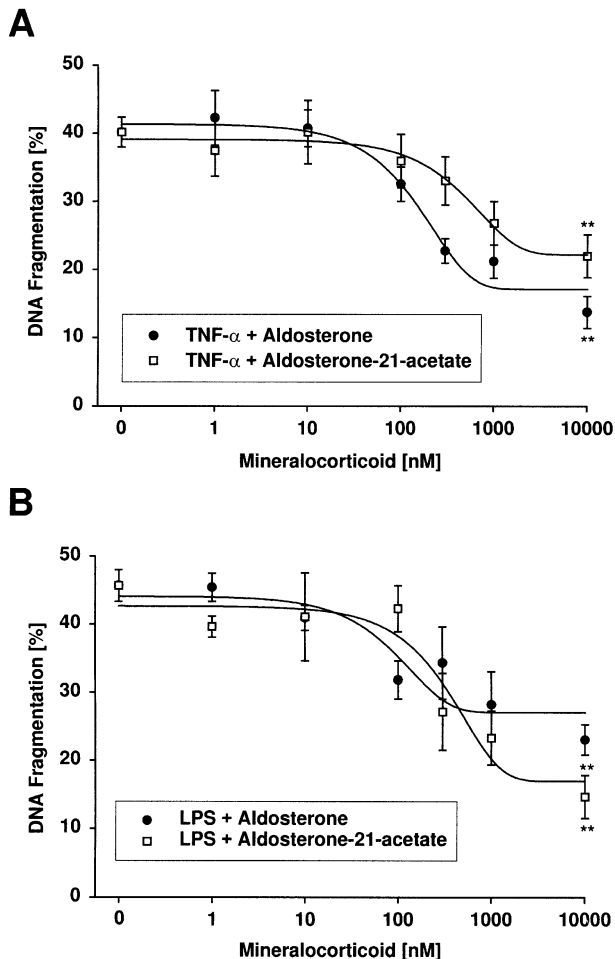


Figure 2 Effects of mineralocorticoids on apoptotic DNA fragmentation induced by TNF- α and bacterial LPS. Bovine glomerular endothelial cells were incubated for 24 h with 10 ng ml⁻¹ TNF- α (A) and 10 ng ml⁻¹ LPS (B) in the absence or presence of increasing concentrations of aldosterone or aldosterone-21-acetate. DNA fragmentation was quantitated by the diphenylamine reaction. Data are means \pm s.e.mean of four separate experiments. ***P* < 0.05 (ANOVA and for multiple comparison the data were corrected by Dunn's Method).

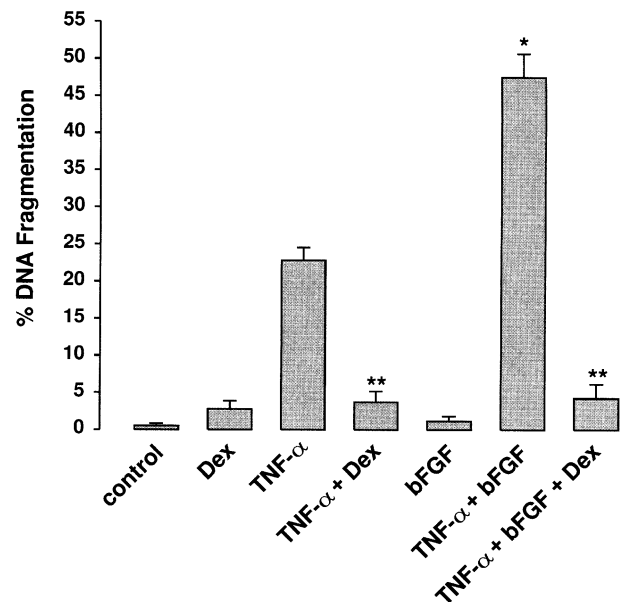


Figure 3 The synergistic action of TNF- α and bFGF was blocked by co-treatment with dexamethasone. Incubations were done for 24 h with 10 nM dexamethasone (Dex), 10 ng ml⁻¹ TNF- α (TNF- α), 10 ng ml⁻¹ TNF- α + 10 nM dexamethasone (TNF- α + Dex), 10 ng ml⁻¹ bFGF (bFGF), 10 ng ml⁻¹ TNF- α + 10 ng ml⁻¹ bFGF (TNF- α + bFGF), and 10 ng ml⁻¹ TNF- α + 10 ng ml⁻¹ bFGF + 10 nM dexamethasone (TNF- α + bFGF + Dex). DNA fragmentation was quantitated using the diphenylamine reaction as described in the Methods section. Values are means \pm s.e.mean of three independent experiments. bFGF significantly potentiated TNF- α -induced apoptosis (**P* \leq 0.01) and dexamethasone blocked apoptosis induction by TNF- α or TNF- α /bFGF (***P* \leq 0.001) (ANOVA and for multiple comparison the data were corrected by Dunn's Method).

~500 nM for LPS (Figure 2). Comparing the potencies of the natural, membrane permeable isoform aldosterone or the more hydrophilic aldosterone-21-acetate revealed no significant difference between both analogues.

Since estradiol was described as a survival factor of HUVECs resulting in a dose-dependent, receptor-mediated inhibition of TNF- α -induced cell apoptosis (Spyridopoulos *et al.*, 1997) we also evaluated sex hormones as potential apoptosis modifiers in glomerular endothelial cells. However, neither estradiol in a concentration range up to 10 μ M nor testosterone up to 1 μ M significantly modulated the development of programmed cell death in response to TNF- α or LPS in our cells. Taken together, our results clearly demonstrate that glucocorticoids but not sex hormones efficiently suppress apoptosis of bovine glomerular endothelial cells triggered either by TNF- α or LPS.

During our search for physiological relevant apoptosis preventing signals we also tested different growth factors and cytokines. Treatment of glomerular endothelial cells with growth factors such as bFGF alone has no significant effect on cell viability or the basal apoptosis rate. Surprisingly, although bFGF is reported as an antiapoptotic factor in endothelial cells, co-treatment of glomerular

endothelial cells with low doses of TNF- α and 1–10 ng ml⁻¹ bFGF resulted in a synergistic bFGF-mediated potentiation of apoptotic DNA fragmentation (Figure 3). Again, dexamethasone (10 nM) suppressed the synergistic signal of TNF- α /bFGF in a similar fashion as it blocked TNF- α alone.

Involvement of the glucocorticoid receptor in the effect of dexamethasone

To address the effect of dexamethasone mechanistically, we first investigated whether the regulation of apoptosis by dexamethasone is mediated by the glucocorticoid receptor. Therefore, we investigated the effect of the glucocorticoid receptor antagonist RU-486 on glomerular endothelial cell survival. In Figure 4 it is demonstrated that addition of RU-486 led to a dose-dependent neutralization of the inhibitory effect of dexamethasone on TNF- α or LPS-induced glomerular endothelial cell apoptosis. To neutralize the inhibitory effect of 10 nM dexamethasone 50–100 nM RU-486 appeared to be sufficient. The neutralizing effect of RU-486 was not caused by direct toxicity as the addition of 1 μ M did not alter basal survival rate.

Furthermore, we tested whether the effect of aldosterone could be affected by the glucocorticoid receptor specific antagonist RU-486. As shown in Figure 5, coinubation of glomerular endothelial cells with 10 ng ml⁻¹ TNF- α , 1000 nM aldosterone, and 100 or 1000 nM RU-486 revealed a neutralization of the antiapoptotic effect of aldosterone. These results may indicate that the effect of high aldosterone concentrations depend on a partially agonistic binding of aldosterone to the glucocorticoid receptor.

Effect of protein synthesis inhibitors in the anti-apoptotic effect of glucocorticoids

Next, we questioned whether the glucocorticoid-induced suppression of apoptosis required continuous protein biosynthesis. As it was demonstrated for several other systems in

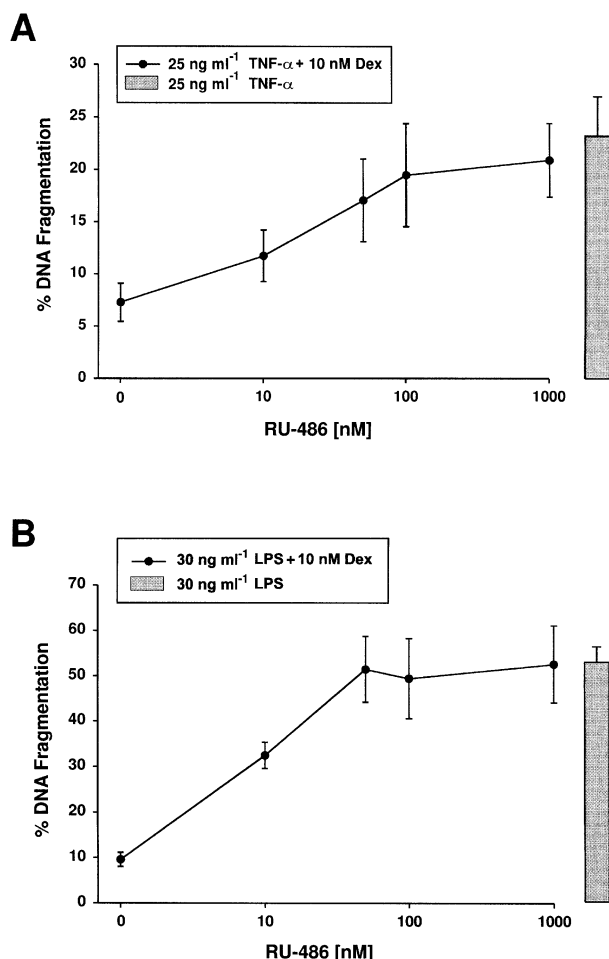


Figure 4 The effect of dexamethasone depends on glucocorticoid receptor binding. Cells were incubated for 24 h with 25 ng ml⁻¹ TNF- α and 10 nM dexamethasone (A) or with 30 ng ml⁻¹ LPS and 10 nM dexamethasone (B) in the absence or presence of increasing concentrations of the glucocorticoid receptor antagonist RU-486 as indicated. In parallel, control incubations were done for 24 h with 25 ng ml⁻¹ TNF- α (A) or 30 ng ml⁻¹ LPS (B) (bars). DNA fragmentation was determined using the diphenylamine reaction. Values are means \pm s.e. mean of at least four individual experiments.

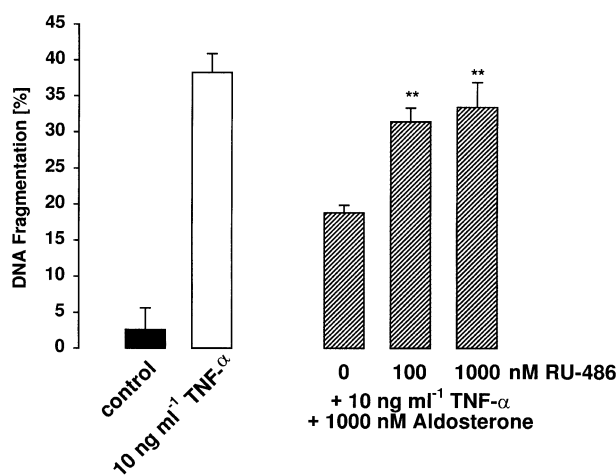


Figure 5 The effect of aldosterone is antagonized by the glucocorticoid receptor antagonist RU-486. Cells were incubated for 24 h with 10 ng ml⁻¹ TNF- α , 10 ng ml⁻¹ TNF- α and 1000 nM aldosterone or with 10 ng ml⁻¹ TNF- α , 1000 nM aldosterone and either 100 or 1000 nM of the glucocorticoid receptor antagonist RU-486 as indicated. DNA fragmentation was determined using the diphenylamine reaction. Values are means \pm s.e. mean of at least four individual experiments. ** P < 0.05 compared to the incubations without RU-486 (ANOVA and for multiple comparison the data were corrected by Dunn's Method).

the past, co-treatment of glomerular endothelial cells with 1 ng ml^{-1} TNF- α and increasing concentrations of cycloheximide synergistically potentiated the TNF- α -induced apoptotic DNA fragmentation reaching optimal effects with $1 \mu\text{M}$ cycloheximide. Cycloheximide alone up to $10 \mu\text{M}$ has only a marginal apoptosis-inducing effect (Figure 6A). As shown in Figure 6A, co-incubation of 1 ng ml^{-1} TNF- α and $1 \mu\text{M}$ cycloheximide revealed $54.8 \pm 3.8\%$ in comparison to $10.9 \pm 2.8\%$ DNA cleavage (means \pm s.e.mean, $n=4$) with TNF- α alone. The addition of fluocinolone to 1 ng ml^{-1} TNF- α / $1 \mu\text{M}$ cycloheximide suppressed the synergistic effect to $16.5 \pm 1.5\%$ DNA fragmentation (mean \pm s.e.mean, $n=4$). Only if the concentration of cycloheximide was increased up to $10 \mu\text{M}$ the protective effect of fluocinolone diminished and DNA degradation raised up to $37.4 \pm 3.8\%$ DNA cleavage (mean \pm s.e.mean, $n=3$). Similar, but less pronounced effects were observed by using LPS. In contrast to TNF- α , a synergistic effect was only hardly seen if cycloheximide was combined with LPS. Comparably, 5 ng ml^{-1} LPS plus $1 \mu\text{M}$ cycloheximide was blocked by fluocinolone whereas LPS plus $10 \mu\text{M}$ cycloheximide was essentially ineffective (Figure 6B).

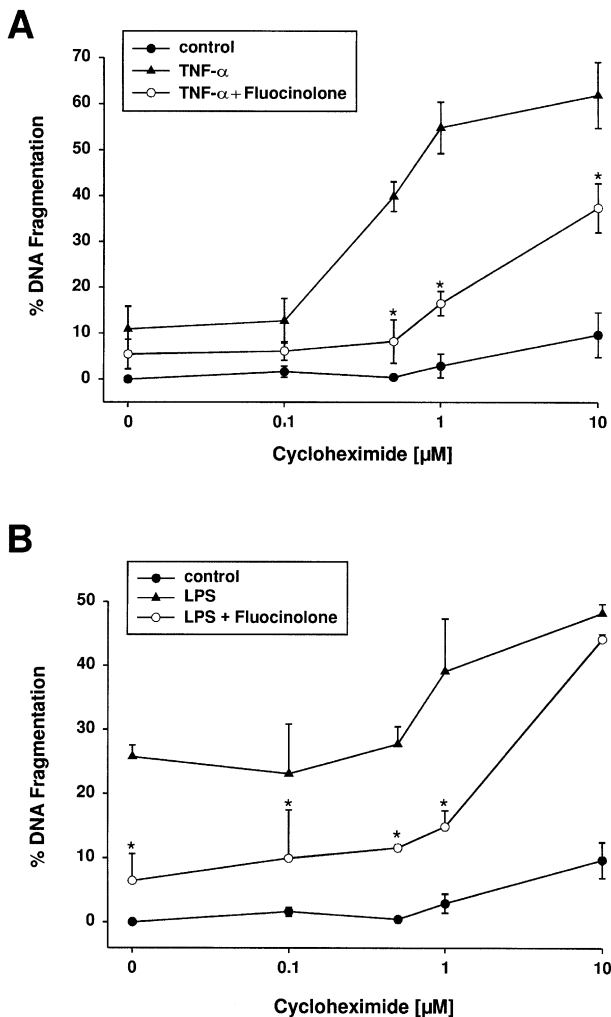


Figure 6 Fluocinolone suppressed apoptosis triggered by co-treatment with TNF- α and cycloheximide. Bovine glomerular endothelial cells were exposed to increasing concentrations of cycloheximide (control), 1 ng ml^{-1} TNF- α plus cycloheximide, and 1 ng ml^{-1} TNF- α plus cycloheximide plus 10 nm fluocinolone (A); or 5 ng ml^{-1} LPS plus cycloheximide, and 5 ng ml^{-1} LPS plus cycloheximide plus 10 nm fluocinolone (B). The amount of DNA fragmentation was determined with the diphenylamine reaction. Values are means \pm s.e.mean of four individual experiments.

Effects of glucocorticoids on caspase-3 activation

The activation of the caspase protease family is recognized as one of the final signalling pathways leading to cell death. We next questioned whether glucocorticoids prevented TNF- α - and LPS-induced apoptosis in glomerular endothelial cells upstream or downstream of caspase-3-activation. Caspase-3-like protease activation starts within 12 h following TNF- α addition and within 8–10 h following LPS stimulation (Meßmer *et al.*, 1999). Caspase-3-like protease activity following its initial activation increased continuously up to 24 h. Within the 24 h incubation period TNF- α elicited caspase-3-like protease activity amounting to around $400 \text{ nmoles AMC min}^{-1} \times \text{mg protein}$ whereas LPS induced within the same time period about 3 fold of the TNF- α -induced caspase-3-like activity (Table 2). Interestingly, dexamethasone totally suppressed the very low basal caspase activity and, moreover, prevented TNF- α -induced caspase-3 activation and also significantly suppressed LPS-induced caspase-3 activation. These results document that glucocorticoids block TNF- α - and LPS-induced apoptosis upstream of caspase-3 activation.

Discussion

In our current work we clearly demonstrated that glucocorticoids and high concentrations of mineralocorticoids but not sex hormones potently suppressed bovine glomerular endothelial cell death induced by cytokines and bacterial endotoxin. Death protection depends on glucocorticoid receptor binding and blocks apoptotic signalling upstream of caspase-3-like protease activation. Conclusively, glucocorticoids should be considered as physiological modulators of glomerular endothelial cell death during glomerular inflammatory diseases.

Cell proliferation, differentiation, and apoptosis are dynamic processes regulating cell homeostasis in physiology and pathophysiology. During severe forms of glomerulonephritis apoptosis of glomerular endothelial cells may contribute to the development of glomerulosclerosis (Kitamura *et al.*, 1998), whereas angiogenic capillary regeneration with endothelial proliferation occurred among mesangiolytic lesions in Thy-1 glomerulonephritis with a recovery of the damaged glomerulus to its normal structure (Shimizu *et al.*, 1996). Endogenous regulation of glomerular endothelial cell apoptosis in response to cytokines such as TNF- α and bacterial endotoxin (Meßmer *et al.*, 1999) may be achieved by glucocorticoids as it was shown for acinar cell apoptosis during cerulein pancreatitis in rats (Kimura *et al.*, 1998). We have documented for the first time that glucocorticoids protect bovine glomerular endothelial cells *in vitro* from cytokine and

Table 2 Prevention of TNF- α - and LPS-mediated caspase-3-like protease activation by dexamethasone

	(nmoles AMC min $^{-1}$ \times mg protein $^{-1}$)		
	Control	TNF- α (25 ng ml $^{-1}$)	LPS (30 ng ml $^{-1}$)
Control	10 \pm 14	412 \pm 271	1415 \pm 494
10 nm Dex	0 \pm 0	31 \pm 35	354 \pm 273

Glomerular endothelial cells were exposed for 24 h to 25 ng ml^{-1} TNF- α or 30 ng ml^{-1} LPS in the absence or in the presence of 10 nm dexamethasone. Caspase-3-like activity was determined using the fluorogenic substrate DEVD-AMC. Data are means \pm s.d. of three independent experiments. ** $P < 0.01$ (ANOVA and for multiple comparison the data were corrected by Dunn's Method).

endotoxin-induced apoptotic cell death. This is in line with a few other reports documenting that glucocorticoids suppressed apoptosis in other cell types such as in rat hepatocytes exposed to aflatoxin B1 (Iida *et al.*, 1998), in lung epithelial cells exposed to IFN- γ and Fas (Wen *et al.*, 1997), in malignant glioma cells exposed to anticancer drugs (Naumann *et al.*, 1998), in a human gastric cancer cell line exposed to actinomycin D (Chang *et al.*, 1997), in L929 mouse fibroblasts activated by TNF- α (Pagliacci *et al.*, 1993), and in human neutrophils (Cox, 1995; Cox & Austin, 1997). Natural and synthetic forms of glucocorticoids differ in their ability to induce gene expression or to repress NF- κ B-dependent transcription in thymocytes (Hofmann *et al.*, 1998). Similarly, the anti-apoptotic potency of the tested compounds can be ordered in the following sequence flucocinolone > dexamethasone > prednisolone > hydrocortisone > corticosterone, thus correlating well with their anti-inflammatory activities.

In addition to glucocorticoids, mineralocorticoids such as aldosterone or aldosterone-21-acetate also revealed anti-apoptotic activities. However, as the glucocorticoid-specific receptor antagonist RU-486 blocked both, the effects of glucocorticoids and mineralocorticoids on TNF- α or LPS-induced endothelial cell apoptosis and mineralocorticoids exhibited their anti-apoptotic effects only at high concentrations, we suggest in both cases a glucocorticoid receptor-dependent action.

In contrast, oestrogen and testosterone exhibited no significant effect on glomerular endothelial cell apoptosis. In this point, microvascular endothelial cells from the glomerulus differ from macrovascular HUVECs (Spyridopoulos *et al.*, 1997) or the breast cancer cell line MCF-7 (Leung *et al.*, 1998) which respond to estradiol as a survival factor. The difference in the reactivity of the certain endothelial cell subtypes to estradiol may depend on the presence or absence of estradiol receptors in the respective cells or an unresponsiveness or difference in the specific apoptotic pathways.

The mechanism of the survival effect of glucocorticoids on glomerular endothelial cells remains to be clarified. In contrast, dexamethasone was intensively investigated in regard to its anti-inflammatory and immunosuppressive activity and in this context found to be a strong inducer of thymocyte apoptosis. Induction of apoptosis in immature thymocytes by dexamethasone involves a modulation of I κ B α and I κ B β expression and downregulation of NF- κ B DNA binding. Downregulation of NF- κ B DNA binding preceded cell death, suggesting that NF- κ B may be important for the survival of immature thymocytes (Wang *et al.*, 1999). Reduction of NF- κ B mediated transactivation may be achieved either by an induction of I κ B α and I κ B β expression (Wang *et al.*, 1999) or by an impairment of TNF- α -induced degradation of I κ B α (Hofmann *et al.*, 1998). A decreased activity of NF- κ B probably results in a lower expression of anti-apoptotic proteins such as XIAP (X-chromosome-linked inhibitor of apoptosis) and IAP1/IAP2 (inhibitor of apoptosis) (Stehlik *et al.*, 1998) followed by an activation of pro-apoptotic pathways such as loss of

mitochondrial membrane potential and the activation of terminal proteases such as the caspase-3 family of proteases (Miyashita *et al.*, 1998).

Suppression of apoptosis also involves modulation of gene expression as it is the case for estradiol which inhibits Bak expression in MCF-7 cells (Leung *et al.*, 1998) and induces caspase-1 expression in HUVECs (Spyridopoulos *et al.*, 1997). Similarly, glucocorticoids on one hand were identified to induce expression of an inhibitor of apoptosis, human inhibitor of apoptosis (hIAP-1) also known as cIAP-2 (Wen *et al.*, 1997), and induce the expression of MnSOD in glomerular cells (Yoshioka *et al.*, 1994). On the other hand, dexamethasone suppressed the expression of the proapoptotic bcl-x_s gene while it also enhanced the basal level of the antiapoptotic Bcl-x_L (Chang *et al.*, 1997). Moreover, apart from its known effect on NF- κ B transcription factor activity, dexamethasone inhibits binding to the Ikaros and AP-1 regulatory elements of the granzyme B promoter and therefore downregulates human granzyme B expression (Wargnier *et al.*, 1998).

In respect to the findings that glucocorticoids either block NF- κ B activity or also regulate other transcription factors one can speculate that glucocorticoids may promote apoptosis e.g. in lymphocytes by blocking NF- κ B activity which is required for the synthesis of short lived survival proteins. On the other hand, glucocorticoids also block apoptosis in several other cells, such as glomerular endothelial cells which may be mediated at least in part by similar mechanisms. For example, TNF- α is able to elicit pro- and anti-apoptotic signals at the same time (Van Antwerp *et al.*, 1998) where the latter one depends on the activation of NF- κ B (Stehlik *et al.*, 1998). One can imagine that glucocorticoids either block the induction of the anti-apoptotic as well as the pro-apoptotic pathways by blocking NF- κ B and other transcription factors. It will be of interest to investigate whether and, if so, which cell specific set of transcription factors determines whether glucocorticoids display either a pro- or anti-apoptotic activity. In this context, we have shown for glomerular endothelial cells that dexamethasone suppressed the terminal activation of caspase-3-like proteases. Therefore, possible inhibitory mechanisms lie upstream of caspase-3-like protease activation and are independent of the stimulus as it is active in the case of TNF- α , TNF- α /bFGF and LPS. The ability of dexamethasone to prevent cytokine- and LPS-induced apoptotic signalling is partially observed even in the presence of protein biosynthesis inhibitors. This suggests that several complex mechanisms may be involved in the anti-apoptotic effect displayed by glucocorticoids in glomerular endothelial cells.

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References

- AUPHAN, N., DIDONATO, J.A., ROSETTE, C., HELMBERG, A. & KARIN, M. (1995). Immunosuppression by glucocorticoids: inhibition of NF- κ B activity through induction of I κ B synthesis. *Science*, **270**, 286–290.
- BALLERMANN, B.J. (1989). Regulation of bovine glomerular endothelial cell growth in vitro. *Am. J. Physiol.*, **256**, C182–C189.
- BAUD, L., FOUQUERAY, B., PHILIPPE, C. & AMRANI, A. (1992). Tumor necrosis factor alpha and mesangial cells. *Kidney Int.*, **41**, 600–603.
- BAUD, L., OUDINET, J.P., BENS, M., NOE, L., PERALDI, M.N., RONDEAU, E., ETIENNE, J. & ARDAILLOU, R. (1989). Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide. *Kidney Int.*, **35**, 1111–1118.

- BERTANI, T., ABBATE, M., ZOJA, C., CORNA, D., PERICO, N., GHEZZI, P. & REMUZZI, G. (1989). Tumor necrosis factor induces glomerular damage in the rabbit. *Am. J. Pathol.*, **134**, 419–430.
- BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.
- BRINER, V.A. & KERN, F. (1994). ATP stimulates Ca^{2+} mobilization by a nucleotide receptor in glomerular endothelial cells. *Am. J. Physiol.*, **266**, F210–F217.
- BURTON, K. (1956). A study of the conditions and mechanisms of the diphenylamine reaction for the estimation of deoxyribonucleic acid. *Biochem. J.*, **62**, 315–323.
- CHANG, T.-C., HUNG, M.-W., JIANG, S.-Y., CHU, J.-T., CHU, L.-L. & TSAI, L.-C. (1997). Dexamethasone suppress apoptosis in a human gastric cancer cell line through modulation of bcl-x gene expression. *FEBS Lett.*, **415**, 11–15.
- COX, G. (1995). Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J. Immunol.*, **154**, 4719–4725.
- COX, G. & AUSTIN, R.C. (1997). Dexamethasone-induced suppression of apoptosis in human neutrophils requires continuous stimulation of new protein synthesis. *J. Leukoc. Biol.*, **61**, 224–230.
- DE BOSSCHNER, K., SCHMITZ, M.L., VANDEN BERGHE, W., PLAISANCE, S., FIERS, W. & HAEGEMAN, G. (1997). Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 13504–13509.
- DUMONT, A., HEHNER, S.P., SCHMITZ, M.L., GUSTAFSSON, J.-A., LIDÉN, J., OKRET, S., VAN DER SAAG, P.T., WISSINK, S., VAN DER BURG, B., HERRLICH, P., HAEGEMAN, G., DE BOSSCHER, K. & FIERS, W. (1998). Cross-talk between steroids and NF- κ B: which language. *Trends Biochem. Sci.*, **23**, 233–235.
- EGIDO, J., GÓMEZ-CHIARRI, M., ORTÍZ, A., BUSTOS, C., ALONSO, J., GÓMEZ-GUERRERO, C., GÓMEZ-GARRE, D., LÓPEZ-ARMADA, J., PLAZA, J. & GONZALEZ, E. (1993). Role of tumor necrosis factor- α in the pathogenesis of glomerular diseases. *Kidney Int.*, **43**, S59–S64.
- HOFMANN, T.G., HEHNER, S.P., BACHER, S., DROGE, W. & SCHMITZ, M.L. (1998). Various glucocorticoids differ in their ability to induce gene expression, apoptosis and to repress NF- κ B-dependent transcription. *FEBS Lett.*, **441**, 441–446.
- IIDA, N., SUGIYAMA, A., MYOUBUDANI, H., INOUE, K., SUGAMATA, M., IHARA, T., UENO, Y. & TASHIRO, F. (1998). Suppression of arachidonic acid cascade-mediated apoptosis in aflatoxin B1-induced rat hepatoma cells by glucocorticoids. *Carcinogenesis*, **19**, 191–202.
- KIMURA, K., SHIMOSEGAWA, T., SASANO, H., ABE, R., SATOH, A., MASAMUNE, A., KOIZUMI, M., NAGURA, H. & TOYOTA, T. (1998). Endogenous glucocorticoids decrease the acinar cell sensitivity to apoptosis during cerulein pancreatitis in rats. *Gastroenterology*, **114**, 372–381.
- KITAMURA, H., SHIMIZU, A., MASUDA, Y., ISHIZAKI, M., SUGISAKI, Y. & YAMANAKA, N. (1998). Apoptosis in glomerular endothelial cells during the development of glomerulosclerosis in the remnant-kidney model. *Exp. Nephrol.*, **6**, 328–336.
- LEUNG, L.K., DO, L. & WANG, T.T.Y. (1998). Regulation of death promoter Bak expression by cell density and 17β -estradiol in MCF-7 cells. *Cancer Lett.*, **124**, 47–52.
- MEßMER, U.K., BRINER, V.A. & PFEILSCHIFTER, J. (1999). Tumor necrosis factor- α and lipopolysaccharide potently induce apoptotic cell death in bovine glomerular endothelial cells. Involvement of cytochrome c release, Bak upregulation, Bcl-x_L downregulation, and caspase-3 activation. *Kidney Int.*, Vol 55 in press.
- MEßMER, U.K., REIMER, D.M. & BRÜNE, B. (1998). Protease activation during nitric oxide-induced apoptosis: comparison between poly(ADP-ribose) polymerase and U1-70kDa cleavage. *Eur. J. Pharmacol.*, **349**, 333–343.
- MIYASHITA, T., NAGAO, K., KRAJEWSKI, S., SALVESEN, G.S., REED, J.C., INOUE, T. & YAMADA, M. (1998). Investigation of glucocorticoid-induced apoptotic pathway: processing of caspase-6 but not caspase-3. *Cell Death Differ.*, **5**, 1034–1041.
- NAUMANN, U., DURKA, S. & WELLER, M. (1998). Dexamethasone-mediated protection from drug cytotoxicity: association with p21^{WAF1/CIP1} protein accumulation. *Oncogene*, **17**, 1567–1575.
- NEWTON, R., HART, L.A., STEVENS, D.A., BERGMANN, M., DONNELLY, L.E., ADCOCK, I.M. & BARNES, P.J. (1998). Effect of dexamethasone on interleukin-1 β (IL-1 β)-induced nuclear factor- κ B (NF- κ B) and κ B-dependent transcription in epithelial cells. *Eur. J. Biochem.*, **254**, 81–89.
- ORTIZ, A., GÓMEZ-CHIARRI, M., ALONSO, J., BUSTOS, C., GÓMEZ-GUERRERO, C., LÓPEZ-ARMADA, M.J., GÓMEZ-GARRE, D., PALACIOS, I., RUÍZ-ORTEGA, M., GUTIERREZ, S., GONZALEZ, E. & EGIDO, J. (1994). The potential role of inflammatory and fibrogenic cytokines in the glomerular diseases. *J. Lipid Mediat. Cell Signal.*, **9**, 55–74.
- OSBORNE, B.A., SMITH, S.W., MCLAUGHLIN, K.A., GRIMM, L., KALLINCH, T., LIU, Z. & SCHWARTZ, L.M. (1996). Genes that regulate apoptosis in the mouse thymus. *J. Cell. Biochem.*, **60**, 18–22.
- PAGLIACCI, M.C., MIGLIORATI, G., SMACCHIA, M., GRIGNANI, F., RICCARDI, C. & NICOLETTI, I. (1993). Cellular stress and glucocorticoid hormones protect L929 mouse fibroblasts from tumor necrosis factor alpha cytotoxicity. *J. Endocrinol. Invest.*, **16**, 591–599.
- PFEILSCHIFTER, J. (1994). Mesangial cells orchestrate inflammation in the renal glomerulus. *News Physiol. Sci.*, **9**, 271–276.
- POLUNOVSKY, V.A., WENDT, C.H., INGBAR, D.H., PETERSON, M.S. & BITTERMAN, P.B. (1994). Induction of endothelial apoptosis by TNF- α : modulation by inhibitors of protein synthesis. *Exp. Cell Res.*, **214**, 584–594.
- SHIMIZU, A., KITAMURA, H., MASUDA, Y., ISHIZAKI, M., SUGISAKI, Y. & YAMANAKA, N. (1996). Glomerular capillary regeneration and endothelial cell apoptosis in both reversible and progressive models of glomerulonephritis. *Contrib. Nephrol.*, **118**, 29–40.
- SHIMIZU, A., KITAMURA, H., MASUDA, Y., ISHIZAKI, M., SUGISAKI, Y. & YAMANAKA, N. (1997). Rare capillary regeneration and subsequent capillary regression with endothelial cell apoptosis in progressive glomerulonephritis. *Am. J. Pathol.*, **151**, 1231–1239.
- SHIMIZU, A., MASUDA, Y., KITAMURA, H., ISHIZAKI, M., SUGISAKI, Y. & YAMANAKA, N. (1998). Recovery of damaged glomerular capillary network with endothelial cell apoptosis in experimental proliferative glomerulonephritis. *Nephron*, **79**, 206–214.
- SPYRIDOPOULOS, I., SULLIVAN, A.B., KEARNEY, M., ISNER, J.M. & LOSORDO, D.W. (1997). Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis. Estrogen as a survival factor. *Circulation*, **95**, 1505–1514.
- STEHLIK, C., DE MARTIN, R., KUMABASHIRI, I., SCHMID, J.A., BINDER, B.R. & LIPP, J. (1998). Nuclear factor (NF)- κ B-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor alpha-induced apoptosis. *J. Exp. Med.*, **188**, 211–216.
- VAN ANTWERP, D.J., MARTIN, S.J., VERMA, I.M. & GREEN, D.R. (1998). Inhibition of TNF-induced apoptosis by NF- κ B. *Trends Cell Biol.*, **8**, 107–111.
- WANG, W., WYKRZYKOWSKA, J., JOHNSON, T., SEN, R. & SEN, J. (1999). A NF- κ B/c-myc-dependent survival pathway is targeted by corticosteroids in immature thymocytes. *J. Immunol.*, **162**, 314–322.
- WARGNIER, A., LAFAURIE, C., LEGROS-MAIDA, S., BOURGE, J.-F., SIGAUX, F., SASPORTES, M. & PAUL, P. (1998). Down-regulation of human granzyme B expression by glucocorticoids. *J. Biol. Chem.*, **273**, 35326–35331.
- WEN, L.P., MADANI, K., FAHRNI, J.A., DUNCAN, S.R. & ROSEN, G.D. (1997). Dexamethasone inhibits lung epithelial cell apoptosis induced by IFN- γ and Fas. *Am. J. Physiol.*, **273**, 921–929.
- WIEGERS, G.J. & REUL, J.M.H.M. (1998). Induction of cytokine receptors by glucocorticoids: functional and pathological significance. *Trends Pharmacol. Sci.*, **19**, 317–321.
- WISSINK, S., VAN HEERDE, E.C., VAND DER BURG, B. & VAN DER SAAG, P.T. (1998). A dual mechanism mediates repression of NF- κ B activity by glucocorticoids. *Mol. Endocrinol.*, **12**, 355–363.
- XIE, H., SEWARD, R.J. & HUBER, B.T. (1997). Cytokine rescue from glucocorticoid induced apoptosis in T cells is mediated through inhibition of I κ B α . *Mol. Immunol.*, **34**, 987–994.
- YOSHIOKA, T., KAWAMURA, T., MEYRICK, B.O., BECKMAN, J.K., HOOVER, R.L., YOSHIDA, H. & ICHIKAWA, I. (1994). Induction of manganese superoxide dismutase by glucocorticoids in glomerular cells. *Kidney Int.*, **45**, 211–219.

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