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Biochimica et Biophysica Acta 1362 (1997) 252–262

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Suppression of NF-κB and AP-1 activation by glucocorticoids in experimental glomerulonephritis in rats: molecular mechanisms of anti-nephritic action

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Received 14 July 1997; accepted 29 July 1997

Abstract

Transcription factors nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) play an important role in the induction of pro-inflammatory factors such as cytokines and cell adhesion molecules, which could be involved in the pathogenesis of glomerulonephritis. We have recently reported the pathogenic significance of NF-κB activation in experimental glomerulonephritis in rats. In this study, we investigated the pathogenic relevance of AP-1 activation in nephrotoxic serum (NTS)-induced glomerulonephritis. Increased AP-1 DNA-binding activity was detected in nephritic glomeruli by a gel shift assay. The kinetics of AP-1 activation was similar to that of NF-κB. Activation of both NF-κB and AP-1 preceded proteinuria, an important pathophysiological parameter for glomerulonephritis. Treatment with prednisolone, a glucocorticoid hormone, prevented activation of both NF-κB and AP-1 in glomeruli and subsequent mRNA expression of NF-κB- and AP-1-regulated genes. Prednisolone was also effective therapeutically and reduced DNA-binding activities of NF-κB and AP-1 which are already activated in nephritic glomeruli. These results suggest that activated NF-κB and AP-1 may play an important pathogenic role in glomerulonephritis and the anti-nephritic action of glucocorticoids may be mediated through the suppression of these transcription factors. © 1997 Elsevier Science B.V.

Keywords: Transcription factor; NF-κB; AP-1; Glucocorticoid; Glomerulonephritis; Pathogenesis

1. Introduction

Pathophysiological studies of glomerulonephritis have demonstrated the pathogenic significance of pro-inflammatory factors such as cytokines [1–5], cell adhesion molecules [6–8], growth factors [9–12], extracellular matrix components [13] and matrix metalloproteinases [14]. These pro-inflammatory factors induce proliferation of renal mesangial cells and matrix accumulation in nephritic glomeruli [15,16] which, as a consequence, can lead to renal insufficiency.

Studies examining the gene regulation of these pro-inflammatory factors in nephritic glomeruli are important for the understanding of the pathogenic mechanisms of glomerulonephritis. Promoter analyses, using cultured cells, have shown that the expression of most of these pro-inflammatory factors is transcriptionally regulated. Since several transcription

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PII S0925-4439(97)00068-9

factors, especially NF-κB and AP-1, could play pivotal roles in their gene expression [17–30], attention has been focused on the regulation and function of transcription factors in inflammatory lesions. Recently, we reported the in vivo activation of NF-κB in nephritic glomeruli of nephrotoxic serum (NTS)-injected rats [31]. We demonstrated that pyrrolidine dithiocarbamate, a potent inhibitor of NF-κB, suppressed both NF-κB activation and development of glomerulonephritis [31]. However, there is not enough information on the pathogenic role of transcription factors in glomerulonephritis.

Glucocorticoids are widely used for the treatment of human chronic inflammatory diseases including glomerulonephritis. Glucocorticoids are also effective in experimental animal models of inflammatory diseases. Possible mechanisms of their anti-inflammatory effects include the inhibition of gene expression of several pro-inflammatory factors such as cytokines and cell adhesion molecules [32,33]. In vitro studies have suggested that the repression of NF-κB- and AP-1-dependent transcription is a major component of the glucocorticoid-mediated inhibition of gene expression [34–38]. However, the precise molecular mechanisms of the therapeutic effects of glucocorticoids on human and experimental inflammatory diseases are not yet fully understood.

In this study, we investigated the pathophysiological significance of AP-1 activation and the effects of glucocorticoid on the activation of NF-κB and AP-1, using a rat NTS-nephritis model. We found that AP-1 was activated in nephritic glomeruli with similar kinetics to NF-κB activation and that glucocorticoid suppressed the activation of NF-κB and AP-1 in parallel with the suppression of glomerulonephritis.

2. Materials and methods

2.1. Induction of glomerulonephritis

Rabbit anti-glomerular basement membrane (GBM) antisera (nephrotoxic sera; NTS) were prepared by the procedure described previously [31]. In brief, rabbits were immunized subcutaneously with GBM of WKY rats (Charles River Japan). After three boosters, sera were prepared and heat-decomplemented. The specificity of NTS was confirmed by immunofluorescence on the frozen sections of kidney using fluorescein isothiocyanate (FITC) conjugated anti-rabbit IgG (Jackson). Immunofluorescence was strongly detected along the GBM in rats injected with NTS. Glomerulonephritis was induced in male WKY rats (180–280 g body weight) by intravenous injection through a tail vein of 0.5 ml of NTS (diluted eighty-fold with PBS) per 200 g body weight of rats.

2.2. Preparation of glomerular nuclear extracts

At the indicated time after NTS injection, kidneys were perfused with saline under ether anesthesia to eliminate circulating blood cells. Renal cortex from one animal was minced in small pieces and glomeruli were isolated by a fractional sieving technique. In brief, the cortex was first sieved using a mesh with a pore size 355 μm. The sieved cortex was collected with 20 ml PBS and passed through a second mesh with a pore size 125 μm. The fraction from this mesh was further sieved using a 75 μm mesh. Glomeruli trapped on the final mesh were collected with PBS and centrifuged at $2000 \times g$ for 10 min at 4°C.

The procedure used for the preparation of nuclear extracts from isolated glomeruli was described previously [31] with slight modifications. In brief, glomeruli were suspended in 500 μl of buffer A [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin] and lysed by homogenization in a Dounce homogenizer of B-type pestle with 50 strokes. The homogenates were chilled on ice for 15 min. 30 μl of 10% Nonidet P-40 was added and samples were vortexed vigorously for 10 s. The nuclear fraction was precipitated by centrifugation at $15\,000 \times g$ for 5 min at 4°C and suspended in 50–100 μl of buffer B [20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin]. The mixture was left on ice for 15 min with frequent agitation. Nuclear extracts were prepared by centrifugation at $15\,000 \times g$ for 5 min at 4°C and stored at −80°C. Protein concentration was determined by the DC protein assay reagent (Bio-Rad).

2.3. Gel shift assay

Gel shift assay was performed as described previously [31]. Sequences of oligonucleotides for DNA probes were as follows: AP-1, 5′-GATTCGTGACTCAGCACAGG-3′; Octamer binding protein (Oct), 5′-GATTCGATTTGCATGCACAG-3′; NF-κB, 5′-GTTCGACAGAGGGGACTTTCCGAGAGGCAAC-3′. Where indicated, the DNA binding reaction was performed in the presence of non-radiolabeled oligonucleotide competitors.

2.4. Pharmacological study of glucocorticoid

A synthetic glucocorticoid, prednisolone (Nacalai tesque), suspended in H_2O was administered orally, once a day, 30 min before NTS injection at a dose of 2 mg/kg. Rats were administered 1 ml of the suspended prednisolone (0.4 mg/ml) per 200 g body weight. Control animals received the same volume of H_2O. Urine samples were collected for 24 h on days 3 and 8 by keeping each animal in an individual metabolic cage. Urinary protein concentration was determined by protein assay reagent using bovine serum albumin as a standard (Bio-Rad). When therapeutic effects were investigated, daily treatment with prednisolone was started on the third day and urinary protein excretion was determined on days 0, 3, 5 and 7.

2.5. Effects of glucocorticoid on NF-κB and AP-1 activation

Glomerular nuclear extracts were prepared from isolated glomeruli of one rat and gel shift assays for NF-κB and AP-1 were carried out according to the procedure described above. When therapeutic effects were investigated, treatment with prednisolone (2 mg/kg) was started on day 3 and nuclear extracts were prepared 2 days after prednisolone treatment.

2.6. Northern blot analysis

Total RNA was isolated from glomeruli of two rats by using Isogen (Nippon gene). 15 μg of total RNA was electrophoresed in a 1% agarose–formaldehyde gel and transferred onto a Hybond–N + nylon filter (Amersham). The filter was hybridized with cDNA probes. cDNA fragments for murine interleukin-1β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), rat intercellular adhesion molecule-1 (ICAM-1), murine transforming growth factor-β1 (TGF-β1) and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham) by DNA labeling kit with random primers (TaKaRa).

2.7. Immunohistochemistry

Kidney was immediately frozen in powdered dry ice. Cryostat sections (7 μm) were mounted onto APS coated slides (Matsunami) and stored at −80°C. The sections were thawed and the tissues were fixed in buffered 2% paraformaldehyde, 0.2% glutaraldehyde and 0.02% NP-40 for 5 min. To inactivate the endogenous peroxidase, the sections were treated with 0.3% hydrogen peroxide in methanol for 30 min. Nonspecific binding was blocked with normal horse serum (Vector). The sections were incubated with biotinylated anti-rabbit IgG (Vector) at room temperature for 2 h. After washes with PBS for three times, the sections were incubated with streptavidin biotin peroxidase complex (Vector), washed again, and incubated in 0.02% 3,3′-diaminobenzidine (Wako) with 0.006% hydrogen peroxide. The sections were counter stained with hematoxylin.

2.8. Enzyme-linked immunosorbent assay (ELISA)

Anti-rabbit IgG level in the rat serum was determined by ELISA. 100 μl of 10 μg/ml normal rabbit IgG (ICN) was coated on a 96-well plate at 4°C overnight. After blocking, 100 μl of test sera, diluted twenty-fold with PBS, was added to each well and incubated at room temperature for 2 h. The wells were washed and incubated with horseradish peroxidase-conjugated goat anti-rat IgG (Jackson). The peroxidase activity was measured using 2,2′-azinobis(3-ethyl-benzothazoline-6-sulfonic acid) (Wako) as a substrate and V_{max} value of the reaction was calculated.

3. Results

3.1. Kinetics of proteinuria

Proteinuria was measured as a parameter for the development of glomerulonephritis. Urinary protein

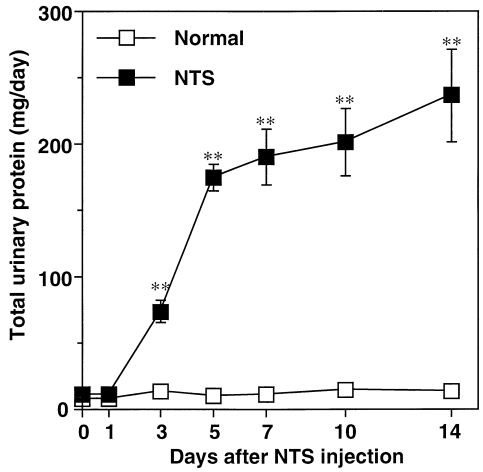


Fig. 1. Proteinuria in NTS-nephritis in WKY rats. Urine samples were collected for 24 h on day 0, 1, 3, 5, 7, 10 and 14 from normal (□) and NTS-treated (■) rats. Data are mean ± S.E. of five rats. ** $P < 0.01$ compared to the normal control (Student's *t*-test).

excretion for 24 h from day 1 was at a normal level (Fig. 1). All animals showed significant proteinuria on day 3 and thereafter (Fig. 1). Proteinuria and other parameters such as blood urea nitrogen and serum cholesterol level gradually increased at least to 6 weeks (data not shown). Finally, rats showed chronic renal failure with glomeruloscrelosis [39]. Herein, pathophysiological analysis was further conducted on an earlier stage (from the onset to day 14), where inflammatory reaction is actively on-going.

3.2. AP-1 DNA-binding activity in nephritic glomeruli

In the previous study, we monitored the DNA-binding activity of transcription factors in isolated glomeruli by gel shift assays [31]. Using this system, we determined AP-1 DNA-binding activity in the nuclear extracts of glomeruli isolated from NTS-treated rats (Fig. 2(A)). AP-1 DNA-binding activity

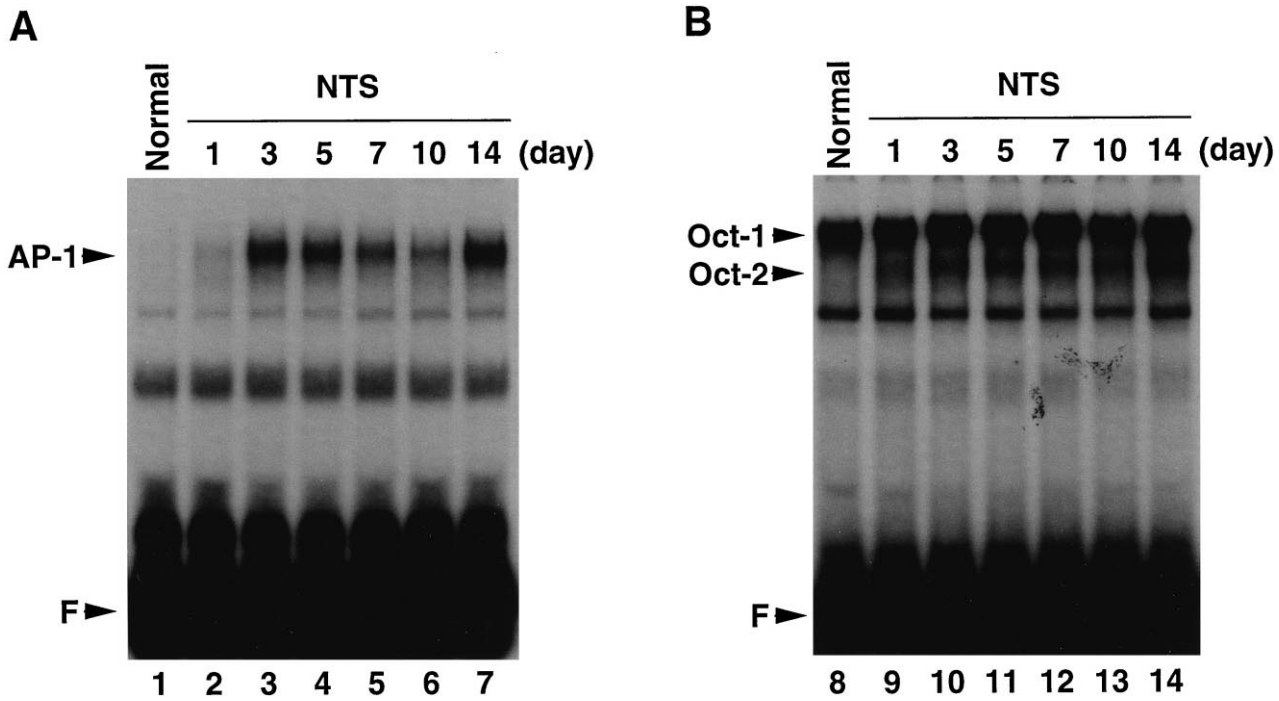


Fig. 2. Kinetic analysis of AP-1 and Oct DNA-binding activities in nephritic glomeruli treated with NTS. AP-1 (A) and Oct (B) DNA-binding activities were determined by the gel shift assays with nuclear extracts (5 μg of protein) prepared from isolated normal glomeruli (lanes 1 and 8) and nephritic glomeruli on days 1 (lanes 2 and 9), 3 (lanes 3 and 10), 5 (lanes 4 and 11), 7 (lanes 5 and 12), 10 (lanes 6 and 13) and 14 (lanes 7 and 14). One specific band for AP-1 and two specific bands for Oct are indicated as AP-1, Oct-1 and Oct-2, respectively. The lower arrow head, F, indicates free probes. One animal was used on each day and similar results were obtained in another experiment.

in normal glomeruli was at a low level (Fig. 2(A), lane 1). In contrast, increased AP-1 DNA-binding activity was detected in the glomeruli of NTS-treated rats (Fig. 2(A), lanes 2–7). Kinetic analysis indicated that significant AP-1 activation was detected on day 1 after NTS injection (Fig. 2(A), lane 2) and the activation was sustained, at least, to day 14 (Fig. 2(A), lane 7). On days 3, 5, and 14, activation was at the highest level (Fig. 2(A), lanes 3, 4 and 7). The kinetics of AP-1 activation was similar to that of NF-κB activation [31]. Activation of both NF-κB and AP-1 preceded proteinuria (Fig. 1). These results indicate that both NF-κB and AP-1 are activated in nephritic glomeruli during the development of glomerulonephritis.

To evaluate the specificity of NF-κB and AP-1 activation, we also monitored DNA-binding activities of ubiquitously expressed Octamer binding proteins (Oct) using the same nuclear extracts as the AP-1 assay (Fig. 2(B)). Two specific bands were detected by gel shift assay. Intensity of the slower migrating band, an Oct-1 like protein, was not changed during the experiment (Fig. 2(B), lanes 8–14). In contrast, the faster migrating band, an Oct-2 like protein, was only detected in nephritic glomeruli (Fig. 2(B), lanes 9–14). These results indicate that some transcription factors are selectively activated in nephritic glomeruli.

A competition gel shift assay was performed to determine the specific AP-1 DNA-binding activity (Fig. 3). The AP-1 DNA-binding activity was competed out by a non-radiolabeled oligonucleotide containing an AP-1 binding site (Fig. 3, lane 2). However, AP-1 binding activity was not affected by an Oct binding site (Fig. 3, lane 3), indicating specific DNA-binding of the AP-1 protein.

3.3. Effect of glucocorticoid on glomerulonephritis

We determined the effect of prednisolone, a synthetic glucocorticoid used for the treatment of glomerulonephritis, on NTS-nephritis. Urinary protein excretion on days 3 and 8 was suppressed by prednisolone at 2 mg/kg (Fig. 4). Other serum and urine parameters such as creatinine clearance and serum cholesterol level were also within the normal range on day 10 (data not shown). These results confirmed that glucocorticoids are effective in NTS-nephritis in WKY rats.

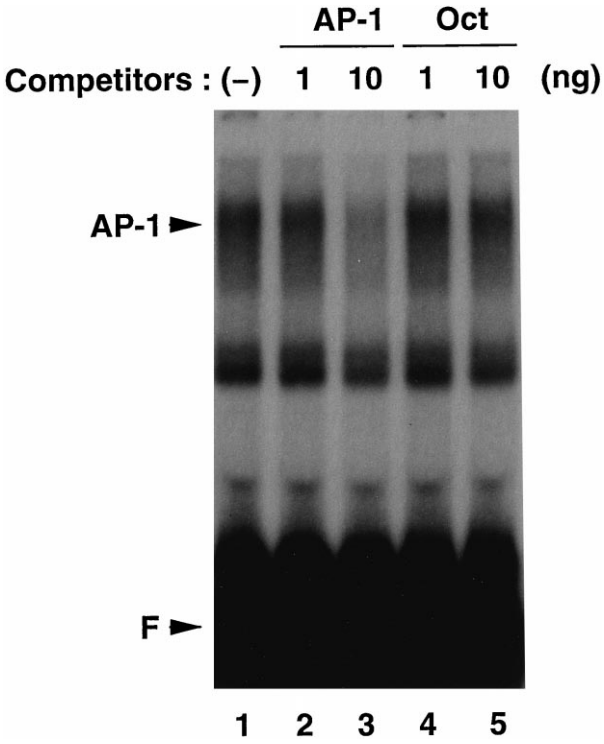


Fig. 3. A competition gel shift assay for AP-1 DNA-binding activity. A gel shift assay with nuclear extracts on day 3 was carried out in the absence (lane 1) or presence of AP-1 (lanes 2 and 3) and Oct (lanes 4 and 5) oligonucleotide competitors; 1 ng (lanes 2 and 4) and 10 ng (lanes 3 and 5) of oligonucleotides were added, respectively. Similar results were obtained in two independent experiments.

3.4. Effects of glucocorticoid on NF-κB and AP-1 activation

Several lines of evidence indicate that glucocorticoids repress NF-κB- and AP-1-dependent transcription in cultured cells. However, the molecular mechanisms of glucocorticoid-mediated anti-inflammatory action in vivo is not well understood. To see whether glucocorticoid can inhibit the transcription factors in vivo, we studied the effects of glucocorticoid on the glomerular NF-κB and AP-1 activation. Gel shift assays were performed using nuclear extracts prepared from glomeruli after treatment with prednisolone on day 3. As shown in Fig. 5, the NTS-induced increases of both NF-κB and AP-1 DNA-binding activities were suppressed by prednisolone treatment. These results suggest that glucocorticoids can suppress NF-κB and AP-1 activation in vivo as well as in vitro.

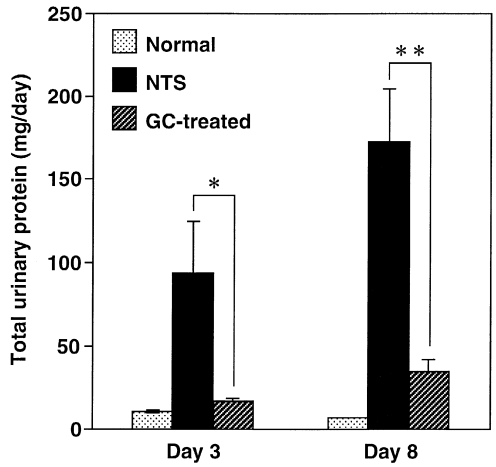


Fig. 4. Preventive effect of glucocorticoid on proteinuria. Prednisolone (2 mg/kg) was administered orally once a day for 30 min before NTS injection on day 0. On days 3 and 8, urine samples were collected for 24 h and total protein excretion was determined. Each bar shows normal (▨), NTS-treated (■), [NTS + prednisolone]-treated (▨) rats, respectively. Data are mean ± S.E. of 5 rats. * $P < 0.05$, ** $P < 0.01$ compared with NTS-treated control (Bonferroni's method).

To determine whether the glucocorticoid-mediated suppression of NF-κB and AP-1 results in the inhibition of NF-κB and AP-1 dependent gene expression, Northern blot analysis was performed by using total RNAs prepared from glomeruli on day 3 after treatment with prednisolone. Fig. 6 shows that mRNA expression of IL-1β, MCP-1, ICAM-1 and TGF-β1, which suggested to be regulated by NF-κB and/or AP-1 in cultured cells, was inhibited by prednisolone treatment. In contrast, GAPDH mRNA expression was not affected by prednisolone treatment. These results suggest that NF-κB and AP-1 might regulate the induction of pro-inflammatory genes in nephritic glomeruli in vivo.

3.5. NTS binding and anti-rabbit IgG production

In NTS-nephritis, glomerular injury is triggered by the binding of rabbit anti-GBM antibody (NTS) in glomerulus and it might be accelerated by the attack

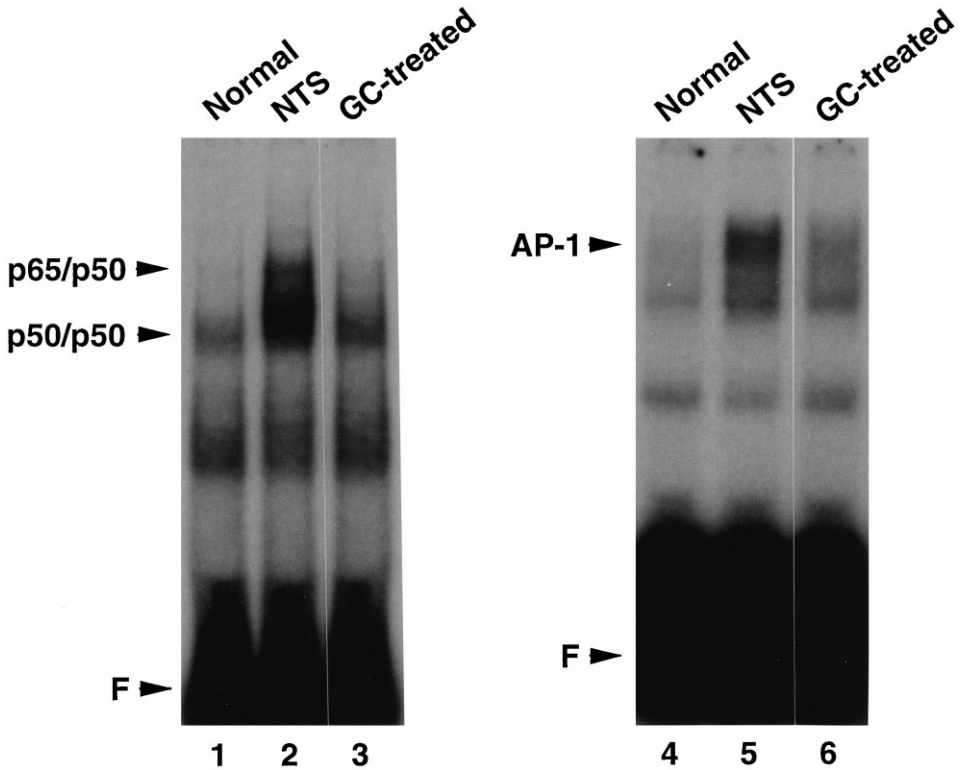


Fig. 5. Preventive effects of glucocorticoid on NF-κB and AP-1 activation. Prednisolone (2 mg/kg) was administered before NTS injection. Gel shift assays for NF-κB (lanes 1–3) and AP-1 (lanes 4–6) were carried out using nuclear extracts prepared from glomeruli of normal (lanes 1 and 4), NTS-treated (lanes 2 and 5) and [NTS + prednisolone]-treated (lanes 3 and 6) rats on day 3. Representative data of four rats is shown.

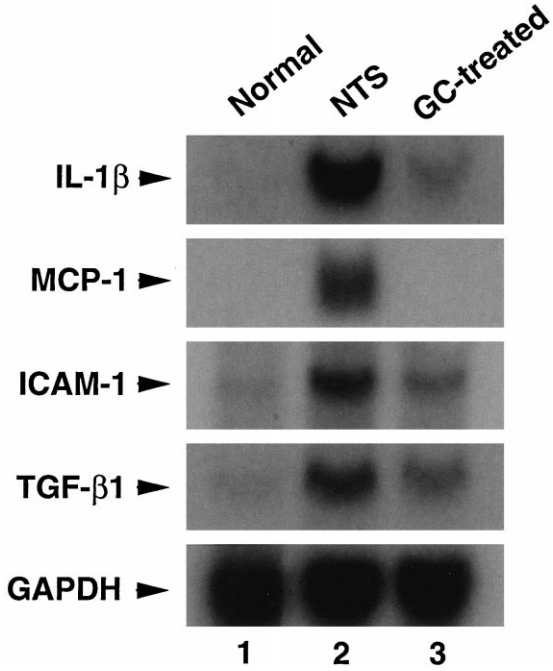


Fig. 6. Glucocorticoid-mediated inhibition of the target gene expression for NF-κB and AP-1. Isolated glomeruli from two animals were collected for the preparation of RNA in each group. Total RNAs were prepared from glomeruli of normal (lanes 1), NTS-treated (lanes 2) and [NTS + prednisolone]-treated (lanes 3) rats on day 3. Northern blot analysis was performed as described under Section 2.

of autologous rat anti-rabbit IgG to the rabbit antibody on GBM. To demonstrate that these upstream events are not affected by glucocorticoid treatment, we investigated the anti-GBM antibody binding to glomeruli immunohistochemically and measured serum anti-rabbit IgG level. The anti-GBM antibody binding to glomeruli on day 1 (Fig. 7(A)) and the serum anti-rabbit IgG production on day 10 (Fig. 7(B)) were not significantly inhibited by glucocorticoid treatment.

3.6. Therapeutic effect of glucocorticoid

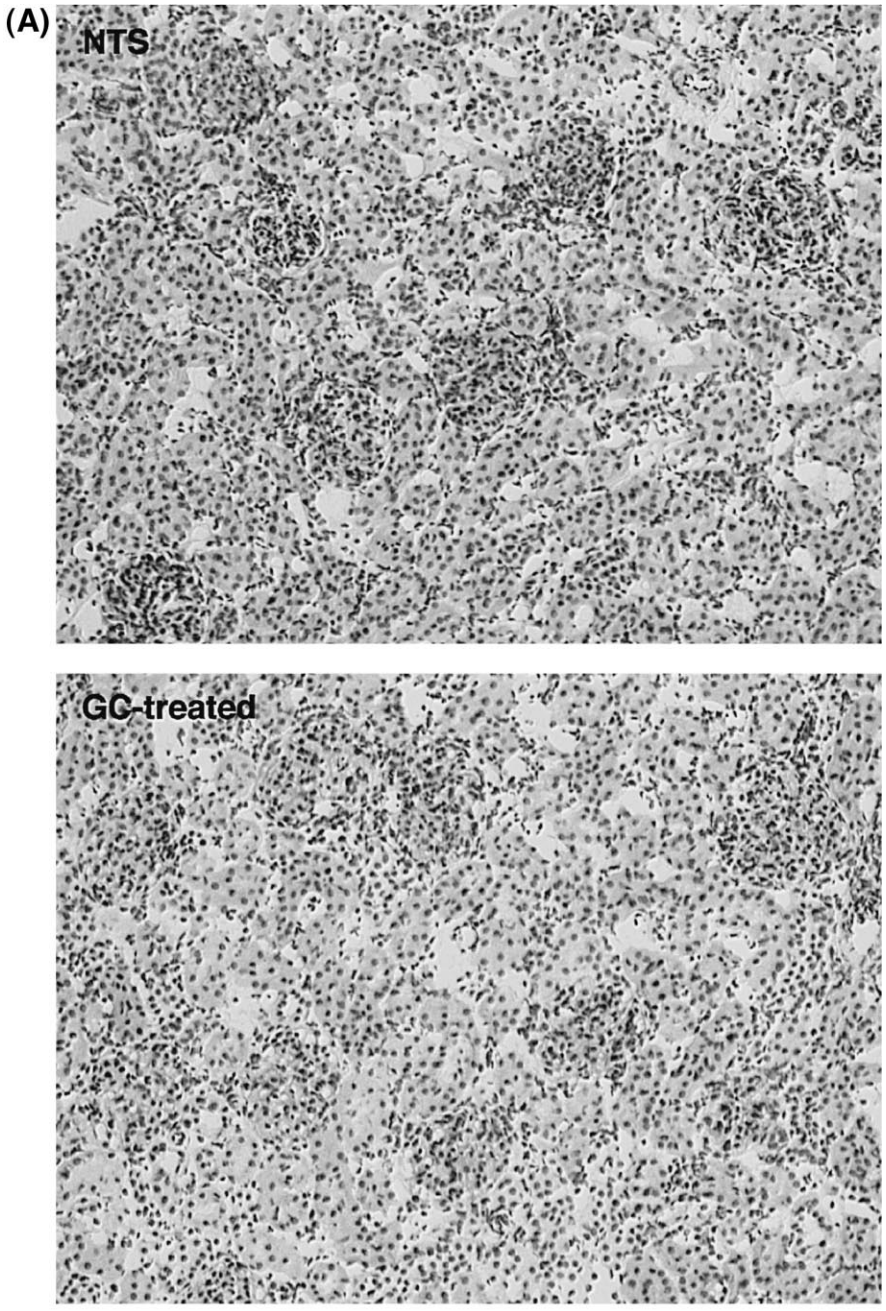
In addition to the preventive effects, we examined the therapeutic effect of glucocorticoid on glomerulonephritis. Prednisolone treatment was started on day 3, where moderate proteinuria (Fig. 1) and full NF-κB [31] and AP-1 activation (Fig. 2) were observed. Both the nephritic group and the glucocorticoid-treated group showed the same level of proteinuria before the glucocorticoid treatment was started on day 3 (Fig. 8(A)). In contrast, progression of proteinuria on days 5 and 7 was suppressed by glucocorticoid treatment as compared to the non-treated nephritic group. In addition, all animals in the nephritic group showed hematuria on day 7, which was not observed in the glucocorticoid-treated group (data not shown). These results indicate that glucocorticoids have a therapeutic effect on NTS-nephritis as well as a preventive effect.

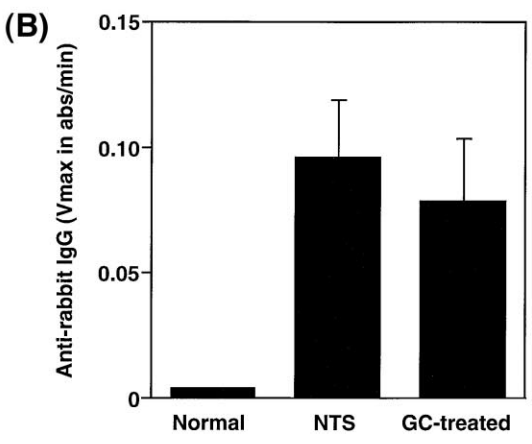
Finally, we investigated the effects of glucocorticoid on the already activated NF-κB and AP-1 in nephritic glomeruli. Significant increases of NF-κB and AP-1 DNA-binding activities in glomeruli of nephritic animals were detected on day 5 (Fig. 8(B), lanes 3 and 4). In contrast, the NTS-induced increases of both NF-κB and AP-1 DNA-binding activities were completely suppressed by therapeutic administration of glucocorticoid from day 3 (Fig. 8(B), lanes 5 and 6). Suppression of NF-κB, but not AP-1, was observed on day 4 (data not shown). These results further support the idea that glucocorticoid-mediated suppression of NF-κB and AP-1 activation may be the major components of the anti-nephritic action of glucocorticoids.

4. Discussion

In this study, we used an experimental NTS-nephritis model in WKY rats. This strain develops proliferative and crescentic glomerulonephritis, in response to a small amount of NTS compared to other strains [40]. The susceptibility to NTS might be due to the increased activity of the natural killer (NK) cells [40]. Kawasaki et al. reported that CD8-positive

Fig. 7. Effects of glucocorticoids on NTS binding to glomeruli and serum anti-rabbit IgG production. (A) Anti-GBM antibody binding to glomeruli was determined by immunohistochemistry on frozen sections prepared from NTS-treated and [NTS + prednisolone]-treated rats on day 1. Data shows a representative of three animals in each group. (B) Serum titers of anti-rabbit IgG were determined by ELISA. Sera were prepared from normal, NTS-treated and [NTS + prednisolone]-treated rats on day 10. Data are mean ± S.E. of five rats.


(A)
NTS
GC-treated


(B)
Anti-rabbit IgG (Vmax in abs/min)
0.15
0.10
0.05
0
Normal
NTS
GC-treated

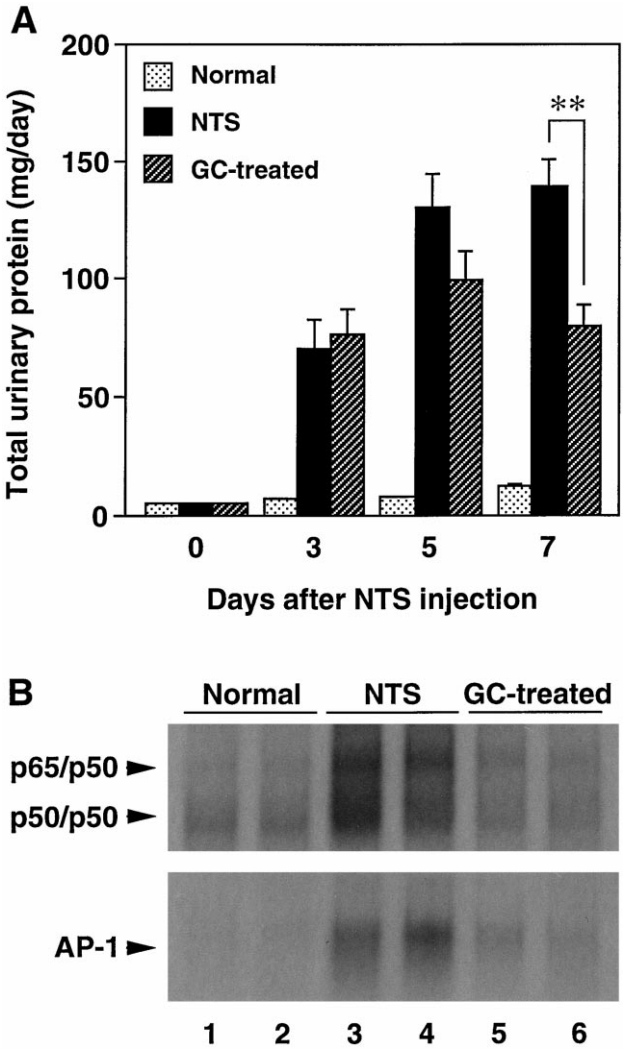


Fig. 8. Therapeutic effects of glucocorticoid on developed glomerulonephritis and activated NF-κB and AP-1. Daily prednisolone treatment (2 mg/kg) was started on the third day after NTS injection. (A) Urinary protein excretion was determined on days 0, 3, 5 and 7. Each bar shows normal (▨), NTS-treated (■), [NTS + prednisolone]-treated (▨) groups, respectively. Data are mean ± S.E. of five rats. $^{**}P < 0.01$ compared with NTS-treated control on day 7 (Bonferroni's method). (B) the DNA-binding activities of NF-κB and AP-1 were determined 2 days after prednisolone treatment (day 5). The experiment was carried out by using two independent rats. The data are obtained from normal (lanes 1 and 2), NTS-treated (lanes 3 and 4) and [NTS + prednisolone]-treated (lanes 5 and 6) rats. Similar results were obtained in another experiment.

cells, probably NK cells, infiltrate into glomeruli [41]. Depletion of the CD8-positive cells prevented proteinuria and reduced the severity of histopathological changes such as a crescent formation in this nephritis model. The infiltration of CD8-positive cells can be detected at least after 4 h until day 11 after NTS injection and the peak infiltration is observed on day 3. In addition, macrophage infiltration gradually increases from 4 h to day 11. NF-κB and AP-1 activation was also detected at this stage, suggesting a contribution of these cells as a source of NF-κB and AP-1 activities in nephritic glomeruli. An Oct-2 like protein detected in nephritic glomeruli (Fig. 2(B)) might be derived from the infiltrated cells because Oct-2 is known to be expressed preferentially in lymphoid cells [42,43]. Future histochemical analysis will provide the information on NF-κB and AP-1 expressing cells and pathophysiological significance of NF-κB and AP-1 activation in glomerulonephritis.

In our previous report, we detected a single NF-κB-derived complex using a gel shift assay [31]. The complex included the p50 NF-κB subunit which co-migrated with human recombinant p50 homodimer. However, one additional complex was detected in this study. The detection of this complex depended on the use of protease inhibitors, aprotinin and leupeptin, in buffers for the extraction of glomerular nuclei. The additional complex was probably derived from p65/p50 heterodimer, because it migrated slowly with the p50 homodimer (Figs. 5 and 8(B)) and reacted with both anti-p65 and anti-p50 antibodies (data not shown). These results indicate that both p65/p50 heterodimer and p50/p50 homodimer are activated during the development of glomerulonephritis.

Several target genes for NF-κB and AP-1 have been shown to be expressed in glomeruli of NTS-treated WKY rats. For example, enhanced ICAM-1 expression is detected on day 1 and thereafter and the peak expression is observed on day 3 [8]. Neutralization of ICAM-1 by a monoclonal antibody can decrease glomerular injury, showed the pathophysiological significance of ICAM-1 [8]. In addition, mRNA expression of other target genes such as IL-1β, MCP-1 and TGF-β1 were induced in nephritic glomeruli. The kinetics of the expression of target genes for NF-κB and AP-1 were similar to that of NF-κB and AP-1 activation. We also showed that glucocorticoid inhibited IL-1β, MCP-1, ICAM-1 and TGF-β1 mRNA expression (Fig. 6). These results suggest that the activated NF-κB and AP-1 might regulate the expression of the pro-inflammatory genes

in nephritic glomeruli. In contrast to gene expression, NF-κB and AP-1 activation is shown to precede the histopathological changes such as mesangial proliferation and crescent formation, which can be observed on day 6 and thereafter [41]. This evidence suggests that activation of NF-κB and AP-1 results in histopathological changes through the transcriptional induction of genes encoding pathogenic pro-inflammatory factors.

We demonstrated that glucocorticoids was able to suppress NF-κB and AP-1 activation in nephritic glomeruli in vivo (Figs. 5 and 8(B)). It is noteworthy that the suppressive effects were also observed against NF-κB and AP-1, which were already in the active state (Fig. 8(B)). However, mechanisms of the suppression of NF-κB and AP-1 activation are not clear. Immunohistochemistry showed that glucocorticoids did not interfere with rabbit anti-GBM antibody (NTS) binding to glomeruli (Fig. 7(A)). Autologous anti-rabbit IgG production is important for the progression of glomerular injury in NTS-nephritis. Unexpectedly, the autologous antibody production on day 10 was not affected by glucocorticoids at the effective dose to suppress proteinuria (Fig. 7(B)). In addition, suppression of NF-κB and AP-1 by glucocorticoids was detected on day 3 and 5 (Figs. 5 and 8), where the serum anti-rabbit IgG was not increased (data not shown). These results suggest that glucocorticoids might suppress glomerulonephritis by inhibiting activation of NF-κB and AP-1 selectively, but not through the systemic immunosuppression.

In summary, we have demonstrated that NF-κB and AP-1 are activated in nephritic glomeruli and that NF-κB and AP-1 may have a regulatory role in the expression of pro-inflammatory factors in nephritic glomeruli. In vivo suppression of NF-κB and AP-1 activation by glucocorticoids in nephritic glomeruli may be one mechanism by which glucocorticoids suppress glomerulonephritis. These findings also provide a novel molecular approach for treatment of glomerulonephritis by selectively controlling NF-κB and AP-1 activities.

Acknowledgements

We thank Dr. Angela Southey for critical reading of the manuscript.

References

[1] H.T. Cook, C. Avey, G.M. Taylor, Int. J. Exp. Pathol. 75 (1994) 157–163.
[2] J. Egido, M. Gomez Chiarri, A. Ortiz, C. Bustos, J. Alonso, C. Gomez Guerrero, D. Gomez Garre, M.J. Lopez Armada, J. Plaza, E. Gonzalez, Kidney Int. 39 (1993) S59–S64.
[3] Y. Horii, M. Iwano, E. Hirata, M. Shiiki, Y. Fujii, K. Dohi, H. Ishikawa, Kidney Int. 39 (1993) S71–S75.
[4] B.H. Rovin, M. Rumancik, L. Tan, J. Dickerson, Lab. Invest. 71 (1994) 536–542.
[5] T. Wada, N. Tomosugi, T. Naito, H. Yokoyama, K. Kobayashi, A. Harada, N. Mukaida, K. Matsushima, J. Exp. Med. 180 (1994) 1135–1140.
[6] J.A. Bruijn, N.J. Dinklo, Lab. Invest. 69 (1993) 329–335.
[7] P.A. Hill, H.Y. Lan, D.J. Nikolic Paterson, R.C. Atkins, Kidney Int. 45 (1994) 700–708.
[8] K. Kawasaki, E. Yaoita, T. Yamamoto, T. Tamatani, M. Miyasaka, I. Kihara, J. Immunol. 150 (1993) 1074–1083.
[9] H.E. Abboud, Kidney Int. 43 (1993) 252–267.
[10] L. Gesualdo, E. Ranieri, G. Pannarale, S. Di Paolo, F.P. Schena, Kidney Int. 39 (1993) S86–S89.
[11] R.J. Johnson, E.W. Raines, J. Floege, A. Yoshimura, P. Pritzl, C. Alpers, R. Ross, J. Exp. Med. 175 (1992) 1413–1416.
[12] K. Yoshioka, T. Takemura, K. Murakami, M. Okada, S. Hino, H. Miyamoto, S. Maki, Lab. Invest. 68 (1993) 154–163.
[13] N.D. Rosenblum, Kidney Int. 45 (1994) S73–S77.
[14] D.H. Lovett, R.J. Johnson, H.P. Marti, J. Martin, M. Davies, W.G. Couser, Am. J. Pathol. 141 (1992) 85–98.
[15] E. Eng, J. Floege, B.A. Young, W.G. Couser, R.J. Johnson, Kidney Int. 45 (1994) S45–S47.
[16] J. Floege, E. Eng, B.A. Young, R.J. Johnson, Kidney Int. 39 (1993) S47–S54.
[17] M. Ahmad, N. Marui, R.W. Alexander, R.M. Medford, J. Biol. Chem. 270 (1995) 8976–8983.
[18] C.K. Asiedu, L. Scotto, R.K. Assoian, M. Ehrlich, Biochim. Biophys. Acta 1219 (1994) 55–63.
[19] K.Y. Chung, A. Agarwal, J. Uitto, A. Mauviel, J. Biol. Chem. 271 (1996) 3272–3278.
[20] J.P. Cogswell, M.M. Godlevski, G.B. Wisely, W.C. Clay, L.M. Leesnitzer, J.P. Ways, J.G. Gray, J. Immunol. 153 (1994) 712–723.
[21] R. Gum, E. Lengyel, J. Juarez, J.H. Chen, H. Sato, M. Seiki, D. Boyd, J. Biol. Chem. 271 (1996) 10672–10680.
[22] L.M. Khachigian, J.W. Fries, M.W. Benz, D.T. Bonthron, T. Collins, J. Biol. Chem. 269 (1994) 22647–22656.
[23] D.V. Kuprash, I.A. Udalova, R.L. Turetskaya, N.R. Rice, S.A. Nedospasov, Oncogene 11 (1995) 97–106.
[24] A. Maatta, V. Glumoff, P. Paakkonen, D. Liska, R.P. Penttinen, K. Elima, Biochem. J. 294 (1993) 365–371.
[25] T. Matsusaka, K. Fujikawa, Y. Nishio, N. Mukaida, K. Matsushima, T. Kishimoto, S. Akira, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 10193–10197.
[26] C.L. Newell, A.B. Deisseroth, G. Lopez Berestein, J. Leukoc. Biol. 56 (1994) 27–35.

[27] B.H. Rovin, J.A. Dickerson, L.C. Tan, C.A. Hebert, Kidney Int. 48 (1995) 1263–1271.
[28] U. Schindler, V.R. Baichwal, Mol. Cell. Biol. 14 (1994) 5820–5831.
[29] A. Takeshita, Y. Chen, A. Watanabe, S. Kitano, S. Hanazawa, J. Immunol. 155 (1995) 419–426.
[30] G. Voraberger, R. Schafer, C. Stratowa, J. Immunol. 147 (1991) 2777–2786.
[31] H. Sakurai, Y. Hisada, M. Ueno, M. Sugiura, K. Kawashima, T. Sugita, Biochim. Biophys. Acta 1316 (1996) 132–138.
[32] P.J. Barnes, I. Adcock, Trends Pharmacol. Sci. 14 (1993) 436–441.
[33] A.C. Cato, E. Wade, Bioessays 18 (1996) 371–378.
[34] E. Caldenhoven, J. Liden, S. Wissink, A. Van de Stolpe, J. Raaijmakers, L. Koenderman, S. Okret, J.A. Gustafsson, P.T. Van der Saag, Mol. Endocrinol. 9 (1995) 401–412.
[35] A. Ray, K.E. Prefontaine, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 752–756.
[36] C. Jonat, H.J. Rahmsdorf, K.K. Park, A.C. Cato, S. Gebel, H. Ponta, P. Herrlich, Cell 62 (1990) 1189–1204.
[37] H.F. Yang Yen, J.C. Chambard, Y.L. Sun, T. Smeal, T.J. Schmidt, J. Drouin, M. Karin, Cell 62 (1990) 1205–1215.
[38] R. Schule, P. Rangarajan, S. Kliewer, L.J. Ransone, J. Bolado, N. Yang, I.M. Verma, R.M. Evans, Cell 62 (1990) 1217–1226.
[39] A. Shimizu, Y. Masuda, H. Kitamura, M. Ishizaki, Y. Sugisaki, N. Yamanaka, Lab. Invest. 74 (1996) 941–951.
[40] R. Granados, D.L. Mendrick, H.G. Rennke, Kidney Int. 37 (1990) 414.
[41] K. Kawasaki, E. Yaoita, T. Yamamoto, I. Kihara, Kidney Int. 41 (1992) 1517–1526.
[42] L.M. Staudt, R.G. Clerc, H. Singh, J.H. LeBowitz, P.A. Sharp, D. Baltimore, Science 241 (1988) 577–580.
[43] S.M. Kang, W. Tsang, S. Doll, P. Scherle, H.S. Ko, A.C. Tran, M.J. Lenardo, L.M. Staudt, Mol. Cell. Biol. 12 (1992) 3149–3154.