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Original Article

The selective mineralocorticoid receptor antagonist eplerenone is protective in mild anti-GBM glomerulonephritis

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Abstract: Background: Growing evidence suggests that blockade of the aldosterone-receptor may preserve kidney function by anti-inflammatory effects independent of the blood pressure. We hypothesized that the selective aldosterone-receptor antagonist eplerenone has a profound anti-inflammatory effect in the autologous phase of anti-glomerular basement membrane (GBM) glomerulonephritis (GN). Methods: Mice received ≈200mg/kg body wt/day eplerenone via supplemented chow diet or standard chow starting at the day of immunization with rabbit IgG. Three days later the anti-GBM antibody was injected and the experiments were stopped at day 7 and 14. Results: Mice receiving eplerenone showed significantly decreased albuminuria and glomerular sclerosis at day 7 and 14 after induction of anti-GBM GN. Eplerenone treatment significantly inhibited the infiltration of CD4+, CD8+ T cells and macrophages into the kidneys. Circulating levels and glomerular deposition of autologous IgG were comparable in both groups. At day 7 the pro-inflammatory cytokines MCP-1 and IL-6 were found to be significantly decreased in regional draining lymph nodes of eplerenone-treated mice, whereas the anti-inflammatory cytokine IL-10 was significantly upregulated. In line, splenocytes from eplerenone-treated nephritic mice produced significantly increased IL-10. Conclusion: Aldosterone-receptor blockade by eplerenone effectively attenuated proteinuria, kidney damage and the inflammatory response in anti-GBM GN by significantly decreasing pro-inflammatory cytokines in the regional draining lymph nodes of the kidney. Our results suggest that this selective aldosterone receptor antagonist is a possible additional tool in the treatment of GN.

Keywords: Aldosterone antagonism, glomerulonephritis, eplerenone, renal inflammation

Introduction

Inhibition of the renin-angiotensin-aldosterone system (RAAS) by the use of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) was shown to successfully delay the progression of renal disease [1-5]. Despite the clear evidence for the role of angiotensin II in promoting renal injury, studies have provided evidence that aldosterone *per se* might also participate in the pathogenesis of renal injury [6, 7]. It was shown that mineralocorticoid receptor antagonists may reduce proteinuria and attenuate renal injury through selective aldosterone blockade independently of effects on blood pressure [8, 9]. A systematic review of clinical trials by Navaneethan *et al.* involving more than 800 patients clearly pointed towards an additional renoprotective effect of mineralocorticoid receptor blockage, even in the context of ACEI and/or ARB [10]. Additionally, spironolactone was shown to suppress the production of the proinflammatory cytokines tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and interferon-γ *in vitro* in peripheral blood mononuclear cells [11] and was successfully applied as anti-inflammatory drug in a phase II study in patients with chronic arthritic diseases [12].

Animal experiments have also indicated that mineralocorticoid receptor antagonism may

directly exhibit antiproteinuric, antifibrotic and antiinflammatory properties [8, 13, 14] and might even induce the regression of preexisting glomerulosclerosis [15]. In streptozotocin-induced diabetic nephropathy spironolactone attenuated renal injury and diminished the inflammatory response with a significantly suppressed glomerular and tubulointerstitial macrophage infiltration [13]. Furthermore, Asai and colleagues [16] reported evidence for an antiproteinuric effect of spironolactone in the rat anti-Thy-1 nephritis.

Due to the binding of spironolactone to progesterone and androgen receptors, its use in humans is limited by its undesirable side effects. This has led to the development of more selective mineralocorticoid receptor antagonists with comparable efficacy such as eplerenone (Epl) [17]. Besides its proven clinical efficacy in heart failure [18, 19], Epl was found to attenuate vascular inflammatory damage in angiotensin II/salt-treated rats independently of blood pressure changes via reduction of macrophage infiltration and diminished expression of the proinflammatory molecules cyclooxygenase 2 as well as osteopontin [20]. In the rat hypertensive nephropathy model induced by aldosterone/salt treatment, Epl significantly reduced renal inflammation and albuminuria [14]. This effect was accompanied by a decreased number of infiltrating macrophages and T cells and a reduced expression of the proinflammatory cytokines osteopontin, MCP-1, IL-1β and IL-6 in Epl-treated animals.

The objective of the present study was to evaluate the influence of Epl in a complement- and Th1-dependent model of accelerated anti-GBM GN [21, 22]. We hypothesized that Epl might exert anti-inflammatory and antiproteinuric effects and therefore be renoprotective in this setting.

Methods

Experimental animals and study design

Eight- to 12-wk-old male C57Bl/6J mice (Charles River, Sulzfeld, Germany) were used throughout the study. Animals were maintained in a pathogen-free central animal facility of the Innsbruck Medical University. All animal experiments were approved by the Austrian veterinary authorities (permit number: BMWF-66.011/0111-II/10b/2008). Accelerated anti-GBM nephritis was induced as described previously [21]. Briefly, mice were preimmunized subcutaneously with 2 mg/ml rabbit IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) dissolved in incomplete Freund´s adjuvant (Sigma, St. Louis, MO, USA) and nonviable desiccated *Mycobacterium tuberculosis* H37a (Difco Laboratories, Detroit, MI, USA). After three days heat-inactivated rabbit anti-mouse GBM antiserum was injected via the tail vein in a concentration of 5 mg/20 g body wt. Animals were randomly allocated to either the Eplerenone (Epl) treatment group or the control group (Co). Epl (Pfizer Inc., New York, NY, USA) was administered orally by regular chow supplemented with eplerenone at 2 mg/g (Ssniff PS M-7, Ssniff Spezialdiäten GmbH, Soest, Germany), resulting in a daily dose of ≈200 mg/kg body wt. [23]. Age- and sex-matched controls received drug-free chow only. Twenty-four-hour urine samples were collected in metabolic cages at days −1, 1, 7 and 14 after induction of anti-GBM nephritis. At days 7 and 14, a set of animals was sacrificed with CO_2 inhalation. Both kidneys were harvested and cut in half: one half was fixed in formalin, the second half was snap-frozen in Tissue Tec® O.C.T.™ Compound (Sakura Finetec, Zoeterwoude, The Netherlands) for immunofluorescence and immunohistochemical staining. The remaining tissue was stored in RNAlater (Ambion, Austin, TX, USA) at −20°C for subsequent RNA isolation. Additionally, draining lymph nodes for RNA isolation and the spleen for the preparation of splenocytes of treated and untreated animals were harvested.

Urinary albumin and creatinine detection

Urinary albumin was determined by a double-sandwich ELISA (Abcam, Cambridge, MA, USA) as reported elsewhere [21]. Urinary creatinine was quantified spectrophotometrically using a commercially available kit (Sigma). Urine albumin excretion was expressed in mg albumin per mg urinary creatinine to standardize for urine concentration.

Evaluation of mouse-anti rabbit IgG

Serum was prepared from peripheral blood 7 and 14 days after the induction of anti-GBM GN. Microtiter plates (96-well; Greiner, Kremsmuenster, Austria) were coated with 100 µg/ml of rabbit IgG (Jackson ImmunoResearch Laborato-

ries Inc.) in carbonate/bicarbonate buffer (pH 9.5) overnight at 4°C, and the plate was blocked with 1% BSA. Plates were washed with PBS containing 0.1% Tween-20 and then incubated with serial-doubling dilutions of mouse serum, starting at 1:100. After further washing, bound mouse IgG was detected with HRP-conjugated goat anti–mouse IgG (Dako, Glostrup, Denmark) at a dilution of 1:2000 and 3,3',5,5'-tetramethybenzidine (Sigma) as a substrate. Serum from non-immunized mice was used as control.

Histology and immunohistochemistry

Formalin-fixed renal tissue was embedded in paraffin, cut in 4 µm sections and stained with periodic acid Schiff (PAS) for histological analysis. In all cases, equatorial glomerular cross-sections (minimum of 60 per case) were evaluated as described previously [22, 24]. Glomerulosclerosis was scored as follows: G0 for a normal glomerulus without deposits of PAS-positive material; G1, mild sclerosis with PAS-positive material up to one third of the glomerulus; G2, moderate segmental sclerosis with up to two thirds PAS-positive material; and G3, severe segmental to global glomerulosclerosis with more than two thirds of the glomerular cross-section stained positive for PAS. The glomerulosclerosis score was then calculated in each group as (0 x number of G0 + 1 x number of G1 + 2 x number of G2 + 3 x number of G3)/(number of G0 + G1 + G2 + G3).

Sections cut from frozen tissue (4 µm) were used for immunofluorescence or immunoperoxidase staining using standard techniques as described earlier [25]. For the detection of heterologous IgG deposition, sections were stained by direct immunofluorescence with a FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories Inc.). For the detection of autologous IgG, FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc.) was used. Semiquantitative assessment of the glomerular deposition of IgG was performed by determining the end point positive titer for detection of staining using serial dilutions of each antibody.

The three-layer immunoperoxidase technique was used for the detection of macrophages and T cells in the kidney sections as described previously [21]. Macrophages were stained using a rat anti-mouse macrophage antibody (working dilution 1:500, clone F4/80; Serotec, Oxford, UK), and a semiquantitative scoring system was performed as follows: 0 = 0-4 cells stained positive, 1+ = 5 – 10 cellls, 2+ = 11-50 cells, 3+ = 51-200 cells, and 4+ = >200 cells stained positive per low-power field. For the detection of $CD4^+$ T cells, a rat anti-mouse CD4 mAb (working dilution 1:500, clone YTS191.1; Serotec) was used, and for $CD8^+$ T cells, a rat anti-mouse CD8α mAb (working dilution 1:500, clone KT15; Serotec) was used. In all cases, an IgG2a isotype antibody (working dilution 1:500, clone G155-178; Pharmingen, San Diego, CA, USA) served as negative control. Biotin-conjugated goat anti-rat IgG antibody (working dilution 1:200, Jackson ImmunoResearch Laboratories Inc.) was used as a secondary antibody, followed by incubation with an avidin-biotin complex and subsequent development with 0.4% 3-amino-9-ethylcarbazole for 10 min and counterstaining with Gill´s Hematoxylin No. 3 (Polysciences Inc., Warrington, PA, USA). Quantification of T cells was done by counting the number of positively stained cells in six adjacent high-power fields of renal cortex and medulla. Histological evaluation was performed in a blinded fashion with the treatment groups unknown to the investigator.

Reverse transcription real-time PCR in renal tissue and draining lymph nodes

Total RNA was isolated using TRI-Reagent (Molecular Research Center Inc., Cinncinati, OH, USA) according to the manufacturer's protocol. Thereafter, 2 µg of total RNA was reverse transcribed using Omniscript Reverse Transcription Kit (Qiagen, Hilden, Germany) and random primers (Roche, Basel, Switzerland). Real-time PCR was performed on an ABI Prism 7700 (Applied Biosystems, Foster City, CA, USA) using SYBR Green Master Mix (Invitrogen, Carlsbad, CA, USA) or TaqMan Master Mix (Applied Biosystems). For the detection of TNFα the following primers were used: forward 5´-GAACTGGCAGAAGAGGCACT-3´, reverse: 5´-AGGGTCTGGGCCATAGAACT-3´. As a reference gene in mouse kidneys β-actin was used (forward: 5´-GAAGTGTGACGTTGACATCCG-3´; reverse: 5´-TGCTGATCCACATCTGCTGGA-3´). For the detection of IL-10 (Mm00439616_m1), IL-6 (Mm00446190_m1), MCP-1 (Mm00441242_m1) and IL-17 (Mm00439619_m1) TaqMan Gene Expression

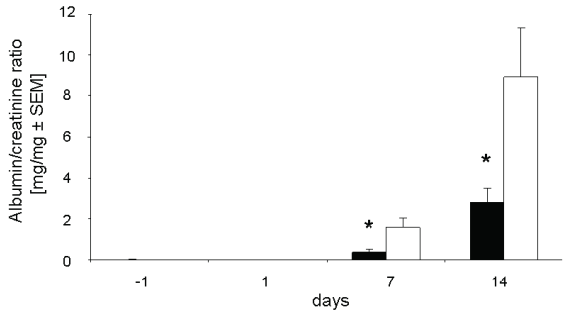


Figure 1. Eplerenone significantly reduces proteinuria. Proteinuria was evaluated on days -1, 1, 7 (n = 22 per group) and 14 (n = 14 per group) after the induction of anti-GBM GN. Urine albumin excretion (in mg) was determined and expressed per mg of urinary creatinine to standardize for glomerular filtration rate. Mice receiving Epl (■) showed significantly reduced albuminuria, whereas controls (□) presented with prominent albuminuria (*p<0.05).

assays (Applied Biosystems) were used. For gene expression analysis in lymph nodes a TaqMan Gene Expression assay glucuronidase β (Mm00446953_m1; Applied Biosystems) was used as reference gene.

Detection of cytokine production from restimulated murine splenocytes

Single-cell suspensions of freshly isolated splenocytes (2 x 10^6 white blood cells/ml) were prepared in RPMI 1640 (Invitrogen) supplemented with 2mM L-glutamine, penicillin (100U/ml, Sigma), streptomycin (0.1 mg/ml, Sigma) and 10% FCS. Cells were seeded in 24-well plates and tested for cytokine response to rabbit IgG (1 mg/ml, preincubated o/n; Jackson ImmunoResearch Laboratories Inc.). Supernatants were harvested after incubation at 37° C for 16 hours, and IL-10 (BD OptEIA™, Pharmingen) and IL-17 (R&D Systems, Minneapolis, MN, USA) were determined by using commercially available ELISA kits.

Statistical analysis

Results are presented as mean values ± SEM. Normal distribution of data was assessed by the Kolmogorov-Smirnov test with Lilliefors correction. Both groups (Epl and Co) were compared by either nonparametric Mann-Whitney-U-test or unpaired Student´s t-test as appropriate, depending on the distribution of the tested variable. A two-tailed p<0.05 was considered statistically significant. All statistical analyses were done with SPSS 12.0.1 for Windows (SPSS, Inc., Chicago, IL).

Results

Albuminuria and glomerular injury are significantly diminished by eplerenone treatment

As an indicator of glomerular injury albuminuria was assessed on days –1, 1, 7 and 14 after initiation of anti-GBM GN. Albuminuria was significantly reduced in Epl-treated animals compared to untreated controls on day 7 (0.39 ± 0.11 vs 1.57 ± 0.50 mg/mg; p<0.05) and 14 (2.77 ± 0.69 vs 8.90 ± 2.4 mg/mg; p<0.05; **Figure 1**). Glomerulosclerosis, as indicated by PAS-positive glomerular deposits (**Figure 2**), was markedly attenuated by Epl-treatment and was found to be significantly diminished 14 days after induction of GN (**Table 1**). Of note, the humoral response evaluated by detection of circu-

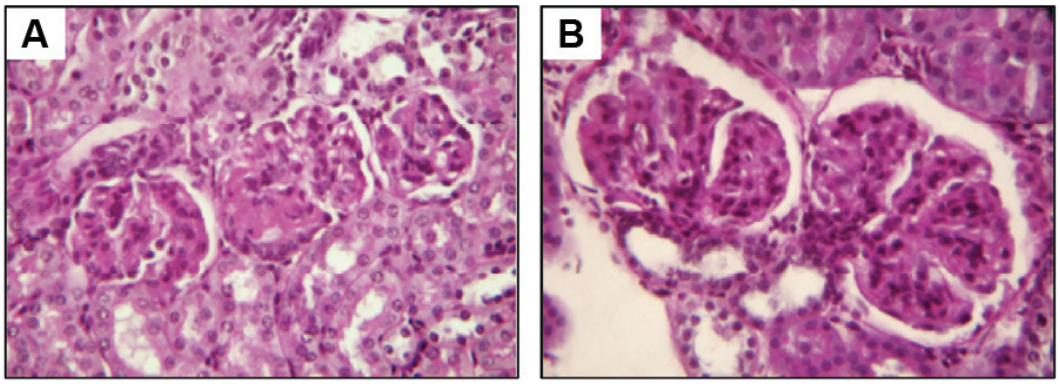
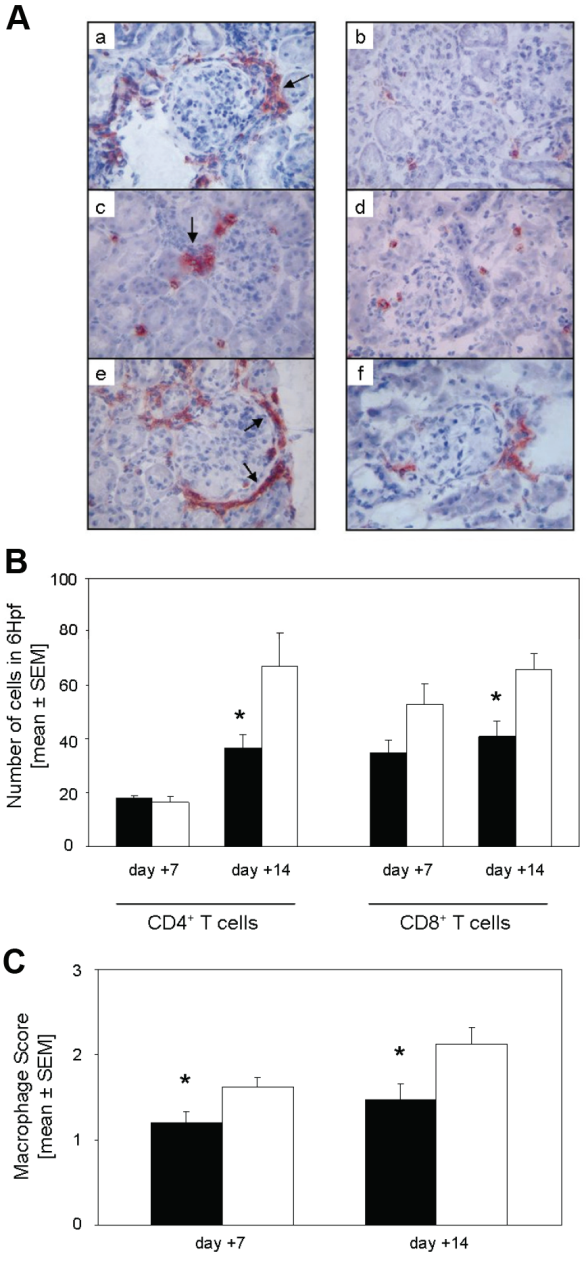


Figure 2. Representative PAS-positive glomerular stainings. PAS-positive deposits were markedly more accentuated in kidneys of control animals (**A**) as compared to Epl-treated animals (**B**).

Table 1. Eplerenone attenuates glomerular sclerosis without influencing the B cell response. PAS-positive deposits were scored in Epl-treated mice and control mice on day 7 (n = 8 per group) and 14 (n = 14 per group). Circulating mouse anti-rabbit IgG levels (†given in arbitrary units) on day 7 (n = 8 per group) and 14 (n = 8 per group) are shown.

	Day 7		Day 14	
	Eplerenone	Vehicle	Eplerenone	Vehicle
Glomerular sclerosis	0.49 ± 0.06	0.71 ± 0.14	0.82 ± 0.05*	1.03 ± 0.07*
Circulating mouse anti-rabbit IgG	0.46 ± 0.10†	0.58 ± 0.19†	0.74 ± 0.15†	0.69 ± 0.17†

* p<0.05 between Epl- and vehicle-treated mice



lating mouse anti-rabbit IgG and the deposition of nephritogenic rabbit IgG did not differ between Epl-treated and untreated mice (**Table 1**).

Eplerenone affects the infiltration of inflammatory cells

Our anti-GBM GN model is characterized by a marked tubulointerstitial and periglomerular leukocyte infiltration, which primarily consists of T cells and macrophages. In control mice we found a prominent accumulation of F4/80$^+$ cells, CD4$^+$ as well as CD8$^+$ T cells, which was most prominent in the periglomerular region (**Figure 3A**). Epl-treated animals showed a significantly reduced infiltration of CD4$^+$ and CD8$^+$ T cells 14 days after the induction of anti-GBM GN (**Figures 3A** and **B**), as well as a significantly lowered number of infiltrating macrophages 7 and 14 days after the onset of disease (**Figures 3A** and **C**). The decreased infiltration of inflammatory cells was also reflected by a decreased

Figure 3. Eplerenone inhibits the infiltration of inflammatory cells. **(A)** Representative pictures of CD4 (a, b), CD8 (c, d) and F4/80 (e, f) immunohistochemistry 14 days after induction of nephritis are shown (magnification x400). In control mice an accentuated interstitial and periglomerular infiltration (arrows) of CD4$^+$ (a) and CD8$^+$ (c) T cells as well as of macrophages (e) was found, which was reduced by Epl treatment ((b) for CD4$^+$, (d) for CD8$^+$ and (f) for macrophages). **(B)** The infiltration of CD4$^+$ T cells, counted in six adjacent high-power fields in cortex and medulla, was significantly diminished in Epl-treated mice (■) at day 14 (*p<0.05), CD8$^+$ T cell infiltration was significantly reduced on day 14 (*p<0.05) and showed a strong trend towards reduction on day 7 (p=0.087) compared to control mice (□). **(C)** Macrophage infiltration was significantly reduced in Epl-treated mice (■) on days 7 and 14 (*p<0.05) in comparison to controls (□).

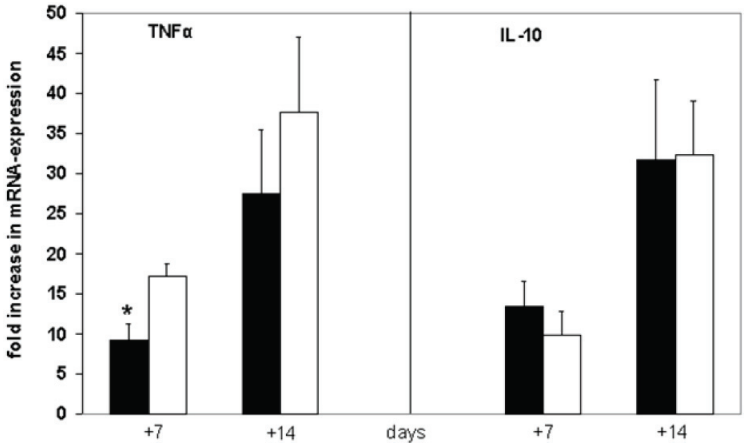


Figure 4. Eplerenone treatment lowers renal TNFα mRNA expression in kidneys. The expression of TNFα- and IL-10 mRNA was evaluated by real-time PCR of total RNA from kidneys of Epl-treated (■) (n = 8) and control mice (□) (n = 8). The fold increase compared to the mRNA expression in kidneys of healthy mice is given. On day 7 TNFα mRNA was significantly downregulated in Epl mice (*p<0.05), whereas the reduction on day 14 did not reach statistical significance. No significant differences in IL-10 gene expression were detected in renal tissue between both groups.

TNF-α mRNA expression in kidneys of Epl-treated mice as compared to control mice 7 days after induction of anti GBM-GN (**Figure 4**). Interestingly, the IL-10 transcription did not differ in the kidneys of Epl-treated and control mice 7 and 14 days after induction of GN (**Figure 4**). In contrast to the expression in the draining lymph nodes (see below), MCP-1 and IL-6 mRNA expression did not differ in kidneys of Epl-treated mice as compared to controls (data not shown).

Eplerenone treatment shifts the balance from a pro-inflammatory towards an anti-inflammatory milieu in lymph nodes and spleen

Recent data provide evidence that the immune response in our anti-GBM GN model is mainly modulated in the regional draining lymph nodes of the kidneys [22, 26]. Therefore, we determined the tissue mRNA expression of pro- and anti-inflammatory cytokines in the regional draining lymph nodes and evaluated the production in isolated splenocytes. We detected significantly diminished expression of the pro-inflammatory cytokines MCP-1 and IL-6 in lymphoid tissue of Epl-treated mice 7 days after induction of anti-GBM GN as compared to respective controls. In line, the expression of the pro-inflammatory cytokine IL-17 was reduced in lymph nodes of Epl-treated compared to control mice, but this difference did not reach statistical significance. In parallel to the reduced expression levels of the pro-inflammatory cytokines, the anti-inflammatory cytokine IL-10 was found to be significantly increased in lymph nodes of Epl-treated mice (**Figure 5A**).

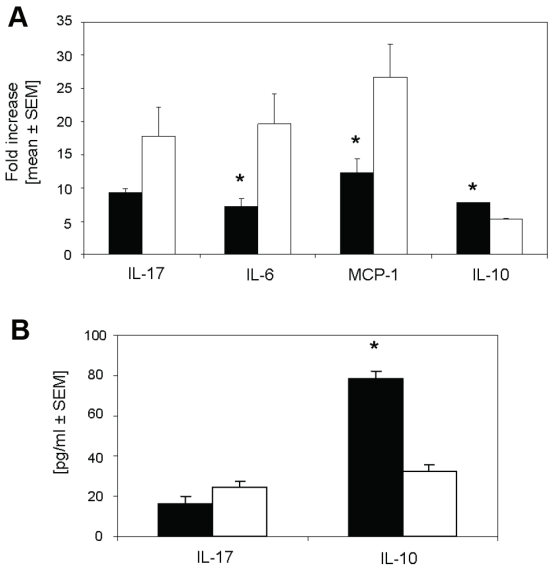


Figure 5. Eplerenone treatment decreases pro-inflammatory cytokines and increases the anti-inflammatory Th2 cytokine IL-10 in the draining lymph nodes. **(A)** The expression of IL-17, IL-6, MCP-1 and IL-10 mRNA was evaluated by real-time PCR of total RNA from regional draining lymph nodes of Epl-treated (■) (n = 8) and control mice (□) (n = 8). The fold increase compared to the mRNA expression in lymph nodes of healthy mice is given. IL-6 and MCP-1 mRNA expression was significantly decreased in Epl-treated animals (■) on day 7 (*p<0.01), whereas IL-10 transcription was significantly increased. **(B)** Splenocytes from Epl-treated (■) and control (□) animals (n = 8 per group) were isolated on day 7, seeded in 24-well plates and stimulated with rabbit IgG (1 mg/ml) for 16 h. In Epl mice (■) a significant increase in rabbit IgG-induced IL-10 levels (*p<0.01) was found, whereas IL-17 levels were decreased.

These data were further proven by restimulating splenocytes from Epl-treated or control mice 7 days after induction of anti GBM GN with rabbit IgG, the antigen used for immunization prior to induction of anti-GBM GN. In line with the cytokine expression in the lymph nodes, rabbit-IgG-restimulated splenocytes from Epl-treated mice displayed significantly increased IL-10, but decreased IL-17 production as compared to control mice (**Figure 5B**).

Discussion

Previous studies provide evidence that aldosterone antagonism can improve proteinuria and kidney function by exerting anti-inflammatory and renoprotective properties independently from the blood-pressure lowering effect [8, 9, 27, 28]. In the present study we show that the specific aldosterone receptor antagonist eplerenone (Epl) has the potency to protect mice from anti-GBM GN by shifting the balance between pro- and anti-inflammatory factors towards an anti-inflammatory profile in the secondary lymphoid organs. Thereby, Epl leads to a decreased infiltration of inflammatory cells into kidneys and to a significantly decreased renal damage.

Epl, a selective mineralocorticoid receptor antagonist, is known to have anti-inflammatory capacity independent of its anti-hypertensive effect [9, 29-32]. The majority of studies evaluating the influence of Epl on inflammatory cells revealed that Epl down-regulates the expression of monocyte/macrophage-specific cytokines, such as MCP-1, IL-6 and osteopontin [14, 29, 30, 33, 34]. In accordance Epl protected mice from anti-GBM GN by reducing the infiltration of inflammatory cells into the kidney without affecting the B cell-response.

Recently, we demonstrated that the balance of pro- and anti-inflammatory cells and cytokines in the local draining lymph node is essential for the infiltration of pro-inflammatory cells into the kidney and for the outcome of our model of anti-GBM GN [22, 26]. Here, Epl significantly decreases the mRNA expression of the pro-inflammatory proteins MCP-1 and IL-6 in regional draining lymph nodes. These cytokines have been shown to be down-regulated by either Epl [14, 29] or spironolactone [13, 35] in other animal models. Interestingly, apart from the monocyte/macrophage-specific cytokines we also detected classical as well as newly described T cell cytokines in lymph nodes and spleen to be influenced by Epl treatment. T cells have been described to play a crucial role in the autologous phase of anti-GBM GN (reviewed by Tipping et al. [36]). The expression of the anti-inflammatory Th2 cytokine IL-10 was found to be increased in the lymph node as well as in restimulated splenocytes of Epl-treated mice. In contrast, Epl treatment decreased the expression of the T cell cytokine IL-17 which has been found to have pro-inflammatory capacity (reviewed by Miossec et al. [37]). We now speculate that Epl might shift the balance towards an anti-inflammatory response in the secondary lymphoid organs and thereby decrease kidney infiltration of effector cells, such as $CD4^+$ and $CD8^+$ T cells as well as macrophages. Probably reflecting the decreased infiltration of macrophages, TNF-α mRNA was decreased in kidneys of Epl-treated mice. In contrast, Epl treatment does not seem to increase infiltration of Th2 cells since no differences in the IL-10 mRNA levels in kidneys of Epl-treated mice compared to respective controls were detected.

Previous studies report that several cells in cardiac and renal tissues produce MCP-1 and other pro-inflammatory cytokines due to NFκB-activation after stimulation with aldosterone, which can be inhibited by spironolactone [35, 38]. But contrary to these reports, we did not detect any differences in the mRNA expression of MCP-1 and IL-6 in renal tissues of Epl-treated mice as compared to respective controls. From our point of view, this is not very surprising, since we have provided compelling evidence that regulation of the inflammatory cell infiltration into the kidney is not orchestrated locally in the kidney, but rather in the regional draining lymph nodes [22, 26]. Inflammatory cells, which have regulatory properties in the model of anti-GBM GN, such as regulatory T cells and mast cells, were only found in the draining lymph nodes, but not in the kidney [22, 26]. Therefore, we speculate that Epl might exert its anti-inflammatory effects directly on inflammatory cells in the secondary lymphoid organs, such as dendritic cells, macrophages and T cells. Dendritic cells might be the main source of MCP-1 production [39], while IL-6 is mainly produced by macrophages and T cells (reviewed by Kishimoto [40]). The latter both express the mineralocorticoid receptor [41, 42]. Nevertheless, further studies are necessary to evaluate the

effects of Epl on the various inflammatory cells involved in the pathogenesis of anti-GBM GN.

As a limitation of our study we acknowledge the lack of blood pressure detection in the animals. However, as several studies have documented a blood pressure-independent anti-inflammatory and nephroprotective effect of mineralocarticoid receptor antagonists [16, 27, 28, 32, 43], it appears unlikely that the effect seen in the present study is largely due to a decrease in blood pressure. Nagase et al [27] elegantly demonstrated the antiproteinuric and nephroprotective effect in a model of hypertensive nephropathy to be independent from hypotensive effects. Furthermore, an antiinflammatory mechanism was suggested by decreased levels of proinflammatory cytokines. Additionally, in a recent investigation Koh *et al.* did not find a significant effect on blood pressure using a comparable dose of Epl [44]. Recently, Brandish *et al.* [32] compared effects of Epl and losartan on inflammatory foci in the hearts of spontaneously hypertensive rats and observed that Epl produced similar antiinflammatory effects to losartan, without a comparable decrease in blood pressure.

Taken together, the selective aldosterone antagonist Epl shifts the balance towards antiinflammatory signals in secondary lymphoid organs thereby leading to decreased kidney infiltration of inflammatory cells and protecting mice from anti-GBM GN. These data provide evidence that Epl might be a new attractive therapeutic option in autoimmune-mediated glomerulonephritis.

Conflict of interest statement

None to declare.

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