Myeloperoxidase deficiency ameliorates progression of chronic kidney disease in mice

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1Division of Nephrology, Department of Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany; 2Division of Nephrology, Department of Pediatrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; 3Department of Physiology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; 4Clinical Pharmacology, University of Halle, Halle, Germany; and 5Division of Cardiology, University of Cologne, Cologne, Germany

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Lehners A, Lange S, Niemann G, Rosendahl A, Meyer-Schwesinger C, Oh J, Stahl R, Ehmke H, Benndorf R, Klinke A, Baldus S, Wenzel UO. Myeloperoxidase deficiency ameliorates progression of chronic kidney disease in mice. Am J Physiol Renal Physiol 307: F407–F417, 2014. First published July 2, 2014; doi:10.1152/ajprenal.00262.2014.—Myeloperoxidase (MPO) is an enzyme expressed in neutrophils and monocytes/macrophages. Beside its well-defined role in innate immune defence, it may also be responsible for tissue damage. To identify the role of MPO in the progression of chronic kidney disease (CKD), we investigated CKD in a model of renal ablation in MPO knockout and wild-type mice. CKD was induced by 5/6 nephrectomy. Mice were followed for 10 wk to evaluate the impact of MPO deficiency on renal morbidity. Renal ablation induced CKD in wild-type mice with increased plasma levels of MPO compared with controls. No difference was found between MPO-deficient and wild-type mice regarding albuminuria 1 wk after renal ablation, indicating similar acute responses to renal ablation. Over the next 10 wk, however, MPO-deficient mice developed significantly less albuminuria and glomerular injury than wild-type mice. This was accompanied by a significantly lower renal mRNA expression of the fibrosis marker genes plasminogen activator inhibitor-I, collagen type III, and collagen type IV as well as matrix metalloproteinase-2 and matrix metalloproteinase-9. MPO-deficient mice also developed less renal inflammation after renal ablation, as indicated by a lower infiltration of CD3-positive T cells and F4/80-positive monocytes/macrophages compared with wild-type mice. In vitro chemotaxis of monocyte/macrophages isolated from MPO-deficient mice was impaired compared with wild-type mice. No significant differences were observed for mortality and blood pressure after renal ablation. In conclusion, these results demonstrate that MPO deficiency ameliorates renal injury in the renal ablation model of CKD in mice.

myeloperoxidase (MPO), a heme protein abundantly expressed in neutrophils, monocytes, and macrophages. Recently, our view of MPO has evolved from a key constituent in innate immunity to an enzyme critically involved in inflammatory vascular disease (19). MPO-derived oxidants, such as hypochlorous acid, interfere with various cell functions and contribute to tissue injury. Recent data have also suggested that MPO can exert proinflammatory properties independent of its catalytic activity (31).

Increased levels of MPO protein and its products have been detected in various kidney diseases, including pyleonephritis and glomerulonephritis (9, 24, 25). Infusion of MPO into the renal artery of experimental animals has demonstrated a pathogenic role of MPO in glomerular dysfunction (17). In addition, systemic autoimmunity to MPO is associated with crescentic glomerulonephritis, in which the presence of circulating antibodies to MPO (antineutrophilic cytoplasmatic antibodies) strongly correlates with disease. Moreover, a recent study (32) has identified an immunodominant MPO T cell epitope and redefined how effector responses can induce injury in MPO-antineutrophilic cytoplasmatic antibody–associated microscopic polyangiitis. Polymorphisms in the MPO gene locus are associated with CKD (18), and the plasma MPO concentration has a prognostic value in patients with renal disease (40). While these observations implicate a general role for MPO in the pathogenesis and progression of renal disease, the role of MPO in the pathogenesis of CKD has not yet been investigated directly. We hypothesized that the absence of MPO will influence the progression of CKD. In the present study, CKD was induced by 5/6 nephrectomy. This model features all clinical hallmarks of CKD, including substantial albuminuria, uremia, glomerulosclerosis, and renal fibrosis.

MATERIALS AND METHODS

Mice. All experiments started in 10- to 12-wk-old male FVB/N mice. Since C57BL/6 mice are resistant against the effects of renal ablation, we backcrossed MPO-deficient mice of the C57BL/6 strain (Jackson Laboratories) for >10 generations into our FVB/N colony. After mice had been backcrossed, experiments were performed using MPO−/− FVB/N mice and mice from the FVB/N colony used for backcrossing. MPO−/− FVB/N mice and wild-type FVB/N mice received background strain characterization testing via single-nucleotide polymorphism markers using a mouse 384 single-nucleotide polymorphism complete background analysis panel (Charles River Laboratories). The markers are spread across the genome at ~7 Mbp. The testing showed that MPO−/− mice were 99.35% FVB. As to be expected, the region in close proximity to the MPO gene on chrono-
Table 1. PCR primers

<table>
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<th>Primer</th>
<th>18S rRNA Forward</th>
<th>Reverse</th>
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<td>5′-TGCGGCTATGAATCGAGAAAG-3′</td>
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PAI, plasminogen activator inhibitor; MMP, matrix metalloproteinase; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand.

some 11 had a non-FVB region. All animal procedures were approved by the local animal committee and were in accordance with national and institutional animal care guidelines.

Renal ablation. In 10-wk-old male FVB/N mice, a flank incision was made to obtain retroperitoneal access to expose the kidney as previously described by us (2, 8). Next, a small incision of the capsule at the upper pole of the kidney was performed. A sling using 4-0 silk tightened, and a purse-string suture was made to close the kidney. The over the kidney to get rid of remained tissue was weighed with a precision balance (ED153, Sarstedt). Fourteen days later, renal ablation was completed by constricting the remnant kidney (8).

After the second surgery since at this time point a robust renal injury was found in the remnant kidney (8). Mice were euthanized 10 wk after the second surgery since at this time point a robust renal injury was found in the remnant kidney (8).

Albuminuria. Mice were placed into metabolic cages for a 6-h urine collection. Urine albumin was measured using a commercially available mouse-specific ELISA (E90–134, Bethyl Laboratories), and urine creatinine was measured by an autoanalyzer (Hitachi 717, Roche). Albuminuria was calculated as milligrams of albumin per milligram of creatinine.

Plasma analysis. Five weeks after renal ablation, mice were slightly anesthetized with CO₂, and the retroorbital sinus was punctured with a glass capillary tube for heparinized blood collection. At death, blood was drawn intracardially. Urea-N was determined by an autoanalyzer (Hitachi 717). MPO plasma concentrations were determined by ELISA (Mouse MPO ELISA Kit HK210, Hycult) according to the manufacturer’s recommendation.

Blood pressure measurements. Systolic blood pressure was measured in conscious mice using tail-cuff plethysmography (Process Control Blood Pressure 2900 series, TSE Systems) as previously described by our laboratory (12). Mice were trained to get used to this procedure in advance.

Histopathological analysis. Kidney tissue was fixed with 4% neutral buffered formaldehyde, embedded in paraffin, and sectioned. Sections were stained for light microscopy with periodic acid-Schiff (PAS) reagent. Glomerular injury was evaluated using a semiquantitative scale from 0 to 3 as previously described by our laboratory (8).

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<td>5′-TGCGGCTATGAATCGAGAAAG-3′</td>
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PAI, plasminogen activator inhibitor; MMP, matrix metalloproteinase; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand.

Tubulointerstitial injury was quantified by a semiquantitative scale from 0 to 3 as previously described by our laboratory (8). Tubulointerstitial injury was defined as dilated or atrophic tubules, intratubular cast formation, sloughing of tubular epithel cells, thickening of the tubular basement membrane, and widening of the intertubular space and was scored from 0 to 4 as follows: 0 = no tubulointerstitial injury, 1 = 25% of the tubulointerstitium was injured, 2 = 50% of the tubulointerstitium was injured, 3 = 75% of the tubulointerstitium was injured, and 4 = >75% of the tubulointerstitium was injured. Renal infiltration of CD3-positive T cells and F4/80-positive monocytes/macrophages was examined immunohistochemically as previously described by our laboratory (34). Infiltration of these cells was quantified by scoring 20 high-power fields (0 = <10 cells, 1 = 10–30 cells, and 2 = >30 cells/field).

Neutrophils were quantified by counting the number of granulocyte differentiation antigen 1 (GR-1)-positive cells per field. All histopathologies were analyzed in a blinded manner.

Autophagy. Autophagy was evaluated as previously described by our laboratory (22). Primary antibodies used for the evaluation of the autophagosomal/lysosomal compartment were rabbit lysosome membrane protein 2 (Limp2; 1:1,000, Paul Safﬁg, Kiel, Germany) and rabbit microtubule-associated protein 1 light chain (LC)3B (1:100, Cell Signaling). Podocytes were stained using guinea pig anti-nephin antibody (1:100, Acris), and DRAQ5 (1:1,000, Molecular Probes) was used as a nuclear counterstain. All secondary antibodies used were ﬂuorescent dye-conjugated afﬁnity-puriﬁed donkey antibodies (Jackson ImmunoResearch). In brief, parafﬁn sections were deparafﬁnized, and antigen retrieval was performed by microwave boiling (30 min, 800 W, 10 mM citrate buﬀer, pH 6.1). Unspeciﬁc binding was blocked (5% horse serum and 0.05% Triton X-100 in PBS, 30 min at room temperature). Primary antibody incubations (in blocking buﬀer, overnight, at 4°C) were followed by incubations with Alexa ﬂuor 488- or Cy3-coupled secondary antibodies (1:400, 30 min, at room temperature) and DRAQ5. Stainings were evaluated with a LSM 510 meta microscope using LSM software (all Zeiss, Jena, Germany).

Fig. 1. Myeloperoxidase (MPO) plasma concentrations were measured 10 wk after renal ablation. A: plasma levels were 2.5-fold increased after renal ablation compared with control mice (P < 0.05). B: mortality started ~3–6 wk after renal ablation. Despite a tendency for earlier mortality in wild-type (WT) mice, no signiﬁcant difference was found in mortality between wild-type and MPO-deﬁcient (MPO−/−) mice. C: 7 days after renal ablation, albuminuria was signiﬁcantly increased in WT and MPO−/− mice compared with controls. D: albuminuria was not signiﬁcantly different between WT and MPO−/− mice after the exclusion of all mice with an albumin-to-creatinine ratio of <0.5 mg/mg. E: throughout the experiment, MPO−/− mice developed signiﬁcantly less albuminuria than WT mice (*P < 0.05, WT vs. MPO−/− mice). Signiﬁcance is shown for the whole experimental duration of 10 wk, and the progression of albuminuria was analyzed with a linear mixed model ﬁxed to the data. F: increased plasma urea-N was found 5 and 10 wk after renal ablation in both groups without a signiﬁcant difference between genotypes. All data with the exception of survival are shown with logarithmic scaling.
**A**

Control Ablation WT

MPO (ng/ml)

10000 1000 100 10

Control Ablation WT

**B**

Cumulative survival (%)

Days after nephrectomy

Control Ablation MPO<sup>−/−</sup> Ablation WT

**C**

Albumin-to-creatinine ratio (mg/mg)

7 days after nephrectomy

Control Ablation WT Ablation MPO<sup>−/−</sup>

**D**

Albumin-to-creatinine ratio (mg/mg)

7 days after nephrectomy

Control Ablation WT Ablation MPO<sup>−/−</sup>

**E**

Albumin-to-creatinine ratio (mg/mg)

Weeks after nephrectomy

Control Ablation WT Ablation MPO<sup>−/−</sup>

* p < 0.05

**F**

Urea-N (mg/dl)

5 weeks after nephrectomy 10 weeks after nephrectomy

Control Ablation WT Ablation MPO<sup>−/−</sup>
Table 2. Body and kidney weights

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<tr>
<td></td>
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<tr>
<td>Control mice</td>
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</tr>
<tr>
<td>Median</td>
<td>28.2, 31.7</td>
<td>3.5</td>
</tr>
<tr>
<td>25th percentile, 75th percentile</td>
<td>26.3, 30.3</td>
<td>2.9, 3.7</td>
</tr>
<tr>
<td>WT mice with ablation</td>
<td>28.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Median</td>
<td>26.1, 28.8</td>
<td>3.1, 4.2</td>
</tr>
<tr>
<td>25th percentile, 75th percentile</td>
<td>28.2, 31.7</td>
<td>2.9, 3.7</td>
</tr>
</tbody>
</table>

WT mice, wild-type mice; MPO−/− mice, myeloperoxidase-deficient mice. *P < 0.05 vs. WT mice with ablation.

Real-time PCR analyses. Total RNA of the renal cortex was prepared according to standard laboratory methods. Quantitative real-time PCR analysis (StepOnePlus, Applied Biosystems) was performed using SYBR green dye (Qiagen) as previously described (8). The primers used are shown in Table 1. Renal RNA expression was measured using the ΔΔCt method (where Ct is threshold cycle) and expressed as expression relative to untouched controls and normalized to the housekeeping 18S rRNA. Quantification of every single mouse was obtained by the comparative Ct method at which ΔΔCt values of treated mice were referred to the mean value of control mice.

Peritonitis-elicited monocytes/macrophages and chemotactic assay. Peritoneal monocytes/macrophages were isolated as previously described by our laboratory (17). Mice were injected with thioglycollate (Sigma, Steinheim, Germany) intraperitoneally. On day 3, the peritoneal cavity was lavaged with RPMI 1640 (Life Technologies) containing 10% FBS, 1% penicillin-streptomycin, 1% pyruvate, and 1.5% HEPES. Cells were centrifuged at 300 g for 10 min at 4°C. Lower chambers of a 24-well Transwell plate were filled with 600 μl chemotactic buffer (RPMI containing 5 mg/ml highly purified BSA and 2% of 1 M HEPES) alone or containing 100 ng/ml chemokine (C-C motif) ligand (CCL)2 (Peprotech, Rocky Hill, NJ). Transwell inserts (8.0-μm pore size, Costar, Amsterdam, The Netherlands) were filled with 80 μl chemotactic buffer. Monocytes/macrophages were diluted at 1 × 106 cells/ml, and 100 μl were added to the Transwell inserts. Chemotaxis was performed over 1.5 h. The inserts were removed and flushed sideways with ice-cold PBS. Cells were collected together with cells from their corresponding well and resuspended in 300 μl PBS. Quantification of the added and migrated cells was performed by FACS (90-s counts) and expressed as a percentage of migrated cells.

Statistical analysis. Data are presented as medians (25th percentile, 75th percentile) unless otherwise indicated. To address the question of whether significant changes were driven by data outliers, we performed a Kolmogorov-Smirnov test on our data to check for uneven distribution. Data sets with a Dallal-Wilkinson-Lilliefor P value lower than 0.05 in this test were considered unevenly distributed and therefore logarithmically transformed to approach a normal distribution. Student’s t-test was then performed on the transformed data to check for significances. Adjusting α-errors for multiple testing was not necessary because only wild-type and MPO-deficient mice after ablation were compared. Survival curves by Kaplan-Meier test were compared using the log-rank test. To evaluate the progression of albuminuria, a general linear mixed model was fit to the data, with fixed effects of group, time point, interaction, repeated-measure effects, and random intercepts of the subjects. Since multiple groups were compared in the monocyte migration assay, one-way ANOVA and post hoc analyses by Newman-Keuls multiple comparison test was performed. Statistical significance was defined as P < 0.05 (IBM SPSS Statistics, 20SPSS).

RESULTS

MPO plasma levels. To evaluate the role of MPO in CKD, we first measured MPO plasma concentrations 10 wk after renal ablation. Plasma levels were ~2.5-fold increased after renal ablation compared with control mice [CKD: 671 (535, 1,687) ng/ml vs. control: 261 (173, 438) ng/ml, P < 0.05; Fig. 1A]. MPO was not detectable in the plasma of MPO knockout mice. Next, we aimed to further elucidate whether MPO is just a marker of renal injury or plays a pathophysiological role in CKD. Hence, we analyzed the impact of MPO deficiency on CKD-related morbidity and mortality.

Body and kidney weights. No significant difference in body weight was found between MPO knockout and wild-type mice at the time of surgery. The amount of removed kidney tissue was not different between wild-type and MPO knockout mice, indicating that the surgical procedure to induce renal injury was identical in both groups. Ten weeks after renal ablation, body weight was slightly higher in MPO-deficient mice compared with wild-type mice. No significant difference was found for the relative weight of the remaining kidney tissue (Table 2). Table 3 shows the number of mice throughout the experiment.

Survival. Mortality started ~3–6 wk after renal ablation. Although there was a tendency for earlier mortality in the wild-type group, no significant difference between both genotypes was found after 70 days (Fig. 1B).

Albuminuria. Seven days after renal ablation, albuminuria was significantly increased in wild-type and MPO-deficient mice compared with control mice. The effect of renal ablation on albuminuria showed a broad variance including a substantial overlap with the control group [control group: 0.15 (0.11, 0.24) mg/mg, wild-type group: 0.91 (0.43, 3.51) mg/mg, and MPO knockout group: 1.31 (0.52, 2.89) mg/mg; Fig. 1C]. A certain percentage of mice did not develop CKD after renal ablation. To avoid studying mice without renal injury, mice with an albumin-to-creatinine ratio below 0.5 mg/mg 1 wk after surgery were excluded from the experiment. Albuminuria

Table 3. Numbers of mice

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<th>Survived First 7 Days After Second Surgery</th>
<th>Albuminuria Over the Cutoff of 0.5 g/g Albumin/Creatinine</th>
<th>Survived the 10-Week Observation Period</th>
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<tbody>
<tr>
<td>WT mice</td>
<td>44</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>MPO−/− mice</td>
<td>44</td>
<td>35</td>
<td>25</td>
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</table>
was not significantly different between wild-type and MPO-knockout mice after exclusion of all mice with an albumin-to-creatinine ratio of <0.5 mg/mg [control group: 0.15 (0.11, 0.24) mg/mg, wild-type group: 1.25 (0.79, 15.21) mg/mg, and MPO knockout group: 1.56 (0.90, 4.81) mg/mg; Fig. 1D]. Throughout the experiment, albuminuria remained increased in both nephrectomized groups compared with the control group. However, MPO-deficient mice developed significantly less albuminuria than wild-type mice (P < 0.05; Fig. 1E). Ten weeks after renal ablation, the albumin-to-creatinine ratio was approximately sixfold higher in the wild-type group than in the MPO knockout group [15.61 (0.95, 78.72) vs. 2.74 (0.52, 20.17) mg/mg, P < 0.05]. Increased urea-N plasma levels were found 5 and 10 wk after renal ablation. No significant difference was found between wild-type and MPO knockout mice [5 wk after nephrectomy: control group, 27.0 (22.0, 31.0) mg/dl; wild-type group, 51.0 (40.3, 75.3) mg/dl; and MPO knockout group, 49.5 (43.0, 56.8) mg/dl; and 10 wk after nephrectomy: control group, 26.0 (23.8, 31.3) mg/dl, wild-type group: 68.0 (49.5, 127.5) mg/dl, and MPO knockout group, 62.5 (50.5, 73.0) mg/dl; Fig. 1F].

Glomerular and interstitial damage. Whereas control animals presented normal renal histology, PAS-stained kidney sections revealed severe histopathological abnormalities with enlarged glomeruli and glomerulosclerosis after renal ablation (Fig. 2A). Semiquantitative analysis of these changes showed a significantly decreased level of glomerular injury in MPO-deficient mice compared with wild-type mice (Fig. 2B). No significant difference was found for interstitial damage (Fig. 2C). Autophagy in podocytes influences albuminuria (11). LC3 and Limp2 are markers of the autophagosomal/lysosomal system. In the present study, an upregulation of Limp2 was observed in podocytes and tubules after renal ablation compared with controls by immunohistochemical stainings. Fewer Limp2-positive vesicles were found in podocytes and tubules of MPO knockout mice compared with wild-type mice after renal ablation (Fig. 2D). The same pattern was observed for LC3B staining of podocytes (Fig. 2E).

Gene expression in the renal cortex. RT-PCR analysis of mRNA deriving from the whole kidney cortex showed significantly decreased expression of the fibrosis markers plasminogen activator inhibitor (PAI)-I, collagen type III, and collagen type IV in MPO-deficient mice, suggesting decreased renal injury in these mice after renal ablation (Fig. 3, A–C). Furthermore, expression of matrix metalloproteinase (MMP)-2 and MMP-9 were also significantly lower in MPO-deficient mice than in wild-type mice (Fig. 3, D and E).

Inflammation. The decreased renal injury observed in MPO-deficient mice compared with wild-type mice was also accompanied by a significantly decreased infiltration of monocytes/macrophages (Fig. 4A) and T cells (Fig. 4B). Fewer infiltrating neutrophils were found, but the difference between wild-type and MPO-deficient mice was not statistically significant (Fig. 4C). Given the decreased infiltration of monocytes/macrophages into the remnant kidneys of MPO-deficient mice, we carried out real-time RT-PCR mRNA expression analyses of CCL2 in mRNA derived from the whole kidney cortex. Significantly lower expression was found in MPO-deficient mice compared with wild-type mice [wild-type mice: 20.13 (5.89, 81.10) and MPO knockout mice: 6.32 (1.64, 20.08); Fig. 4D]. No significant difference was found for the mRNA expression of neutrophil chemokines such as chemokine (C-X-C motif) ligand (CXCL1), CXCL2, and CXCL5 (Table 4).

Chemotaxis of monocyte/macrophages. Since MPO-deficient mice showed decreased renal infiltration of monocytes/macrophages, we next performed a chemotactic assay to explore the chemokine-mediated recruitment of monocytes/macrophages. Peritonitis-elicted macrophages were isolated from MPO−/− and wild-type mice. Chemotaxis of monocyte/macrophages derived from wild-type mice could be induced by CCL2. However, chemotaxis was significantly lower toward CCL2 in MPO−/− mouse-derived monocytes/macrophages than in wild-type mouse-derived monocytes/macrophages (Fig. 4E).

Systolic blood pressure and cardiac changes. Slightly increased systolic blood pressure was found after renal ablation without significant differences between MPO-deficient and wild-type mice (Fig. 5A). Along this line, the relative heart weight was also increased similarly in both genotypes after renal ablation without a significant difference between MPO-deficient and wild-type mice (Fig. 5B).

Basal phenotype. We finally confirmed that MPO-deficient mice have no basal renal phenotype. Light microscopy showed no glomerular or tubulointerstitial abnormalities. No significant difference in albuminuria was found [MPO−/− mice: 0.20 (0.18, 0.42) mg albumin/mg creatinine and wild-type mice: 0.17 (0.13, 0.26) mg albumin/mg creatinine]. In addition, RT-PCR analysis of mRNA deriving from the whole kidney cortex showed no significant difference between MPO-deficient and wild-type mice for PAI-I [1.30 (0.88, 2.05) vs. 0.95 (0.75, 1.38)], CCL2 [0.84 (0.59, 1.18) vs. 1.00 (0.64, 1.67)], collagen type IV [1.19 (0.93, 1.84) vs. 1.03 (0.87, 1.15)], or MMP-2 [1.33 (1.05, 1.50) vs. 1.03 (0.77, 1.29)]. For albuminuria, n = 7 MPO−/− animals and 8 wild-type animals; for RT-PCR analysis, n = 8 MPO−/− animals and 10 wild-type animals.

DISCUSSION

To date, no study has investigated the role of MPO in the initiation and progression of CKD. In the present study in mice, we found threefold higher MPO plasma concentrations 10 wk after renal ablation. This suggests but does not prove that MPO plays a role in CKD. Therefore, we next examined whether the absence of MPO would attenuate the renal injury in CKD. The finding of reduced albuminuria, glomerular injury, and diminished invasion of immune cells into the remnant kidney in MPO knockout mice provides the first evidence for a pathogenic role of MPO released from neutrophils or monocytes/macrophages in the progression of CKD induced by renal ablation.

Renal ablation, a mainstay in experimental kidney research, was used for CKD induction (2). This model features all the clinical hallmarks of CKD, including substantial albuminuria, uremia, and glomerulosclerosis. The amount of removed kidney tissue was nearly identical in wild-type and MPO knockout mice, indicating that the primary experimental stimulus to induce CKD did not differ between both genotypes. Interestingly, albuminuria was identical in both genotypes 1 wk after renal ablation, indicating that MPO deficiency has no effect on the acute responses to renal mass reduction. We used a number of quantitative and semiquantitative measures to gauge the extent of renal injury induced by renal ablation. Renal injury,
as assessed by scoring of glomerular changes in PAS-stained renal sections, was confirmed by upregulation of markers of renal injury and fibrosis such as collagen and PAI-1. To further characterize the molecular basis of MPO-dependent glomerular injury, we determined the renal expression of MMP-2 and MMP-9. MMPs comprise a group of proteases responsible for the degradation of extracellular matrix proteins, which can result in augmented turnover of the extracellular matrix and release of bioactive fragments and growth factors (28) and is considered a profibrotic event. In a murine model of angiotensin II-induced atrial fibrillation, MPO has been shown to promote the activation of MMP-2 and MMP-9 in cardiac tissue.

Fig. 2. A: representative micrographs of periodic acid-Schiff-stained sections (400-fold magnification). Compared with controls, enlarged glomeruli with glomerular sclerosis and tubulointerstitial damage were found 10 wk after ablation. The glomerular injury was less prominent in MPO−/− mice compared with WT mice. B: scoring of the glomerular injury showed a significantly lower level of injury in MPO−/− mice compared with WT mice. C: no significant difference was found for interstitial damage. D and E: representative confocal micrographs of double immunohistochemical stainings against the podocyte marker nephrin (red) and the lysosomal marker lysosome membrane protein 2 (Limp2; green; D) or nephrin and the autophagosomal marker microtubule-associated protein 1 light chain (LC)3B (green; E) in control mice and mice after renal ablation in both genotypes. DRAQ5 (blue) was used as a nuclear counterstain. D: an increased number of Limp2-positive lysosomes in podocytes (*) was found after renal ablation, and the number was higher in WT mice than in MPO−/− mice. A similar pattern was found for the lysosomes in tubules. The tubules are shown in black and white as well as color for better discriminatory power. Glomeruli are marked by circles. E: a similar glomerular pattern was found for the autophagosomal marker LC3. Because of weak staining, LC3 was not evaluated in tubules at low power. Graphs are shown with logarithmic scaling.
since a reduction of both proteinases was found in MPO-deficient mice (35). A similar reduction of MMP-2 and MMP-9 expression was observed in the present study in the kidney of MPO-deficient mice.

Podocyte injury is a key determinant of glomerular injury and progression of CKD. Recent data have suggested that autophagy in podocytes influences glomerular disease susceptibility and albuminuria (11). We therefore evaluated the podocyte autophagosomal/lysosomal system in the present study by immunohistochemical staining of LC3 and Limp2. In correlation with renal injury and albuminuria, an increased amount of autophagosomal and lysosomal vesicles was found after renal ablation in both genotypes, but a lower number of vesicles was found in MPO-deficient mice. A similar pattern was found for tubular lysosomal vesicles. The exact role of autophagy in renal disease is unclear. Recent data have suggested a protective role of autophagy in proximal tubular cells since transgenic or pharmacological inhibition of autophagy aggravated in vitro and in vivo injury (14, 15). The following possibilities can be envisioned: by digesting cellular constituents, autophagy generates substrates for ATP production. Autophagy has a housekeeping function by removing potentially cytotoxic components. In addition, antiinflammatory effects have been described for autophagy (15). MPO-derived reactive species induce apoptosis in neutrophils and neutrophil-like cells (29) and in endothelial cells (36). Controversially, it has been proven that the MPO protein, by binding to CD11b/CD18 integrins, delays apoptosis of neutrophils and prolongs their lifespan, thereby aggravating inflammation (7).

For a long time, apoptosis was considered the sole form of programmed cell death during disease, whereas necrosis was regarded as an unregulated process. Recent data have revealed that necrosis can also occur in a regulated manner (23, 38, 39). The initiation of programmed necrosis, called necroptosis, requires the kinase activity of receptor-interacting proteins. Necroptosis has been described in ischemia-reperfusion injury, but no data are available on the role of necroptosis in CKD and whether MPO plays a role in necroptosis. More work has to be done on MPO and cell death pathways.

One of the central mechanisms in inflammation is the recruitment of leukocytes from the vasculature into the surrounding tissue. This process follows a well-defined cascade of events and is orchestrated by a complex interplay of adhesion molecules, cell surface receptors, and chemokines. Increasing
Fig. 4. Infiltration of inflammatory cells was measured by immunohistochemistry. A–C: representative micrographs (400-fold magnification) and scoring of F4/80-positive monocytes/macrophages (A), CD3-positive T cells (B), and granulocyte differentiation antigen 1 (GR-1)-positive neutrophils (C). Scoring revealed a significant decreased infiltration of monocytes/macrophages and T cells in MPO−/− mice compared with WT mice. D: in line with this finding, renocortical RNA expression of chemokine (C-C motif) ligand (CCL)2 was also lower in MPO−/− mice compared with WT mice. E: monocytes/macrophages isolated from MPO−/− mice showed significantly less migration toward CCL2 than WT monocytes/macrophages. Data are shown with logarithmic scaling.
Evidence supports a role of MPO and MPO-derived oxidants in leukocyte recruitment (31). In vitro data have suggested that catalytically active as well as catalytically inactive MPO participates in leukocyte recruitment (31). The in vivo relevance of this observation was recently confirmed by demonstrating proadhesive effects of catalytically active as well as inactive MPO in different models of inflammation (20). In the present study, MPO knockout mice exhibited significantly less renal tissue infiltration of monocytes/macrophages and T cells than wild-type mice. Consistent with a dampened immune response to renal ablation, induction of the chemokine CCL2 was reduced in MPO knockout mice.

The association of monocytes/macrophages and tissue damage in renal disease has been established (5, 21). In our study, reduced mRNA expression of CCL2 and a lower number of monocytes/macrophages were found in the kidney of MPO-deficient mice. To address the question of whether this was due to a primary impairment of chemotaxis, we performed chemotactic assays using peritoneal monocytes/macrophages. Monocytes/macrophages derived from a MPO-deficient source showed impaired migratory capacity compared with wild-type cells. In contrast, no significant difference was found in vivo for the induction of CXCL1, CXCL2, and CXCL5, which cause recruitment of neutrophils. MPO released by activated neutrophils has been shown to be important in the activation and adhesion of other neutrophils. MPO facilitates neutrophil extravasation via integrins (16). Moreover, decreased migration of MPO-deficient neutrophils in vitro in response to LPS has been shown (10). Decreased infiltration of MPO-deficient neutrophils has also been shown in vivo in renal as well as hepatic ischemia-reperfusion injury and LPS-induced pulmonary injury (10, 20, 26). The role of neutrophils in CKD is largely unknown. Recent data have suggested that granulocyte colony-stimulating factor has beneficial effects in kidney injury (42). Protective functions (i.e., enhancement of barrier function of epithelial cells) for neutrophils have been described during colitis (43).

Severe glomerular and tubulointerstitial injury is found after renal ablation. Interestingly, whereas glomerular damage and albuminuria were significantly attenuated in MPO-deficient mice, no significant differences were found for interstitial injury and plasma levels of urea-N. We found exactly the same separation of glomerular and interstitial injury as well as renal function in a recent study in which mice were treated with aliskiren and losartan after renal ablation. Both treatments lowered glomerular injury and albuminuria but had no significant effect on interstitial injury and urea-N (8). Since blockade of the renin-angiotensin system is the best available nephroprotective strategy in CKD, this suggest important differences between the pathogenesis and treatment of glomerular sclerosis and albuminuria and the pathogenesis and treatment of interstitial injury and renal function in the renal ablation model. Completely different treatment approaches (blockade of the renin-angiotensin system and MPO deficiency) do not influence tubulointerstitial injury, whereas they positively influence glomerular injury and albuminuria.

Our results are in line with recent data showing a MPO-dependent acceleration of injury in immune-mediated inflammatory syndromes, such as multiple sclerosis (30), acute coronary syndrome (1), ischemia-reperfusion (26), and atrial fibrillation (35). Furthermore, MPO contributes to the dysfunction of the local vasculature during inflammation by modifying local nitric oxide production and availability (6). However, MPO has also been shown to exert protective effects in different infectious diseases. Brennan et al. (4) found increased atherosclerosis in MPO-deficient mice. The same investigators (3) also reported that MPO−/− mice are more susceptible to experimental autoimmune encephalomyelitis, most likely due to an enhanced proliferation of T cells. Similarly, enhanced lung inflammation was found in MPO−/− recipients after allogenic bone marrow transplantation (27).

In the present study, MPO-deficient mice showed significantly less albuminuria and glomerular damage than control mice after renal ablation, but the MPO deficiency did not completely prevent the renal injury. However, this cannot be expected in this model since we have recently shown that even maximal blockade of the renin-angiotensin system lowers but does not normalize renal injury (8). Given the impact of MPO on inflammatory processes, pharmacological strategies to in-

![Fig. 5. A and B: systolic blood pressure (A) and relative heart weight (B). No significant differences were found between WT and MPO−/− mice for both parameters. Data are shown with logarithmic scaling.](http://ajprenal.physiology.org/)

### Table 4. Expression of neutrophil chemokines

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Ablation</th>
<th>Ablation MPO−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative mRNA Expression</td>
<td>CXCL1</td>
<td>CXCL2</td>
<td>CXCL5</td>
</tr>
<tr>
<td>Median</td>
<td>0.81</td>
<td>1.01</td>
<td>1.46</td>
</tr>
<tr>
<td>25th percentile, 75th percentile</td>
<td>0.55, 1.69</td>
<td>0.41, 2.68</td>
<td>0.35, 2.84</td>
</tr>
</tbody>
</table>

**Fig. 5.** A and B: systolic blood pressure (A) and relative heart weight (B). No significant differences were found between WT and MPO−/− mice for both parameters. Data are shown with logarithmic scaling.
hibit MPO have been developed and tested in different animal models (37, 41). It has been shown that MPO inhibition leads to a reduction of oxidative stress in animal models of inflammation (37). It will be of interest to investigate the combined blockade of the renin-angiotensin system and MPO in CKD.

What is the mechanistic role of MPO in CKD? Plasma levels of MPO were threefold elevated after 5/6 nephrectomy. The beneficial effect of MPO deficiency is most likely not due to differences between resident renal cells of wild-type and MPO-deficient mice since these cells do not express MPO. Expression of MPO is restricted to the myeloid lineage of hematopoietic cells, where it is mainly found in neutrophils and monocyes/macrophages (31). Monocyte/macrophages and neutrophils are found abundantly in the kidney after 5/6 nephrectomy in the stage where CKD is in full development, as shown in Fig. 4, B and C. Interestingly, renal cells, like endothelial cells, can uptake MPO released from neutrophils. This occurs by a cell contact-dependent, \( \beta_2 \)-integrin-mediated transfer from neutrophils (13). Taken together, the evidence provided here, documenting elevated serum levels of MPO after renal ablation and less proteinuria and glomerular injury in MPO-deficient mice, suggests that MPO is involved in the pathophysiology of CKD. Given that current pharmacological strategies to treat CKD are, for the most part, of limited clinical efficacy and directed mainly to the renin-angiotensin system, MPO may serve as a potential new target for the treatment of CKD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


