Supplemental Figure 1. Circulating myeloid cells and anti-MPO IgG titer in CCR2$^{-/-}$ and C/EBPβ$^{-/-}$ chimeric mice. (**A**) Gating strategy for myeloid cells analyzed by flow cytometry. CD45^{+} immune cells were further characterized after exclusion of doublets. Neutrophils were determined as CD11b^{+}Ly6G^{+} cells. Ly6C^{hi} classical (CM) and Ly6C^{lo} non-classical monocyte (NCM) subsets were defined from monocytes gated as CD11b^{+}Ly6G^{-}CD115^{+}. (**B**) Circulating neutrophils, classical (CM), and non-classical (NCM) monocytes determined by flow cytometry in CCR2$^{-/-}$ and WT chimeric mice. CM and NCM were decreased in CCR2$^{-/-}$ chimeric mice. Cells were defined using the gating strategy above and expressed as percentage (%) of CD45^{+} cells. (**C**) Anti-MPO titer in CCR2$^{-/-}$ and WT chimeric mice was determined in serum by ELISA at week 4 and 8 after bone marrow transplantation. (**D**) Circulating neutrophils, classical (CM), and non-classical (NCM) monocytes determined by flow cytometry in CCR2$^{-/-}$ and WT chimeric mice. CM and NCM were decreased in C/EBPβ$^{-/-}$ chimeric mice. Cells were defined using the gating strategy above and expressed as percentage (%) of CD45^{+} cells. (**E**) Anti-MPO titer in C/EBPβ$^{-/-}$ and WT chimeric mice was determined in serum by ELISA at week 4 and 8 after transplantation. **P<0.01; ***P<0.001.

Supplemental Figure 2. Gating strategy for kidney-infiltrating myeloid cells analyzed by flow cytometry. CD45^{+} immune cells were analyzed after exclusion of doublets and dead cells. Neutrophils were defined as CD11b^{+}Ly6G^{+} cells, classical monocytes (CM) as CD11b^{+}Ly6G^{-} Ly6C^{hi} cells, and non-classical monocytes (NCM) as CD11b^{+}Ly6G^{-}Ly6C^{-}MHC-II^{-}CD11c$^{mid/+}$ cells. Counting beads were used for quantification.

Supplemental Figure 3. Renal immune cell recruitment in mice with anti-MPO IgG-mediated NCGN. IHC staining for Gr-1, CD68, F4/80 and CD3 in renal sections from MPO$^{-/-}$/WT, MPO$^{-/-}$

/CCR2$^{-/-}$ and MPO$^{-/-}$/Csf2rb$^{-/-}$ chimeric mice. A representative image for each group and marker is shown.

Supplemental Figure 4. Circulating myeloid cells and anti-MPO IgG titer in Csf2rb$^{-/-}$ chimeric mice. (**A**) Circulating lymphocytes, neutrophils and monocytes were determined in Csf2rb$^{-/-}$ and WT chimeric mice by flow cytometry. (**B**) Anti-MPO titer in Csf2rb$^{-/-}$ and WT chimeric mice was determined in serum by ELISA at week 4 and 8 after bone marrow transplantation.

Supplemental Figure 5: **Differentiation and stimulation of human monocyte-derived macrophages.** (A) Characteristic morphology of monocyte-derived macrophages following 6 days of differentiation with either GM-CSF or M-CSF. A representative image for each subset is presented. (B) Representative immunoblot showing a strong MPO expression in neutrophils (PMN) and monocytes, but not in monocyte-derived macrophages differentiated 6 days with either GM-CSF or M-CSF. Actin served as loading control. (C) TNFα-primed monocyte-derived macrophages were stimulated 4 hours with either isotype IgG or anti-MPO IgG. Unstimulated cells and cells stimulated with LPS (1 µg/ml)/ATP (2 mM) were used as negative and positive control, respectively. IL-1β release was detected in the culture medium by ELISA. (D) TNFα-primed monocyte-derived macrophages were stimulated 1 hour with either isotype IgG or anti-MPO IgG. Unstimulated cells and cells stimulated with PMA (100 ng/ml) were used as negative and positive control, respectively. Extracellular reactive oxidative species (ROS) were detected by ferricytochrome reduction assay.

Supplemental Figure 6. Gating strategy for the detection of myeloid cells in urine from AAV patients and healthy controls. Dead cells were excluded from the analysis. Monocytes and

granulocytes were first identified on forward versus sideward scatter (FSC vs SSC) gating. Neutrophils were further characterized as $CD11b^+CD66b^+CD16^+$ cells. To exclude any contaminating lymphocytes, monocytes were discriminated based on their HLA-DR and CD14 expression. Then, classical (CM), intermediate (IM) and non-classical (NCM) monocytes were determined based on their expression of CD14 and CD16 cell surface receptors.

Supplemental Table 1. Summary of population characteristics. Values as medians (inter-quartile range). kAAV, kidney ANCA-associated vasculitis; Rem, remission; HC, healthy control; BVAS, Birmingham Vasculitis Activity Score; CRP, C-reactive protein; SCrea, serum creatinine; UPCR, urinary protein creatinine ratio

Supplemental Materials and Methods

Materials

Complete and incomplete Freund´s adjuvant, liberase, DNAse, fetal bovine serum (FBS), bovine serum albumin (BSA), phorbol 12-myristate 13-acetate (PMA) and tumor necrosis factor-α (TNF-α) were from Sigma-Aldrich (Steinheim, Germany). Ionomycin was purchased from Calbiochem-Merck (Darmstadt, Germany), RPMI 1640 medium and PBS without magnesium and calcium ions from Invitrogen by Thermo Fisher Scientific (San Diego, CA, USA), and 2-mercaptoethanol from Gibco by Thermo Fisher Scientific.

Human Samples

Blood from healthy human donors was obtained after approval by Charité and after written informed consent. Urine was collected from patients with either active AAV or in remission,

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and from healthy controls. Collection and analysis of human samples were approved by the local ethics committee (EA3/011/08, EA3/011/06, EA2/103/17, and EA1/034/10). The use of archived renal specimens was approved by the local Ethics committee (reference number 4415).

Animal Experiments

Mice were kept under specific pathogen-free conditions at the Max Delbrück Center for Molecular Medicine (Berlin, Germany) animal facility. The purification of murine MPO from WEHI-3 cells and polyclonal anti-MPO IgG from immunized MPO-deficient mice, immunization, and irradiation protocols for $MPO^{-/-}$ were performed as previously described.[1] In brief, $MPO^{-/-}$ mice were immunized intraperitoneally with murine MPO in complete Freund's adjuvant, boosted intraperitoneally after 4 weeks with murine MPO in incomplete Freund's adjuvant, lethally irradiated and subsequently transplanted intravenously with bone marrow cells ($1.5x10^7$) from either C57BL/6J WT (The Jackson Laboratory), $CCR2^{-/-}$ (kindly provided by Dr. Susan Brandenburg, Charité-Universitätsmedizin Berlin, Germany), $C/EBP\beta^{-/-}$ (kindly provided by Prof. Achim Leutz, MDC, Berlin, Germany) or $Csf2rb^{-/-}$ (kindly provided by Prof. Dr. Michael Sieweke, Center for Regenerative Therapies Dresden (CRTD), Dresden, Germany) mice. All mice were on Bl6 background except the $C/EBP\beta^{-/-}$ mice which had a 129S1/Sv*C57BL/6J mixed genetic background. Nephrotoxic nephritis (NTN) was induced in mice by intraperitoneal injection of 10µl/g of nephrotoxic sheep serum (Probetex, San Antonio, TX, USA) per mouse. For CSF2 neutralization, mice were treated daily with 200 µg rat anti-mouse GM-CSF (Clone MP1-22E9 purified in vivo GOLD, Leinco Technologies, St. Louis, MO, USA) antibody intraperitoneally. Control mice were injected intraperitoneally with an equal amount of rat IgG2a isotype antibody (clone 2A3, BioXCell, Lebanon, NH, USA). Antibody

treatment started the day of NTN induction. Animals were sacrificed 6 days later. Animal experiments were approved by the local authorities (Landesamt für Gesundheit und Soziales, Berlin, Germany) and followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.[2]

Human and Murine Monocyte Isolation

Human monocytes were isolated as described previously.[3] Monocytes were prepared from the PBMC fraction by culture for 1 hour at 37°C in DMEM in cell culture dishes; attached cells were washed twice in PBS and resuspended in RPMI medium. Cell viability by trypan blue exclusion was found to be greater than 95% in every experiment. Cells were >95% monocytes.

Mouse monocytes were isolated from bone marrow of mice using the Monocyte Isolation Kit (BM) from Miltenyi Biotec (Bergisch Gladbach, Germany), following the manufacturer's instructions.

Histologic Examination of Renal Damage

Renal tissues were collected at the time of euthanasia in cold PBS, fixed in 4% formalin overnight, and embedded in paraffin using routine procedures. Sections were stained with Periodic acid–Schiff and all glomeruli on each kidney section assessed by light microscopy using an Axio Imager M2 microscope (Zeiss, Jena, Germany). Glomerular crescents and necrosis were expressed as the mean percentage of glomeruli with crescents and necrosis in each animal and scored in a blinded manner.

Histologic Examination of Renal Damage and Leukocyte Infiltration

For the detection of myeloid cells by immunohistochemistry, sections of snap-frozen kidney tissues (4 µm thick) were stained with antibodies for neutrophil Gr-1 (#553126, BD Biosciences) and for monocyte/macrophage CD68 (#MCA1957F, Serotec-BioRad, Feldkirchen, Germany) and F4/80 (#123124, Biolegend). Rat antibody binding was detected using horseradish peroxidase-labeled secondary rabbit anti-rat IgG and tertiary goat anti-rabbit IgG antibodies from Dako (Glostrup, Denmark). 3-amino-9-ethylcarbazole and hydrogen peroxide (Sigma-Aldrich) were used as substrates. Sections were counterstained with hematoxylin and mounted in Faramount aqueous mounting medium from Dako. For the detection of T cells by immunohistochemistry, paraffin-embedded sections were deparaffinized, rehydrated and stained with anti-CD3 antibody (#A0452, Dako). Antigen retrieval was performed in citrate buffer, pH 6.0, for 20 min. Antibody binding was detected using the rabbit specific HRP/AEC IHC detection kit from Abcam following the manufacturer's instructions.

Renal Immunofluorescence of Human Biopsies

In situ hybridization, for detection of GM-CSF RNA in FFPE tissue, was performed using RNAscope® Probe-Hs-CSF2 (313981; ACD, Hayward, CA, USA) and the RNAscope 2.5 HD RED Kit (322350; ACD). After deparaffination in xylene and dehydration in ethanol, the 4 µm thick FFPE sections were blocked with peroxidase for 10 min. Using the kit-provided antigen retrieval buffer, slides were boiled at 95°C for 15 min and digested wit protease at 40°C for 30 min. The kidney sections were hybridized with the target probe for CSF2 in the HybEZ hybridization oven (ACD) at 40°C for 2h. The following pre-amplification and amplification steps were conducted according to manufacturer´s recommendations. Signals were detected by incubation of the sections with Fast Red substrate for 10 min at room temperature, counterstaining was conducted with hematoxylin, followed by drying at 60°C for 15 min and

mounting with Eco Mount (ACD). For quality check, additional samples were hybridized with probes for PPIB as positive control and DapB as negative control.

Functional Measurement of Renal Damage

Mice were transferred to metabolic cages with free access to water and food 1 day before they were euthanized. Urine was collected over 16 hours. Proteinuria, erythrocyturia, and leukocyturia were screened in urine with dipsticks from Roche Diagnostics (Indianapolis, IN, USA). Results were expressed on a scale of zero (none) to four (severe) for erythrocyturia, and of zero (none) to three (severe) for proteinuria and leukocyturia. Urine albumin concentration was determined by ELISA (Bethyl Laboratories, Inc., Montgomery, TX, USA). NGAL concentration in urine was determined with the NGAL (LCN2) ELISA Kit from Dianova (Hamburg, Germany).

Monocyte Supernatants

Monocytes were isolated from bone marrow of WT mice using the Monocyte Isolation Kit (BM) from Miltenyi Biotec (Bergisch Gladbach, Germany), following the manufacturer's instructions. Monocytes were primed in RPMI medium for 15 minutes with 5 ng/ml of TNFα and subsequently incubated with or without anti-MPO IgG for 4 hours at 37°C. Unstimulated cells were used as negative control. In some conditions, monocytes were pre-incubated 30 minutes with 10 ng/ml recombinant murine CSF2 (R&D System, Wiesbaden-Nordenstedt, Germany) before TNFα priming. At the end of the experiment, supernatants were collected after centrifugation, stored at -20°C and used for ELISA and co-culture with $CD4^+$ T cells.

$CD4^+$ T Cell Polarization *in vitro*

$CD4^+$ T cells were isolated from spleens of WT mice using the $CD4^+$ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), following the manufacturer's instructions. Isolated $CD4^+$ T cells were seeded in a 96-well plate ($2.5x10^5$ cells/well) coated with anti-CD3 (0.5 µg/ml; clone 145-2C11, BD Biosciences) and anti-CD28 (0.2 µg/ml; clone 37.51, BD Biosciences) antibodies. Cells were cultured in RPMI 1640 medium with 10% FBS, 50 µM 2-mercaptoethanol and supplemented with 10 µg/ml antibodies (anti-IFNγ, anti–IL-2 and anti–IL-4) from the CytoBox T_H17 Kit (Miltenyi Biotec). Supernatants from stimulated monocytes were added (to a final monocyte:T cell ratio of 5:1) at day 0 and 2. After 4 days in culture, cells were collected and T_H17 polarization was assessed by flow cytometry.

Human Macrophage Differentiation and Stimulation

Human monocytes were cultured 6 days in RPMI medium containing 100 ng/ml of either recombinant human M-CSF or GM-CSF (R&D System) for macrophage differentiation. Medium was changed every second day. For the determination of IL-1β production, monocyte-derived macrophages were primed 15 minutes with 2 ng/ml TNFα and subsequently stimulated with anti-MPO monoclonal antibody (clone 2C7, OriGene, Herford, Germany) or control IgG (R&D System). Unstimulated cells and cells stimulated with LPS/ATP were used as negative and positive controls, respectively. After 4 hours stimulation, supernatants were collected and human IL-1β was determined by ELISA (R&D System) following the manufacturer's instructions. Extracellular ROS production was determined after one hour stimulation using the ferricytochrome C reduction assay. Monocyte-derived macrophages were pretreated with 5 mg/ml cytochalasin B (Sigma-Aldrich) for 15 minutes. Cells were further incubated with 300 U/ml superoxide dismutase (Sigma-Aldrich) and then primed with TNFα for 15 minutes before stimulation with antibodies as described above. Unstimulated cells and cells stimulated with

100 ng/ml PMA (Sigma-Aldrich) were used as negative and positive controls, respectively. Samples were incubated in 96-well plates at 37°C and the absorption of samples was scanned repetitively at 550 nm using a Microplate Autoreader (Molecular Devices, Munich, Germany). Results are reported as nmol superoxide/well per hour.

Immunoblotting

Monocytes and macrophages were lysed in buffer containing 50mMTris/hydrochloride, pH 7.5, 150mM sodium chloride, 200 mM PMSF, 1% NP-40, 0.1% SDS, 0.5% deoxycholate, and supplemented with complete protease and phosphatase inhibitor cocktails. Protein lysates were resolved by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were probed with the following antibodies: rabbit anti-MPO (475915, Merck Millipore, Calbiochem, Darmstadt, Germany) and rabbit anti–β-actin (Cell Signaling Technology, Frankfurt am Main, Germany). Horseradish peroxidase–labeled donkey anti-rabbit IgG (GE Healthcare, Little Chalfont, United Kingdom) were used as secondary antibodies. The SuperSignal™ West Dura Chemiluminescent Substrate from Thermo Fisher Scientific was used for the detection.

Isolation of Renal Leukocytes

The isolation of murine renal leukocytes was performed as previously described.[4] In brief, kidney biopsies were minced with scissors and digested for 40 minutes at 37°C with 0.2 mg/ml liberase and 100 U/ml DNase in PBS without magnesium and calcium ions (PBS). Cell suspensions were washed with PBS, filtered through 70-μm meshes, resuspended in PBS/1% FBS, and further processed for flow cytometry.

Isolation of Leukocytes from Human Urine

Fresh urine samples with a volume of at least 20 ml were collected from all patients and were centrifuged within 6 hours at 2000 rpm for 8 minutes at 4°C. Supernatants were stored at -80°C for later detection of soluble proteins. Cell pellets were washed in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA), centrifuged as above and resuspended in PBS/1% BSA. Cell suspensions were kept on ice and further processed for the detection of myeloid cells by flow cytometry.

Flow Cytometry

Renal cell suspensions were incubated 15 minutes with anti-mouse CD16/32 antibody (Biolegend) to block Fc receptors. Cells were then incubated 20 minutes with the following fluorochrome-conjugated antibodies to identify renal myeloid cells by flow cytometry: CD45 (30F11; BD Bioscience, Heidelberg, Germany), CD11b (M1/70), Ly6C (HK1.4), and Ly6G (1A8), all from Biolegend (San Diego, CA, USA). Dead cells were excluded using the LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (ThermoFisher Scientific).

To identify T_H17 T cell subset, isolated $CD4^+$ cells and renal cell suspensions were stimulated for 4 hours with phorbol 12-myristate 13-acetate (50 ng/ml) and ionomycin (1 mg/ml) in the presence of 5µg/ml Brefeldin A (Biolegend) in X-VIVO10 medium (Biozym, Hessisch Oldendorf, Germany). After washing, cells were incubated 15 minutes with anti-mouse CD16/32 antibody cells and further stained 20 minutes with the following antibodies: anti-CD45 (30F11) from BD Biosciences, and anti-CD3 (17A2), anti-CD4 (GK1.5), and anti-CD8 (53-6-7) from Biolegend. After 15 minutes fixation with 4% paraformaldehyde, cells were washed with PBS and permeabilized with 0.1% nonidet P-40 (NP-40) for 20 minutes. For intracellular staining, cells were incubated 25 minutes at 4°C with anti–IL-17A (TC11-18H10.1)

and anti-IFNγ (XMG12) from Biolegend. Cells were then washed, resuspended in PBS and analyzed by flow cytometry.

Isolated human urinary immune cells were incubated 10 minutes on ice with FcR Blocker (Miltenyi Biotec, Bergisch Gladbach, Germany) in PBS/1% BSA and further stained 20 minutes with the following antibodies (all from Biolegend): anti-CD11b (ICRF44), CD14 (63D3), CD16 (3G8), CD11b (G10F5) and HLA-DR (L243). Cells were then washed, centrifuged and fixed 15 minutes with BD Cytofix/Cytoperm Fixation and Permeabilization Solution (BD Biosciences from Thermo Fisher Scientific). Cells were again washed, centrifuged and resuspended in PBS/1% BSA containing DAPI to exclude dead cells, and analyzed by flow cytometry. CountBright Absolute Counting Beads (Invitrogen by Thermo Fisher Scientific) were added to samples to quantify the amount of the different immune cell subsets.

All samples were kept on ice during the whole procedure and acquired using an FACS CANTO II flow cytometer with BD FACSDiva software version 6.1.2 (BD Biosciences). Data were analyzed using FlowJo software version 10 (Treestar, Ashland, OR).

Quantitative RT-PCR

Whole-kidney RNA was extracted with the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and reverse transcription was performed with The SuperScript III One-Step RT-PCR System (Thermo Fisher Scientific). Renal Csf2 gene expression was determined by quantitative RT-PCR using the QuantStudio 3 instrument (Thermo Fisher Scientific) and specific primers synthetized by BioTeZ Berlin-Buch GmbH (Berlin, Germany).

ELISAs

The concentration of murine IL-1β (Mouse IL-1 beta/IL-1F2 Quantikine ELISA Kit from R&D System) and human IL-1β (Human IL-1 beta/IL-1F2 Quantikine ELISA Kit from R&D

System) in monocyte cell culture supernatants was determined by ELISA, as per the manufacturer's guidelines. The concentration of CSF2 in mouse (Mouse GM-CSF DuoSet® ELISA from R&D Systems) and human (Human GM-CSF Quantikine HS ELISA Kit from R&D Systems) urines was measured by ELISA following the manufacturer's protocol.

Statistical analysis

Results are given as means ± SEM. Comparisons were made using ANOVA with post-hoc analysis, comparisons between two groups were done by unpaired t-test using GraphPad Prism8 software. Differences were considered significant at $p<0.05$.

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