**UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF**

Institut für Experimentelle Immunologie und Hepatologie

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# RENAL PROXIMAL TUBULAR EPITHELIAL CELLS EXERT IMMUNOMODULATORY FUNCTION BY DRIVING INFLAMMATORY CD4$^+$ T CELL RESPONSES.

**Dissertation**

zur Erlangung des Grades eines Doktors der Medizin
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vorgelegt von:

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***Deus in minimis maximus.***

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**RESEARCH ARTICLE** | *Inflammatory Mediators in Kidney and Bladder Diseases and Hypertension*

## Renal proximal tubular epithelial cells exert immunomodulatory function by driving inflammatory CD4$^+$ T cell responses

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[1]*Institute of Experimental Immunology and Hepatology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [2]*Institute of Pathology, University Hospital Eppendorf, Hamburg, Germany;* [3]*Institute of Cellular and Integrative Physiology, University Hospital Eppendorf, Hamburg, Germany; and* [4]*III, Medical Clinic University Hospital Eppendorf, Hamburg, Germany*

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**Breda PC, Wiech T, Meyer-Schwesinger C, Grahammer F, Huber T, Panzer U, Tiegs G, Neumann K.** Renal proximal tubular epithelial cells exert immunomodulatory function by driving inflammatory CD4$^+$ T cell responses. *Am J Physiol Renal Physiol* 317: F77–F89, 2019. First published April 24, 2019; doi:10.1152/ajprenal.00427.2018.—In immune-mediated glomerular diseases like crescentic glomerulonephritis (cGN), inflammatory CD4$^+$ T cells accumulate within the tubulointerstitial compartment in close contact to proximal and distal tubular epithelial cells and drive renal inflammation and tissue damage. However, whether renal epithelial cell populations play a role in the pathogenesis of cGN by modulating CD4$^+$ T cell responses is less clear. In the present study, we aimed to investigate the potential of renal epithelial cells to function as antigen-presenting cells, thereby stimulating CD4$^+$ T cell responses. Using a FACS-based protocol that allowed comparative analysis of cortical epithelial cell populations, we showed that particularly proximal tubular epithelial cells (PTECs) express molecules linked with antigen-presenting cell function, including major histocompatibility complex class II (MHCII), CD74, CD80, and CD86 in homeostasis and nephrotoxic nephritis, a murine model of cGN. Protein expression was visualized at the PTEC single cell level by imaging flow cytometry. Interestingly, we found inflammation-dependent regulation of epithelium-expressed CD74, CD80, and CD86, whereas MHCII expression was not altered. Antigen-specific stimulation of CD4$^+$ T cells by PTECs in vitro supported CD4$^+$ T cell survival and induced CD4$^+$ T cell activation, proliferation, and inflammatory cytokine production. In patients with antineutrophil cytoplasmic antibody-associated glomerulonephritis, MHCII and CD74 were expressed by both proximal and distal tubules, whereas CD86 was predominantly expressed by proximal tubules. Thus, particularly PTECs have the potential to induce an inflammatory phenotype in CD4$^+$ T cells in vitro, which might also play a role in the pathology of immune-mediated kidney disease.

antigen presentation; CD4$^+$ T cell stimulation; crescentic glomerulonephritis; proximal tubular epithelial cells

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### INTRODUCTION

In immune-mediated kidney disease like crescentic glomerulonephritis (cGN), lymphocytes infiltrate the kidney and localize within the tubulointerstitial compartment in close contact to proximal and distal tubules. This enables direct interaction between leukocytes and renal epithelial cell populations that might contribute to the pathogenesis of kidney disease (48, 1, 32). Major histocompatibility complex class II (MHCII)-driven antigen presentation by epithelial cells has been suggested to play a role in the initiation and/or regulation of CD4$^+$ T cell responses during inflammation (26). In the kidney, several studies have demonstrated low expression of MHCII by renal proximal tubular epithelial cells (PTECs) of healthy humans (15) and naive mice and rats (19, 36), which was upregulated in patients with renal cell carcinoma (15) as well as murine lupus nephritis (57), graft-versus-host disease (49), and kidney transplants (2, 29). Moreover, antigenic stimulation of T cells by activated PTECs has been shown in vitro (19).

Beside MHCII-triggered T cell receptor (TCR) signaling, a second signal is required for full T cell activation. The second signal is induced by CD28-mediated T cell interactions with the costimulatory molecules CD80 and CD86 expressed by antigen-presenting cells (APCs). There are conflicting data regarding renal epithelial cell expression of the costimulatory molecules CD80 and CD86. Several studies did not determine expression of both in homeostasis and lupus nephritis (20, 28, 55), whereas other studies showed expression of CD80 and CD86 by PTECs of patients with interstitial inflammation (41) and CD80 by tubular cells of patients with IgA nephropathy (56). Furthermore, the MHCII invariant chain (CD74) plays a critical role in the process of antigen presentation by directing the transport of MHCII molecules and modulating antigenic peptide loading (52). CD74 expression was found in tubular cells in murine lupus nephritis (61) as well as activated parietal epithelial cells in murine focal segmental glomerulosclerosis (59), murine and human cGN, and patients with IgA nephropathy (11).

However, whether renal epithelial cell populations have the ability to function as APCs in cGN, thereby modulating CD4$^+$ T cell responses, is less clear. In the present study, we assessed the immunomodulatory potential of renal epithelial cell popu-

  

lations in mice with nephrotoxic nephritis (NTN), a murine model of cGN, and patients with antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN) by analyzing epithelial expression of MHCII, CD74, and costimulatory molecules in both kidney homeostasis and inflammation and by determining the phenotype of PTEC-activated $CD4^+$ T cells in vitro.

## MATERIALS AND METHODS

*Animals.* Male C57BL/6 and OT-II mice were bred in the animal facility of the University Medical Center Hamburg-Eppendorf (Hamburg, Germany). Mouse experiments were conducted according to the German animal protection law and approved by the institutional review board (Behörde für Gesundheit und Verbraucherschutz, Hamburg, Germany, G20/13). Mice received humane care according to national guidelines of the National Institutes of Health in Germany.

*Human samples.* Kidney biopsies were obtained from five anonymized patients with diagnosed ANCA-associated cGN. All human samples are handled in accordance with the Declaration of Helsinki.

*Animal treatment.* NTN was induced by intraperitoneal injection of 2.5 mg nephrotoxic sheep serum per gram of mouse body weight, as previously described (30). Mice were euthanized 4, 8, or 14 days after NTN induction. To assess serum creatinine levels, heart blood was drawn from individual mice. One day before euthanization, mice were housed in metabolic cages for urine collection. Albuminuria was determined by ELISA (Mice-Albumin Kit, Bethyl, Montgomery, TX).

*Histological analysis.* Paraffin-embedded human kidney sections were stained with anti-MHCII, anti-CD74, and anti-CD86 antibodies (all Bioss Antibodies, Woburn, MA). The ZytoChem-Plus AP Polymer-Kit (Zytomed Systems, Berlin, Germany) was used for antigen detection. Before MHCII and CD86 staining, heat-induced epitope retrieval was done using Dako Target Retrieval Solution (Agilent Technologies, Santa Clara, CA). Before CD74 staining, antigen retrieval was done using citric-acid citrate buffer (pH 6). Immunohistochemistry of murine kidney tissue was performed by routine procedures as previously described (42). Crescent formation was assessed in 30 glomeruli/mouse in a blinded fashion in paraffin-embedded, periodic acid-Schiff-stained kidney sections. Murine kidney sections were stained with anti-CD3 antibody (A0452, Dako, Hamburg, Germany). Tissue sections were developed with the Vectastain ABC-AP kit (Vector Laboratories, Burlingame, CA).

*FACS of renal epithelial cell populations.* Kidneys were harvested from naive and nephritic mice 4, 8, or 14 days after the induction of NTN. The renal medulla and adrenal structures were removed, and the remaining cortexes were finely minced. Renal cortex tissue was digested in DMEM-F-12-GlutaMAX medium (ThermoFisher Scientific, Waltham, MA) containing 0.25% BSA (Serva Electrophoresis, Heidelberg, Germany) and 0.01% collagenase from *Clostridium histolyticum* (Sigma-Aldrich, St. Louis, MO) at 37°C for 17 min. Thereafter, renal tissue was passed through a 250-μm sieve. Epithelial cells were separated from other cells by Percoll density gradient centrifugation using a solution that contains 45% Percoll (GE Healthcare Life Sciences, Chicago, IL) and 55% of 2× PBS-glucose. Epithelial cells, which accumulated in the interphase of the gradient, were removed and washed four times with 2× PBS-glucose. To prevent unspecific antibody binding, cells were incubated with anti-CD16/32 antibody solution (93, BioLegend, San Diego, CA). Epithelial cells were then stained with fluorochrome-labeled antibodies specific to E-cadherin (DECMA-1, PE), CD45 (30-F11), CD11c (N418), and CD11b (M1/70, all PerCP-Cy5.5, all BioLegend) as well as FITC-conjugated *Lotus tetragonolobus* lectin from the asparagus pea (Vector Laboratories) binding to α-linked L-fucose in the brush-border glycocalyx of proximal tubular epithelial cells. Subsequently, CD45/CD11b/$CD11c^-$ E-cadherin$^+$ *Lotus tetragonolobus* agglutinin $(LTA)^+$ PTECs and $LTA^-$ non-PTECs were isolated by FACS (BD FACSAria Fusion and a BD FACSAria III, Becton Dickinson, Franklin Lakes, NJ).

*Isolation of $CD4^+$ T cells and dendritic cells.* Ovalbumin (OVA)-specific $CD4^+$ T cells were isolated from the spleen and lymph nodes of naive OT-II mice. Splenic dendritic cells (DCs) were isolated from naive C57BL/6 mice. Tissue was passed through 70-μm nylon meshes before erythrocyte lysis using $NH_4Cl$. Thereafter, cells were incubated with anti-CD16/32 antibody solution. $CD4^+$ T cells were isolated by magnetic-activated cell sorting using the $CD4^+$ T cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The purity of 7 isolated $CD4^+$ T cells was ~96%. $CD11c^+$ DCs were isolated by magnetic-activated cell sorting using CD11c Microbeads (Miltenyi Biotec).

*In vitro culture experiments.* OVA-specific $CD4^+$ T cells were isolated from the spleen and lymph nodes of OT-II mice and cocultured with PTECs or splenic DCs from C57BL/6 mice in the presence of $OVA_{323–339}$ peptide (5 μg/ml) for 5 days. To block MHCII, cocultures were done in the presence of anti-MHCII antibody (M5/114.15.2, 5 μg/ml, ThermoFisher). To block costimulatory molecules, cocultures were done in the presence of anti-CD80 (16-10A1) and anti-CD86 (GL-1, both BioXCell, West Lebanon, NH) antibodies. Recombinant murine transforming growth factor (TGF)-β (BioLegend) was used at a concentration of 4 ng/ml.

*Flow cytometry.* Epithelial cells, splenocytes, and $CD4^+$ T cells were incubated with anti-CD16/32 antibody solution before antibody staining to prevent unspecific binding. LIVE/DEAD Fixable Staining Kits (ThermoFisher Scientific) were used to exclude dead cells. Cells were surfaced stained with fluorochrome-labeled antibodies specific to E-cadherin (DECMA-1, PE), CD74 (In1/CD74, Alexa Fluor 647), CD86 (GL-1, APC-Cy7), MHCII (M5/114.15.2, APC-Cy7), CD25 (PC61, BV605), TCRβ (H57-597, PE-Cy7), CD45 (30-F11, PerCP), CD11c (N418, PerCP, BV605), CD11b (M1/70, PerCP, all BioLegend), CD80 (16-10A1, BV421), Ki-67 (B56, FITC), and CD44 (IM7, BV421, all BD PharMingen). $CD4^+$ T cell were also stained with annexin V (FITC, BD PharMingen) and 7-aminoactinomycin D (7-AAD; PerCP, BioLegend). Renal epithelial cells were further stained with FITC-conjugated *Lotus tetragonolobus* lectin (Vector Laboratories). For intracellular cytokine staining, $CD4^+$ T cells were restimulated with phorbol myristate acetate (10 ng/ml) and ionomycin (250 ng/ml) for 6 h with the addition of brefeldin A (1 μg/ml, all Sigma-Aldrich) and monensin (BioLegend) after 60 min. After surface and Live/Dead staining, cells were fixed in Fix/Perm solution before incubation in Perm/Wash buffer (Foxp3 Transcription Factor Staining Buffer Set, ThermoFisher Scientific) with antibodies specific to interferon (IFN)-γ (XMG1.2, FITC), IL-2 (JES6-5H4, BV605, both BioLegend), TNF-α (MP6-XT22, PE), and Foxp3 (FJK-16s, FITC, both ThermoFisher Scientific).

*Imaging flow cytometry analysis of PTECs and splenic DCs.* Renal epithelial cells were isolated as described above and incubated with anti-CD16/32 antibody solution. Cells were stained with antibodies directed against E-cadherin (DECMA-1, PE), CD74 (In1/CD74, Alexa Fluor 647), CD86 (GL-1, APC-Cy7), MHCII (M5/114.15.2, APC-Cy7), CD80 (16-10A1, BV421), and CD44 (IM7, BV421). Epithelial cells were further stained with FITC-conjugated *Lotus tetragonolobus* lectin. Splenocytes were stained with anti-CD16/32 antibody solution and antibodies specific to CD45 (30-F11, PerCP), CD11c (N418, BV605), CD44 (IM7, BV421), CD74 (In1/CD74, Alexa Fluor 647), MHCII (M5/114.15.2, APC-Cy7), CD80 (16-10A1, BV421), and CD86 (GL-1, APC-Cy7). Cells were resuspended in PBS containing 0.5% BSA and 2 mM EDTA and analyzed using Amnis ImageStream (Millipore Sigma, Billerica, MA). Image files were analyzed with Amnis IDEAS software (Millipore Sigma) as previously described (37).

*Quantitative real-time RT- PCR analysis.* Total RNA was isolated from FACS-sorted renal epithelial cells using the RNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was transcribed into cDNA using the Verso cDNA

Table 1. *Sequences of the primers used for the analysis of mRNA expression*

| Target | Primers | | Amplicon Length, bp | Annealing Temperature, °C |
|---|---|---|---|---|
| | Forward | Reverse | | |
| *β-Actin* | 5′-TGGAATCCTGTGGCATCCATGAAA-3′ | 3′-TAAAACGCAGCTCAGTAACAGTCCG-5′ | 348 | 56 |
| *Aqp1* | 5′-GCATCACCTCCTCCCTAGTC-3′ | 3′-GTGTAGTCAATCGCCAGCAG-5′ | 220 | 60 |
| *Aqp2* | 5′-CTTTGCCTCCACTGATGAGC-3′ | 3′-GAGGGGAACAGCAGGTAGTT-5′ | 243 | 60 |
| *Calb1* | 5′-TGAGCTGGATGCTTTGCTGA-3′ | 3′-GTACAGCTTCCCTCCATCCG-5′ | 118 | 60 |
| *Cxadr* | 5′-ATCGTTTACCTGCAAGCCAC-3′ | 3′-CAGACGGGGATATCAGCCAT-5′ | 226 | 60 |
| *H2-Ab1* | 5′-TCTGTGGAGGTGAAGACGAC-3′ | 3′-GGCCAAACTCAGGAAGCATC-5′ | 171 | 60 |
| *Cd74* | 5′-TGACCATCACCTCCCAGAAC-3′ | 3′-GTAACGTTCTTCACAGGCCC-5′ | 154 | 60 |
| *Cd86* | 5′-GCACGTCTAAGCAAGGTCAC-3′ | 3′-CATATGCCACACACCATCCG-5′ | 167 | 60 |
| *Cd80* | 5′-TAGTTTCTCTTTTTCAGGTTGTGAA-3′ | 3′-AGAGTTGTAACGGCAAGGCA-5′ | 395 | 60 |
| *Cd44* | 5′-CGCAGGTGTATTCCATGTGG-3′ | 3′-GCACAGATAGCGTTGGGATG-5′ | 204 | 60 |

Aqp, aquaporin; Calb1, calbindin 1; Cxadr, coxsackievirus and adenovirus receptor; H2-Ab1, major histocompatibility complex class II.

Synthesis Kit (Life Technologies, Carlsbad, CA) on a MyCycler thermal cycler (BioRad, München, Germany). Quantitative RT-PCR was performed using ABsolute qPCR SYBR Green Mix (Thermo Scientific). Relative mRNA levels were calculated using the $\Delta\Delta C_T$ method (where $C_T$ is threshold cycle) after normalization to the reference gene β-actin. Primers were obtained from Metabion (Martinsried, Germany). The sequences of the primers used are shown in Table 1.

*Statistical analyses.* Data were analyzed using GraphPad Prism software (GraphPad, San Diego, CA). Statistical comparison was carried out using a Mann-Whitney *U*-test or one-way ANOVA with post analysis by a Tukey-Kramer test. Data are expressed as means ± SE. *P* values of $<0.05$ were considered statistically significant.

## RESULTS

*Establishment of a FACS-based protocol for the isolation of murine primary PTECs.* To perform comparative expression analysis of epithelial cell populations from healthy and inflamed kidneys, we established a sorting strategy using FACS, in which we also excluded potentially contaminating professional APCs like DCs and macrophages. After dissection of the kidney medulla, renal cortex cells were digested with collagenase and subjected to a Percoll gradient to enrich epithelial cells by ultracentrifugation. Thereafter, cells were stained with fluorochrome-labeled antibodies directed against the epithelial cell-specific protein E-cadherin, the leukocyte-specific protein CD45, and the macrophage- and DC-specific proteins CD11b and CD11c. To distinguish between proximal and distal tubular epithelial cell populations, cells were further stained with the fluorochrome-labeled PTEC-specific marker LTA (51), which binds to brush-border glycoproteins of proximal tubuli. To remove professional APCs, we generated a dump channel that excluded $CD45^+$ leukocytes and $CD11b^+/CD11c^+$ macrophages and DCs. We then sorted for E-cadherin$^+$ LTA$^+$ PTECs and E-cadherin$^+$ LTA$^-$ epithelial cells, which we termed non-PTECs (Fig. 1*A*).

By performing mRNA expression analysis, we showed that E-cadherin$^+$ LTA$^+$ cells highly express aquaporin-1 (*Aqp1*), which forms a water channel specifically expressed in proximal tubules, whereas very low expression of *Aqp1* mRNA was

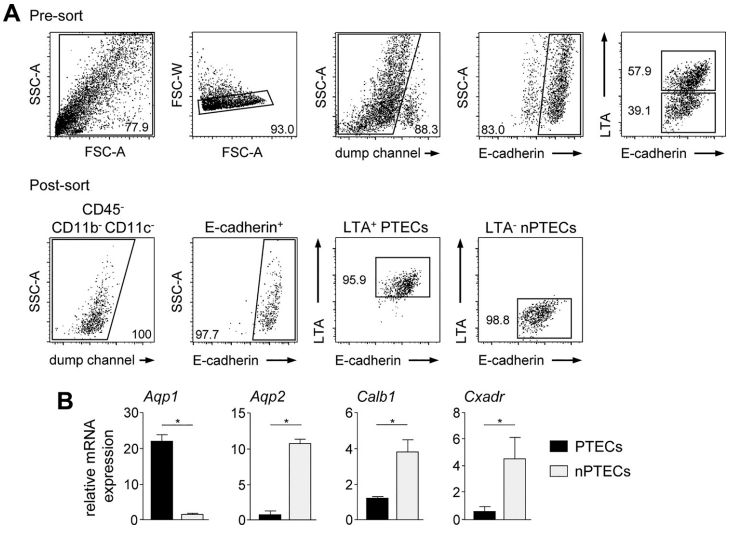


Fig. 1. Isolation of proximal tubular epithelial cells (PTECs) and non-PTECs (nPTECs) by FACS. *A*: renal cortex cells were subjected to a Percoll gradient. Cells were then stained with *Lotus tetragonolobus* agglutinin (LTA) and antibodies against E-cadherin, CD45, CD11b, and CD11c. Subsequently, CD45$^-$ CD11b$^-$ CD11c$^-$ E-cadherin$^+$ LTA$^+$ PTECs and LTA$^-$ nPTECs were isolated by FACS. Representative dot plots from at least four independent experiments are shown. Numbers show frequencies of cells in the respective gates. *B*: PTEC and nPTEC mRNA expression was analyzed by quantitative RT-PCR and normalized to the reference gene β-actin ($n = 4$; Mann-Whitney *U*-test). Bar graphs show means ± SE. $^*P < 0.05$. *Aqp1*, aquaporin-1; *Aqp2*, aquaporin-2; *Calb1*, calbindin 1; *Cxadr*: coxsackievirus and adenovirus receptor; SSC, side scatter.

detected in E-cadherin$^+$ LTA$^-$ cells, confirming pure isolation of PTECs using the FACS-based protocol. In E-cadherin$^+$ LTA$^-$ cells, we showed high mRNA expression of aquaporin-2 (*Aqp2*) and of the $Ca^{2+}$-binding protein calbindin 1 (*Calb1*), both predominantly expressed in distal tubules. Moreover, mRNA expression of coxsackievirus and adenovirus receptor (*Cxadr*), a gene expressed by parietal epithelial cells and a distinct population of distal tubular epithelial cells, was strongly increased in E-cadherin$^+$ LTA$^-$ cells compared with E-cadherin$^+$ LTA$^+$ cells (Fig. 1*B*). Thus, E-cadherin$^+$ LTA$^-$ non-PTECs comprise distal tubular epithelial cells and potentially parietal epithelial cells that were hardly present in the E-cadherin$^+$ LTA$^+$ PTEC population.

*Predominantly PTECs express molecules linked with APC function at steady state.* To determine the capacity of renal epithelial cell populations for antigen-specific CD4$^+$ T cell activation, we analyzed mRNA and protein expression of molecules linked with APC function in naive mice. For mRNA expression analysis, we used FACS-sorted PTECs and non-PTECs. Protein expression analysis was done by flow cytometry, and the gating strategy was the same as that shown in Fig. 1*A*. We found that PTECs express mRNA of *H2-Ab1* (MHCII), essential for peptide presentation to CD4$^+$ T cells, *Cd74*, which is critical for peptide loading of MHCII molecules, and costimulatory molecules *Cd86*, *Cd80*, and *Cd44*. Compared with non-PTECs, mRNA expression of *H2-Ab1*, *Cd74*, and *Cd80* was strongly increased in PTECs, whereas expression of *Cd86* and *Cd44* was not different (Fig. 2*A*).

Flow cytometry analysis revealed that distinct subsets of PTECs express MHCII (25.1 ± 7.9%), CD74 (14.8 ± 4.7%), CD80 (13.2 ± 5.7%), and CD44 (8.1 ± 1.4%) at steady state. Interestingly, we showed a high frequency of PTECs expressing CD86 (56.9 ± 2.2%). In comparative analysis, we found that all analyzed proteins were significantly less expressed in non-PTECs. We detected a low frequency of non-PTECs expressing MHCII (11.1 ± 6.4%) and CD74 (3.7 ± 3.1%), whereas CD80 (0.6 ± 0.2%) and CD44 (0.4 ± 0.1%) were not expressed. We also demonstrated expression of CD86 at an intermediate frequency in non-PTECs (34.2 ± 9.4%; Fig. 2*B*).

In addition, we compared protein expression levels of PTECs with those of splenic CD11c$^+$ DCs, a well-defined population of professional APCs that potently induce T cell activation. We demonstrated that the frequencies of PTECs expressing MHCII, CD74, CD80, and CD44 were substantially reduced compared with splenic DCs, whereas the frequency of CD86$^+$ PTECs was elevated (Fig. 2*B*). Thus, PTECs rather than non-PTECs express molecules linked with APC function at steady state, although their protein levels are below the protein levels of these molecules expressed by splenic DCs.

*Imaging flow cytometry analysis of PTEC protein expression at the single cell level.* Using an Amnis ImageStream flow cytometer that combines features of flow cytometry and fluorescent microscopy, we visualized protein expression at the PTEC single cell level. Single cells were selected using a dot plot of the bright-field aspect ratio versus bright-field area. The aspect ratio is a value calculated by dividing the height of each cell by the width. Cell debris has a high aspect ratio, whereas the area is very small (37). On the basis of single cells, PTECs were identified through binding of PE-labeled anti-E-cadherin antibody and FITC-labeled LTA (Fig. 3, *A* and *B*). The same analysis was done with splenic DCs identified through binding of PerCP-labeled anti-CD45 antibody and PE-Cy7-labeled anti-CD11c antibody (Fig. 3, *C* and *D*). Amnis ImageStream analysis revealed expression of MHCII, CD74, CD86, CD80, and CD40 by PTEC and DC single cells. Comparable to flow cytometry, we also detected PTECs and DCs that did not express the analyzed proteins (Fig. 3, *B* and *D*).

*Immunomodulatory properties of PTECs.* Having shown that PTECs express molecules linked with APC function, we then analyzed the phenotype of PTEC-stimulated CD4$^+$ T cells. For this purpose, OVA-specific CD4$^+$ T cells from OT-II mice were cocultured with PTECs in the presence of OVA. After 5 days, CD4$^+$ T cell activation and cytokine production were analyzed. When PTECs and OVA were present, the frequency of CD4$^+$ T cells expressing the activation marker CD25 was increased compared with CD4$^+$ T cells cultured only in the presence of OVA. We also detected an elevated frequency of IL-2- and IFN-γ-expressing CD4$^+$ T cells after coculture with PTECs. Interestingly, PTECs induced strong expression of the inflammatory cytokine TNF-α in CD4$^+$ T cells. In contrast, we did not detect expression of the T helper 2 cytokines IL-5, IL-13, and IL-4 or the anti-inflammatory cytokine IL-10 in PTEC-activated CD4$^+$ T cells (data not shown). Compared with splenic DC-activated CD4$^+$ T cells, induction of CD25, IL-2, and IFN-γ expression was much less pronounced in PTEC-stimulated CD4$^+$ T cells, whereas induction of TNF-α was enhanced (Fig. 4*A*).

To assess the contribution of MHCII to PTEC-mediated stimulation of CD4$^+$ T cells, cocultures were performed in the presence of anti-MHCII antibody, resulting in strongly reduced activation and cytokine production of CD4$^+$ T cells in the presence of OVA (Fig. 4*B*).

We further analyzed proliferation of PTEC-stimulated CD4$^+$ T cells by staining for the proliferation marker Ki-67. We detected induction of Ki-67 expression in CD4$^+$ T cells when PTECs and OVA were present, whereas PTECs alone did not induce Ki-67 expression. Moreover, PTEC-induced CD4$^+$ T cell proliferation was abrogated after blockage of MHCII by anti-MHCII antibody (Fig. 4*C*). By comparing Ki-67 and CD25 expression, we demonstrated that PTECs induced proliferation of activated CD25$^+$ CD4$^+$ T cells (Fig. 4*D*). In addition, blockage of the costimulatory pathway using anti-CD80 and anti-CD86 antibodies also decreased PTEC-induced CD4$^+$ T cell activation, proliferation, and inflammatory cytokine production (Fig. 4*E*).

Activation-induced cell death has been proposed as an important mechanism to limit immunity by induction of apoptosis in TCR-stimulated T cells (17). To assess whether PTECs induce apoptosis in CD4$^+$ T cells as a mechanism to limit T cell responses, we performed annexin V/7-AAD staining with CD4$^+$ T cells cultured in the presence and absence of PTECs for 5 days. We determined a high frequency of viable annexin V$^-$ 7-AAD$^-$ CD4$^+$ T cells as well as a low frequency of early apoptotic annexin V$^+$ 7-AAD$^-$ and late apoptotic/dead annexin V$^+$ 7-AAD$^+$ CD4$^+$ T cells when PTECs were present. In contrast, almost all CD4$^+$ T cells were dead in the absence of PTECs. Antigenic stimulation of CD4$^+$ T cells by PTECs did not alter the frequency of viable and dead CD4$^+$ T cells and induced a slight increase in the frequency of early apoptotic CD4$^+$ T cells (Fig. 5*A*). Thus, PTECs do not substantially induce activation-induced cell death in stimulated CD4$^+$ T cells and instead support CD4$^+$ T cell survival.

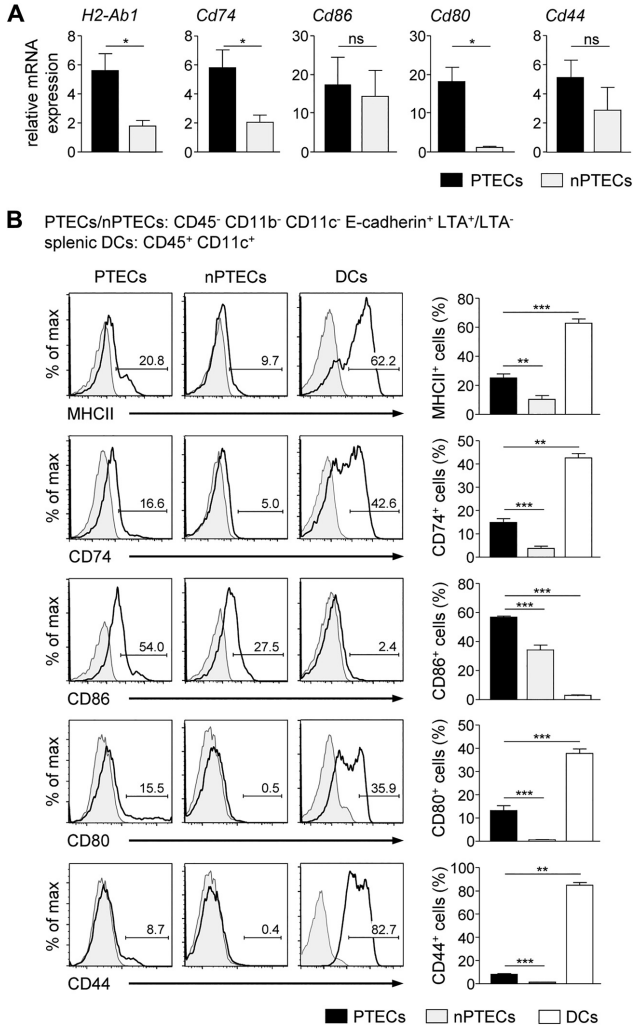


Fig. 2. Expression of molecules linked with antigen-presenting cell (APC) function by proximal tubular epithelial cells (PTECs) and non-PTECs (nPTECs) at steady state. *A*: PTEC and nPTEC mRNA expression was analyzed by quantitative RT-PCR and normalized to the reference gene β-actin ($n = 6$; Mann-Whitney *U*-test). *B*: PTECs, nPTECs, and splenic dendritic cells (DCs) were stained with antibodies against major histocompatibility complex class II (MHCII), CD74, CD86, CD80, and CD44 and analyzed by flow cytometry ($n = 8$; one-way ANOVA with post analysis by Tukey-Kramer test). Representative histograms are shown. Bar graphs show means ± SE. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ns, not significant; *H2-Ab1*, MHCII.

We further asked whether PTECs may drive the generation of regulatory T cells (Tregs) expressing the transcription factor Foxp3 to regulate the inflammatory immune response. Conversion of conventional $CD4^+$ $Foxp3^-$ T cells into $CD4^+$ $Foxp3^+$ Tregs in the periphery is favored by low-dose TCR stimulation in the presence of TGF-β (13, 16, 39). To assess the capacity of PTECs for Treg induction, we performed coculture experiments with OVA-specific $CD4^+$ T cells in the presence or absence of TGF-β. In general, $CD4^+$ T cells cultured in the absence of PTECs showed no induction of Foxp3 expression and, also, unstimulated $CD4^+$ T cells did not express Foxp3. When PTECs and OVA were present, there was a slight increase in the frequency of $Foxp3^+$ Tregs that was not altered in the presence of TGF-β. The situation was different when TCR stimulation was increased by anti-CD3 antibody instead of OVA. Here, $Foxp3^+$ Treg induction was favored in the presence of PTECs and further increased by exogenous TGF-β (Fig. 5*B*). Tregs express the high-affinity IL-2 receptor and depend on IL-2 signaling for maintenance and function (34). We showed that the frequency of IL-2-expressing conventional $CD4^+$ T cells was elevated after anti-CD3 antibody stimulation compared with OVA-stimulated $CD4^+$ T cells (Fig. 5*C*). Together with an upregulated expression of the IL-2 receptor α-subunit CD25 by anti-

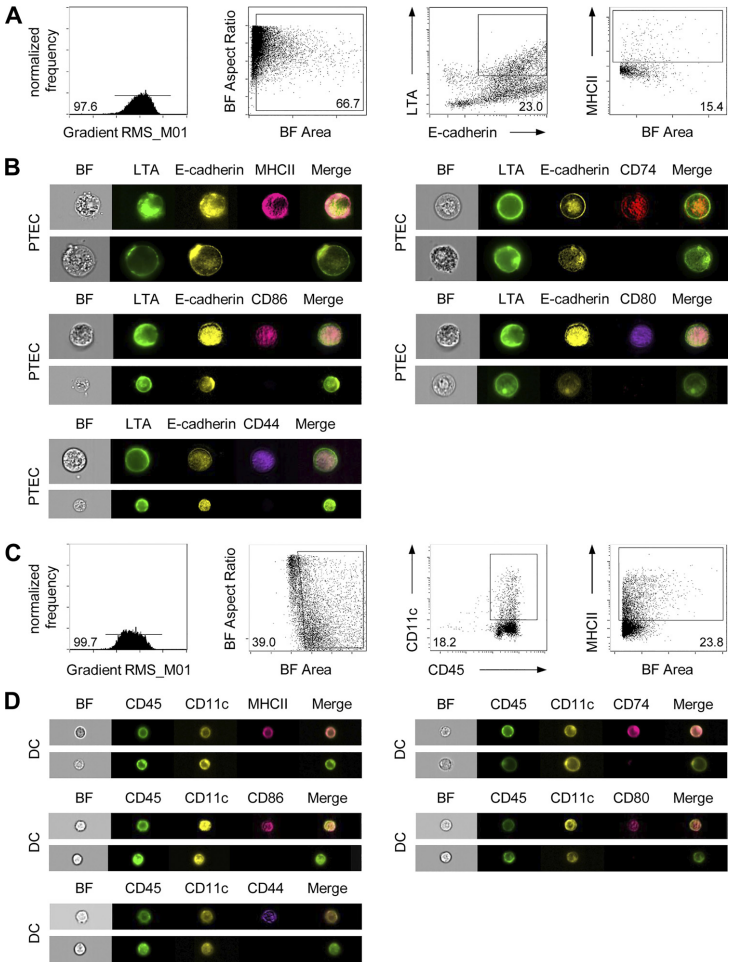


Fig. 3. Expression of molecules linked with antigen-presenting cell function at the proximal tubular epithelial cell (PTEC) single cell level. *A*: renal epithelial cells were stained with *Lotus tetragonolobus* agglutinin (LTA) and antibodies against E-cadherin, major histocompatibility complex class II (MHCII), CD74, CD86, CD80, and CD40. The gating strategy for the selection of single PTECs is shown. BF, bright field. *B*: coexpression of E-cadherin and MHCII, CD74, CD86, CD80, and CD44, respectively, is shown on LTA$^+$ individual PTECs. *C*: splenocytes were stained with antibodies against CD45, CD11c, MHCII, CD74, CD86, CD80, and CD40. The gating strategy for the selection of single dendritic cells (DCs) is shown. *D*: expression of MHCII, CD74, CD86, CD80, and CD40 was shown on individual CD45$^+$ CD11c$^+$ DCs. Representative images from two independent experiments are shown.

CD3 stimulated CD4$^+$ Foxp3$^+$ Tregs (Fig. 5*C*), this might account for better Treg induction after anti-CD3 antibody stimulation. In summary, PTECs support CD4$^+$ T cell survival, induce activation, proliferation, and inflammatory cytokine production in CD4$^+$ T cells, and exhibit a low capacity to convert conventional CD4$^+$ T cells into inducible Tregs upon antigen-specific stimulation in vitro.

*PTECs upregulate expression of CD74 and downregulate expression of costimulatory molecules during NTN.* Having shown that PTECs express MHCII, CD74, and costimulatory molecules under homeostatic conditions, we now analyzed mRNA and protein expression of these molecules during cGN. Therefore, NTN was induced in C57BL/6 mice by injection of nephritogenic serum, and the phenotype of epithelial cell populations was analyzed at different time points of kidney inflammation. Glomerular damage was determined by quantification of crescent formation in periodic acid-Schiff-stained kidney sections (43). Four days after NTN induction, we detected crescent formation in NTN-treated mice that was further increased 8 days after the induction of NTN (Fig. 6*A*). Glomerular filtration barrier integrity was assessed by measurement of the albumin-to-creatinine ratio in urine 1 day before epithelial cell analysis. Kidney function of NTN-treated mice was severely affected, since we detected strong proteinuria 3 days after NTN induction, which was further present at *day 7* of NTN (Fig. 6*B*). At the same time, we detected a massive renal infiltration of CD3$^+$ T cells that localized in the tubulointerstitium in direct contact to proximal and distal tubuli (Fig. 6*C*). Moreover, renal mRNA expression of the epithelial cell-activating cytokine IFN-γ was highly elevated (Fig. 6*D*),

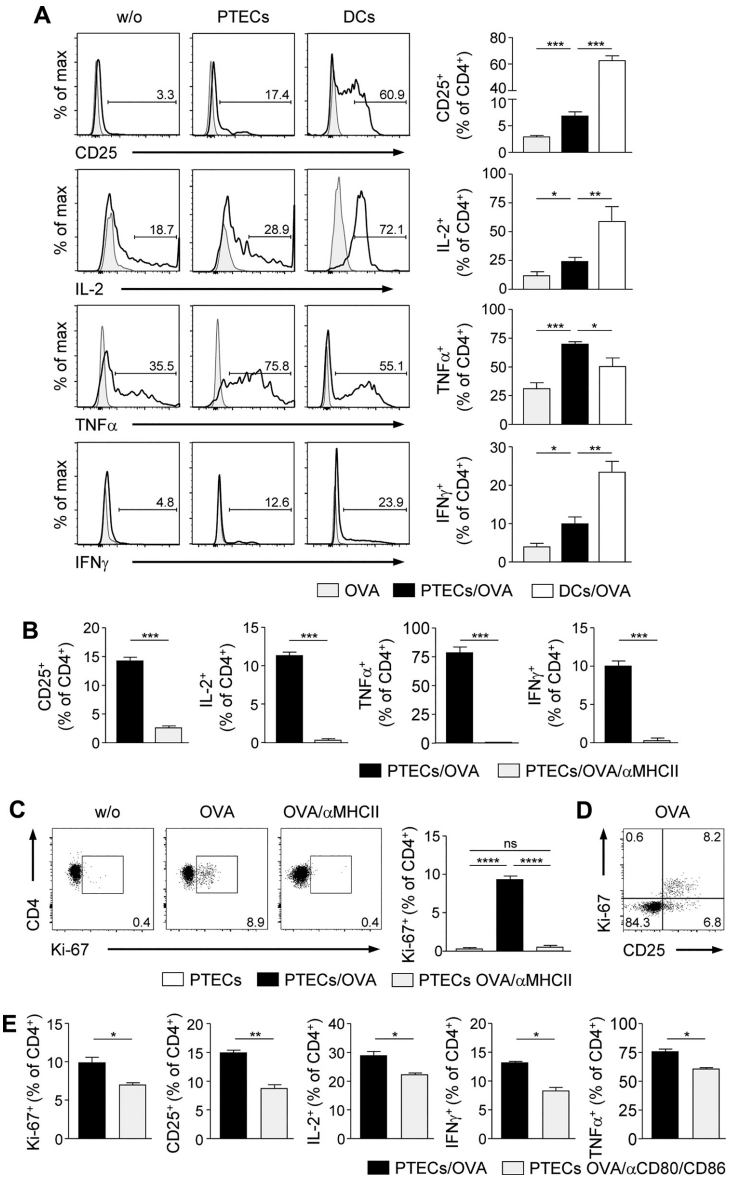


Fig. 4. Phenotype of proximal tubular epithelial cell (PTEC)-stimulated CD4$^+$ T cells. *A*: ovalbumin (OVA)-specific CD4$^+$ T cells were cocultured with PTECs or splenic dendritic cells (DCs) and OVA for 5 days. CD4$^+$ T cells were stained for CD25, IL-2, TNF-α, and interferon (IFN)-γ and analyzed by flow cytometry ($n$ = 9–10; one-way ANOVA with post analysis by Tukey-Kramer test). *B*: OVA-specific CD4$^+$ T cells were cocultured with PTECs, OVA, and anti-major histocompatibility complex class II (MHCII) antibody for 5 days. Cells were stained with CD25, IL-2, TNF-α, and IFN-γ and analyzed by flow cytometry ($n$ = 6; Mann-Whitney *U*-test). *C* and *D*: CD4$^+$ T cells were stained for Ki-67 and CD25 and analyzed by flow cytometry ($n$ = 6; one-way ANOVA with post analysis by Tukey-Kramer test). *E*: OVA-specific CD4$^+$ T cells were cocultured with PTECs and OVA in the presence or absence of anti-CD80 and anti-CD86 antibodies for 5 days ($n$ = 6; Mann-Whitney *U*-test). Representative dot plots and histograms are shown. Bar graphs show means ± SE. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. ns, not significant; w/o, without.

and, within renal CD4$^+$ T cells, the frequency of IFN-γ-expressing T helper 1 cells was enhanced (Fig. 6*E*).

We demonstrated increased *MHCII* mRNA expression by both PTECs and non-PTECs 8 days after NTN induction, but the frequency of MHCII-expressing PTECS and non-PTECs did not enhance during NTN. However, the frequency of MHCII$^+$ PTECS remained elevated compared with non-PTECs during renal inflammation. Interestingly, particularly PTECs showed upregulated mRNA and protein expression of CD74 8 days after NTN induction. CD74 protein expression was also increased by non-PTECs but, compared with PTECs, the frequency of CD74$^+$ non-PTECs remained significantly

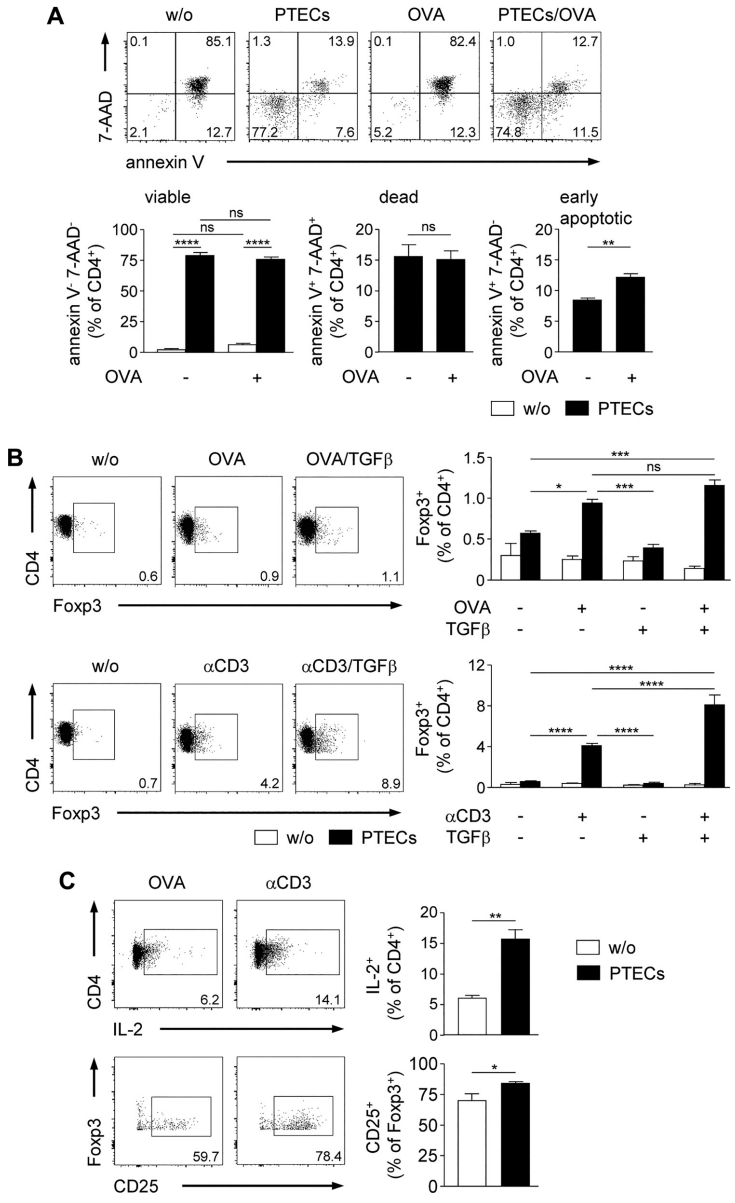


Fig. 5. Apoptotic cell death and regulatory T cell induction in proximal tubular epithelial cell (PTEC)-stimulated $CD4^+$ T cells. *A*: ovalbumin (OVA)-specific $CD4^+$ T cells were cocultured with PTECs and OVA for 5 days. $CD4^+$ T cells were stained for annexin V and 7-aminoactinomycin D (7-AAD) and analyzed by flow cytometry ($n = 6$; one-way ANOVA with post analysis by Tukey-Kramer test and Mann-Whitney *U*-test). *B* and *C*: OVA-specific $CD4^+$ T cells were cocultured with PTECs in the presence of OVA, anti-CD3 antibody, and/or transforming growth factor (TGF)-β for 5 days. $CD4^+$ T cells were stained for Foxp3, IL-2, and CD25 and analyzed by flow cytometry [$n = 6$; one-way ANOVA with post analysis by Tukey-Kramer test (*B*) and Mann-Whitney *U*-test (*C*)]. Representative dot plots are shown. Bar graphs show means ± SE. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. ns, not significant; w/o, without.

reduced. Furthermore, PTECs downregulated *Cd86* mRNA expression 4 days after NTN induction. This correlated with a decreased frequency of PTECs expressing CD86 at this time point of NTN, which was further reduced at *day 8* of NTN. We also detected a strong reduction in CD86 protein expression in non-PTECs 8 days after the induction of NTN. Differences were determined in the expression of CD80 by PTECs and non-PTECs. While the frequency of CD80-expressing PTECs was reduced at *day 8* of NTN, non-PTECs showed upregulated *CD80* mRNA and protein expression during NTN. Further-

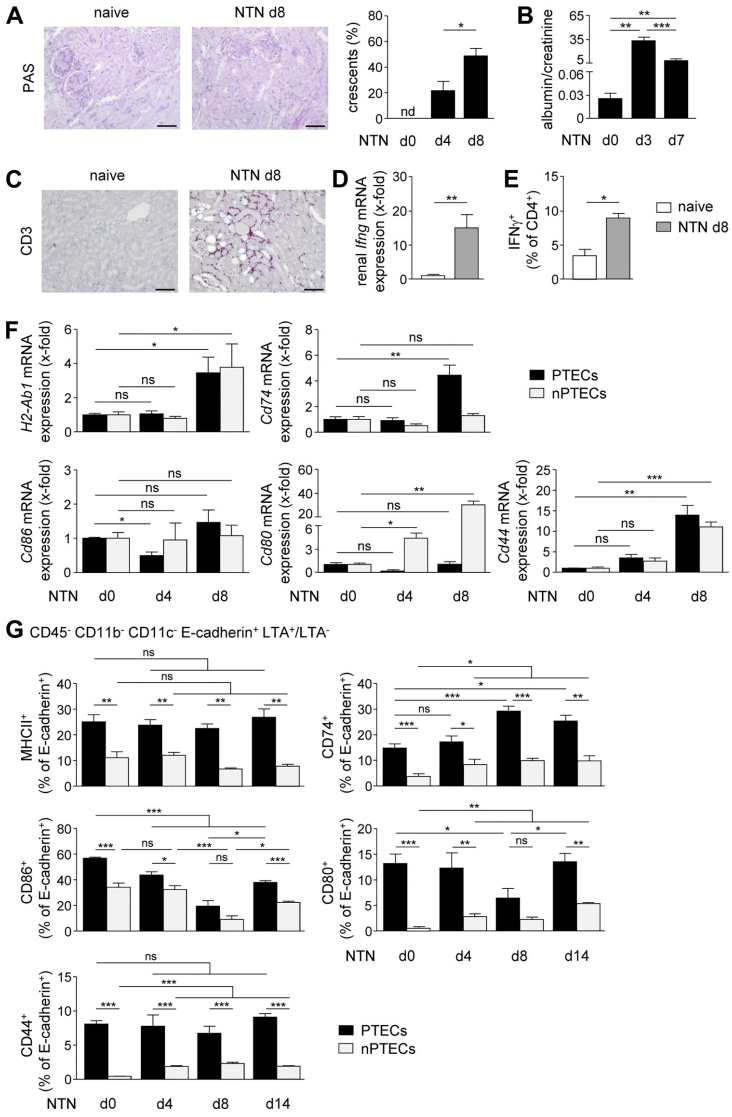


Fig. 6. Expression of molecules linked with antigen-presenting cell function by proximal tubular epithelial cell (PTECs) and non-PTECs (nPTECs) during nephrotoxic nephritis (NTN). NTN was induced in C57BL/6 mice and analyzed 4 and 8 days (d) later. *A*: glomerular crescent formation was quantified in periodic acid-Schiff (PAS)-stained kidney sections of naive and nephritic mice ($n$ = 8; Mann-Whitney *U*-test). *B*: renal dysfunction was assessed by the determination of the albumin-to-creatinine ratio in urine by ELISA ($n$ = 8; one-way ANOVA with post analysis by Tukey-Kramer test). *C*: kidney sections of naive and nephritic mice were stained with anti-CD3 antibody. *D*: renal interferon (IFN)-γ mRNA expression of nephritic mice was analyzed by quantitative RT-PCR and normalized to gene expression of naive mice ($n$ = 8; Mann-Whitney *U*-test). *E*: the frequency of renal IFNγ$^+$ CD4$^+$ T cells was analyzed by flow cytometry in naive and nephritic mice ($n$ = 8; Mann-Whitney *U*-test). *F*: PTEC and nPTEC mRNA expression in nephritic mice was analyzed by quantitative RT-PCR and normalized to gene expression in naive mice ($n$ = 6; one-way ANOVA with post analysis by Tukey-Kramer test). *G*: PTECs and nPTECs from naive and nephritic mice were stained with antibodies against major histocompatibility complex class II (MHCII), CD74, CD86, CD80, and CD44 and analyzed by flow cytometry ($n$ = 8; Mann-Whitney *U*-test). Representative photomicrographs are shown. Scale bars = 50 μm. Bar graphs show means ± SE. *$P < 0.05$; **$P < 0.01$; ***$P <$ 0.001. ns, not significant; *H2-Ab1*, MHCII; nd, not detectable.

more, we found increased *Cd44* mRNA expression in PTECs and non-PTECs, but only the frequency of CD44-expressing non-PTECs was elevated during NTN (Fig. 6, *F* and *G*). We further did protein expression analysis 14 days after NTN induction. At this late time point of NTN, the frequency of MHCII$^+$ and CD44$^+$ PTECs was still not altered compared with naive mice, whereas the frequency of CD74$^+$ PTECs remained significantly elevated. Interestingly, the frequency of CD86- and CD80-expressing PTECs and non-PTECs increased again from *day 8* to *day 14* of NTN (Fig. 6*G*).

*Expression of molecules associated with APC function by human tubular epithelial cells in ANCA-associated GN.* To assess expression of MHCII, CD74, and CD86 by human renal epithelial cell populations, immunostaining was performed in kidney sections from patients with ANCA-associated GN. Comparable to mice, strong CD86 expression was particularly

shown in proximal tubular epithelial cells. In contrast, MHCII and CD74 expression was determined in distinct proximal and distal tubular epithelial cells (Fig. 7). Thus, renal tubular epithelial cells express molecules associated with APC function in the chronically inflamed kidney.

## DISCUSSION

cGN is a life-threatening disease triggered by so far poorly defined mechanisms. Thus, identifying molecular and cellular pathways involved in the pathology of cGN is of high clinical relevance. In this study, we showed regulated epithelial expression of molecules linked with APC function in NTN. Moreover, we demonstrated that PTECs induce an antigen-specific inflammatory $CD4^+$ T cell activation in vitro. Whether PTECs exert immunomodulatory function in vivo, particularly in cGN, needs to be further addressed.

Full $CD4^+$ T cell activation requires both MHCII-mediated TCR signaling and CD80/CD86-mediated costimulatory signaling. To analyze the potential of renal epithelial cell populations to function as APCs in mice, we established an isolation protocol based on E-cadherin expression and LTA staining in renal cortical tissue where the tubular epithelial cells are located. An important point was the exclusion of potential contaminating $CD45^+$ professional APCs that would affect our expression analysis. One limitation of the isolation method is the focus on E-cadherin to distinguish epithelial from nonepithelial cells. Epithelial cells constitutively express E-cadherin; however, expression levels can be downregulated, e.g., by inflammatory cytokines. Thus, staining of additional epithelial cell-specific markers, preferable not regulated by inflammation, would be helpful in future studies. Another critical point is the discrimination of PTECs and non-PTECs using LTA, which binds to glycoproteins within the brush borders of proximal tubules (51). We cannot exclude that PTECs lose parts of their brush border during the isolation procedure or during renal inflammation, which would lead to the inclusion of PTECs in the non-PTEC population. Therefore, staining of other surface proteins, which are not expressed in the brush border of PTECs, would improve the isolation method. To strengthen the basis of the PTEC/non-PTEC discrimination and particularly to define which epithelial cell populations comprise the non-PTEC population, further analysis of proteins expressed by the distinct renal epithelial cell populations would be desirable, together with the development of new antibodies suitable for cell sorting and flow cytometry.

In comparative analysis, we provided evidence that predominantly PTECs expressed molecules essential for $CD4^+$ T cell activation. Compared with the non-PTEC population, the frequency of PTECs expressing MHCII, CD74, CD80, and CD86 was significantly increased. Comparative analysis with splenic DCs, regarded as professional APCs, further revealed that PTECs expressed low levels of MHCII, CD74, and CD80, whereas CD86 was stronger expressed. Low expression of MHCII has been previously described for cell populations other than professional APCs, including intestinal epithelial cells (35), vascular endothelial cells (44), and keratinocytes (12) as well as liver sinusoidal endothelial cells (LSECs) and hepatocytes (21, 38). These cell populations have the capacity to stimulate T cell responses by antigen presentation and, therefore, are defined as nonprofessional APCs. Based on our findings from the phenotype analysis and in vitro functional experiments, we suggest that PTECs are a population of nonprofessional APCs in the kidney. That nonprofessional APCs can exert immunomodulatory functions beside professional APCs has been particularly demonstrated in the liver. Here, LSECs were shown to promote Treg induction (8, 31), inhibit inflammatory cytokine production (9), induce T cell anergy (10), and tolerize professional APCs, thereby leading to insufficient T cell activation (47). Thus, hepatic nonprofessional APCs contribute to tolerance induction in the liver, and it needs to be assessed whether renal nonprofessional APCs use similar or different mechanisms to modulate immune responses in immune-mediated kidney disease.

As shown for LSECs and hepatocytes in the liver (38), nonprofessional APCs induce a distinct phenotype in stimulated $CD4^+$ T cells. Defining the type of PTEC-induced effector $CD4^+$ T cell population is important for the understanding how renal epithelial cells might modulate immune responses in kidney disease. A previous study (19) demonstrated that PTECs were able to process and present antigens via MHCII, a prerequisite for $CD4^+$ T cell stimulation. We showed that PTECs induced antigen-specific activation, proliferation, and inflammatory cytokine production in $CD4^+$ T cells in vitro. In detail, especially expression of the proinflammatory cytokine TNF-α was induced. So far, the function of TNF-$\alpha^+$ $CD4^+$ T cells in cGN has not been addressed. In general, TNF-α was found to be pathogenic in kidney disease. In NTN (27, 53, 54), ANCA-associated GN (33), and lupus nephritis (5, 45, 60), renal TNF-α levels were elevated and interference with the TNF-α signaling pathway had beneficial effects on disease

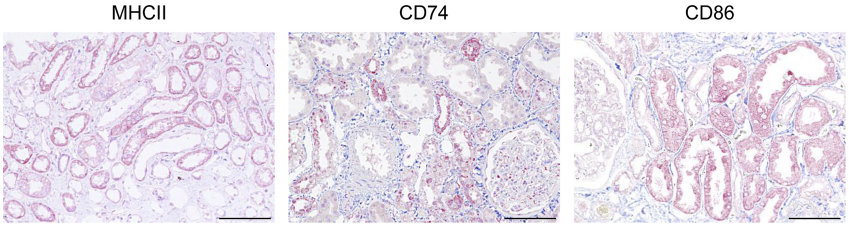


Fig. 7. Expression of major histocompatibility complex class II (MHCII), CD74, and CD86 in the human kidney. Kidney sections from patients with antineutrophil cytoplasmic antibody-associated glomerulonephritis were stained for MHCII, CD74, and CD86. Representative microphotographs of five individual donors are shown. Scale bar = 100 μm.

pathology (3, 27, 33). However, it was also reported that patients with systemic lupus erythematosus with low TNF-α levels were more prone to lupus nephritis, whereas patients with high levels were not predisposed (23). Regarding cellular TNF-α expression in cGN, there is one study (22) showing renal TNF-$\alpha^+$ $CD4^+$ T cells in experimental autoimmune GN, but the function of these cells was not analyzed. Since several studies have correlated elevated TNF-α levels with increased renal cell infiltration and elevated expression of chemokines and adhesion molecules by renal parenchymal cells (3, 47, 53), one might speculate that PTEC-induced TNF-$\alpha^+$ $CD4^+$ T cells could promote recruitment of inflammatory cells into the kidney.

We further detected induction of IFN-γ in PTEC-stimulated $CD4^+$ T cells, albeit at low levels. Here, we confirmed data of another study (55) that addressed weak IFN-γ induction by PTECs to low expression of costimulatory molecules, a finding that we did not determine. In contrast, particularly CD86 was strongly expressed by PTECs. However, comparative analysis of $CD4^+$ T cells stimulated either by PTECs or splenic DCs revealed a rather weak activation of $CD4^+$ T cells through PTECs. As with other nonprofessional APCs, PTECs constitutively expressed low levels of MHCII, which might be responsible for weak $CD4^+$ T cell activation despite high expression of the costimulatory molecule CD86. This assumption is supported by the finding that a stronger TCR signal induced through anti-CD3 antibody increased expression of IL-2 and CD25 in PTEC-stimulated $CD4^+$ T cells.

Nonprofessional APCs like LSECs (8, 31) and hepatocytes (6, 7) have been shown to favor Treg induction, thereby facilitating immune regulation in the liver. Therefore, we assessed whether PTECs might also exert immune regulatory function through induction of $Foxp3^+$ Tregs in vitro. Our data did not support this assumption, as PTEC-mediated induction of $Foxp3^+$ Tregs was low, even in the presence of the Foxp3-inducing cytokine TGF-β. Amplification of the TCR signal through anti-CD3 antibody in conjunction with TGF-β increased the frequency of $Foxp3^+$ Tregs; however, the efficiency of PTEC-induced Treg generation remained low compared with the ability of LSECs and hepatocytes to induce Tregs (6-8, 31), strongly indicating that Treg induction is not a predominant feature of PTECs.

Increased epithelial expression of MHCII has been associated with the pathology of diseases like inflammatory bowel disease (35), graft-versus-host disease (4), autoimmune hepatitis, and primary sclerosing cholangitis (50). Previous studies have reported IFN-γ-mediated upregulation of MHCII expression by PTECs in vitro (24, 58) and in vivo upon LPS treatment or infection with *Listeria monocytogenes* (18, 19). We analyzed expression of MHCII by renal epithelial cells in NTN, in which we showed elevated IFN-γ levels in the inflamed kidney. However, despite an increased MHCII mRNA expression of both PTECs and non-PTECs during NTN, epithelial expression of MHCII protein was not enhanced demonstrating that at least in NTN, IFN-γ produced in vivo did not alter MHCII expression by PTECs and non-PTECs. Whether this has implications for the pathology of cGN still remains unclear since induction of constitutive high MHCII expression in proximal tubules was shown to be insufficient for inducing kidney injury in murine lupus nephritis (25). Nevertheless, the question remains open as to whether additional, renal epithelial cell-derived signals might favor immunity. In this regard, we demonstrated an elevated frequency of PTECs expressing CD74 during NTN. So far, a function of PTEC-derived CD74 has not been described. Since CD74 is crucial for antigenic peptide loading on MHCII molecules (52), it could be speculated that enhanced CD74 expression during renal inflammation supports MHCII-mediated antigen presentation by PTECs. However, the knowledge about mechanisms that regulate expression of MHCII and CD74 by renal tubular epithelial cells in cGN is very limited yet, and further studies are required to understand the inflammation-induced modulation of PTEC-expressed MHCII and CD74 and the consequences for $CD4^+$ T cell activation and subsequent disease pathology.

CD44 has been suggested as an activation marker of parietal epithelial cells whose expression was shown by activated parietal epithelial cells in patients with cGN (40), focal segmental glomerulosclerosis (14), and IgA nephropathy (46). Since CD44 protein expression was only increased in the non-PTEC population during NTN, this indicated that potentially parietal epithelial cells respond to renal inflammation by enhanced CD44 expression.

In contrast to previous studies (20, 28, 55), we determined high expression of CD86 by PTECs of mice and humans. Interestingly, epithelial expression of CD86 and CD80 was downregulated in the acute, T cell-dependent phase of murine cGN, whereas at a later time point of the disease, expression of both molecules was again increased, suggesting an inflammation-dependent regulation of epithelium-expressed costimulatory molecules. So far, there is no explanation for this observation, but based on the in vitro finding that blockage of the costimulatory pathway reduced PTEC-induced $CD4^+$ T cell activation and inflammatory cytokine production, it could be speculated that during the T cell-dependent phase of cGN, downregulation of costimulatory molecule expression might dampen overwhelming T cell responses through excessive T cell activation. However, strong CD86 expression by renal epithelial cells is somehow surprising, and it would be of interest to know whether epithelium-derived CD86 exerts other functions than costimulation.

In summary, this study revealed expression of molecules essential for T cell activation by renal epithelial cell populations in healthy and diseased mice and humans; PTECs were particularly identified to induce an inflammatory phenotype in $CD4^+$ T cells. The predominant in vitro data of this study open the possibility for hypotheses about the in vivo relevance of renal epithelial cell-mediated $CD4^+$ T cell activation in the disease pathology of cGN. However, suitable in vivo models need to be developed first, e.g., mouse lines in which PTECs specifically do not express MHCII, CD74, or CD80/CD86 and antigen-specific kidney disease models, to further analyze this important question.

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## DISCLOSURES

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

## AUTHOR CONTRIBUTIONS

P.C.B., T.W., C.M.-S., and K.N. performed experiments; P.C.B., T.W., C.M.-S., and K.N. analyzed data; P.C.B., T.W., C.M.-S., F.G., T.B.H., and K.N. interpreted results of experiments; P.C.B. and K.N. drafted manuscript; T.W., C.M.-S., F.G., T.B.H., U.P., and G.T. edited and revised manuscript; F.G., T.B.H., U.P., G.T., and K.N. conceived and designed research; G.T. and K.N. approved final version of manuscript; K.N. prepared figures.

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## II. Zusammenfassende Darstellung der Publikation

### II.1 Einführung

Bereits in vergangenen Untersuchungen konnte beispielsweise für intestinale Epithelzellen, respiratorisches Epithel sowie für Keratinozyten gezeigt werden, dass eine Haupthistokompatibilitätskomplex-Klasse II (MHCII)-vermittelte Antigenpräsentation durch Epithelzellen bei bestimmten Entzündungsreaktionen eine $CD4^+$ T-Zell-Immunantwort auslösen beziehungsweise beeinflussen kann (Kambayashi et al. 2014). Neben dem Mechanismus des MHCII-vermittelten T-Zell-Rezeptor-Signalings bedarf es noch eines weiteren Signals für die vollständige Aktivierung von T-Zellen, nämlich der CD28-vermittelten Interaktion von T-Zellen mit den kostimulatorischen Molekülen CD80 und CD86, welche üblicherweise von professionellen antigenpräsentierenden Zellen (APCs), also dendritischen Zellen, Monozyten, Makrophagen sowie B-Zellen, exprimiert werden. Zellen, die nicht unter die Definition der professionellen APCs fallen, können demnach als nicht-professionelle APCs bezeichnet werden. Außerdem kommt der MHCII-Histokompatibilitätsantigen γ-Kette (CD74) eine wichtige Bedeutung bei der Antigenpräsentation zu, da sie den Transport von MHCII innerhalb der Zelle steuert und an der Beladung von MHCII mit Antigenpeptiden beteiligt ist (Stumptner-Cuvelette et al. 2002). In einer früheren Studie wurde bereits gezeigt, dass proximale Tubulusepithelzellen (PTECs) Antigene über MHCII prozessieren und präsentieren können (Hagerty et al. 1994). Außerdem wurde bereits über eine IFN-γ-vermittelte Hochregulation der MHCII-Expression durch PTECs *in vitro* (Jevnikar et al. 1990; Wüthrich et al. 1990) und *in vivo* (Haas et al. 1995; Hagerty et al. 1994) berichtet. Dennoch ist noch weitgehend unklar, ob renale Epithelzellen die Pathogenese der rapid-progressiven Glomerulonephritis (RPGN) durch Modulation der $CD4^+$ T-Zell-Immunantwort beeinflussen. In der vorliegenden Arbeit etablierten wir eine FACS-basierte Methode zur Isolation einzelner, definierter renaler Epithelzellpopulationen. Wir untersuchten die immunmodulierenden Fähigkeiten renaler Epithelzellen in Mäusen mit nephrotoxischer Nephritis (NTN), einem Mausmodell der RPGN, sowie in Patienten mit anti-neutrophilen cytoplasmatischen Antikörper (ANCA)-assoziierten Glomerulonephritiden hinsichtlich der Expression von MHCII, CD74 und

kostimulatorischer Moleküle. Außerdem untersuchten wir *in vitro* den Phänotyp von $CD4^+$ T-Zellen, welche durch PTECs aktiviert wurden.

## II.2 Material und Methoden

### II.2.1 Sortierung von renalen Epithelzellpopulationen durch fluorescence activated cell sorting (FACS)

Die Etablierung einer FACS-basierten Methode zum Sortieren von kortikalen Tubulusepithelzellpopulationen war ein wesentlicher Bestandteil dieser Arbeit. Dazu entnahmen wir die Nieren von gesunden C57BL/6 Mäusen an Tag 4, Tag 8 und Tag 16 nach Induktion der NTN. Nach Entfernung nicht-kortikaler Anteile wurde das kortikale Gewebe in einer Lösung mit 0,01% Kollagenase aus *Clostridium histolyticum* verdaut. Anschließend wurde das Gewebe durch einen 250 µm-Filter gesiebt. Dann wurden die Epithelzellen von anderen Zellpopulationen durch eine Percoll-Dichtegradienten-Zentrifugation voneinander getrennt. Die Epithelzellen wurden anschließend mit anti-E-Cadherin-Antikörpern zur Markierung des epithelzellspezifischen E-Cadherins sowie mit Lotus tetragonolobus agglutinin (LTA), welches spezifisch an die Glycocalyx des Bürstensaums von PTECs bindet, markiert. Außerdem wurden durch Markierung mit Antikörpern gegen CD45, CD11c sowie CD11b restliche, verbleibende Lymphozyten-Populationen, welche auch professionelle APC-Populationen enthalten, durch die Generation eines *dump channels* aus der Analyse ausgeschlossen. So konnten wir letztendlich E-Cadherin-positive LTA-positive CD45/CD11c/CD11b-negative PTECs mit einer Reinheit von ca. 96% sowie E-Cadherin-positive LTA-negative CD45/CD11c/CD11b-negative „non-PTECs“ (nPTECs) mit einer Reinheit von ca. 99% sortieren und anschließend die verschiedenen Tubulusepithelzellpopulationen hinsichtlich ihrer Genexpression von MHCII, CD74 und von kostimulatorischen Molekülen analysieren.

### II.2.2 Isolation von $CD4^+$ T-Zellen und dendritischen Zellen (DCs)

Wir isolierten Ovalbumin$_{323-339}$(OVA)-spezifische $CD4^+$ T-Zellen aus Milz und Lymphknoten von naiven OT-II Mäusen. Diese OT-II-Mäuse exprimieren einen für $OVA_{323-339}$ -spezifischen T-Zell-Rezeptor (TCR). DCs wurden aus Milz und Lymphknoten von naiven C57BL/6 Mäusen isoliert. Das Gewebe wurde anschließend

durch einen 70 µm-Nylonfilter gesiebt und die verbliebenen Erythrozyten wurden mit $NH_4Cl$ lysiert. Dann wurde die Zellsuspension mit anti-CD16/CD32-Antikörpern inkubiert, um eine unspezifische Bindung von Antikörpern an Fc-Rezeptoren zu verhindern. Schließlich wurden die $CD4^+$ T-Zellen mit dem $CD4^+$ T Cell Isolation Kit (Miltenyi Biotec, Bergisch-Gladbach, Deutschland) durch magnetic-activated cell sorting (MACS) sortiert. Die DCs wurden mit Hilfe von CD11c Microbeads (Miltenyi Biotec) ebenfalls über MACS sortiert. Die Reinheit der auf diese Weise sortierten $CD4^+$ T-Zellen betrug circa 96%.

### II.2.3. Zellkulturen

Um eine antigenspezifische Aktivierung von $CD4^+$ T-Zellen durch PTECs oder DCs zu untersuchen, inkubierten wir OVA-spezifische $CD4^+$ T-Zellen zusammen mit PTECs oder DCs und unter Hinzugabe von $OVA_{323-339}$-Peptid (5 µg/ml) für fünf Tage. Zum Blockieren von MHCII- beziehungsweise CD80/CD86-Molekülen benutzten wir anti-MHCII- und anti-CD80/CD86-Antikörper. Rekombinantes murines TGF-β zur Induktion von regulatorischen T-Zellen (Tregs) benutzten wir in einer Konzentration von 4 ng/ml.

### II.2.4. Durchflusszytometrie

Die Phänotypen der renalen Epithelzellen, DCs und $CD4^+$ T-Zellen wurden mittels Durchflusszytometrie untersucht. Um eine unspezifische Antikörperbindung zu vermeiden, inkubierten wir die Epithelzellen, die DCs sowie die $CD4^+$ T-Zellen zunächst mit einem anti-CD16/CD32-Antikörpergemisch. Anschließend markierten wir die Zellen mit Antikörpern gegen E-Cadherin, CD74, CD86, MHCII, CD25, TCR, CD45, CD11c, CD11b, CD80 und CD44. Um auch apoptotische bzw. tote Zellen nachweisen zu können, wurden die $CD4^+$ T-Zellen außerdem mit Annexin-V sowie 7-AAD markiert. Die Epithelzellen wurden zusätzlich mit dem FITC-konjugierten LTA inkubiert. Zum Nachweis der Expression der Proteine IFN-γ, IL-2, TNF-α und Foxp3 führten wir eine intrazelluläre Markierung mit den entsprechenden Antikörpern durch.

### II.2.5 Bildgebende Durchflusszytometrie (ImageStream®)

Um die Expression von Proteinen zu visualisieren, die an der antigenspezifischen Aktivierung von $CD4^+$ T-Zellen durch APCs beteiligt sind, wurde eine bildgebende Durchflusszytometrie durchgeführt. Wir isolierten die renalen Epithelzellen wie zuvor

beschrieben. Zur Markierung nutzten wir Antikörper gegen E-Cadherin, CD74, CD86, MHCII, CD80 sowie CD44. Außerdem wurden die Epithelzellen mit FITC-konjugiertem LTA markiert. Die DCs wurden mit Antikörpern gegen CD45, CD11c, CD44, CD74, MHCII, CD80 und CD86 markiert. Die Zellen wurden dann im Amnis ImageStream® Durchflusszytometer (Millipore Sigma, Billerica, MA, USA) und mit Hilfe der Amnis IDEAS Software (Millipore Sigma) analysiert.

### II.2.6 Quantitative real-time RT-PCR-Analyse

Um die Genexpression renaler Epithelzellen zu untersuchen, wurde eine quantitative real-time RT-PCR-Analyse durchgeführt. Wir isolierten die RNA von FACS-sortierten renalen Epithelzellen mit Hilfe des RNeasyMicro Kit (Qiagen, Hilden, Deutschland). Die RNA wurde dann mit Hilfe des cDNA Synthesis Kit (Life Technologies, Carlsbad, CA, USA) und dem MyCycler Thermocycler (BioRad, München, Deutschland) in cDNA transkribiert. Die quantitative RT-PCR wurde mit dem ABsolute qPCR SYBR Green Mix durchgeführt. Die Berechnung der relativen mRNA-Expression wurde mit Hilfe der $\Delta\Delta$CT-Methode nach Normalisierung zum Referenzgen ß-Actin durchgeführt.

### II.2.7 Statistische Auswertung

Zur Datenauswertung nutzten wir die GraphPad Prism Software (GraphPad software, San Diego, CA, USA). Die statistische Analyse basiert auf dem Mann-Whitney-U-Test oder einer ANOVA mit anschließendem Tukey-Kramer-Test. Die Darstellung der Daten erfolgte als Mittelwert mit Angabe des Standardfehlers. Ein *p*-Wert von $< 0{,}05$ wurde dabei als statistisch signifikant gewertet, wobei folgende Abstufungen vorgenommen wurden: *p< 0.05, ** p< 0.01, *** p< 0.001 und **** p< 0.0001.

## II.3 Ergebnisse

### II.3.1 Methodenetablierung zur Isolation muriner PTECs

Um die antigenpräsentierenden Fähigkeiten der verschiedenen Tubulusepithelzellpopulationen analysieren zu können, erarbeiteten wir eine auf FACS basierende Methode zur Zellisolation, in welcher wir professionelle APCs, wie beispielsweise DCs und Makrophagen, durch Markierung der Zellen mit Antikörpern gegen CD45, CD11b und CD11c durch die Generierung eines *dump channels* aus der Isolation ausschließen konnten.

Durch das FITC-konjugierte LTA, welches als PTEC-spezifischer Marker an die Glycocalyx des Bürstensaums von PTECs bindet, konnten wir proximale von distalen Tubulusepithelzellen unterscheiden und getrennt voneinander untersuchen. Die auf diese Weise isolierten E-Cadherin$^+$ LTA$^+$ PTECs zeigten in der mRNA-Analyse eine starke Expression von Aquaporin-1 (Aqp1), welches spezifisch in proximalen Tubuli vorkommt. Die E-Cadherin$^+$ LTA$^-$ nPTECs zeigten hingegen nur eine sehr gering ausgeprägte Expression von Aqp1, aber eine starke Expression der in distalen Tubuli exprimierten Moleküle Aquaporin-2 (Aqp2) und Calbindin-1 (Calb1), was die Reinheit der auf diese Weise sortierten PTECs und nPTECs bestätigt.

### II.3.2 Expression von MHCII, CD74 sowie kostimulatorischen Molekülen durch PTECs und DCs

Die mRNA-Analysen konnten zeigen, dass PTECs sowohl MHCII, CD74, als auch die kostimulatorischen Moleküle CD80, CD86 und CD44 exprimieren. Die mRNA-Expression von MHCII, CD74 und CD80 in PTECs war deutlich stärker als die Expression dieser Moleküle in nPTECs. Keine Unterschiede zwischen PTECs und nPTECs zeigten sich jedoch in der mRNA-Expression von CD44 und CD86. In der durchflusszytometrischen Analyse der PTECs aus gesunden Mäusen zeigte sich, dass 25,1 ± 7,9% der PTECs MHCII, 14,8 ± 4,7% CD74, 13,2 ± 5,7% CD80 und 8,1 ± 1,4% CD44 exprimierten. Eine sehr hohe Anzahl an PTECs, 56,9 ± 2,2%, exprimierten CD86. Alle untersuchten Moleküle wurden in nPTECs signifikant geringer exprimiert, während CD80 und CD44 in nPTECs nicht exprimiert wurden. Dementsprechend exprimierten also PTECs in höherem Ausmaß als nPTECs diejenigen Moleküle, welche mit der Funktion von APCs assoziiert sind. Wir haben außerdem vergleichende

Analysen der Proteinexpression der erwähnten Moleküle mit DCs - also professionellen APCs - durchgeführt, um die Stärke der Proteinexpression von PTECs besser einordnen zu können. Hier konnten wir zeigen, dass PTECs in deutlich geringerem Ausmaß als DCs APC-assoziierte Moleküle exprimieren.

### II.3.3 Bildgebende durchflusszytometrische Analyse (ImageStream®)

Die bildgebende durchflusszytometrische Analyse (Amnis ImageStream®) zeigte auf Einzelzellebene die Expression von MHCII, CD74, CD80, CD86 und CD44 sowohl in PTECs als auch in DCs. Analog zur durchflusszytometrischen Analyse fanden wir sowohl PTECs als auch DCs, welche die untersuchten Moleküle nicht exprimierten.

### II.3.4 Immunmodulierende Eigenschaften von PTECs

Um die immunmodulierenden Eigenschaften von PTECs analysieren zu können, untersuchten wir sowohl die Expression verschiedener Aktivierungs-, Proliferations- sowie Apoptosemarker, als auch die Expression phänotyp-spezifischer Zytokine. Die Expression des Aktivierungsmarkers CD25 auf $CD4^+$ T-Zellen war deutlich höher bei Ko-Kultivierung von $CD4^+$ T-Zellen mit PTECs und OVA, verglichen mit der Ko-Kultivierung von $CD4^+$ T-Zellen mit OVA alleine. Es zeigte sich ebenso eine stärkere Expression von IL-2 und IFNγ in $CD4^+$ T-Zellen nach Ko-Kultivierung mit PTECs. Interessanterweise induzierten PTECs eine starke Expression von TNF-α in $CD4^+$ T-Zellen. Ebenso konnten wir eine starke MHCII-Abhängigkeit der T-Zellaktivierung durch PTECs feststellen. In Ko-Kulturen von PTECs und OVA zeigte sich eine deutlich geringere Aktivierung und Zytokinproduktion der $CD4^+$ T-Zellen nach Inkubation mit einem anti-MHCII-Antikörper. Die Proliferation der $CD4^+$ T-Zellen, gemessen am Proliferationsmarker Ki-67, war in Anwesenheit des anti-MHCII-Antikörpers aufgehoben. Durch Markierung der Apoptose- und Zelltodmarker Annexin V und 7-AAD in $CD4^+$ T-Zellen konnten wir zeigen, dass PTECs das Überleben der $CD4^+$ T-Zellen begünstigen. Nach Blockade der kostimulatorischen Signalwege mit Antikörpern gegen CD80 und CD86 zeigte sich eine verminderte PTEC-induzierte Aktivierung, Proliferation und Zytokinproduktion der $CD4^+$ T-Zellen. Desweiteren konnten wir *in vitro* nachweisen, dass PTECs in geringem Ausmaß dazu in der Lage sind, eine Umwandlung von $CD4^+$ T-Zellen zu Tregs zu begünstigen. Dieser Effekt war jedoch bei Stimulation der $CD4^+$ T-Zellen durch anti-CD3-Antikörper stärker

ausgeprägt als bei Stimulation der $CD4^+$ T-Zellen durch OVA. Bei Stimulation der $CD4^+$ T-Zellen durch OVA zeigte sich auch kein Unterschied in der Frequenz der induzierten Tregs durch die An- oder Abwesenheit von TGF-β, bei der Stimulation durch anti-CD3-Antikörper kam es jedoch durch hinzugefügtes TGF-β zu einer verstärkten Treg-Induktion.

### II.3.5 Expression von CD74 und APC-assoziierten Molekülen durch PTECs in der NTN

Um analysieren zu können, ob und wie die Expression der APC-assoziierten Moleküle durch PTECs unter inflammatorischen Bedingungen reguliert wird, untersuchten wir PTECs auch in C57BL/6 Mäusen zu unterschiedlichen Zeitpunkten nach Induktion der NTN. Somit konnten sowohl die frühe, akute, T-Zell-abhängige Phase als auch die späte, chronische Phase der NTN hinsichtlich der Expression APC-assoziierter Moleküle durch PTECs im Verlauf abgebildet werden. Acht Tage nach der Induktion der NTN konnten wir eine erhöhte mRNA-Expression von MHCII sowohl durch PTECs als auch durch nPTECs feststellen. Allerdings änderte sich die Frequenz der MHCII-positiven PTECs und nPTECs nicht während des Verlaufs der NTN. PTECs zeigten jedoch auch in der NTN eine höhere MHCII-Expression als nPTECs. Insbesondere PTECs zeigten auch eine stärkere Expression von CD74 an Tag acht und an Tag 14 nach NTN-Induktion. Interessanterweise zeigte sich die Expression von CD86 durch PTECs als auch durch nPTECs an Tag vier und Tag acht vermindert. Die Expression von CD80 verhielt sich bei PTECs und nPTECs gegenläufig. Während PTECs die Expression von CD80 während des Verlaufs der NTN herunterregulierten, zeigte sich bei nPTECs ein Anstieg der Expression.

### II.3.6 Expression APC-assoziierter Moleküle durch humane Tubulusepithelzellen in ANCA-assoziierten Vaskulitiden

Durch immunhistochemische Färbungen von Präparaten aus Nierenbiopsien von Patienten mit ANCA-assoziierten Vaskulitiden konnten wir - analog zu den murinen Analysen - eine starke Expression von CD86 in proximalen Tubuli nachweisen. Allerdings ließ sich in den humanen Zellen - im Gegensatz zu den murinen Zellen - die Expression von MHCII und CD74 sowohl in proximalen als auch distalen Tubulusepithelzellen darstellen. Dementsprechend exprimieren renale Tubulusepithelzellen in der chronischen Nierenentzündung Moleküle, welche mit der

Funktion von APCs verknüpft sind.

## II.4 Diskussion

In dieser Arbeit konnten wir zeigen, dass renale Tubulusepithelzellen APC-assoziierte Moleküle exprimieren. Darüber hinaus haben wir gezeigt, dass PTECs *in vitro* eine antigenspezifische, inflammatorische $CD4^+$ T-Zell-Aktivierung induzieren. Die Frage, ob PTECs auch *in vivo* immunmodulierende Eigenschaften haben, muss weiter untersucht werden.

Da die Reinheit der isolierten Epithelzellpopulationen eine wichtige Rolle für alle darauffolgenden Zellanalysen spielt, muss die ausschließliche Verwendung von E-Cadherin und LTA zur Markierung und Isolation der PTECs unter den im Folgenden genannten Aspekten differenziert betrachtet werden. Die Expression des zur Unterscheidung zwischen Epithelzellen und Nicht-Epithelzellen herangezogenen E-Cadherins zeigt eine Abhängigkeit unter anderem von bestimmten Zytokinen. In einer entzündlichen Umgebung könnte es also zu einer Herunterregulation von E-Cadherin auf Epithelzellen kommen. Daher wäre es sinnvoll, in zukünftigen Untersuchungen auch weitere, weniger beeinflussbare Marker zur Differenzierung zwischen Epithelzellen und Nicht-Epithelzellen bei der Isolation dieser Zellen heranzuziehen. Ebenso kann nicht ausgeschlossen werden, dass PTECs - entweder während der Isolationsaufbereitung oder in der entzündlichen Umgebung - Teile ihres Bürstensaums verlieren. Das zur spezifischen Markierung von PTECs verwendete LTA, welches an die Glykokalyx des Bürstensaums bindet, würde in einem solchen Fall an PTECs nicht mehr binden und demnach diese Zellen fälschlicherweise der nPTEC-Population zuordnen. Aus diesem Grund müssen zukünftig weitere PTEC-spezifische Marker gefunden und genutzt werden, welche durch Aufbereitungsmethoden oder Änderungen des umgebenden Milieus nicht beeinflussbar sind.

Wir konnten zeigen, dass PTECs *in vitro* eine antigenspezifische Aktivierung, Proliferation und die Produktion inflammatorischer Zytokine in $CD4^+$ T-Zellen induzieren. Insbesondere die Expression des proinflammatorischen Zytokins TNF-α wurde stark induziert. Bisher wurde die Funktion von $TNF\text{-}\alpha^+$ $CD4^+$ T-Zellen in der RPGN noch nicht untersucht. In der NTN (Khan et al. 2005, Timoshanko et al. 2003, Vielhauer et al. 2005), den ANCA-assoziierten Nierenschädigungen (Little et al. 2006) sowie der Lupusnephritis (Boswell et al. 1988, Qing et al. 2018, Yokoyama et al. 1995)

waren die TNF-α-Spiegel in der Niere erhöht. Eingriffe in den TNF-α-Signalweg wirkten sich hier günstig auf den Erkrankungsverlauf aus (Bethunaickan et al. 2012, Khan et al. 2005, Little et al. 2006). Bezüglich der zellulären TNF-α-Expression in der RPGN gibt es bislang lediglich eine Studie (Hünemörder et al. 2015), die die Existenz von TNF-$\alpha^+$CD4$^+$ T-Zellen in der experimentellen autoimmunvermittelten Glomerulonephritis zeigt. Allerdings wurde hier die Funktion dieser Zellen nicht weiter untersucht. Da bislang mehrere Studien erhöhte TNF-α-Spiegel mit einer erhöhten Infiltration von Entzündungszellen in die Niere und einer erhöhten Expression von Chemokinen und Adhäsionsmolekülen durch Nierenparenchymzellen in Verbindung brachten (Bethunaickan et al. 2012, Schildberg et al. 2008, Timoshanko et al. 2003), könnte man auch für PTEC-induzierte TNF-$\alpha^+$ CD4$^+$ T-Zellen annehmen, dass sie die Infiltration der Niere durch Entzündungszellen steigern.

Wie andere nicht-professionelle APCs (Kambayashi et al. 2014) exprimierten auch PTECs in geringem Ausmaß MHCII und könnten demnach trotz hoher Expression des kostimulatorischen Moleküls CD86 für eine schwache Aktivierung von CD4$^+$ T-Zellen verantwortlich sein.

Wir konnten in dieser Studie zeigen, dass die Frequenz CD74-exprimierender PTECs im Verlauf der NTN zunimmt. Eine Funktion von CD74 auf PTECs wurde bisher allerdings noch nicht beschrieben. Da CD74 für die Beladung von MHCII-Molekülen mit antigenen Peptiden von entscheidender Bedeutung ist (Stumptner-Cuvelette et al. 2002), könnte man die Annahme treffen, dass eine verstärkte CD74-Expression durch PTECs während einer Nierenentzündung die MHCII-vermittelte Antigenpräsentation durch PTECs unterstützt. Die Erkenntnisse über die Mechanismen, die die Expression von MHCII und CD74 durch renale Tubulusepithelzellen in der RPGN regulieren, sind jedoch noch sehr begrenzt. Daher sind weitere Untersuchungen erforderlich, um die entzündungsbedingte Modulation von MHCII und CD74 in PTECs und die Konsequenzen für die Aktivierung von CD4$^+$ T-Zellen und den anschließenden Krankheitsverlauf nachvollziehen zu können.

Zusammenfassend konnten wir in dieser Studie zeigen, dass renale Epithelzellen sowohl in gesunden als auch in nephritischen Mäusen sowie auch im Menschen Moleküle exprimieren, welche eine wichtige Funktion für die T-Zell-Aktivierung haben. Insbesondere konnten wir zeigen, dass PTECs einen inflammatorischen Phänotyp in CD4$^+$ T-Zellen induzieren. Die hier präsentierten *in-vitro*-Daten bieten Raum für

Hypothesen über die *in-vivo*-Relevanz der durch renale Epithelzellen vermittelten Aktivierung von $CD4^+$ T-Zellen im Krankheitsverlauf der RPGN. Allerdings ist die Entwicklung geeigneter *in-vivo*-Modelle für weitere Untersuchungen essentiell, wie beispielsweise Mauslinien, in denen PTECs die Moleküle MHCII, CD74, CD80 oder CD86 nicht exprimieren sowie die Etablierung antigenspezifischer Modelle für renale inflammatorische Erkrankungen.

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## IV. 1 Zusammenfassung

Bei immunvermittelten glomerulären Erkrankungen bzw. Krankheitsverläufen, wie beispielsweise der rapid-progressiven Glomerulonephritis (RPGN), akkumulieren $CD4^+$ T-Zellen im Tubulointerstitium in engem Kontakt zu proximalen und distalen Tubulusepithelzellen und vermitteln Nierenentzündung und Gewebeschädigung. Dennoch ist noch weitgehend unklar, ob renale Epithelzellen die Pathogenese der RPGN durch Modulation der $CD4^+$ T-Zell-Immunantwort beeinflussen. Die vorliegende Studie zielt darauf ab, zu untersuchen, ob renale Tubulusepithelzellen als antigenpräsentierende Zellen (APCs) fungieren und inwiefern sie eine $CD4^+$ T-Zell-Immunantwort hervorrufen können. Durch Etablierung einer FACS-basierten Isolationsmethode konnten wir verschiedene renale Epithelzellpopulationen sortieren und zeigen, dass insbesondere proximale Tubulusepithelzellen (PTECs) Moleküle exprimieren, die mit der Funktion von APCs assoziiert sind, wie beispielsweise MHCII, CD74, CD80 und CD86. Dies konnte sowohl in Zellen aus naiven Mäusen, als auch in Mäusen mit nephrotoxischer Nephritis (NTN), einem Mausmodell für die RPGN, gezeigt werden. Die Proteinexpression dieser Moleküle konnten wir in PTECs auf Einzelzellebene durch die Nutzung von bildgebender Durchflusszytometrie (ImageStream®) darstellen. Interessanterweise konnten wir in den Epithelzellen eine entzündungsabhängige Regulation von CD74, CD80 und CD86, jedoch keine Regulation von MHCII nachweisen. Die antigenspezifische Stimulation von $CD4^+$ T-Zellen *in vitro* durch PTECs förderte das Überleben von $CD4^+$ T-Zellen und induzierte deren Aktivierung, Proliferation und die Produktion inflammatorischer Zytokine. Bei Patienten mit ANCA-assoziierten Glomerulonephritiden konnten wir die Expression von MHCII und CD74 sowohl in proximalen als auch in distalen Tubulusepithelzellen nachweisen, während CD86 hauptsächlich von proximalen Tubulusepithelzellen exprimiert wurde. Demzufolge haben vor allem PTECs die Fähigkeit, *in vitro* einen inflammatorischen Phänotyp in $CD4^+$ T-Zellen zu induzieren und ihnen könnte daher eine Rolle in der Pathogenese immunvermittelter Nierenerkrankungen zuteilwerden.

## IV.2 Abstract

In immune-mediated glomerular diseases like crescentic glomerulonephritis (cGN), inflammatory $CD4^+$ T cells accumulate within the tubulointerstitial compartment in close contact to proximal and distal tubular epithelial cells and drive renal inflammation and tissue damage. However, whether renal epithelial cell populations play a role in the pathogenesis of cGN by modulating $CD4^+$ T-cell responses is less clear. In this study, we aim at investigating the potential of renal epithelial cells to function as antigen-presenting cells (APCs) thereby stimulating $CD4^+$ T-cell responses. By using a FACS-based protocol that allowed comparative analysis of cortical epithelial cell populations, we showed that particularly proximal tubular epithelial cells (PTECs) expressed molecules linked with APC function including MHCII, CD74, CD80, and CD86 in homeostasis and nephrotoxic nephritis, a murine model of cGN. Protein expression was visualized on PTEC single cell level by imaging flow cytometry (ImageStream®). Interestingly, we found an inflammation-dependent regulation of epithelial-expressed CD74, CD80, and CD86 while MHCII expression was not altered. Antigen-specific stimulation of $CD4^+$ T cells by PTECs *in vitro* supported $CD4^+$ T-cell survival and induced $CD4^+$ T-cell activation, proliferation, and inflammatory cytokine production. In patients with ANCA-associated GN, MHCII and CD74 were expressed by both proximal and distal tubules whereas CD86 was predominantly expressed by proximal tubules. Thus, particularly PTECs have the potential to induce an inflammatory phenotype in $CD4^+$ T cells *in vitro*, which might also play a role in the pathology of immune-mediated kidney disease.

## V. Erklärung des Eigenanteils an der Publikation

Hiermit erkläre ich, Philippe Christophe Breda, folgenden Eigenanteil an der Publikation:

Ich führte Experimente durch, analysierte Daten, interpretierte die Ergebnisse der durchgeführten Experimente und war am Verfassen von Teilen (Materials and Methods) des Manuskripts beteiligt. Ebenso war ich an der Methodenentwicklung- und Etablierung für das Sortieren von PTECs durch FACS sowie an der Einzelzellanalyse von PTECs durch bildgebende Durchflusszytometrie beteiligt.

## VI. Danksagung

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## VII. Curriculum Vitae

**Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.**

## VIII. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....