Podocytes Are Nonhematopoietic Professional Antigen-Presenting Cells

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ABSTRACT

Podocytes are essential to the structure and function of the glomerular filtration barrier; however, they also exhibit increased expression of MHC class II molecules under inflammatory conditions, and they remove Ig and immune complexes from the glomerular basement membrane (GBM). This finding suggests that podocytes may act as antigen-presenting cells, taking up and processing antigens to initiate specific T cell responses, similar to professional hematopoietic cells such as dendritic cells or macrophages. Here, MHC–antigen complexes expressed exclusively on podocytes of transgenic mice were sufficient to activate CD8+ T cells in vivo. In addition, deleting MHC class II exclusively on podocytes prevented the induction of experimental anti-GBM nephritis. Podocytes ingested soluble and particulate antigens, activated CD4+ T cells, and crosspresented exogenous antigen on MHC class I molecules to CD8+ T cells. In conclusion, podocytes participate in the antigen-specific activation of adaptive immune responses, providing a potential target for immunotherapies of inflammatory kidney diseases and transplant rejection.


Adaptive immune responses are initiated by the stimulation of naïve T lymphocytes by antigen-presenting cells (APCs). Briefly, dendritic cells (DCs) and macrophages are present throughout the body; on antigen capture, these cells enter local lymph nodes or the spleen and present the antigens to naïve T cells. Whether antigen presentation results in immunity or tolerance depends on additional factors, such as danger signals, the type of APCs, and the local microenvironment.¹ T cells recognize antigenic peptides bound to the MHC on the surface of APCs that are presented by three different mechanisms. (I) Intracellularly synthesized antigens, mainly derived from defective ribosomal products of intracellular pathogens or self-proteins, are presented by MHC class I molecules and may activate CD8+ cytotoxic T cells.² (II) Peptide fragments from extracellular antigens, which are endocytosed and then degraded by proteases, are presented on MHC class II molecules by professional APCs to CD4+ helper T cells. (III) Endocytosed antigens can also be presented to CD8+ lymphocytes through a process called crosspresentation. Depending on the endocytic process and the activation of APCs, alternative intracellular sorting of the antigen cargo may result in the loading and surface presentation of MHC class I peptide complexes.³,⁴ Professional APCs such as DCs are, thereby, capable of activating CD8+ cells to provide immunity against viruses that do not infect APCs or nonhematopoietic tumors. Other than the ability for antigen presentation, the activation of naïve T cells by antigen presentation implies additional

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qualities. First, the APCs have to exhibit high phagocytic capacity and the mechanism to channel the antigen into the according pathway. Thus, antigen presentation requires cell–cell contact; the APCs must have the ability to sense danger signals, like microbiobal components or innate proinflammatory secretions, and after activation, the APCs have to attract the T cells by chemokines. Finally, after T cell receptor ligation, they ensure T cell activation by the expression of several costimulatory receptors.

The main function of the kidney glomerulus is to filter blood plasma into primary urine for subsequent handling by the renal tubular system. Small molecules, such as water, sugars, electrolytes, and small proteins, pass through, whereas the barrier retains high-molecular weight proteins and cells in circulation. The glomerular filtration barrier consists of a specialized fenestrated endothelium, the glomerular basement membrane, and filtration slits, which are generated by intricately interdigitated foot processes from terminally differentiated visceral epithelial cells known as podocytes. Several studies defined the critical importance of podocytes for kidney function, and it is now widely accepted that damage to the podocytes is a key event that initiates progression to many glomerular diseases.

Under inflammatory conditions, podocytes display enhanced expression of MHC class II molecules, which was first described for pauci-immune necrotizing crescentic GN. B7-1, a costimulatory molecule that is essential for complete activation of T cells, is likewise upregulated during local renal inflammation (e.g., lipopolysaccharide-induced nephritic syndrome). Because podocytes are in continuous contact with blood plasma and hence, foreign antigens present in the blood and because podocytes remove Ig and immune complexes from the glomerular basement membrane through immune globulin receptor type N, we suspected that podocytes could act as professional APCs like macrophages. This finding was underlined by the fact that podocytes and macrophages may share lineage commitment (e.g., reflected by their expression of the transcription factor MafB) as well as other sets of genes.

RESULTS

Podocytes Phagocytose-Labeled Latex Beads and Soluble Ovalbumin

To examine the antigen presentation capacity of podocytes, we used isolated primary podocytes isolated from mouse glomerulus and a conditionally immortalized murine podocyte cell line (PCL) developed and kindly provided by Karlhans Endlich (University of Greifswald, Germany). Presentation of antigenic peptides in MHC class II complexes or MHC class I molecules by crosspresentation requires the uptake of extracellular antigens by phagocytosis, pinocytosis, or receptor-mediated mechanisms. We found that podocytes could ingest both labeled latex beads and soluble fluorescence-labeled ovalbumin by phagocytosis. This incorporation was analyzed by FACS or visualized by microscopy (Figure 1, A–F). After intravenous injection, the uptake of labeled beads could also be shown to occur in vivo (Figure 1, G and H). Supplemental Figure 1 shows that PCL cells expressed MHC class I and II molecules in densities quantitatively comparable with peritoneal exudate macrophages (PEMs). Furthermore, PCL cells express costimulatory molecules, such as CD80 and intercellular adhesion molecule, as well as the podocyte-specific molecule podocalyxin. The analysis of previously published microarray data revealed that the PCL cells as well as sorted primary murine podocytes express all of the genes necessary for MHC class I and II functions and expression, like the transcription factors Rfxap, Rfx5, Rfxant, and NF-κ. In addition, primary podocytes are positive for several other macrophage markers like emr1, sfp1, MafB, Mpeg1, and Runx1 (Supplemental Figure 2).

Podocytes Activate Naive OT-II Cells

We next addressed the question of whether proteins taken up by podocytes were processed as peptide–MHC complexes for presentation to T cells. PCL cells loaded with ovalbumin induced proliferation of ovalbumin-specific CD4+ T cells in a dose-dependent manner (Figure 2A). As expected, MHC-disparate bone marrow-derived macrophages (BMMs) from BALB/c mice did not, whereas BMM from C57BL/6 mice activated the OT-II cells. OT-II T cells also upregulated the activation marker CD25. A representative histogram is shown in Figure 2C, and a summary of three experiments is shown in Figure 2D. In addition to undergoing activation and proliferation, the CD4+ T cells secreted the Th1 cytokines IL-2 and IFN-γ (Figure 2B).

We next asked whether podocytes could also activate CD8+ T cells. In the mixed lymphocyte reactions performed, podocytes were also able to activate allogeneic CD8+ T cells. In comparison, LPS-activated DCs were the best activators of allogeneic CD4+ and CD8+ T cells, whereas macrophages were inefficient in our experiments (Figure 3). Also, the observed activation of T cell by DCs in the syngeneic setting may reflect presentation of xenogeneic protein antigens contained in FCS as observed in previous studies. Interestingly, podocytes mainly activated allogeneic CD8+ T cells, whereas their capacity to activate CD4+ T cells was markedly lower (Figure 3, C and D). This strong allogeneic activation was also seen in experiments with unsorted spleen cells from OT-II mice. Because the PCL cells were generated from CBA (H2k) × C57BL/10 (H2b) mice, we were able to analyze the activation of alloreactive cells and ovalbumin-reactive T cells in a mixture of unsorted spleen cells from OT-II transgenic C57BL/6 (H2b) mice simultaneously in one experimental setting (Supplemental Figure 3). In the presence of ovalbumin (Supplemental Figure 3, A and D), a very strong allogeneic reaction of the Vα2-negative nontransgenic cells, together with the ovalbumin-specific activation of the transgenic Vα2-positive OT-II T cells, was detected as CD62L down- and CD69 upregulation on the T cells. In contrast, in the absence of ovalbumin, we
detected only an allospecific reaction (Supplemental Figure 3, B and E).

**Crosspresentation by Podocytes**

We next wanted to know if podocytes are able to take up exogenous antigen and crosspresent it to CD8+ T cells. For the purpose of comparison, DCs and BMMs were equally loaded for 1 or 2 days with different concentrations of ovalbumin and cocultivated with a mouse T cell hybridoma line, which sensitively recognizes H2Kd loaded with the ovalbumin peptide. LPS-stimulated DCs were very potent activators of antigen-specific CD8+ T cells (Figure 4A). Antigen crosspresentation by BMMs was less potent than for DCs (Figure 4, C and D). We also observed antigen crosspresentation by PCL cells (Figure 4E). The PCL cell capacity was lower than the capacity observed for DCs and BMMs, but it was significantly enhanced by the addition of TNF-α (Figure 4F). Toll-like receptors (TLRs) are essential in DCs for sensing microbial molecules and consequently, inducing maturation. We found that PCL cells expressed high levels of mRNA for almost all TLRs (Supplemental Figure 4). Furthermore, we found that six mostly T cell-attracting chemokines were expressed by PCL cells (Supplemental Figure 4). Interestingly, the subunits of the immunoproteasome (Lmp-2 and Lmp-7) and two proteins essential for efficient crosspresentation (nox-2 and pg91) are also expressed by PCL cells.

**Freshly Isolated Murine Podocytes Have Features Similar to PCL Cells**

To complete the in vitro experiments, we repeated selected experiments with freshly isolated murine podocytes. We isolated glomeruli from murine kidneys by sieve filtration.12 The outgrowing cells were analyzed by immunostaining (Supplemental Figure 5). Thus, our data (Supplemental Figure 6) show that primary podocytes also possess the capacity to activate T cells by MHC classes I and II presentation as well as crosspresentation.

**Podocyte/T Cell Contact In Vivo**

One essential requirement for antigen presentation is close contact between the T cell and the APC. In healthy glomeruli, podocytes are separated from T cells by the glomerular basement membrane (GBM). However, we detected colocalization of T cells in close contact with podocytes in...
Figure 2. Podocytes activate CD4+ T cells by MHC II presentation. BMMS or PCLs were cultivated for 1 day in the presence or absence of ovalbumin. The cells were intensely washed, and 5×10⁴ OT-II cells, purified by magnetic cell sorting, were added at a ratio of 1:1. (B) Supernatants were collected after 48 hours and analyzed for IL-2 and IFN-γ expression by ELISA. Proliferation was measured by ³H uptake, and the CD25 upregulation was analyzed after 48 hours. (A) PCL cells loaded with ovalbumin induced proliferation of ovalbumin-specific MHC class II-restricted CD4+ T cells from OT-II mice in a dose-dependent manner comparable with C57BL/6 BMMS, whereas BALB/c BMMS did not. *Significant differences to medium alone or podocytes without ovalbumin (P<0.05, t test). C shows a representative FACS staining, and D shows quantification of three experiments determining surface expression of the T cell activation marker CD25.

infamed kidneys. In a glomerulus section from an NZB mouse with lupus nephritis (Figure 5, A and B) and a C57BL/6 mouse 21 days after induction of anti-GBM nephritis (Figure 5, C and D), we observed CD3+ cells surrounded by the podocalyxin-positive foot processes of podocytes (Figure 5, A–D). This contact was laminin-free, indicating a hole in the glomerular membrane. The observation was confirmed by confocal microscopy (Supplemental Movies 1–3). We analyzed several rat models of inflammatory glomerular disease, including the passive Heymann nephritis model (not shown), the 5/6 nephrectomy model (Figure 5E), and a renal transplantation model (Figure 5F). In all models, CD8+ T cells were surrounded by ezrin-positive podocytes and found outside the blood vessel. In tissue sections of human kidneys from a patient with lupus GN (International Society of Nephrology/Renal Pathology Society [ISN/RPS] IV), we also detected close contacts between synaptopodin-stained podocytes and CD3+ T cells (Figure 5, G and H).

Activation of CD8 OT-I T Cells by Podocytes In Vivo
We show that podocytes can act as APCs. To validate this phenomenon also occurs in vivo, an appropriate model was established. Mice expressing ovalbumin exclusively in their podocytes were irradiated, and the hematopoietic system was reconstituted with bone marrow from B6.C.H2b⁻/⁻ mice. In these mice, a mutation in the binding groove of the MHC class I molecule prevents the binding of the ovalbumin peptide. After bone marrow reconstitution, only nonhematopoietic cells of the chimeric mice are able to present the ovalbumin peptide, and within the nonhematopoietic cells, only podocytes express the antigen. To facilitate podocyte/T cell contacts, we induced aGBM nephritis in the recipient animals. The experimental setup is illustrated in Figure 6A. The development of proteinuria is shown in Figure 6B. Extensive FACS analyses were performed to exclude radiation-resistant hematopoietic cells in the recipient mice. The exemplified FACS blot of spleen cells in Figure 6C indicates the success of the bone marrow transplantation. The antibody 5F1 recognizes the H2k and not the mutated H2kb⁻/⁻. Only the transferred Carboxyfluorescein succinimidyl ester (CFSE)-labeled OT-I cells are 5F1-positive. A representative experiment of two mice is shown in Figure 6, D and E. OT-I T cell proliferation was only detected in the nephrin Ova-transgenic mice. The difference in the frequency of proliferating OT-I cells in the spleen was significant (1.9%±1.11% versus 8.2%±1.09%, P=0.02). In the renal lymph nodes, the difference in the percentages of proliferating OT-I cells in transgenic or control recipient mice was even more pronounced (3.6%±1.3% versus 37.0%±9.5%, P=0.05). The summary is shown in Figure 6F. As a control, bone marrow DCs from the bone marrow chimeric recipient mice were generated and incubated with 0.5 mg/ml ovalbumin and LPS (0.1 μg/ml) for 1 day, and OT-I and -II cells were added in different ratios. As expected, bone marrow DCs from bone marrow chimeric mice did not activate OT-I cells (Figure 6G). In contrast, the bone marrow DCs were able to activate CD4+ transgenic OT-II T cells (Figure 6G), because MHC class II presentation is not altered in these mice. The inability of the hematopoietic cells (like DCs) to induce OT-I T cell proliferation in these animals supports the conclusion that the podocyte–T cell interaction is responsible for the observed antigen-specific T cell activation in vivo.

MHC II Expression on Podocytes Is Essential to Induce a Severe Anti-GBM Nephritis
To circumstantiate these results, a second in vivo model was established. Homozygote mice with a loxP-flanked exon 1 of the MHC class II β1 locus were bred with a transgenic mouse line expressing Cre recombinase exclusively in podocytes. The F3 offspring from this breeding have a loss of MHC class II only in the podocytes, whereas all other MHC class II-restricted Ag presentation is unchanged (Supplemental Figure 9). To study the role of podocyte MHC class II expression in the pathogenesis of GN, we induced an anti-GBM disease in these mice. Most of the control animals developed a focal and segmental nephrosclerosis and glomerular crescent formation, whereas the animals without MHC class II in the podocytes developed only a very moderate...
disease (Figure 7, A–F). In addition, in the kidneys from nephritic mice lacking MHC class II on podocytes, we found decreased numbers of CD45+ and CD45/CD4 double-positive leukocytes compared with kidneys from mice with podocytic MHC class II expression (Figure 7, H, J, and K). Furthermore, CD4 activation, as assessed by the appearance of a population, expressing the effector memory phenotype (CD62Llow/CD44high) was significantly stronger in control animals (80.17% ± 5.0% versus 54.9% ± 3.4% of all intra-renal CD4 cells) compared with animals lacking MHC class II on podocytes (Figure 7, I and L). These experiments strongly suggest that T cell activation by podocytes occurs in vivo and plays an important role in CD4-driven immune response.

DISCUSSION

For decades, podocytes have been considered to be a major passive antigenic target in many different glomerular diseases in man, such as membranous glomerulopathy, minimal change disease, and FSGS as well as many other glomerulonephritides. Our study is the first to show that podocytes in vitro and in vivo are able to act as activating specific T cell responses by all three of the modes usually used by professional hematopoietic APCs. Based on our findings, we propose a new and unexpectedly active role for podocytes in the adaptive immune responses, which may have broad importance for initiation and progression of immunologic kidney disease as well as alloimmune responses after renal transplantation.

Recent studies showed that podocytes can acquire macrophage-like functions such as expression of CD80 on LPS stimulation as well as upregulation/activation of TLRs leading to the local release of chemokines. Using immortalized as well as primary murine podocytes, we found expression of MHC classes I and II molecules and costimulatory molecules in amounts quantitatively comparable with macrophages. In addition, primary podocytes are positive for several other macrophage markers (Supplemental Figure 7). This finding is in agreement with a published gene array analysis that showed the expression of several macrophage transcripts (CD68, F4/80, CD206, DEC205, and the transcription factor PU.1) in podocytes (Supplemental Figure 7). Transcriptome analysis of laser-captured glomeruli from patients with lupus nephritis revealed that myeloid lineage transcripts were expressed in the biopsy samples. In crescentic GN, podocytes transform into crescent-forming cells, which are double-positive for podocalyxin and CD68, showing several characteristics of macrophages, such as hydrogen peroxide and cytokine production. This finding was first described for rat glomeruli and subsequently, idiopathic collapsing glomerulopathy in humans. Uptake of latex beads was, so far, thought to be an identifying exclusive feature of professional macrophages. We showed a high phagocytic activity in cultured podocytes and in vivo as well. We also showed that not just macrophages but also podocytes express many TLRs being used to recognize danger signals and specifically, modulated on TNF-α stimulation, leading to the release of T cell-attractive chemokines. A microarray study analysing the transcriptional profile of podocyte cell line from Peter Mundel using challenge with TNFα also shows the upregulation of these chemokines.

There are increasing data supporting a role of TLRs in inflammatory kidney diseases. Endocytosed antigens usually enter the MHC class II pathway to permit CD4+ T cell activation. Only professional APCs are able to also pass
presentation by the podocytes is sufficient to activate CD8+ OT-I cells in the murine kidney and furthermore, that MHC class II antigen presentation by podocytes is necessary to induce the CD4+ T cell-driven glomerular disease. The fact that intrinsic renal cells expressing MHC class II are required for the development of immune-mediated renal injury was shown before,29 and our results show that these intrinsic renal cells are the podocytes.

Although we performed very extensive FACS control staining, the persistence of a very small population of host DCs in the kidney, acting as a potential confounding factor for ovalbumin presentation to OT-I cells in the bone marrow chimeras, cannot be excluded. Because the newly generated podocyte-specific MHC class II-deficient mice were virtually resistant to the induction of anti-GBM nephritis, MHC II presentation by podocytes plays a decisive role in this T cell-driven disease. Thus, we propose a new kidney-intrinsic T cell activation pathway through the antigen-presenting properties of podocytes. Our data suggest an important direct role of podocytes in glomerular-mediated immune reaction leading to glomerular diseases and during rejection of kidney grafts, where T cell-mediated immune mechanisms are mandatory for renal tissue damage.13,30,31 However, the relative contribution of renal DCs presenting podocyte antigens or alternatively, podocytes themselves functioning as APCs for the initiation and perpetuation of a T cell immune response remains an important question to be solved by future studies. We consider it very likely that antigen presentation by DCs and podocytes is not mutually exclusive. It has been shown previously in elegant studies that renal DCs present glomerular autoantigens to T cells after ingestion and migration to the draining renal lymph nodes.13,32 A putative transfer of MHC II molecules from podocytes to the DCs or the migration of podocytes to the lymph nodes cannot be completely excluded. However, because activated DCs are extremely potent MHC class II expressors, the additive cargo of some podocyte MHC molecules by trohcytosis or exosome transfer would most probably play only a minor role. Under conditions where the deterioration of the glomerular basal membrane facilitates the podocyte T cell contact in vivo, podocytes might contribute to the tissue-intrinsic activation of T cells recruited by podocyte-derived chemokines. This glomerular preconditioning may be operative early on whenever kidney damage is caused by drugs, toxins, ischemia, or infections, even before DC migration from the renal tissue to the draining lymph nodes has occurred. Whether this newly described kidney intrinsic pathway of T cell activation is specific for podocytes needs to be evaluated in future studies, because within the kidney, different nonhemopoetic cells with potential antigen-presenting capacity have been described, such as renal proximal tubular cells33 as well as activated vascular endothelial cells.34

In summary, our studies suggest that podocytes should be regarded not only as victims but also culprits of immune-mediated extracellular material in the MHC class I pathway, allowing the generation of effector CD8+ cells, a process denominated crosspresentation. So far, crosspresentation capacity has been shown for macrophages and DCs and only a few other hematopoietic (e.g., B cells25 and neutrophils26) and nonhematopoietic (liver sinusoidal endothelial cells27) cell types. In contrast to liver sinusoidal endothelial cells, which induce a liver-specific T cell tolerization,28 we found that activated podocytes are potent activators of CD8+ T cells. Next, we showed that this finding could also happen in vivo. Double staining for podocytes together with T cells showed that, in many inflammatory kidney disease models in rats, man, and mice, T cells can be observed next to podocytes. This result could also been shown by FACS analysis of cell doublets from kidney (Supplemental Figure 8).

Finally, using two in vivo model systems, we proved that, in a situation of anti-GBM nephritis, that the MHC class I antigen

Figure 4. Podocytes activate T cells by crosspresentation. Crosspresentation and activation of the ovalbumin-specific H2-Kb-restricted T cell hybridoma B3Z. DCs, BMMs, or PCLs were incubated with different concentrations of ovalbumin for 18 or 44 hours. After antigen uptake, serial dilutions of the cells were cocultured in 96-well plates with 50,000 B3Z T cells (APC/T cell ratios from 1:1 to 0.01:1). After 16 hours, the T cell activation was analyzed as described. (A and B) LPS-activated DCs incubated with ovalbumin for 18 or 44 hours in the presence of 0.1 μg/ml LPS. (C and D) BMMs incubated with ovalbumin for 18 or 44 hours. (E) Unstimulated PCLs incubated with ovalbumin for 44 hours. (F) PCLs stimulated with TNF-α (20 ng/ml) and incubated with ovalbumin for 44 hours. Individual values present the mean of three individual experiments ± SD.
glomerular diseases and potentially, kidney transplant rejection, being able to directly activate T cells as newly defined kidney APCs.

CONCISE METHODS

Full methods are in Supplemental Material.

The murine PCL,\textsuperscript{11} developed from the H-K\textsuperscript{Kb}-tsA58 transgenic mouse strain,\textsuperscript{35} was kindly provided by Karl-Hans Endlich (University of Greifswald, Greifswald, Germany). The PCLs were maintained and differentiated as described.\textsuperscript{11}

To isolate primary murine podocytes, glomeruli from the kidney cortices of approximately 30–40 mice were purified by the sieving method based on the original work of Krakower and Greenspon.\textsuperscript{12,36} The primary cellular outgrowth was harvested and analyzed by immunostaining.

The preparation and culture of bone marrow cells from C57BL/6 and BALB/c mice to generate DCs and macrophages, respectively, have been previously described.\textsuperscript{37} Granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor (PeproTech/Tebu) were used at 200 U/ml.

PEMs were elicited by intraperitoneal injection of thioglycollate (Sigma, Steinheim, Germany). PEMs were harvested 4 days post-intraperitoneal injection by peritoneal lavage with 10 ml sterile PBS and enriched by adhesion. The purity of the resulting cell population was analyzed by FACS.

Immunostaining

Primary cells grown in chamber slides and frozen on paraffin-embedded sections of kidney biopsies were used and stained with the indicated antibodies. The slides were embedded with 4',6-diamidino-2-phenylindole–containing mounting medium and evaluated with a fluorescence microscope (Zeiss Axiovert 220M with AxioCam MRm and ApoTome together with the software AxioVision 4.7.1; Oberkochen, Germany) or confocal microscopy (Leica SP5 together with the software LAS 2.3.0; Mannheim, Germany).

To visualize podocyte–T cell contacts, renal biopsies were taken from rats 12 weeks after 5/6 nephrectomy, 5 days after induction of passive Heymann nephritis, 11 days after puromycin injection, day 6 of the anti-Thy1 model, and 11 days after renal transplantation of a Fischer rat kidney into a Lewis rat recipient. In addition, renal biopsies from 24-week-old NZB/W mice with proven lupus nephritis and human kidney biopsies with proven immune complex GN were analyzed. Biopsies from pauci-immune crescentic GN and normal renal tissues served as controls.

Figure 5. T cell–podocyte contacts are detected in vivo. (A–D) Immunofluorescence showing triple staining for GBM positive for laminin (green), podocalyxin-positive podocytes (blue), and CD3+ T cells (red) in (A and B) the glomerulus of an NZB/W mouse with lupus nephritis or (C and D) a C57BL/6 mouse 21 days after induction of anti-GBM nephritis. (A, B, and D) The close contact (without laminin) between two CD3+ T cells and podocytes is marked with a yellow arrow. Immunohistological double staining of ezrin (blue) as a podocyte marker and CD8 (brown) as a T cell marker in rat models of renal disease. Close contact between CD8+ T cells and podocytes is marked with an arrow in (E) a 5/6 nephrectomy model (a nephron loss model) and (F) the allogeneic Fischer–Lewis renal transplantation model with signs of rejection. Immunohistological double staining of synaptopodin (blue; podocyte marker) and CD3 (brown; T cell marker) in human lupus GN (ISN/RPS IV). (G and H) Close contact between a CD3+ T cell and the foot processes of a podocyte (blue) is visible.
Flow Cytometry (FACS)

To block Fc receptors, ex vivo-prepared or -cultured cells were incubated with Fc block (BD Biosciences) or 5 μl normal mouse serum for 10 minutes on ice. Subsequently, cells were stained with 1:100 dilutions of the monoclonal antibodies for 30 minutes on ice. All FACS analyses were done on an FACSCalibur (BD Biosciences) equipped with Cell Quest Pro 4.1 and FlowJo 9.3.1 software.

OT-II Cell Stimulation Assays

For stimulation assays, defined populations of bone marrow macrophages, podocytes, DCs, or PEMs were cultured for 1 day in the presence or absence of ovalbumin. The cells were washed intensively at least five times with PBS, and 5 × 10^5-purified CD4+ cells from OT-II mice^38 were added at a ratio of 1:1. Because of the limited numbers of primary podocytes, the effector/target cell ration E/T ratio was diminished in these assays to 0.2:1. CD4+ OT-II cells were purified from spleens by magnetic cell sorting. The purity of the resulting cell population was analyzed by FACS as described above, and it was >95%. After 24 hours of coculture, an aliquot was used to measure the upregulation of CD69 by FACS. After 48 hours, the supernatants of cultures were collected and analyzed for the presence of IL-2 and IFN-γ by ELISA using the DuoSet ELISA development system according to the manufacturer’s instructions (R&D Systems).
Additionally, proliferation was measured by $^3$H uptake; downregulation of CD62L and upregulation of CD25 were analyzed by FACS.

**Crosspresentation and B3Z Activation**

To analyze the MHC class I-specific T cell activation, the SL-H2-K$^b$-specific, murine CD8+ T cell B3Z hybridoma was used. These cells were kindly provided by Nilabh Shastri at the University of California at Berkeley (Berkeley, CA). The antigen-specific activation was monitored by a colorimetric assay. APCs were incubated with indicated concentrations of ovalbumin, and serial dilutions of these cells were then cocultured with B3Z cells. To exclude nonspecific reactions, serial dilutions of TNF-α (PeproTech/Tebu), LPS, or ovalbumin alone were tested in parallel. Ionomycin served as a positive control.

**OT-I Cell Activation In Vivo**

The podocin ovalbumin (NOH) or nontransgene littermates were radiated two times with 3 Gy. Immediately, 30 mio fresh isolated bone marrow cells from a B6.C.H2bm1 mouse (Jackson Laboratories) were injected in the tail vain. After reconstitution, the nephrotoxic nephritis was induced by a standard protocol. First, the mice were immunized subcutaneously with 50 μg rabbit IgG together with 50 μl Freund’s complete adjuvant. After 6 and 7 days, a rabbit serum reacting with the glomerular basal membrane was injected intravenously. With the beginning signs of proteinuria (on average, 10 days post-induction), 10⁷ CFSE-labeled OT-I cells were transferred intravenously. To display the in vivo T cell activation, the spleen and the renal lymph node were removed, and cells were analyzed by FACS as described above. Additionally, the bone marrow was used to generate bone marrow DCs. After differentiation, these cells were loaded with ovalbumin, and serial dilutions were used in the proliferation experiments. After 2 days coculture with either OT-I or -II cells, the thymid uptake was measured.

**Anti-GBM GN in Mice with MHC Class II-Deficient Podocytes**

C57BL6 mice with a podocyte-specific MHC class II knockout were generated by breeding mice with a loxP-flanked exon 1 of the MHC class II $β$-1 locus and another C57BL/6 transgenic mouse line expressing the Cre recombinase exclusively in podocytes. The genotype was

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**Figure 7.** MHC II expressed on podocytes is essential for the induction of severe anti-GBM nephritis. Transgenic mice expressing the Cre recombinase exclusively in podocytes were crossed in a mouse line expressing a floxed MHC class II gene. In the F3 offspring and control littermates, an anti-GBM nephritis was induced as described in Figure 6. The kidneys were analyzed by histology and FACS. Exemplified periodic acid–Schiff staining is shown from mice without MHC class II (A and C) on podocytes and (B and D) from control animals. The quantification of three independent experiments is shown in E (percent crescentic glomeruli) and F (glomerulosclerosis index). The FACS gating strategy is shown in G–I. (J–L) The quantifications are shown as box plots (box-and-whisker diagrams graphically depicting the lowest number observed, lower quartile, median, upper quartile, and largest observation). **P<0.001. (G and J) First, the relative frequencies of CD45+ leukocytes in the kidneys were calculated; (H and I) subsequently, the main subpopulations of the leukocytes were analyzed, and within the CD45/CD4 double-positive populations, the frequencies of effector memory cells (CD44 high/CD62L low) were determined. Please note that most control animals displayed glomerulosclerosis, pronounced and frequent crescent formation, and infiltration of CD4+ effector memory cells. This result was not seen in mice lacking MHC class II expression selectively on podocytes.
determined by PCR. All experimental mice are homozygote for the floxed MHC II gene. One group was double transgenic (causing no MHC II expression on podocytes) or control single transgenic littermates (no Cre expression results in an unchanged MHC II expression). In these mice, we induced an anti-GBM disease and analyzed the disease progression by FACS or histologic evaluation.

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DISCLOSURES

None.

REFERENCES

21. Li S, Kurs C, Köng F, Holdsworth SR, Tipping PG: Major histocompatibility complex class II expression by intrinsic renal cells is

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