Human proximal tubule epithelial cells modulate autologous dendritic cell function

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Abstract

Background. We have previously demonstrated that human kidney proximal tubule epithelial cells (PTEC) are able to modulate autologous T and B lymphocyte responses. It is well established that dendritic cells (DC) are responsible for the initiation and direction of adaptive immune responses and that these cells occur in the renal interstitium in close apposition to PTEC under inflammatory disease settings. However, there is no information regarding the interaction of PTEC with DC in an autologous human context.

Methods. Human monocytes were differentiated into monocyte-derived DC (MoDC) in the absence or presence of primary autologous activated PTEC and matured with polyinosinic:polycytidylic acid [poly(I:C)], while purified, pre-formed myeloid blood DC (CD1c+ BDC) were cultured with autologous activated PTEC in the absence or presence of poly(I:C) stimulation. DC responses were monitored by surface antigen expression, cytokine secretion, antigen uptake capacity and allogeneic T-cell-stimulatory ability.

Results. The presence of autologous activated PTEC inhibited the differentiation of monocytes to MoDC. Furthermore, MoDC differentiated in the presence of PTEC displayed an immature surface phenotype, efficient phagocytic capacity and, upon poly(I:C) stimulation, secreted low levels of pro-inflammatory cytokine interleukin (IL)-12p70, high levels of anti-inflammatory cytokine IL-10 and induced weak Th1 responses. Similarly, pre-formed CD1c+ BDC matured in the presence of PTEC exhibited an immature tolerogenic surface phenotype, strong endocytic and phagocytic ability and stimulated significantly attenuated T-cell-stimulatory ability.

Conclusions. Our data suggest that activated PTEC regulate human autologous immunity via complex interactions with DC. The ability of PTEC to modulate autologous DC function has important implications for the dampening of pro-inflammatory immune responses within the tubulointerstitium in renal injuries. Further dissection of the mechanisms of PTEC modulation of autologous immune responses may offer targets for therapeutic intervention in renal medicine.

Keywords: dendritic cells; immune modulation; proximal tubule epithelial cells

Introduction

Tubulointerstitial damage and fibrosis is considered the hallmark of chronic progressive kidney diseases, often characterized by the infiltration of pro-inflammatory mononuclear leucocytes in the local environment [1]. Proximal tubule epithelial cells (PTEC) of the kidney are thought to mediate this disease process in many human kidney diseases [2]. Indeed, chemokines secreted by activated PTEC in the perturbed disease state play a pivotal role in the recruitment of immune cells into the interstitium of the kidney [3–6]. Despite the established chemotactic role of PTEC, the nature of direct interactions between PTEC and immune infiltrates in the tubulointerstitium is still poorly understood. Our group has recently demonstrated for the first time the ability of activated primary human PTEC to inhibit autologous immune responses [7]. However, it still remains unclear whether they modulate autologous immune responses via direct T-and B-cell interactions or through a modulation of professional antigen-presenting cell (APC) function.

Dendritic cells (DC) are professional APC that play an essential role in the induction and regulation of immune responses. Triggering and activation of DC by danger signals can lead to the up-regulation of co-stimulatory (CD86) and co-inhibitory (PD-L1) molecules, activation markers (CD83), production of pro-inflammatory [tumour necrosis factor-α (TNF-α), interleukin (IL)-12] and regulatory (IL-10) cytokines and priming of CD4+ T helper (Th) cell and CD8+ cytotoxic T-cell responses. Although
all DC are capable of antigen uptake and priming of naive T cells, it is clear that DC actually represent a heterogeneous population of cells [8] with subset-specific functional specializations now emerging [9, 10]. Human DC mobilized to peripheral tissues during disease can be broadly categorized into (i) inflammatory DC that rapidly develop from peripheral blood monocytes in response to infection and inflammation (monocyte-derived DC; MoDC) [11] and (ii) lineage (CD3, 14, 15, 19, 20, 56) negative, MHC class II (HLA-DR)-positive blood DC (BDC) that can be divided into CD11c+ CD123hi plasmacytoid DC (pDC) and CD11c+ myeloid DC (mDC) [12]. These myeloid DC are further delineated into CD1c (BDCA-1)+ and CD141 (BDCA-3)+ subsets [13].

Monocytes capable of differentiation into inflammatory MoDC have been detected in the tubulointerstitium of patients with proliferative glomerulonephritis (GN) [14]. Consistent with this study, Segerer et al. [17] observed a strong and localized tubulointerstitial infiltration of DC-SIGN+ cells (expressed by inflammatory MoDC and myeloid DC [15, 16]) in patients with proliferative GN. Notably, Fiore et al. [18] also showed a significant reduction in circulating CD1c+ BDC in patients with active lupus nephritis (LN) compared to those in remission, with minimal to no evidence for their localization within the glomerular compartment [19]. The restricted localization of infiltrating DC within the diseased tubulointerstitium would suggest they are ideally positioned to receive signals from activated PTEC.

However, there is no information available regarding the interaction of PTEC with DC in an autologous human context. In particular, the influence of PTEC on DC differentiation and maturation in the inflammatory disease setting and how this interaction impacts on the functional capacity of DC to initiate immune responses is unknown. In this present study, we monitored in an in vitro coculture system the effects of activated PTEC on the phenotype and function of autologous MoDC and CD1c+ BDC.

Materials and methods

Subjects

Kidney tissue from the healthy portion of malignant and non-malignant nephrectomies was obtained with informed consent after approval by the Queensland Institute of Medical Research (P293) and Royal Brisbane and Women’s Hospital (2002/011) Ethics Committees. Peripheral blood was obtained from these same donors 3 to 6 months post-nephrectomy.

Isolation and culture of PTEC

Cortex tissue was dissected from macroscopically/microscopically normal portions of the kidney and processed for PTEC purification within 1 h. PTEC were purified following the method of Glynn and Evans [20] and cultured in defined medium (DM). DM comprised a 1:1 mixture of Dulbecco’s Modified Eagle’s Medium and Ham’s F12 containing 15 mM HEPES buffer, L-glutamine and pyridoxine hydrochloride (Invitrogen, Grand Island, NY). The medium was supplemented with epidermal growth factor (10 ng/mL), insulin (10 μg/mL), transferrin (5 μg/mL), selenium (5 ng/mL), hydrocortisone (36 ng/mL), triiodothyronine (4 μg/mL), penicillin (50 U/mL) and streptomycin (50 μg/mL). Cell stocks were frozen at Passage 1 (P1) and all PTEC were used in experiments between P2 and P3. PTEC were characterized on the basis of (i) strong staining for cytokeratin-18, (ii) strong staining for alkaline phosphatase activity using the naphthol AS-MX method and (iii) characteristic cobblestone morphology.

Activation of PTEC

PTEC were cultured in DM until 70–80% confluence. As previously established [7], PTEC were then exposed to 100 ng/mL interferon (IFN)-γ (R&D Systems, Minneapolis, MN) for 24 h to mimic an inflammatory disease setting. To prevent further proliferation, PTEC were irradiated with 3000 cGy prior to co-culture with monocytes/DC.

MoDC generation

Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll–Paque™ Plus density gradient centrifugation (GE Healthcare, Uppsala, Sweden). All leucocyte cultures were performed in complete medium (CM) consisting of RPMI 1640, supplemented with 10% heat-inactivated human AB serum, 100 U/mL penicillin, 100 μg/mL streptomycin 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 10 mM HEPES buffer solution (all from Invitrogen) and 50 μM 2-mercaptoethanol (Sigma–Aldrich, St Louis, MO).

Monocytes were isolated from PBMC by CD14+ immunomagnetic selection (Mitenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. For differentiation into MoDC, purified monocytes were cultured for 5–7 days in CM supplemented with 800 U/mL granulocyte-macrophage colony-stimulating factor [GM-CSF (Milenyi Biotec)] and 1000 U/mL IL-4 (Milenyi Biotec) in 24-well plates alone (Ctrl-MoDC) or in the presence of IFN-γ-activated, irradiated PTEC (PTEC-MoDC).

Following differentiation, MoDC were separated from adherent PTEC by consecutive gentle washing with CM and matured on their own in CM for 24 h in the presence of 50 μg/mL polynosinic-polycytidylic acid [poly(I:C); Sigma–Aldrich], a synthetic double-stranded RNA analogue.

CD1c+ BDC isolation

CD1c+ BDC were isolated by positive immunomagnetic selection using the CD1c (BDCA-1)+ DC isolation kit (Miltenyi Biotec). Where indicated, CD1c+ BDC were cultured without (Ctrl-BDC) or with autologous IFN-γ-activated PTEC (PTEC-BDC) in CM for 24 h alone or in the presence of 50 μg/mL poly(I:C). Cells were then separated from adherent PTEC by consecutive gentle washing with CM for downstream analysis and T cell assays.

Flow cytometry phenotyping

Cells were labelled with combinations of PE-, fluorescein isothiocyanate (FITC)-, PerCP-, APC-, PE-Cy7-, V450- and Pacific Orange-conjugated mouse anti-human lineage (CD3, CD14, CD19, CD20, CD34 and CD56), CD11c, CD45, CD83, CD86, DC-SIGN (CD209), PD-L1 (CD274) (all from BD Biosciences, Franklin Lakes, NJ) and HLA-DR (Invitrogen) antibodies and appropriate isotype controls. For IL-10 staining, cells were pre-incubated with GolgiStop (BD Biosciences) for 12 h and then fixed and permeabilized using Fixation/Permeabilization buffers (BD Biosciences), before intracellular labelling with IL-10 or IgG isotype control (BD Biosciences) according to the manufacturer’s instructions. Cell acquisition was performed on a BD FACSCanto II flow cytometer (BD Biosciences) and analysis of flow data was performed using FlowJo 7.6.4 (Tree Star, Inc., Ashland, OR). For viability staining, cells were labelled with a Near-IR dead cell stain kit (Invitrogen) according to the manufacturer’s instructions and analysed by flow cytometry.

Cytokine secretion assays

Culture supernatants were harvested and levels of IL-10, TNF-α and IL-12/70 were determined using flow cytometric bead arrays (BD Biosciences) according to the manufacturer’s instructions.

Antigen uptake

Cells were incubated at 2 × 10^4 in the presence of 1 mg/mL FITC-dextran (Sigma–Aldrich) or fluorescent latex beads (Sigma–Aldrich) at a cell/bead ratio of 1:1500 for 60 and 90 min, respectively, at both 4 and 37°C. Following incubation, cells were washed three times in phosphate-buffered saline and analysed by flow cytometry and results calculated as delta mean florescence intensity (delta MFI) (MFI at 37°C – MFI at 4°C) for dextran and percentage uptake for latex.
Quantitative reverse transcription–polymerase chain reaction

Total RNA was isolated from cells with the Absolutely RNA MicroPrep Kit (Stratagene, Santa Clara, Germany) according to the manufacturer’s instructions. Reverse transcription was performed using an oligo-(dT)15 primer (Roche, Mannheim, Germany) and Superscript III Reverse Transcriptase (Invitrogen). Real-time polymerase chain reactions (PCR) for toll-like receptor 3 (TLR3), TBX21 (T-bet), Foxp3, GATA3 and ubiquitin C (UBC) were performed with SuperArray RT² qPCR Primers (QIAGEN, Hilden, Germany) and RT² SYBR Green qPCR master mix (QIAGEN). UBC was used for normalization of complementary DNA input, and real-time PCR were performed using a Rotor-Gene Q thermal cycler (QIAGEN) according to the manufacturer’s instructions: initial denaturation at 95°C for 10 min, followed by 45–50 cycles at 95°C for 15 s and at 60°C for 60 s. Data analysis was performed using Rotorgene software (QIAGEN) and the delta delta Ct (ΔΔCt) method.

Allogeneic MLR

Allogeneic CD4+ T cells were isolated from PBMC by negative immunomagnetic selection using the CD4+ T-cell isolation kit II (>95% purity) (Miltenyi Biotec). Poly(I:C)-stimulated MoDC and CD1c+ BDC, separated from PTEC, were plated in triplicate in 96-well plates at 1:2 serial dilutions starting at 2.5 × 104 cells per well. Allogeneic CD4+ T-cell responders were added at 1 × 105 cells per well. Cells were cultured for 5 days and proliferation was assessed by the addition of 1 μCi [3H]-thymidine per well (Perkin Elmer, Boston, MA) for the last 8 h of culture. Cytokines IFN-γ, IL-2, IL-4 and IL-10 were measured in the culture supernatants after 5 days using flow cytometric bead arrays (BD Biosciences).

Statistical analysis

Comparisons between two groups were performed using a two-tailed t-test. Statistical tests were performed using GraphPad Prism 5.0 analysis software (GraphPad, San Diego, CA). P-values ≤0.05 were considered statistically significant.

Results

MoDC differentiated in the presence of PTEC express low levels of HLA-DR and CD86, high levels of PD-L1 and IL-10 and display elevated phagocytic capacity

Monocytes were isolated by CD14+ immunomagnetic selection (mean purity 92.9 ± 3.3% SD, n = 8) and differentiated into MoDC in the absence (Ctrl-MoDC) or presence of IFN-γ-activated and irradiated autologous PTEC (PTEC-MoDC). The presence of PTEC during differentiation did not alter MoDC viability or recovery (data not shown). Confirming monocyte-to-MoDC differentiation, both Ctrl- and PTEC-MoDC expressed DC-specific marker DC-SIGN (Figure 1a and b). However, unlike Ctrl-MoDC, PTEC-MoDC retained expression of monocyte marker CD14, indicating a PTEC-mediated inhibition of complete monocyte-to-MoDC differentiation (Figure 1a and c). This population of MoDC differentiated in the presence of PTEC also displayed lower HLA-DR levels in all donors and significantly decreased the expression of co-stimulatory molecule CD86 compared to Ctrl-MoDC (Figure 1d and e). In addition, PTEC-MoDC expressed significantly higher levels of co-inhibitory molecule PD-L1 than Ctrl-MoDC (Figure 1f), with no detectable expression of DC activation marker CD83 on either MoDC population (data not shown). Collectively, these results suggest that MoDC differentiated in the presence of PTEC display a less mature phenotype.

Fig. 1. Surface antigen expression on MoDC differentiated in the absence (Ctrl-MoDC) and presence of PTEC (PTEC-MoDC). (A) Representative experiment for the cell surface expression of CD14 and DC-SIGN. (B–D) Surface expression of DC-SIGN (b), CD14 (c), HLA-DR (d), CD86 (E) and PD-L1 (F) by Ctrl- and PTEC-MoDC in a representative donor (left) and in four to six individual donors (right). Each symbol represents an individual donor. Surface expression was measured by flow cytometry (gated on live, single cells) and expressed as the delta mean fluorescence intensity (delta MFI) (MFI test-MFI isotype control). Statistical comparisons between two groups were performed using a two-tailed t-test.
To examine the functional consequences of this, we monitored antigen uptake and cytokine secretion of these two MoDC populations. While Ctrl- and PTEC-MoDC demonstrated similar levels of receptor-mediated endocytosis of FITC-dextran (Figure 2a), MoDC differentiated in the presence of PTEC exhibited greater phagocytic activity than Ctrl-MoDC (Figure 2b), consistent with their more immature phenotype. The regulatory cytokine IL-10, previously shown to inhibit monocyte-to-MoDC differentiation [21], was detected at significantly higher levels within PTEC-MoDC co-cultures (Figure 2c), with intracellular staining confirming all IL-10 production restricted to differentiating MoDC (Supplementary Figure 1). Secretion of pro-inflammatory cytokines TNF-α and IL-12p70 was not detected from either population (data not shown).

Poly(I:C)-matured PTEC-MoDC express low levels of HLA-DR, CD86, CD83 and IL-12p70 and secrete high levels of IL-10

Both MoDC populations were subsequently matured with the TLR3 agonist, poly(I:C), a double-stranded RNA analogue. Poly(I:C) is a physiologically relevant activator, associated with tissue necrosis during inflammatory events [22, 23]. Levels of TLR3 messenger RNA (mRNA) from both MoDC populations were examined with PTEC-MoDC demonstrating significantly higher TLR3 expression than Ctrl-MoDC (Figure 3a). Despite this, upon poly(I:C) stimulation, PTEC-MoDC expressed significantly less HLA-DR and CD86 than Ctrl-MoDC (Figure 3b and c). Notably, while stimulation with poly(I:C)-induced CD83 expression on Ctrl-PTEC in three of four donors, absent or low levels of CD83 were detected on PTEC-MoDC (Figure 3d). Upon poly(I:C) stimulation, PTEC-MoDC also expressed similar or higher levels of PD-L1 compared to Ctrl-MoDC (Figure 3e). These MoDC phenotypes remained constant whether PTEC were present (data not shown) or absent during the maturation phase, suggesting PTEC exert their modulatory effects primarily during monocyte-to-MoDC differentiation.

We next examined MoDC cytokine secretion upon exposure to poly(I:C) stimulation. Both Ctrl- and PTEC-MoDC produced similar levels of pro-inflammatory cytokine TNF-α (Figure 4a). However, PTEC-MoDC secreted lower levels of Th1-inducing cytokine IL-12p70 in all donors and elevated levels of IL-10 in four of five donors compared to Ctrl-MoDC (Figure 4b and c). These results suggest conditioning of MoDC by PTEC may attenuate their Th1-inducing capabilities.

**MoDC differentiated in the presence of PTEC induce weak Th1 responses**

The ability to induce proliferation of allogeneic CD4+ T cells in a mixed lymphocyte reaction (MLR) is one of the definitive features of DC. We compared the capacity of poly(I:C)-matured Ctrl-MoDC and PTEC-MoDC to induce CD4+ T-cell responses in an allogeneic MLR. PTEC-MoDC were substantially less effective at inducing allogeneic CD4+ T-cell proliferation than Ctrl-MoDC (Figure 5a and b). In addition, PTEC-MoDC stimulated secretion from T cells of significantly lower levels of Th1 cytokine IFN-γ (Figure 5c) and IL-2 (Figure 5d) compared to Ctrl-MoDC. Moreover, PTEC-MoDC induced significantly lower expression of Th1 transcription factor, T-bet, and higher expression of regulatory T-cell (Treg) transcription factor, FoxP3, than Ctrl-MoDC (Figure 5e and f). Both Ctrl- and PTEC-MoDC induced similar expression of Th2 transcription factor, GATA3 (data not shown), while there was little or no detectable production of Th2 cytokine IL-4 and T-cell-derived IL-10 (data not shown). These data demonstrate that PTEC-MoDC are weak inducers of Th1 responses.

**CD1c+ BDC matured in the presence of PTEC express low levels of HLA-DR and CD83 and high levels of PD-L1**

To investigate the effect of PTEC on the phenotype of the pre-formed CD1c+ BDC subset, we isolated CD1c+ BDC by immunomagnetic selection (mean purity 81.6% lineage− HLA-DR+ cells ± 10.8% SD, n = 12; Figure 6a) and examined surface antigen expression on CD1c+ BDC cultured without (Ctrl-BDC) or with autologous IFN-γ-activated PTEC (PTEC-BDC). As CD1c+ BDC are
known to express TLR3 and respond to triggering with poly(I:C) [10, 24], we also maintained these cultures in the presence of poly(I:C) stimulation.

As observed with MoDC, the presence of PTEC did not alter CD1c⁺ BDC viability (data not shown). Both unstimulated Ctrl- and PTEC-BDC up-regulated expression of CD86, HLA-DR, CD83 and PD-L1 following overnight incubation, with PTEC-BDC expressing higher CD86 and PD-L1 and similar levels of CD83 and HLA-DR compared to Ctrl-BDC (Figure 6b–e).

In response to poly(I:C) stimulation, PTEC-BDC expressed significantly higher CD86 but significantly reduced CD83 levels in all donors and lower expression of HLA-DR in 8/9 donors compared to Ctrl-BDC (Figure 6b–d). Moreover, PTEC significantly up-regulated expression of PD-L1 on CD1c⁺ BDC (Figure 6e). Collectively, these data demonstrate that PTEC direct the CD1c⁺ BDC repertoire towards a less mature tolerogenic phenotype.

**PTEC-BDC display elevated endocytic and phagocytic capacity**

To further define the altered maturation state of CD1c⁺ BDC cultured in the presence of PTEC, we assessed the capacity of these cells for receptor-mediated endocytosis and phagocytosis. Both unstimulated and poly(I:C)-matured PTEC-BDC displayed greater levels of endocytosis and phagocytosis than their Ctrl-BDC counterparts (Figure 7a and b), suggesting a PTEC-mediated inhibition of CD1c⁺ BDC maturation.

The production of cytokines by Ctrl- and PTEC-BDC was also examined. Neither unstimulated BDC population produced detectable levels of IL-10, while poly(I:C)
maturation induced significantly lower IL-10 production by PTEC-BDC than Ctrl-BDC (Figure 7c). IL-12p70 was not detected in any CD1c+ BDC culture supernatant (data not shown). As expected, TNF-α levels were increased 2- to 10-fold in poly(I:C)-exposed BDC. However, the effect of PTEC exposure on BDC-derived TNF-α could not be measured as PTEC themselves produced high levels of TNF-α in response to poly(I:C) (data not shown). These data establish a specialized regulatory role for CD1c+ BDC matured in the presence of PTEC to inhibit CD4+ T-cell proliferation.

Discussion

We have recently reported that human PTEC are able to modulate autologous T- and B-lymphocyte responses [7]. Here, we extend these findings to demonstrate that activated PTEC are also able to exert a profound effect upon autologous MoDC development and maturation of both MoDC and pre-formed CD1c+ BDC. Given the central role of DC in initiating and directing T- and B-cell responses, these findings may, in part, explain our earlier observations.

Epithelial cells of non-renal origin have been shown to modulate the functional phenotype of APC in the local microenvironment. Human epithelial cell lines of the lung and intestine have been shown to regulate the differentiation and maturation of allogeneic monocytic precursor cells, promoting the development of more tolerogenic
MoDC [25, 26]. Our data, based on autologous primary PTEC, are in line with these studies. Although DC-SIGN expression on Ctrl- and PTEC-MoDC were comparable, indicating both MoDC populations had progressed along the DC differentiation pathway [15], PTEC-MoDC retained expression of monocyte marker CD14, suggesting an inhibition or delay in monocyte-to-MoDC differentiation in the presence of PTEC. In addition, PTEC-MoDC displayed lower MHC class II and CD86 levels, suggesting these cells were also phenotypically and functionally less mature than Ctrl-MoDC. This concept of PTEC maintaining DC in a less mature state is supported by our observation of elevated phagocytosis by PTEC-MoDC, a function associated with immature DC during the steady state [27]. This attenuated differentiation and maturation may, in part, be IL-10-mediated, as higher levels of this cytokine were detected in PTEC-MoDC culture supernatants following differentiation. IL-10 has been previously shown to suppress monocyte-to-MoDC differentiation, leading to an incomplete loss of CD14 expression [21], as observed with PTEC-MoDC in our study. Notably, in preliminary transwell experiments, we observed no elevated IL-10 production and the complete down-regulation of CD14 by developing MoDC from contact-independent PTEC co-cultures (data not shown). This indicates that PTEC down-modulation of MoDC differentiation is contact-dependent. We anticipate investigating this mechanism further as more autologous donors become available.

TLR3, a sensor of tissue necrosis during inflammatory processes [23], is the only DC-specific TLR among human leucocytes [28]. TLR3 activation with poly(I:C) has been shown to aggravate inflammation in a mouse model of LN [29]. Notably, PTEC-MoDC displayed significantly elevated TLR3 expression, suggesting an increased sensitivity to poly(I:C) stimulation. Despite this, upon poly(I:C) stimulation, PTEC-MoDC maintained a less mature profile than Ctrl-MoDC, expressing lower levels of HLA-DR, CD86 and CD83. In line with this phenotypic profile, poly(I:C)-matured PTEC-MoDC demonstrated substantially decreased T-cell-stimulatory capacity compared to Ctrl-MoDC. This finding is reminiscent of the data of Verkade et al. [30], who observed a phenotypic and functional impairment of mature MoDC in patients with severe chronic kidney disease as compared to control subjects.

In addition to a less mature surface antigen expression profile, poly(I:C)-matured PTEC-MoDC secreted lower levels of IL-12 and elevated IL-10 protein than Ctrl-MoDC. These results concur with a recent human study, focussing on mesenchymal stem cells, which showed a modulation of lipopolysaccharide-matured MoDC phenotype by allogeneic PTEC [31]. Interestingly, this suggests that PTEC modulation of MoDC is independent of the maturation stimuli.

The secretion of regulatory cytokine IL-10 by poly(I:C)-matured PTEC-MoDC is consistent with the less mature...
anti-inflammatory phenotype of the MoDC population. IL-10 has been shown to be protective in various experimental immune-mediated kidney diseases [32–35], while recent mouse studies suggest that renal DC may attenuate kidney injury via IL-10 secretion [36, 37]. Our current study indicates a monocyte-derived DC population differentiated locally under inflammatory conditions may be the source of this anti-inflammatory cytokine.

IL-10 and Th1-inducing cytokine IL-12p70 can act in opposition to each other, with IL-10 shown to impair DC-derived IL-12p70 and downstream Th1 responses [21, 38]. Notably, production of IL-12 was suppressed in PTEC-MoDC, suggesting a weak Th1-polarizing potential. Supporting this concept, PTEC-MoDC were also poor inducers of Th1 cytokine IFN-γ and transcription factor T-bet by CD4⁺ T cells. There is evidence of Th1 nephritogenic immune responses in some forms of human GN, including crescentic GN and membranoproliferative GN [39]. However, a putative role of monocyte-derived DC in regulating this pathogenic immune response requires further investigation. In addition to a weak Th1 response, our PTEC-MoDC induced strong FoxP3 transcription factor expression in allogeneic T cells, a marker of regulatory T cells. The potential of Tregs to suppress pathogenic Th1 immune responses has recently been demonstrated in an experimental model of crescentic GN [40], while proportions of Tregs have been shown to inversely correlate with clinical disease in LN patients [41]. However, until now, a putative involvement of MoDC in priming this regulatory mechanism has not been documented. Together, these results indicate that PTEC drive the differentiation of a less mature tolerogenic MoDC population, possibly by contact-dependant mechanisms.

CD1c⁺ BDC are a major subgroup of the pre-formed blood DC population [24] and they have been reported in the renal interstitium of both healthy [42] and diseased [18] kidneys. We have also isolated and identified these cells from diseased human biopsies using flow cytometry (Kassianos A, Wilkinson R, unpublished data). To understand if PTEC could modulate pre-formed CD1c⁺ BDC towards a tolerogenic phenotype and function, CD1c⁺ BDC were isolated from PBMC and co-cultured with autologous PTEC. The most dramatic PTEC-mediated effects were observed during poly(I:C) stimulation of the BDC population. Poly(I:C)-stimulated PTEC-BDC displayed reduced expression of HLA-DR and CD83, augmented PD-L1 levels, elevated endocytic and phagocytic potential and attenuated allostimulatory capacity compared to poly(I:C)-stimulated Ctrl-BDC alone. These collective results support the concept that PTEC also retain CD1c⁺ BDC in a less mature tolerogenic state. However, in contrast to MoDC, the presence of PTEC increased expression of CD86 and reduced IL-10 secretion by poly(I:C)-matured CD1c⁺ DC, highlighting the emerging complexities and functional specializations of human DC subpopulations [9, 10]. The pleotropic nature of CD86 ligation, where engagement of CD86 with CTLA-4 (rather than co-stimulatory receptor CD28) results in negative T-cell signalling and down-regulation of T-cell responses [43], may explain the apparent dichotomy of increased CD86 expression with decreased allostimulatory capacity by PTEC-BDC. These findings may also offer a mechanism for the protective role CD86 has been reported to play in a mouse model of crescentic GN [44].

Based on our results, we propose that under inflammatory conditions, activated PTEC modulate locally differentiated MoDC and infiltrating, pre-formed CD1c⁺ BDC by conditioning them towards an anti-inflammatory less mature phenotype. We suggest these regulatory DC subsequently mediate suppressive effects on pathogenic T cells and thus minimize tissue damage and the onset of chronic kidney disease. This PTEC-DC interaction is supported by the tubulointerstitial localization of human DC within patient kidney specimens [18, 42]. We have now commenced a study designed to analyse the maturation phenotype of DC isolated from human renal disease biopsies to provide in vivo collaborative data for these findings.

In conclusion, this study enhances our understanding of the role DC play during inflammatory kidney disease and further dissection of the mechanisms of PTEC modulation...
of autologous immune responses may offer targets for diagnostic and therapeutic intervention in renal medicine.

**Supplementary data**

Supplementary data are available online at http://ndt.oxfordjournals.org.

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**Author contributions—** R.W. conceptualized the project, designed the research, performed the studies, analysed the data, interpreted results and wrote the paper. A.J.K. designed the research, performed the studies, analysed the data, interpreted results and wrote the paper. S.S., X.W. and K.E.R. performed the studies and analysed and interpreted the data. K.B. provided intellectual input and review of the manuscript. H.H. provided intellectual input and contributed to writing the paper.

**Conflict of interest statement.** None declared.

(See related article by Disteldorf and Panzer. Is there a role for proximal tubular cells in regulating dendritic cell maturation and function in renal disease? *Nephrol Dial Transplant* 2013; 28: 239–241.)

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