Human plasmacytoid dendritic cells acquire phagocytic capacity by TLR9 ligation in the presence of soluble factors produced by renal epithelial cells

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Plasmacytoid dendritic cells (pDCs) are antigen presenting cells specialized in viral recognition through Toll-like receptor (TLR)7 and TLR9, and produce vast amounts of interferon alpha upon ligation of these TLRs. We had previously demonstrated a strong influx of pDCs in the tubulointerstitium of renal biopsies at the time of acute rejection. However, the role of human pDCs in mediating acute or chronic allograft rejection remains elusive. pDCs are thought to have a limited capacity to ingest apoptotic cells, critical for inducing $CD4^+$ T cell activation via indirect antigen presentation and subsequent activation of antibody producing B cells. Here we tested whether the function of pDCs is affected by their presence within the graft. Maturation and interferon alpha production by pDCs was enhanced when cells were activated in the presence of viable HK2 renal epithelial cells. Importantly, soluble factors produced by cytomegalovirus-infected (primary) epithelial or endothelial cells enhanced pDC activation and induced their capacity to phagocytose apoptotic cells. Phagocytosis was not induced by free virus or soluble factors from non-infected cells. Activated pDCs showed an enhanced $CD4^+$ and $CD8^+$ T cell allostimulatory capacity as well as a potent indirect alloantigen presentation. Granulocyte Macrophage-Colony Stimulating Factor is one of the soluble factors produced by renal epithelial cells that, combined with TLR9 ligation, induced this functional capacity. Thus, pDCs present in the rejecting allograft can contribute to alloimmunity and potentially act as important orchestrators in the manifestation of acute and chronic rejection.

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Plasmacytoid dendritic cells (pDCs) were first classified as dendritic cells (DCs) in the 1990s,[1] and have since been shown to be capable of acting as antigen-presenting cells (APCs).[1–3] pDCs selectively express the endosomal Toll-like receptor (TLR)7 and TLR9,[4] which allows for the detection of viral and bacterial single-stranded RNA and hypomethylated DNA, respectively.[5,6] Ligation of these TLRs leads to the production of vast amounts of interferon alpha (IFNα), and pDCs are hence referred to as the type I IFN-producing cells. These abilities stage pDCs as APCs instrumental for the clearance of infections.[7–9] However, pDCs can also express proteins involved in the induction of T-cell tolerance (e.g., Indoleamine-pyrrole 2,3-dioxygenase, Delta-Like-4, PD-L1, and Granzyme B).[10–13]

APCs are key in the manifestation of both acute and chronic rejection in solid organ transplantation. Passenger APCs of donor origin can induce allo-reactive T-cell activation via the direct antigen (Ag) recognition pathway early after transplantation, whereas APCs of the recipient are involved in chronic rejection by mediating indirect Ag presentation.[14] Although in theory any activated passenger APC can induce direct allo-reactivity, the induction of $CD4^+$ T cells with indirect specificity requires the phagocytosis of donor cell–derived Ag (e.g., apoptotic cells), and subsequent presentation of the acquired Ag in the context of major histocompatibility complex class II by mature APCs.

APC maturation can be induced by infections, via the recognition of pathogen-associated molecular patterns by, for example, TLRs. Transplanted patients are susceptible to opportunistic infections due to their immune-suppressed state, and especially infection with polyomaviruses (BK virus) and cytomegalovirus (CMV) causes morbidity and mortality.[15] Interestingly, multiple studies have suggested a relation between infection (especially with CMV) and allograft rejection, despite remaining inconclusive due to the complexity of this interplay.[16]

Our laboratory has previously shown a strong influx of pDCs in the tubulointerstitium of renal biopsies taken at time of rejection, as compared with those taken at time of transplantation.[17] As such, pDCs could be bridging infection and allograft loss.[18] Despite these observations, the role of pDCs in the manifestation of solid organ rejection remains elusive. Experimental transplantation models have demonstrated that pDCs are capable of allo-Ag presentation.[18–20] Moreover, an IFNα signature was shown clinically during chronic antibody-mediated rejection, which coincided with an increased number of pDCs within the graft.[21] In addition, human pDCs are able to ingest antigen (e.g., virions, synthetic long peptides, and exosomes).[22–24] However, data on the ability of pDCs to ingest apoptotic cells (ACs) remains controversial, and hence their potential role in mediating chronic rejection. It has been suggested that pDCs can phagocytose only infected ACs.[25,26] whereas other studies have demonstrated pDCs to have a very limited capacity to ingest ACs.[27]

Because we observed an influx of pDCs in renal tissue at time of rejection, we set out to assess whether human pDC biology, and especially phagocytosis, is affected by their presence in renal tissue, and whether viral infection alters this interaction. Here we demonstrate that TLR9 ligation of pDC in the presence of tissue cell–produced soluble factors, including granulocyte-macrophase colony-stimulating factor (GM-CSF), initiates the phagocytic capacity of pDCs and strongly enhances their function. Our data position pDCs as APCs capable of mounting allogeneic immune responses via the indirect Ag presentation pathway, following activation by TLR9 ligation in the presence of tissue cell–produced soluble factors.

RESULTS

CpG-mediated pDC phenotypic maturation and IFNα production are enhanced following co-culture with renal epithelial cells

Recently, we demonstrated a strong influx of pDCs in the tubulointerstitium of biopsies of patients with acute allograft rejection.[17] Because pDCs were present in close proximity of the tubules, we investigated whether human kidney proximal tubular epithelial (HK2) cells could affect phenotypic maturation and IFNα production of purified blood-derived human pDCs.

In absence of any activating stimuli, culturing pDCs alone, or in the presence of viable HK2 cells did not lead to clear alterations in phenotypic maturation (Figure 1a) nor IFNα production (Figure 1b). Mimicking infection by the addition of the synthetic TLR9 ligand CpG led to the upregulation of the costimulatory molecule CD86, as well as the co-inhibitory molecule PD-L1 (Figure 1c). As expected, pDC activation by CpG led to a robust IFNα production (Figure 1d). Interestingly, CpG activation of pDCs in the presence of viable HK2 cells led to a significant increase in the expression of the costimulatory molecules CD86 and CD83, which coincided with a markedly reduced increase in the expression of the co-

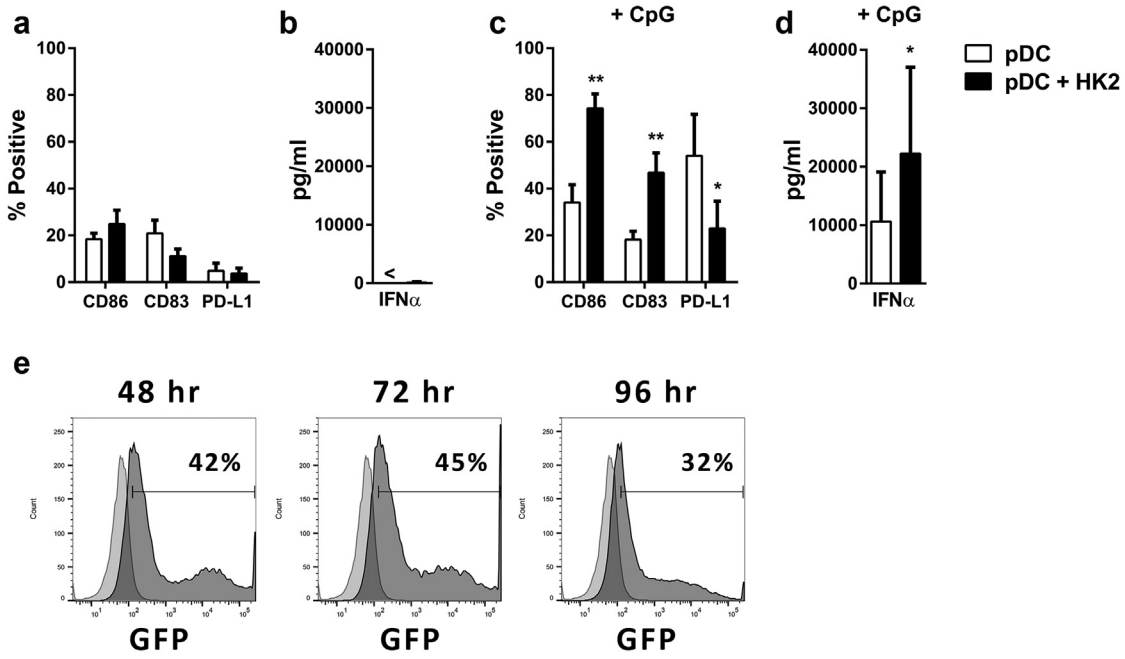

Figure 1 | CpG-mediated plasmacytoid dendritic cell (pDC) phenotypic maturation and IFNα production are enhanced following co-culture with renal epithelial cells. (**a**) pDCs were cultured in the presence (black bars) or absence (white bars) of viable HK2 cells, after which cell surface expression of CD86, CD83, and PD-L1 was determined by flow cytometry and (**b**) interferon (IFN)α production by ELISA. <: Cytokine levels <10 pg/ml. (**c**) pDCs were cultured in absence (white bars) or presence (black bars) of viable HK2 cells and subsequently activated with CpG. The expression of cell surface markers was assessed by flow cytometry and (**d**) IFNα production by enzyme-linked immunosorbent assay. $n = 6$; *$P < 0.05$, **$P = < 0.01$; Student t test. (**e**) HK2 cells were infected with a green fluorescent protein (GFP)–expressing cytomegalovirus strain (multiplicity of infection 200), after which the infection efficiency was analyzed by flow cytometry at 48, 72, and 96 hours.

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