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The renal microenvironment modifies dendritic cell phenotype

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Renal dendritic cells are a major component of the renal mononuclear phagocytic system. In the renal interstitium, these cells are exposed to an osmotic gradient, mainly sodium, whose concentration progressively increases towards inner medulla. Renal allograft rejection affects predominantly the cortex, suggesting a protective role of the renal medullary micromilieu. Whether osmolar variations can modulate the function of renal dendritic cells is currently undefined. Considering the central role of dendritic cells in promoting allorejection, we tested whether the biophysical micromilieu, particularly the interstitial osmotic gradient, influences their alloreactivity. There was a progressive depletion of leukocytes towards the medulla of homeostatic kidney. Only macrophages opposed this tendency. Flow cytometry of homeostatic and post-transplant medullary dendritic cells revealed a switch towards a macrophage-like phenotype. Similarly, bone marrow–derived dendritic cells developed *ex vivo* in sodium chloride–enriched medium acquired a M2-like signature. Microarray analysis of allotransplant dendritic cells posed a medullary downregulation of genes mainly involved in alloantigen recognition. Gene expression profiles of both medullary dendritic cells and bone marrow–derived dendritic cells matured in hyperosmolar medium had an overlap with the macrophage M2 signature. Thus, the medullary environment inhibits an alloimmune response by modulating the phenotype and function of dendritic cells.

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Bone marrow–derived antigen-processing cells of the kidney, referred to as renal mononuclear phagocytes (rMoPh), have a crucial role in regulating renal physiology and pathology.[1–6] rMoPh are conventionally defined through surface receptor expression pattern as macrophages (Mϕ) and dendritic cells (DCs). Renal DCs and Mϕ share a common origin in bone marrow–derived precursors, represented by circulating monocytes and pre-DCs.[1,7–11] Renal DCs ($CD45^+$ $CD11c^+$ $MHCII^+$) act as sentinels by sensing signals from the surrounding tissue and rapidly mount-specific immunogenic or tolerogenic responses.[4,6,12–14] Another hallmark of rMoPh plasticity is the ability of Mϕ to polarize towards either a proinflammatory M1 (tissue damage) or an anti-inflammatory M2 (tissue repair) phenotype.[1,15,16] Genomic, proteomic, and functional approaches are directed to define specific M1/M2 stimuli and responses. As simplified *in vitro* models for generating Mϕ/DC often contradicted *in vivo* situations,[17] the current view is that tissue Mϕ interact with a dynamic complex of pathophysiological factors to generate either a 'proinflammatory' (M1), a 'product of Th2 activation' (M2a), a 'pro-Th2 activation' (M2b), or a 'deactivated/immunoregulatory' (M2c) profile.[18–25] An overlap between these categories as well as between 'classical' Mϕ and DC patterns has been described.[26] Whether biophysical tissue factors can modulate the rMoPh phenotype and function remains unexplored. The kidney represents a suitable model in this regard, as different biophysical conditions exist within the same organ. rMoPh populating the renal parenchyma are exposed to an osmolarity gradient, which progressively increases along the medulla, mainly determined by rising concentration of interstitial substrates such as sodium.[27,28] In allorejected kidney, a cortex-oriented tissue damage and inflammation has been reported,[29] suggesting a protective role of the renal medullary micromilieu. We postulate that tissue-specific non-immunologic factors, particularly interstitial osmolarity, shape the phenotype and gene signature of renal DCs in homeostasis and during allograft rejection.

RESULTS

Homeostatic renal medullary DCs exhibit a macrophage-like phenotype

Renal leukocytes in homeostasis reside in both cortical and medullary interstitium (Figure 1a). The distribution of renal leukocytes in different renal compartments in homeostasis was assessed by flow cytometric analysis of $CD45^+$ cells. Cortical and medullary $CD45^+$ cell sub-populations were detected through the expression of specific surface markers: DCs ($CD11c^+$ $MHCII^+$), Mϕ ($F4.80^+$ $CD11b^+$ $CD11c^-$), monocytes ($Ly6C^+$ $Ly6G^-$), inflammatory monocytes ($Ly6C^{++}$ $Ly6G^-$), granulocytes ($Ly6C^+$ $Ly6G^+$), B cells ($CD19^+$ $CD11c^-$), $CD4^+$ T cells ($CD3^+$ $CD4^+$), $CD8^+$ T cells ($CD3^+$ $CD8^+$), and NK cells ($NK1.1^+$ $CD3^-$). Flow cytometric analysis revealed a significant tendency of the whole leukocyte population to populate the renal cortex (Figure 1b). The number of renal DCs decreased towards the medulla (Figure 1c and d), whereas Mϕ showed a predominant medullary orientation (Figure 1c and d). Renal DCs were further investigated for the coexpression of classical Mϕ markers (F4.80, CD11b) as well as for the intensity of CD11c expression. A 10% increase in $F4.80^+$ DCs, as well as a minor (significant) increase in the percentage of $CD11b^+$ DCs, was observed in the medulla, in comparison with the cortex (Figure 1f, left). The enhanced expression of F4.80 was further revealed by a twofold increase in its median fluorescence intensity (Figure 1e and f, right). The intensity of CD11b expression was slightly increased in medullary DCs, as shown in Figure 1e and f (right). Accordingly, the intensity of CD11c expression in the medulla significantly decreased (Figure 1e and f, right). These observations indicated a switch of homeostatic renal medullary DCs towards a Mϕ-like phenotype.

Acute renal allograft rejection occurs preferentially in the cortex

Studies of transplanted kidneys identified renal cortex as the major site of allograft injury, as the histopathological features consistent with rejection develop predominantly in this compartment (Figure 2a). To further investigate the compartment-specific pattern of renal allograft rejection, flow cytometric analysis was applied to the donor (BALB/c, $CD45.2^+$) and host (B6.SJL, $CD45.1^+$) leukocytes in the renal cortex and medulla in early rejection. Syngeneic kidney transplantation (KTx) served to evaluate the specific effects of ischemia–reperfusion injury on the dynamics of host and donor leukocytes in the different renal compartments (Supplementary Figure S1a and b online). During early allograft rejection (3 days after KTx), host leukocytes strongly and preferentially infiltrated the renal cortex (Figure 2b and c), supporting observations from mouse allograft sections. The compartment-specific distribution of infiltrating leukocytes was best exemplified by host DCs, which were up to eight times more abundant in the renal cortex compared with the medulla (Figure 2d). By contrast, at 3 days after KTx, a substantial loss of renal donor leukocytes was found (Figure 2c and e) in comparison with the number of leukocytes (including DCs) in the cortex and medulla of naive BALB/c kidneys (Supplementary Figure S1c and d online). At 7 days after KTx, host leukocyte infiltration remained significantly higher in the renal cortex compared with that in the medulla (Supplementary Figure S1e online). At this stage, donor leukocytes could not be detected anymore (Supplementary Figure S1e, left online).

Macrophage-like signature is preserved in allograft medullary donor DCs and occurs in host DCs infiltrating the medulla

The phenotypic differences observed between cortical and medullary DCs in native kidneys, consisting in higher expression of F4.80 and CD11b and lower expression of CD11c in the medulla, were conserved in donor DCs under strong inflammatory stimuli (Figure 3a and b). Interestingly, host DCs modulated their phenotype in a manner comparable to donor DCs, as cells in the medulla at 3 days after KTx displayed significantly higher levels of F4.80 and CD11b on their surface and lower expression of CD11c, compared with host DCs infiltrating the cortex (Figure 3c and d). At 7 days after KTx, host DCs in the cortex and medulla showed similar levels of F4.80, whereas CD11b and CD11c expression demonstrated a similar trend at 3 days after KTx (Figure 3e and f). However, immunohistology of kidney sections (7 days after KTx) showed that the complete pool of $F4.80^+$ leukocytes was higher in the medulla compared with that in the cortex (Supplementary Figure S2 online). At the same time point, a significant increase in the percentage of $ArginaseI^+$ (ArgI) mononuclear cells could be observed in the medulla (Supplementary Figure S3 online), indicating an M2-like profile.

DCs gain alternative macrophage-like phenotype upon maturation in hyperosmolar medium *ex vivo*

We tested the influence of an hyperosmolar environment on the DC phenotype *ex vivo*. To imitate the osmolarity gradient through the renal compartments, hypertonic sodium chloride (NaCl)–enriched medium was applied to bone marrow–derived DCs (BMDCs) from day 4 of culture. We induced a Toll-like receptor-2 and -4 (TLR2/4) pathway activation via lipopolysaccharide (LPS) to mimic allorejection-associated damage-associated molecular pattern–triggered signals (illustrated *in situ* by fibrinogen; Supplementary Figure S4a and b online), recognizing that interpretation of this model in the context of sterile damage-associated molecular pattern–induced inflammation has its limitations. The phenotypic differences between cortical and medullary DCs observed *in vivo* could be reproduced *ex vivo* by exposing DCs to increasing concentrations of NaCl, in a range comparable to the osmolarity of the outer medullary interstitium (290–450 mOsm/l). The hypertonicity increased the expression of F4.80; this effect was enhanced under LPS stimulation (Figure 4a and b). The intensity of CD11b expression followed a similar tendency, although unchanged upon LPS treatment (Figure 4c). Moreover, hyperosmolarity induced increased the expression of macrophage mannose receptor by BMDCs (Figure 4d and e); however, LPS treatment abrogated

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