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Dendritic cell function in different renal microenvironments under homeostatic conditions and during allograft rejection

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Dendritic cells (DCs) are the major population of the renal mononuclear phagocytic system, a subgroup of bone marrow-derived leukocytes which also includes renal macrophages. Renal mononuclear phagocytes form a network of stellate cells which spans the interstitial spaces between the renal tubules. Renal DCs are essential for the maintenance of tissue homeostasis, as they sample innocuous antigens from the surrounding renal tissue and present them to T cells in order to induce immune tolerance. Renal DCs are exposed to drastic changes in interstitial osmolarity, which ranges from 300 mOsm/L in the cortex up to 1200 mOsm/L in the inner medulla. A major contribution to the strong osmolarity increase in medulla is given by interstitial sodium. An aspect so far not investigated is whether non-immunologic biophysical factors within the kidney, such as medullary hyperosmolarity, can modulate the function of rMoPh and particularly of DCs.

Dendritic cells play crucial roles in mounting specific immune responses to any organ allograft. The ischemia-reperfusion injury (IRI) associated with renal transplantation triggers donor rDCs to migrate to host lymph nodes where they present alloantigens to host T lymphocytes. This represents a crucial event in the initiation of allograft rejection.

Allograft biopsy studies showed that the histopathological features of kidney allograft rejection predominantly develop in the renal cortex. We hypothesize that renal tissue-specific biophysical factors, particularly interstitial osmolarity, can shape the phenotype and function of dendritic cells. We also postulate that DC alloreactivity is influenced by interstitial osmolarity and varies in relation with their distribution within the renal parenchyma.

This work aims to: 1) describe the leukocyte population residing in renal cortex and medulla under homeostatic conditions and to analyze the distribution of host and donor leukocytes in the different renal compartments during allograft rejection; 2) analyze the phenotype of renal resident DCs at steady-state and of donor/host DCs during allograft rejection in renal cortex and medulla; 3) investigate the gene expression profile of cortical and medullary DCs during homeostasis and kidney allograft rejection, separately analyzing donor and host DCs; 4) test in an *in vitro* model whether a sodium chloride-induced osmolarity gradient affects antigen uptake and presentation

capacity of bone marrow-derived dendritic cells.

Under homeostatic conditions, renal leukocytes were significantly more abundant in renal cortex. Renal DCs followed this tendency whereas macrophages (M ϕ) were mainly detected in renal medulla. Flow cytometric analysis showed that DCs modulate their phenotype in relation to their distribution within the kidney, as medullary DCs exhibited a more macrophage-like phenotype by up-regulating the expression of surface markers F4.80 and CD11b and down-regulating CD11c. The hypothesized effect of medullary hyperosmolarity on the DC phenotype was investigated *ex vivo* by developing bone marrow-derived DCs in different NaCl-induced osmolarity conditions: 290 mOsm/L (osmolarity of renal cortex), 370 and 450 mOsm/L (representative of the osmolarity gradient in renal medulla). Consistent with the *in vivo* phenomenon, increasing osmolarity induced the expression of F4.80 and CD11b while CD11c expression was reduced. Furthermore, qRT-PCR revealed that mRNA levels of the genes Arg1 and Mrc1, hallmarks of M2-macrophage polarization, were significantly increased under hyperosmolarity. Based on these results, we suggest osmolarity of the surrounding environment, especially NaCl concentration, as an important modulator of mononuclear phagocyte phenotype.

It was further investigated whether the DC distribution in renal cortex and medulla (different biophysical microenvironments) could have modulatory effects on their gene expression profile, as a reflection of their functional activity, at steady-state and during allograft rejection. Microarray analysis revealed that, in the steady-state kidney, cortical and medullary DCs maintain a similar gene expression pattern, suggesting minor functional differences in renal homeostasis. During allograft rejection however, a cortex-specific gene signature appeared. Donor and host DCs, despite their different origin, activated a similar gene expression profile when localized in the same renal compartment, suggesting that tissue-specific factors might steer the functional state of DCs. Genes induced in both donor and host DCs in cortex were related to a variety of immunologic processes, such as antigen processing, loading on class I and II MHC molecules and presentation, T cell co-stimulation and pro-inflammatory cytokine signaling. This suggested a remarkably higher allogenicity of cortical DCs and led to postulate that under inflammatory conditions, the efficiency of DCs to activate T cells via antigen presentation might be negatively modulated by hyperosmotic NaCl concentrations. This point was confirmed *ex vivo*, as the development of DCs under hyperosmolar NaCl significantly reduced their capacity to prime T cells, via presentation of antigen-MHCI/MHCII complexes. This effect was particularly significant upon LPS stimulation. Our study proposes that tissue-specific biophysical factors, especially osmolarity, modulate phenotype and functional features of renal mononuclear phagocytes. Both *in vivo* and *ex vivo*, the exposure to an hyperosmotic environment resulted in suppressed DC immunogenicity.

These findings implicate that DC-mediated inflammatory responses, also in pathologies where hyperosmolar microenvironments might occur (e.g. solid carcinoma of different type, hyperosmolar state in diabetes mellitus), might be substantially altered, thereby influencing disease course and outcome.