Function and homeostasis of murine splenic dendritic cell subsets

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The immune system protects us from harmful micro-organisms and tumor cells. In the immune system, dendritic cells (DCs) are specialized Ag-presenting cells that have a dominant role in the initiation of T cell responses. For this, DCs present intracellular Ags in MHC class I molecules to $CD8^+$ T cells or extracellular Ags in MHC class II to $CD4^+$ T cells, respectively. Next to these two classical Ag-presentation pathways, DCs are also able to cross-present extracellular Ags in the context of MHC class I to $CD8^+$ T cells. This cross-presentation pathway is important for the generation of primary $CD8^+$ T cell mediated responses against Ags that are not produced by the presenting DC itself, e.g. cell-specific viruses and tumor cells, which could otherwise not be presented to naive $CD8^+$ T cells.

DCs form a heterogeneous population in the mouse spleen. Splenic DCs can be subdivided into $CD8^+$ and $CD8^-$ DC subsets with different phenotype and localization. The specific localization of $CD8^+$ DCs in the white pulp and of $CD8^-$ DCs in the marginal zone (MZ) is regulated by chemokines, CCR7 and S1P[1-5]. In spleen, the $CD8^-$ DC subset specifically expresses SIRPα, which is a cell surface glycoprotein expressed by myeloid cells that was shown to be involved in migration of several cell types[6-8]. We showed that in the absence of functional SIRPα in SIRPα-Δ87 mutant mice the number of $CD8^-$ DCs in spleen is strongly reduced (chapter 2). Furthermore, SIRPα is involved $CD8^-$ DC migration by regulating integrin-mediated cell adhesion. $CD8^-$ DCs derived from SIRPα-Δ87 mutant mice express lower levels of MMP-9 and MMP-12 and are less mature in phenotype as compared to SIRPα-wt derived $CD8^-$ DCs. The study described in chapter 2 suggests that SIRPα plays an important role in the migration, the localisation and the homeostasis of $CD8^-$ DCs *in vivo*.

Next to differences in their homeostatic regulation splenic DC subsets also display different capacities to activate T cells. $CD8^+$ DCs are described as the cross-presenting DCs involved in $CD8^+$ T cell activation, while $CD8^-$ DCs are mainly involved in $CD4^+$ T cell activation. In chapter 3 of this thesis, we describe that yeast is efficiently cross-presented both *in vivo* and *in vitro*. Interestingly, $CD8^-$ DCs preferentially cross-presented the yeast-derived Ags thereby launching a $CD8^+$ T cell response, while both splenic DC subsets were able to present yeast-derived Ags in the context of MHC class II to $CD4^+$ T cells[9]. Since both DC subsets equally well phagocytosed yeast in a dectin-1 dependent

manner, the observed differences in cross-presentation and cytokine production between the subsets after stimulation with yeast could not be explained by differential uptake of yeast. These results strongly indicate that $CD8^+$ and $CD8^-$ DCs are specialized with respect to their differential capacities to activate naïve $CD4^+$ or $CD8^+$ T cells in response to stimulation with yeast.

Efficient Ag-presentation is the main function of DCs in an organism. It has been shown that NADPH oxidase (NOX2) mediated ROS production in the phagosome is important for efficient cross-presentation, since ROS production controls the phagosomal pH and thereby the breakdown of Ags[10;11]. We therefore investigated in chapter 4 whether differences in ROS production between DC subsets might cause the differences in Ag-presentation capacities. $CD8^-$ DCs show higher mRNA expression of NOX2 subunits and are more efficient in total ROS production after PMA and yeast stimulation as compared to $CD8^+$ DCs. In addition, inhibition of ROS by the chemical compound apocynin reduced the *in vitro* capacity of splenic DCs to cross-present yeast-derived Ags. Taken together these data suggest that the differential Ag-presentation capacities of splenic $CD8^+$ and $CD8^-$ DCs could result from differentially regulated ROS production and its influence on Ag-presentation.

DCs phagocytose as well as endocytose Ags for efficient presentation to T cells. We observed in chapter 5 that $CD8^+$ DCs acquire Ags from Mφ for cross-presentation. Marginal metallophilic macrophages (MMMs) specifically transfer targeted Ags to $CD8^+$ but not to $CD8^-$ DCs. The transfer of Ags between MMMs and $CD8^+$ DCs can either result in T cell activation or T cell tolerance, depending on the use of an adjuvant. Since we could not observe migration of MMMs out of the MZ, we suggest that DCs migrate through the MZ and thereby acquire Ags form MMMs. This study is the first in assigning an important role to MMMs in the induction of primary $CD8^+$ T cell responses.

Based on the data presented in this thesis we conclude that, although $CD8^+$ and $CD8^-$ DCs are closely related in their origin, their homeostasis and Ag-presentation capacities are differentially regulated. Analysis of the T cell stimulation capacities of splenic DC subsets indicates functional specialization of $CD8^+$ and $CD8^-$ DCs in the elicitation of adaptive immune responses *in vivo*.