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Origin and Differential *In vivo* Function of Antigen Presenting Cells

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Scientific background

In vivo T and B lymphocyte biology is now successfully studied using gene and cell ablation, as well as adoptive cell transfer strategies. However, macrophages (MO) and dendritic cells (DC) - although as Antigen Presenting Cells (APC) key players in innate and adaptive immunity - have remained largely refractory to *in vivo* approaches. Both MO and DC belong to the mononuclear phagocyte system, a network of myeloid cells that is seeded throughout the body and includes cells as diverse as liver Kupffer cells, brain microglia and bone osteoclasts. MO are long-lived, sessile cells, which contribute to tissue remodeling and homeostasis. MO play a central role as innate enhancers of anti-microbial resistance, but are poor T cell stimulators and their role in adaptive immune responses remains controversial. In contrast, the short-lived migratory DC have been functionally defined by their unrivaled potency to stimulate naïve T cells. Highlighting their specialization, DC harbor unique antigen presentation pathways that enable them to efficiently prime $CD4^+$ and $CD8^+$ T cells against endogenous and exogenous antigens. Interestingly, however, T cell antigen encounter on DC - both in thymus and periphery - can also result in antigen-specific tolerance. Phenotypic heterogeneity of murine DC with six subsets, including epidermal Langerhans' cells (LC) and type I IFN-producing plasmacytoid DC suggests that immunostimulatory and tolerizing activities could be associated with distinct DC subsets. Solid experimental evidence supporting such a scenario, which would have important implications for DC vaccine development is missing. Moreover, differential functions of MO and DC subsets could encode therapeutic potential of strategies aiming at manipulation of the myeloid composition of the organism. Such approaches are however currently hampered by our poor understanding of the *in vivo* origin and function of the mononuclear phagocyte system.

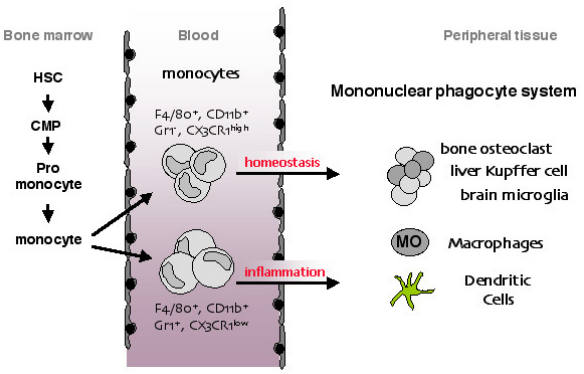

Fig. 1 *Schematic representation of the contribution of "homeostatic" and "inflammatory" peripheral blood monocytes to the mononuclear phagocyte system*

Studying the *in vivo* origin of mononuclear phagocytes

Mononuclear phagocytes are of hematopoetic origin and most subsets rely on lifelong reconstitution by the bone marrow. *In vitro* differentiation assays indicate peripheral blood monocytes as mononuclear phagocyte precursors. With half of these leukocytes leaving the bloodstream in steady-state each day, monocytes could indeed constitute a significant systemic reservoir of myeloid precursors (Fig. 1). We therefore choose to study *in vivo* monocyte homing and differentiation using a mutant mouse strain, in which the CX_3CR1 chemokine receptor gene is replaced with a green fluorescent protein gene (GFP) (CX_3CR1^{GFP} mice). All monocytes of CX_3CR1^{GFP} mice are bright green fluorescently labeled (Fig 2A) and can upon engraftment of congenic recipient mice be traced by flow cytometry and immuno-histochemistry (Fig 2A). CX_3CR1^{GFP}

mice allowed us to identify two distinct monocyte subsets: large, short-lived $CX_3CR1^{lo}Gr1^+$ cells that are actively recruited to sites of inflammation and small $CX_3CR1^{high}Gr1^-$ cells characterized by homing to non-inflamed, resting tissues. Interestingly, this monocyte dichotomy is evolutionary conserved as differential CX_3CR1 expression also characterizes the two known $CD14^+$ and $CD14^{low}CD16^+$ human monocyte subsets. Preliminary studies indicate that both "inflammatory" and "homeostatic" monocytes can differentiate *in vivo* into DC. Focusing on the spleen and the respiratory tract we now define the monocyte contribution to other MPS compartments and investigate the molecular cues that guide *in vivo* monocyte differentiation into DC and MO.

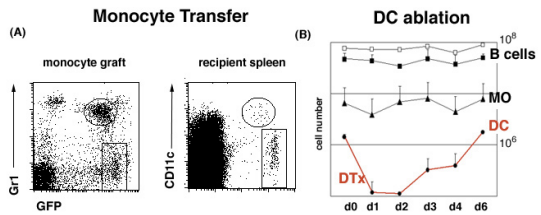

Fig. 2 *FACS analysis of (A) CX_3CR1^{GFP} blood monocyte graft and monocyte recipient spleen 4 days after transfer; (B) DC depletion and splenic APC populations of CD11c-DTR transgenic mice after i.p. injection of 100 ng DTx (day 0)*

Studying *in vivo* functions of dendritic cells

In order to investigate *in vivo* DC functions we generated a mouse model that allows the conditional ablation of $CD11c^+$ DC in the intact organism (Fig 2B). Our model is based on the DC-restricted expression of a diphtheria toxin (DTx) receptor transgene in mice that are naturally resistant to the bacterial exotoxin. DTx injection of CD11c-DTR mice results in the rapid systemic depletion of DC and allowed us to define a first essential *in vivo* DC function, i.e. the priming of cytotoxic $CD8^+$ T cell responses to intracellular pathogens, such as Listeria and Malaria parasites. In our studies at the Weizmann Institute we currently investigate the role of $CD11c^+$ DC in T cell homeostasis, $CD4^+$ T cell priming, T cell memory maintenance and Graft versus Host Disease. In order to probe for differential activities of DC subsets, we are furthermore developing binary transgenic systems that will restrict expression of the DTx receptor to $CD8\alpha^+$ DC, monocyte-derived or plasmacytoid DC.

Selected Publications

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