The Mononuclear Phagocyte System –Origins & Functions

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The body-wide cellular network of mononuclear phagocytes is subdivided into monocytes, macrophages and dendritic cells, which are defined by distinct anatomic location and phenotypes. Monocytes are circulating in the blood, while macrophages and dendritic cells are generally found ubiquitously distributed in tissues. Macrophages include organ-specific representatives in the serosa, alveolar and interstitial spaces of the lung, brain (microglia), bone (osteoclasts) and liver (Kupffer cells). Dendritic cells have been divided into thymic DC, epidermal Langerhans cells, type I IFN-producing Plasmacytoid dendritic cells (PDC), and "conventional" dendritic cells (cDC) defined by high level expression of the β integrin CD11c. Murine CD11c^{high} cDC are furthermore themselves composed of at least three subsets differentiated according to expression of CD11b, CD4 and CD8. Dendritic cells have co-evolved with acquired immunity. They are Antigen Presenting Cells (APC) with unrivaled potency to activate naïve T cells and ensure peptide-specific, protective immunity. In addition dendritic cells can act as critical regulatory cells that control the inherent auto-reactivity of the T cell compartment. Both these activities rely on cognate interactions of antigen presenting dendritic cells with antigen-specific T cells and it remains unknown, how dendritic cells can fulfill opposing tasks in immuno-stimulation and tolerization. Pleiotropic dendritic cell functions could reflect differential activation states. Alternatively they could be associated with dedicated and specialized dendritic cell subsets. The latter scenario would be of particular interest for the clinic, but solid experimental evidence in support of such task division remains rare.

Dendritic cell Functions

To probe for differential functions of murine macrophages, dendritic cells and dendritic cell subsets we use an in vivo system that allows the conditional ablation of defined mononuclear phagocyte subpopulations in the intact mouse. Our model is based on restricted expression of a human diphtheria toxin (DTx) receptor transgene in mice that are naturally resistant to the bacterial exotoxin. DTx injection of the prototype system - CD11c-DTR mice, in which DTR expression is targeted to CD11c^{high} cells (Jung et al. Immunity 17:211) - results in rapid depletion of cDC. CD11c-DTR mice allowed us to confirm the critical role of these cells in the initiation of in vivo T cell responses. Furthermore, they were instrumental in the detection of the unique organ-restricted priming potential of another dendritic cell subset, the Plasmacytoid dendritic cells (Sapoznikov et al.). More recently, the ablation strategy enabled us to unravel novel cDC functions beyond their anticipated role as APC. Assisted by intravital microscopical studies we thus discovered the requirement of novel perivascular cDC clusters in the bone marrow for the maintenance of recirculating B cells (Figure 1). In collaboration with N. Dekel and M. Neeman we observed an unexpected role of the cells in decidual tissue remodeling at the fetal/

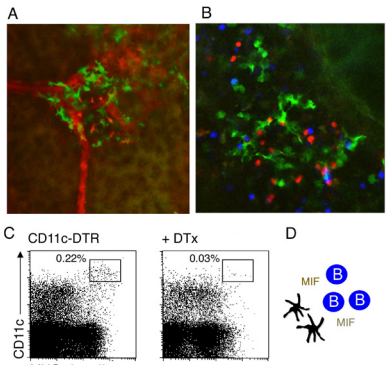


Fig. 1 *The Bone Marrow Immune Niche. The use of compound transgenic mice (CD11c-DTR: Cx3cr1^{GFP}) allowed the in situ visualization and ablation of cDC in the bone marrow. Two-photon microscopy of the murine skull revealed the existence of novel perivascular clusters of dendritic cells (DC green; vessels, red) (A), which co-localize with adoptively transferred B (blue) and T cells (red) and thus demarcate long-sought BM immune niches (B). DTx treatment of CD11c-DTR: Cx3cr1^{GFP}mice results in the ablation of CD11c^{high} bmDC (C) and allowed to establish their requirement as MIF-producing cells for mature B cell survival in the BM (collaboration with I. Shachar) (Sapoznikov et al. NI 9:388).*

maternal interface (Plaks & Birnberg et al. submitted). We are now in the process to extend our ablation strategy to dendritic cell subsets. Here we will use a Cre/loxP technology-based binary transgenic system to restrict DTR expression to CD8α^{+} dendritic cells, which are suspected to play a unique role in cross-presentation and tolerance establishment. In addition we generated a mouse that constitutively lacks cDC to study the role of these cells in development and homeostasis (Birnberg et al. submitted).

Dendritic cell Origins

Differential *in vivo* functions of macrophages, dendritic cells and dendritic cell subsets could potentially be exploited for therapeutic purposes. Rather than transferring terminally differentiated mononuclear phagocytes, which cannot faithfully be generated in vitro and are unlikely to reach the desired physiological microenvironment, manipulation of the mononuclear phagocyte system could rely on the transfer of precursor cells that would differentiate in their physiological context. Such strategies will, however, require an in depth understanding of the underlying differentiation pathways. To study *in vivo* dendritic cell origins we performed adoptive transfer experiments of dendritic cell precursors (MDPs, monocytes) into dendritic cell-depleted mice. These experiments revealed a general dichotomy of the in vivo dendritic cell compartment into lymphoid tissue-resident dendritic cells and dendritic cells residing in peripheral tissues (Varol et al.). Moreover, the ability to efficiently seed given anatomic sites, such as the intestinal lamina

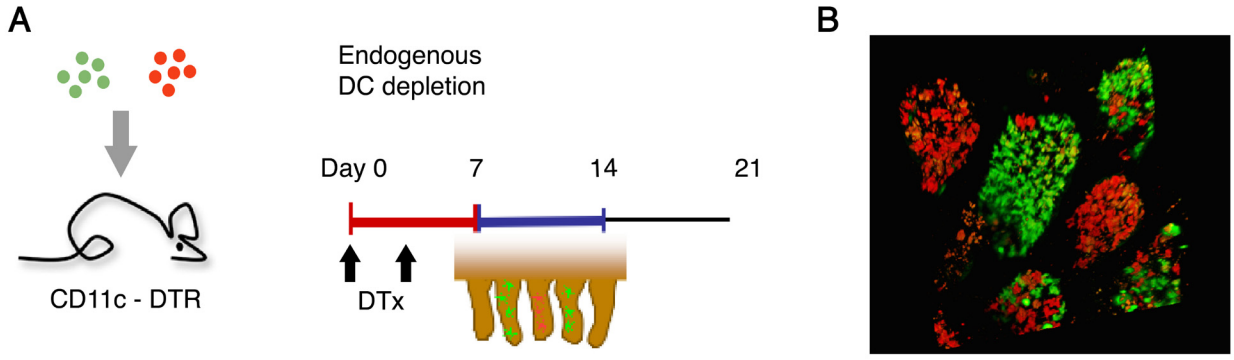


Fig. 2 *In vivo reconstitution of the intestinal lamina propria dendritic cell (lpDC) compartment with monocyte graft-derived cells. Scheme illustrating experimental strategy of precursor cell transfer into dendritic cell-depleted mice (A); Two-photon analysis of lpDC reconstitution. Note that as a result of clonal expansion of the green and red fluorescent protein-expressing precursors individual villi carry a distinct label (Varol, Vallon- Eberhard et al. in preparation).*

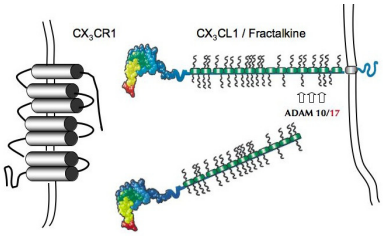
propria, with defined graft-derived cells (Figure 2) provides a novel approach to study in situ dendritic cell functions. We are now in the process to develop a lenti-viral transduction protocol for the mononuclear phagocyte precursors to define molecular determinants governing *in vivo* dendritic cell differentiation and investigate their contribution to local inflammatory dysfunction.

Monocyte studies

Monocytes circulate in the blood and link bone marrow myelopoesis to the peripheral macrophage and dendritic cell pool. Both in men and mice blood monocytes comprise two major phenotypically distinct subsets (Geissman, Jung & Littman, Immunity 2003), which seemingly bear differential functions and fates upon extravasation into the tissues (Landsman et al.). In steady state the monocyte input to the periphery is rather limited and remains poorly understood. In contrast, acute blood monocyte recruitment is well appreciated as major factor in local inflammatory reactions. Moreover, recent studies indicate that monocytes and their immediate macrophage and dendritic cell progenies have a critical impact on the fate of the injury and the decision between resolution and progression into chronicity. To gain further insights into monocyte biology and explore their use in cell therapy we are developing genetic tools to investigate the contribution of these intriguing cells in health and disease with a focus on inflammation, angiogenesis and atherosclerosis.

The physiological role of the CX3CR1 chemokine receptor and its ligand CX3CL1

A third focus of our laboratory is given to the "CX_3C chemokine family", which is comprised of the 7TM receptor CX_3CR1 and its sole ligand Fractalkine/ CX_3CL1. Both CX_3CR1 and CX_3CL1 are widely expressed throughout the organism, but in given tissues expression is highly cell type-restricted. Taking advantage of mice that harbor a targeted replacement of the Cx_3cr1 gene by a GFP reporter (Jung et al. MCB, 2000), we could f.i. show that CX_3CR1 expression in the gut is specific for villus lamina propria DC (see Figure 2) that interact with CX_3CL1-expressing columnar epithelial cells to form trans-epithelial dendrites. In the brain $CX_3CL1/R1$ interactions are restricted to the neuronal-microglial interface, while in the blood CX_3CL1-expressing endothelium seems to provide a constitutive survival signal to circulating monocytes (Landsman et al. submitted). The key to the enigmatic physiological role of $CX_3CR1/L1$ might lie in the unique structure of the ligand CX_3CL1. Unlike classical small peptide chemokines, CX_3CL1 is synthesized as a large, membrane-tethered protein and presented on an extended mucin-like stalk. Constitutive or inducible proteolytic cleavage by the metalloproteases ADAM 10 and 17 (TACE) can however result in release of a soluble CX_3CL1 entity (see scheme). To determine the physiological role of membrane-anchored vs. shed CX_3CL1 we plan to reconstitute CX3CL1-deficient mice with BAC-transgenes encoding cleavable, non-cleavable and secreted CX_3CL1 variants.



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