Deciphering myeloid (progenitor) cell function and communication in (tumor) tissues

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Addendum

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English summary

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Curriculum vitae

PhD portfolio

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English summary

Myeloid cells are derived from myeloid progenitors via a process called myelopoiesis. The myeloid lineage belongs to the innate immune system and includes various cell types, such as monocytes, macrophages (MΦ), and dendritic cells (DC). Among these, DC are specialized in capturing and presenting antigens to T-cells in the lymph nodes (LN), which leads to T-cell activation and differentiation. Therefore, DC are recognized as the initiators of the cancer-immunity cycle, a series of stepwise events resulting in T-cell mediated anti-cancer immune responses. In addition, DC play crucial roles in shaping T-cell states and function in specific niches inside the tumor. However, DC alone are often not enough to prime efficient $CD8^+$ T-cell responses in cancer patients. $CD4^+$ T-cells can promote the functional maturation of DC, allowing them to support effective $CD8^+$ T-cell responses. This process, known as DC licensing by $CD4^+$ T-cell "help", operates at least in part via CD40 signaling.

Cancer immunotherapies have revolutionized cancer treatment. Immunotherapy is focused on harnessing both adaptive and innate immunity to destroy malignant cells. The best example of cancer immunotherapy used in the clinic is immune checkpoint blockade (ICB). Herein, monoclonal antibodies are used to block inhibitory receptors on immune cells, most often T-cells, thereby activating these cells. Apart from ICB, cancer vaccines and cell therapy are explored as cancer immunotherapies. Although $CD8^+$ T-cells have been the main focus of cancer immunotherapy, recent progress highlights the importance of DC and $CD4^+$ T-cells in tumor immunity.

In this thesis, I describe our efforts to elucidate the molecular mechanisms of $CD4^+$ T-cell "help" as a critical signal that can successfully activate human classical (c)DC1. I approach this question from the perspective of both DC- and $CD4^+$ T-cells. I also present the clinical importance of DC licensing, and delineate (pharmacological) targeting strategies for future cancer immunotherapy. Additionally, I outline our endeavor to understand myelopoiesis under stress as an integral component of our exploration into myeloid cell biology. I introduce these concepts in **chapter 1**.

In **chapter 2**, using single-cell RNA sequencing, we demonstrate that human cDC1 uniquely respond to activated $CD4^+$ T-cells by upregulating pathways important for T-cell priming, including migration, antigen processing and presentation, co-stimulation, cytokine and chemokine production. Using an *in vitro* cytotoxic T-lymphocyte (CTL) priming system, we demonstrate that among all human DC types, cDC1 are most capable to transmit $CD4^+$ T-cell "help for optimal CTL responses, particularly in a setting of (tumor) cell-associated antigens. We identify remarkable overlap between the mRNA

signature of "helped" cDC1 and those of tumor-infiltrating mature cDC across a multitude of human cancer types. In melanoma patients, the presence of "helped" cDC1 proved to positively correlate with overall patient survival as well as response to anti-PD-1 immunotherapy. These data show for the first time that $CD4^+$ T cell "help" can be delivered in the tumor via cDC1, which improves the CTL response against the tumor.

In **chapter 3**, as a continuation of chapter 2, I present the work aiming at delineating (novel) ligand-receptor interactions guiding the cross-talk between cDC1 and $CD4^+$ T-helper (Th) cells in human. We show that after T-cell receptor (TCR) triggering and CD28 co-stimulation, $CD4^+$ T-cells can produce type I interferon (IFN-I), specifically IFNβ, via the DNA-sensing STING pathway. Using both gene manipulation in $CD4^+$ T-cells and antibody blocking on cDC1, we uncover that IFN-I signaling entails a CD40-independent pathway of cDC1 licensing, especially by enhancing their antigen (cross)-presentation ability. Moreover, we demonstrate the co-occurrence of IFN-I producing $Ki67^+CXCL13^+CD4^+$ T-cells and mature cDC in human tumors. In cancer patients, the frequencies of $Ki67^+CXCL13^+CD4^+$ T-cells are positively correlated with the frequencies of tumor-reactive $CD8^+$ T-cells as well their clinical outcomes. These data indicate that IFN-I, produced as a result of $CD4^+$ T cell "help" delivery in the tumor, promotes the CTL response against the tumor via cDC1 activation.

Immune checkpoints (IC) are regulators of the immune response and generally divided into stimulatory and inhibitory categories. They include not only molecules acting on adaptive immune cells such as T-cells, but also molecules targeting innate immune cells such as DC. Increasing evidence suggests that therapeutic targeting of immune checkpoints on DC can contribute to the efficacy of cancer immunotherapies. In **chapter 4**, we provide an overview of IC on DC that can augment or inhibit their functions, and summarize the mechanisms of action of several selected IC, according to their functional categories: phagocytosis, immune activation and immune inhibition.

Although many studies, including our own, have demonstrated the pivotal role of cDC1 in T-cell mediated anti-tumor responses, the rarity and fragility of human cDC1 remains an obstacle for clinical application. In **chapter 5**, we try to tackle this problem by developing a method to generate cDC1-like cells *in vitro* from $c\text{-}Kit^+CD34^+$ myeloid progenitors isolated from non-mobilized blood of adult donors. We subsequently demonstrate that in the presence of $CD4^+$ Th cells, these progenitor-derived cDC can relay $CD4^+$ T-cell "help" to promote the CTL response. We also show that this method can be used to improve the detection of tumor-specific $CD8^+$ T-cells, that can be used for adoptive T-cell therapy in cancer patients.

Myeloid progenitors can thus be a great source to generate DC for both research and clinical use. Next to that, these cells can react to pathologic conditions. In **chapter 6**, we demonstrate that an immune response to short-term infection in the airways leads to local generation of MΦ from myeloid progenitors. This process is activated by toll-like receptor 5 stimulation on the progenitors, as we show both *in vitro* and *in vivo*. The myeloid progenitors migrate from the blood to the inflamed lung tissue as a result of CCL2-CCR2 chemokine-receptor interaction. These results indicate that myeloid progenitors can contribute to innate immunity in inflamed tissue by generating MΦ *in situ*.

Apart from perturbation caused by external stress, the functions of myeloid progenitors can also be disturbed by genetic mutation, leading to myeloid neoplasia. In **chapter 7**, we study this aspect in the context of Langerhans cell histiocytosis (LCH). We observe higher frequencies of circulating myeloid progenitors in both high risk and low risk LCH patients compared to healthy donors, indicating disturbed mobilization from the bone marrow. In addition, we develop a method that facilitates detection of mutation-carrying LCH progenitor cells, by culturing the LCH cells from myeloid progenitors. We show that patients with single LCH lesions can have circulating progenitors carrying genetic driver mutation, which may predict LCH dissemination. This is relevant for clinical stratification of LCH patients.

In **chapter 8**, I present my perspective on the theoretical and clinical implications of the findings in this thesis and how they relate to current literature.

Overall, this thesis contributes to an improved understanding of the function and intercellular communication of DC and myeloid progenitors in tissues, particularly in the context of cancer and inflammation.