Characterization of a human dentritic cell line; a prelude to allogeneic dendritic cell-based tumor vaccination

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Summary

Cancer immunotherapy aims to (re)activate the host's immune system to fight cancer. The recognition of DC as the main orchestrators of the immune response has made DC promising targets for the development of anti-cancer immunotherapies. In fact, it has been demonstrated that DC vaccination results in the development of anti-tumor immune responses and subsequent reduction of established tumors in both murine tumor models and cancer patients. However, although early clinical trials indicate that DC vaccination is feasible and safe, clinical results obtained so far are variable. Moreover, it has been demonstrated that immunological and clinical outcome are dependent on the quality of the DC vaccine, indicating that the preparation of a DC vaccine should be standardized. Since the development of such standardized vaccines is often hampered by the relative limited availability of DC precursors and inter- and intra-donor variability it would be preferable to use DC from a readily available and unlimited source, such as cell lines can provide. In this thesis, the applicability of the human CD34+ myelomonocytic cell line MUTZ-3 as a reproducible model for DC differentiation and its possible utility in clinical DC vaccination protocols is investigated.

Chapter 1 provides an overview of the various immunotherapeutic anti-tumor approaches that have been or are currently being investigated. In particular, the role of DC as sentinels of the immune system and their utility in anti-tumor vaccination is discussed.

In **chapter 2,** two populations of DC from skin, i.e. CD1a+ epidermis-derived LC (CD1a+ LC) and CD1a+ dermis-derived DDC (CD1a+ DDC) are analyzed by high density microarray analysis. We were able to demonstrate that CD1a+ DDC specifically express markers associated with DDC phenotype, such as the macrophage mannose receptor (MMR), DC-SIGN, the scavenger receptor CD36, coagulation factor XIIIa (FXIIIa) and chemokine receptor CCR5, whereas CD1a+ LC specifically express Langerin, membrane ATPase (CD39) and CCR6, all hallmarks of the LC lineage. In addition, $CD1a^+$ DDC displayed a more activated pro-inflammatory and migratory profile, and LC exhibited a more quiescent profile, expressing genes involved in cell adhesion and DC retention in the epidermis, indicating that both DC subsets show a remarkable difference in maturation status under steady state conditions. In conclusion, transcriptional profiling is consistent with the notion that CD1a+ DDC and LC represent two distinct DC subsets, and that under steady state conditions CD1a+ DDC and LC represent opposites of the DC activation spectrum

In **chapter 3,** the human CD34+ myelomonocytic leukemia cell line MUTZ-3 is introduced as a human cell line model of myeloid DC differentiation. MUTZ-3 progenitors display the unique ability to differentiate into interstitial DC (IDC) and Langerhans cells (LC) in a cytokine-dependent manner. Phenotypic characterization revealed that the MUTZ-3 cell line consists of three distinct subpopulations. Small CD34+CD14-CD11b- progenitors constitute the proliferative compartment of the cell line with the ability to differentiate through a CD34-CD14-CD11b+ stage to ultimately give rise to a morphologically large, non-proliferating CD14+CD11b+ progeny. Moreover, over the course of differentiation, MUTZ-3 progenitors acquire a more myeloid DC precursor phenotype with up-regulated expression of myeloid differentiation markers and cytokine receptors associated with DC

differentiation, and down-regulated expression of cytokine receptors associated with DC precursor proliferation. Moreover, the CD14+CD11b+ cells could be identified as common, immediate myeloid DC precursors with the ability to differentiate into either LC or IDC, exhibiting characteristic and mutually exclusive expression of Langerin and DC-specific ICAM grabbing non integrin (DC-SIGN), respectively. The identity of the MUTZ-3-derived LC subset was confirmed further by the presence of Birbeck granules. We conclude that the MUTZ-3 cell line provides a ready and continuous supply of common myeloid precursors, which should facilitate further study of the ontogeny of myeloid DC lineages.

In **chapter 4**, an extensive transcriptional analysis of the MUTZ-3-derived IDC and LC is provided. Transcriptional profiling revealed that the MUTZ-3 IDC express the C-type lectins macrophage mannose receptor (MMR) and DC-SIGN, the scavenger receptor CD36, the co-agulation factor XIIIa and the monocyte/macrophage marker CD14, all hallmarks of IDC lineage, whereas MUTZ-3 LC express Langerin, membrane adenosine triphosphatase (ATPase) and CCR6, all hallmarks of the LC lineage. Our data indicate that MUTZ-3-derived IDC and LC on the whole adhere to the proposed definitions of the IDC and LC phenotype, and thus represent a readily available, physiologically relevant and well defined model to investigate DDC and LC biology *in vitro*.

In **chapter 5a**, the applicability of allogeneic MUTZ-3-derived DC to generate tumor-specific cytotoxic T lymphocytes is analyzed and compared to autologous MoDC. The data show comparable induction efficiencies of TAA-specific CTL with MUTZ-3 IDC and MoDC. Functional activity of the MUTZ-3 DC-generated CTL could be confirmed by recognition of endogenously processed and presented epitopes on tumor cells. For this, several adenocarcinoma associated, HLA-A2 restricted TAA-derived peptides were used. One of these peptides is derived from the newly described colon tumor antigen Ebp1. Identification and characterization of this novel antigen, and its derived HLA-A2-restricted peptide, is described in **chapter 5b**.

In **chapter 6**, one way to improve DC induced T cell reactivity is described. By making use of a novel bispecific αCD40/αCD28 diabody, leukemia-derived DC induced increased T cell – DC cluster formation, increased T cell proliferation, as well as an increased TAA-specific T cell priming efficiency, as demonstrated by the use of MUTZ-3 IDC. Overall, the bispecific αCD40/αCD28 diabody strengthens DC induced T cell reactivity and might serve as a potent therapeutic tool to effectively augment anti-tumor T cell responses elicited by DC.

In **chapter 7**, the relative ability of MUTZ-3 IDC and LC to induce a specific T cell response is analyzed in order to reveal the more suitable candidate for use as a clinical vehicle of tumor vaccines. While mature LC and IDC displayed comparable lymph node-homing potential, mature LC showed significantly higher allogeneic T cell stimulatory capacity. IDC supported the induction of tumor antigen-specific CD8+ T cells at an overall higher efficiency as compared to LC, which might be related to the observed inability of LC to release T cell stimulatory cytokines such as IL-12p70, IL-15,

and IL-23. Indeed, transduction with IL-12p70 did significantly improve the priming efficiency of LC and abrogated the difference in tumor-specific CD8+ priming efficiency between the DC subsets. Overall, despite the inability of LC to release type-1 T cell-stimulatory cytokines, *in vitro* functions of LC and IDC suggest comparable abilities of both subsets for the *in vivo* induction of anti-tumor T cells.

Finally, in the concluding **chapter 8**, the different human DC differentiation models described so far are discussed. Evidence is provided that monocytic leukemia cell lines such as THP-1, KG-1, K562, monomac-6 and U-937 are poor models for DC differentiation. The CD34+ acute myeloid leukemia cell line MUTZ-3, in contrast, represents a valuable and sustainable model system for myeloid DC differentiation, reflecting all physiologically observed transitional maturation states. In addition, the MUTZ-3 cell line provides DC that exhibit the phenotypic and functional properties that are essential for the *in vivo* generation of CTL-mediated immunity and thus represents a suitable candidate for both DC differentiation and clinical tumor vaccination studies.