Abstract

Immunotherapy based on dendritic cells (DCs) was first tested in clinical trials for the treatment of cancer in the 1990s. Currently, the ability of DCs to modulate immune responses is also being tested in several clinical studies focusing on autoimmune disease treatment with the aim of suppressing the overactivated immune system and restoring immune tolerance. For this purpose, so-called tolerogenic DCs with considerable suppressive potential are used. Tolerogenic DCs can be generated *ex vivo* from monocytes using pharmacological agents, which in DCs induce a regulatory phenotype with low expression of activation markers, high expression of inhibitory markers and secretion of suppressive cytokines. In the first part of this study, we show that cultivation of human blood monocytes in the presence of glucocorticoid dexamethasone and 19-nor-1,25-dihydroxyvitamin D_2 (paricalcitol) enables *ex vivo* generation of tolerogenic DCs with a highly stable suppressive phenotype characterized by upregulated IL-10 production, inhibitory IL-T3 and PD-L1 molecule expression, the low stimulatory capacity and the ability to induce regulatory T cell development. Moreover, we show that metabolic changes and signaling through NF-κB, p38 MAPK, ERK1/2 molecules and the mTOR/STAT3 pathway play an important role in the maintenance of tolerogenic DC suppressive phenotype and function. In the next part of this study, we show that dexamethasone and vitamin D_2 can also be used to generate tolerogenic DCs of sufficient quality from patients with type 1 diabetes mellitus (T1D), despite their suffering from ongoing autoimmune processes. However, the patients' glycemic control has a crucial impact on the quality of the generated tolerogenic DCs. In fact, long-term hyperglycemia significantly influences not only the tolerogenic DC phenotype but also the possibility to induce stable antigen-specific T cell hyporesponsiveness and promote regulatory T cell differentiation in T1D patients. Thus, these findings provide important information for determination of a group of T1D patients who could benefit from the treatment with the tolerogenic DC-based therapy. In the last part of the study, we evaluate the possibility of generating tolerogenic DCs for the treatment of autoimmune diseases on a scale for clinical testing. We optimize the manufacturing protocol with respect to tolerogenic DC yield, purity, viability, phenotype and function. We also suggest assays that can be routinely used for control of the quality and suppressive capacity of tolerogenic DCs generated for a clinical study. The results summarized in this thesis represent important findings for the generation of tolerogenic DCs in patients with T1D and for the design of a potential clinical trial.