Abstract

Utilization of tolerogenic dendritic cells (tolDCs) as a cell-based therapy represents a promising strategy in treatment of autoimmune diseases including type 1 diabetes (T1D). Numerous protocols have been established to generate tolDCs *ex vivo* and their therapeutic effect has been demonstrated in animal models of autoimmune diseases. In this thesis we compared three different variants of such protocols which are based on the combined treatment of bone marrow-derived DCs with vitamin D and dexamethasone applied at different time points of their maturation towards tolDCs. We assessed the efficiency of these protocols in regards of their effect on the expression of co-stimulatory molecules CD40, CD80, CD86, and MHC II and the chemokine receptor CCR7 on the surface of tolDCs. Then, we evaluated the migration pattern of antigen unloaded tolDCs *in vivo* as well as their effect on the induction of immune responses and cell proliferation of lymph node cells. This was achieved by labelling of tolDCs with membrane dye PKH26 and by following their migration path by flow cytometry after intraperitoneal (i.p) or subcutaneous (s.c.) injection into either left or right side of the body. On day 1, 3, 5, 7, and 9, the presence of $PKH26^+$ tolDCs was examined in spleen, pancreatic, mesenteric, inguinal and axillary lymph nodes of NOD mice. Total cell recoveries from these anatomical sites were used as a measure of their migratory capacity. Flow cytometric analysis readily detected live $PKH26^+CD11c^+CD3^-$ tolDCs in spleens and pancreatic lymph nodes after i. p. administration, whereas s. c. injection led to their accumulation preferentially in inguinal and axillary lymph nodes on the respective application side. In addition, we monitored the impact of the above indicated application routes on the prevention of diabetes by tolDCs in the NOD-SCID model by adoptive co-transfer of tolDCs with NOD-derived splenocytes. Our data provide strong evidence that the type of culture protocol along with application route affect tolerogenic properties of tolDCs. Specifically, we established that the capability of tolDCs to migrate to pancreatic lymph nodes and to prevent diabetes in the NOD-SCID adoptive co-transfer model is the most effective when vitamin D and dexamethasone treated tolDCs are administrated via i.p. route. This original data provides a novel experimental platform for further optimizing this protocol, which in a long run, can be potentially used for therapeutic application in future human trials.

Key words

Type 1 diabetes, tolerogenic dendritic cells, cell-based therapy, non-obese diabetic mouse, non-obese diabetic-severe combined immunodeficiency mouse, application routes, *in vivo* trafficking, protocol optimization