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The characteristics and immunoregulatory functions of regulatory dendritic cells induced by mesenchymal stem cells derived from bone marrow of patient with chronic myeloid leukaemia

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ABSTRACT

Dendritic cells (DCs) are specialised antigen-presenting cells that play crucial roles in the initiation and regulation of immune responses. Recently, mesenchymal stem cells (MSCs) have gained further interest after demonstration of immunomodulatory effects on complicated interactions between T cells and even DCs. However, the mechanisms underlying these immunoregulatory effects of MSCs induced DCs are poorly understood. In addition, it is unclear whether the immunoregulatory functions of MSCs are altered in disease states. In this study, we showed that chronic myeloid leukaemia (CML) patients bone marrow derived MSCs (CML–MSC) could differentiate mature DCs (mDCs) into a distinct regulatory DC population, they had lower expression of CD40, CD80, CD83 and CD86. Similar to immature DCs (imDCs), CML–MSC induced DCs (CML–MSC–DCs) displayed powerful phagocytic capacity. Moreover, CML–MSC–DCs had the capacity to induce T cell anergy, another capacity of regulatory DCs. CML–MSC–DCs could inhibit the proliferation of T cells not only through TGF-β1, but also by inducing the production of Treg cells or T-cell anergy. At last, CML–MSC–DCs could efficiently induce more CD4+CD25+Foxp3+Tregs from naive CD4+CD25−Foxp3−T cells than that of normal-MSC–DCs *in vitro*. CML–MSC–DCs derived TGF-β1 was largely responsible for the increase in CD4+CD25+Foxp3+Tregs based on knockdown studies. The immunoregulatory effects of DCs induced by CML–MSCs enhance the potential use of autologous MSCs in cell therapy.

1. Introduction

Mesenchymal stem cells (MSCs) are multipotential cells that reside within the bone marrow and can be induced to differentiate into various components of the marrow microenvironment, including osteoblasts, adipocytes, myoblasts, chondroblasts, neurons and gliacytes under certain conditions.[1] MSCs are able to support haematopoiesis in long-term bone marrow culture by producing numerous extra-cellular matrix ligands and a number of haematopoietic cytokines. MSCs are able to inhibit T cells proliferation *in vitro* and mediate a systemic immunosuppressive property *in vivo*.[2,3] In addition, Horwitz et al. reports the first use of MSCs for the treatment of children with severe osteogenesis imperfecta based on their ability to

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differentiate to bone cells.[4] Koc et al. demonstrated that autologous MSCs could be infused along with peripheral blood stem cells in advanced breast cancer patients, the infusion could promote the haematopoietic recovery and was not an adverse reaction.[5] Other studies suggest that cotransplantation of MSCs permits HSC alloengraftment and decreases graft-versus-host disease (GVHD) through the approach of regulatory immune response *in vivo*.[6–8] Taking advantage of their immune suppressive effect as well as the ability to support haematopoiesis, both autologous and allogeneic MSCs are of great therapeutic potential in the context of cell-based therapy.

Dendritic cells (DCs), the most potent antigen-presenting cells (APCs), are derived from CD34+ BM stem cells and can be generated from monocytes *in vitro* by incubation with granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4).[9] DCs are key mediators for the initiation and regulation of both innate and adaptive immune responses. DCs exist in at least two different stages: immature and mature. Depending on their different states, DCs possess different functional properties. Mature DCs (mDCs) initiate an immune response depending on its transition from antigen processing to antigen-presenting cell, during which it up-regulates MHC class II and costimulatory molecules (CD80 and CD86) on the cell surface.[10] Immature DCs (imDCs) that are deficient of costimulatory molecules can induce T-cell anergy, generate regulatory T cells and promote alloantigen-specific tolerance.[11] Thus, the unique capacity of DCs to respond to maturation signals and subsequently to activate naive T cells enables these cells to determine the fundamental outcome of immunity.

Compared to allogenetic MSCs, autologous MSCs from patients who needed cell-based therapy may be an ideal alternative stem cell source. Our previous studies demonstrated that CML derived MSCs (CML–MSC) were similar to normal adult derived MSCs in phenotype, morphology, multi-differentiation capacity and haematopoiesis supporting function. CML–MSC did not express BCR/ABL gene and Ph chromosome.[12] Because the immunomodulatory effects of MSCs are critical for the clinical application of CML–MSC, we want to understand the immunomodulatory effects and definitive mechanisms of CML–MSC. Although CML–MSC is able to inhibit T cells proliferation *in vitro*,[13] a definitive mechanism underlying this phenomenon is still lacking. In addition, the effects and mechanisms of CML–MSC on the development of DCs are not clear.

Recently, Zhang et al. showed that MSCs could induce mDCs into a subset of DC population with immunoregulatory property.[14] However, the mechanisms underlying these immunoregulatory effects of MSCs induced DCs are still poorly understood. Moreover, it is unclear whether the immunoregulatory functions of MSCs are altered in disease states. In this study, we showed that CML patients bone marrow derived MSCs could differentiate mDCs into a distinct regulatory DC population. Compared with mDCs, they had lower expression of CD40, CD80, CD83 and CD86, but higher expression of CD11b. CML–MSC induced DCs could efficiently inhibit the proliferation of T-lymphocyte not only through TGF-β1, but also through the induction of Tregs or T cell anergy. In addition, CML–MSC–DCs could efficiently induce more CD4+CD25+Foxp3+Tregs from naive CD4+CD25−Foxp3−T cells than that of normal-MSC–DCs *in vitro*. CML–MSC–DCs derived TGF-β1 was largely responsible for the increase in CD4+CD25+Foxp3+Tregs based on knockdown studies.

2. Materials and methods

2.1. Isolation and culture of MSCs derived from CML patients

Sixteen patients with CML (aged from 23 to 56; median age: 41) were investigated in this study. For CML patients, 13 patients were in their initial chronic phase and 3 patients were in accelerated phase. Thirteen patients were untreated at the time of study. Three patients were receiving hydroxyurea at the time of marrow collection. Eight normal adults (aged from 19 to 35; median age: 32; 4 males and 4 females) were investigated as control. Institutional Review Board approval was obtained for the use of the human bone marrow. Demography and diagnosis of the patients were listed in Table 1. Mononuclear cells (MNCs) were separated by a Ficoll-Paque gradient centrifugation (specific gravity 1.077 g/ml; Sigma Diagnostics, St. Louis, MO, USA), and cultured in an expansion medium at 37 °C with 5% CO_2 in fully humidified atmosphere. After being cultured for 24–48 h, the culture medium was replaced and non-adherent cells were removed. Once cells were more than 80% confluent, they were detached with 0.25% trypsin–EDTA (Sigma), then CD14 positive cells were depleted using CD14 micromagnetic beads (Miltenyi Biotec, Auburn, USA) and CD14 negative cells were replated. MSCs expressed CD105, CD29 and CD44; they did not express haematopoietic markers CD34, CD45 and endothelial markers CD31, vWF. Moreover, CD14 and HLA-DR were also negative. They had the ability to differentiate into, at least, adipocyte, osteoblast, endothelial and neural *in vitro*.[12]

2.2. Differentiation of DCs

Human peripheral blood mononuclear cells (hPBMCs) from healthy donors were isolated by centrifugation over Ficoll-Hypaque gradients (Nycomed Amersham, Uppsala, Sweden). CD14+ monocytes were isolated as the adherent fraction after incubation for 1 h in RPMI 1640 (BioWhittaker, Verviers, Belgium) supplemented with 10% foetal calf serum (FCS) (BioWhittaker), 100 U/mL penicillin/streptomycin (Bristol-Myers Squibb, Sermoneta, Italy), and 50 μM 2-mercaptoethanol (Bio-Rad, Segrate, Italy) (DC medium) at 37 °C. After extensive washing, adherent monocytes were differentiated into DCs by culture in 10 ng/mL recombinant human IL-4 (rhIL-4) (R&D Systems, Minneapolis, MN) and 100 ng/mL recombinant human GM-CSF (rhGM-CSF) (Schering-Plough, Kenilworth, NJ) in DC medium. To induce maturation of monocyte-derived cells, lipopolysaccharide (LPS) (1 μg/mL) was added for another 48 h of culture with GM-CSF and IL-4.

2.3. Isolation of CD4+T cells and their subsets

hPBMC was isolated by Ficoll-Paque (1.077 g/mL) density gradient centrifugation. CD4+T lymphocytes were isolated from hPBMC by using CD4 micromagnetic beads (Miltenyi Biotec, Auburn, USA) according to the manufacturer's instructions.