Cytotherapy, 2015; 17: 1732–1745

International Society for Cellular Therapy
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Xeno-immunosuppressive properties of human decidual stromal cells in mouse models of alloreactivity *in vitro* and *in vivo*

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Abstract

Background aims. Human decidual stromal cells (*h*DSCs) may cure acute graft-versus-host disease (GVHD) in humans. We evaluated immunosuppression by xenogenic *h*DSCs in mice, both *in vitro* and *in vivo*. *Methods.* *h*DSCs inhibited mouse lymphocyte proliferation in allo- and xeno-stimulation assays in mixed lymphocyte culture (MLC) and after mitogenic stimulation. The immunosuppressive effect of *h*DSCs was dose-dependent and strain-independent. Trans-well experiments showed that *h*DSCs needed cell-to-cell contact to be immunosuppressive. In a GVHD model, Balb/c mice were transplanted with bone marrow and splenocytes from C57BL/6 a donor. Varying doses of *h*DSCs (10^5–10^6 per mouse) were infused at different time points. Recipient mice showed lower GVHD scores than untreated mice, but they did not have consistently improved survival. Histopathological investigation of liver, gastrointestinal tract and skin of animals with GVHD did not show any significant improvement from *h*DSC infusion. *Results.* *h*DSCs were transduced with immunosuppressive genes including those encoding interleukin-10, prostaglandin-E2 receptor, indoleamine dioxygenase, interferon-γ and programmed death ligand-1. Transduced and untransduced *h*DSCs showed similar effects *in vitro* and *in vivo*. At a dose of 10^6 *h*DSCs per mouse, the majority of recipients died of embolism. *Conclusions.* *h*DSCs inhibit allo-reactivity, xeno-reactivity and mitogen-induced stimulation in mouse lymphocytes. Although the GVHD score was reduced by *h*DSC infusion, survival and GVHD histopathology were not improved. One reason for failure was fatal embolism.

Key Words: *cell therapy, decidual stromal cells, graft-versus-host disease, mesenchymal stromal cells, mouse model*

Introduction

Graft-versus-host disease (GVHD) is a severe and life-threatening complication after allogeneic hematopoietic stem cell transplantation (A-HSCT). It is an inflammatory condition in which allo-reactive donor T cells attack recipient tissues [1] and is most prominent in the skin, gastrointestinal tract and liver. Without preventive or therapeutic intervention, almost all A-HSCT patients will have acute or chronic GVHD [2]. Despite advances in the development of new immunosuppressive drugs and novel therapeutic methods, GVHD is still a major threat [3]. In severe cases of GVHD in which there is no response to standard treatments, the prognosis is poor and survival is low [3,4].

Several reports have suggested that mesenchymal stromal cells (MSCs) have an immunosuppressive effect both *in vitro* and *in vivo* [4–6]. It has been shown both in humans and mice that MSCs can inhibit T-cell proliferation in mixed lymphocyte culture (MLC) as well as after non-specific mitogenic stimulation [6–8]. Considering the immunomodulatory effects of MSCs [7,9], we used bone marrow (BM) MSCs to treat steroid-resistant GVHD for the first time [4,10]. Subsequently, MSC therapy was extended also to other immunological and inflammatory disorders [11].

Despite the fact that the first cases responded dramatically, with complete reversal of acute GVHD, subsequent observations showed that some patients do not respond at all [4,10]. MSCs with similar cell-surface markers and function can be isolated from various tissues (eg, BM, adipose tissue and umbilical cord) [12,13]. The main common characteristic of MSCs from different sources is their immune-modulatory properties [13,14]. We recently

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(Received 30 March 2015; accepted 3 September 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.09.001

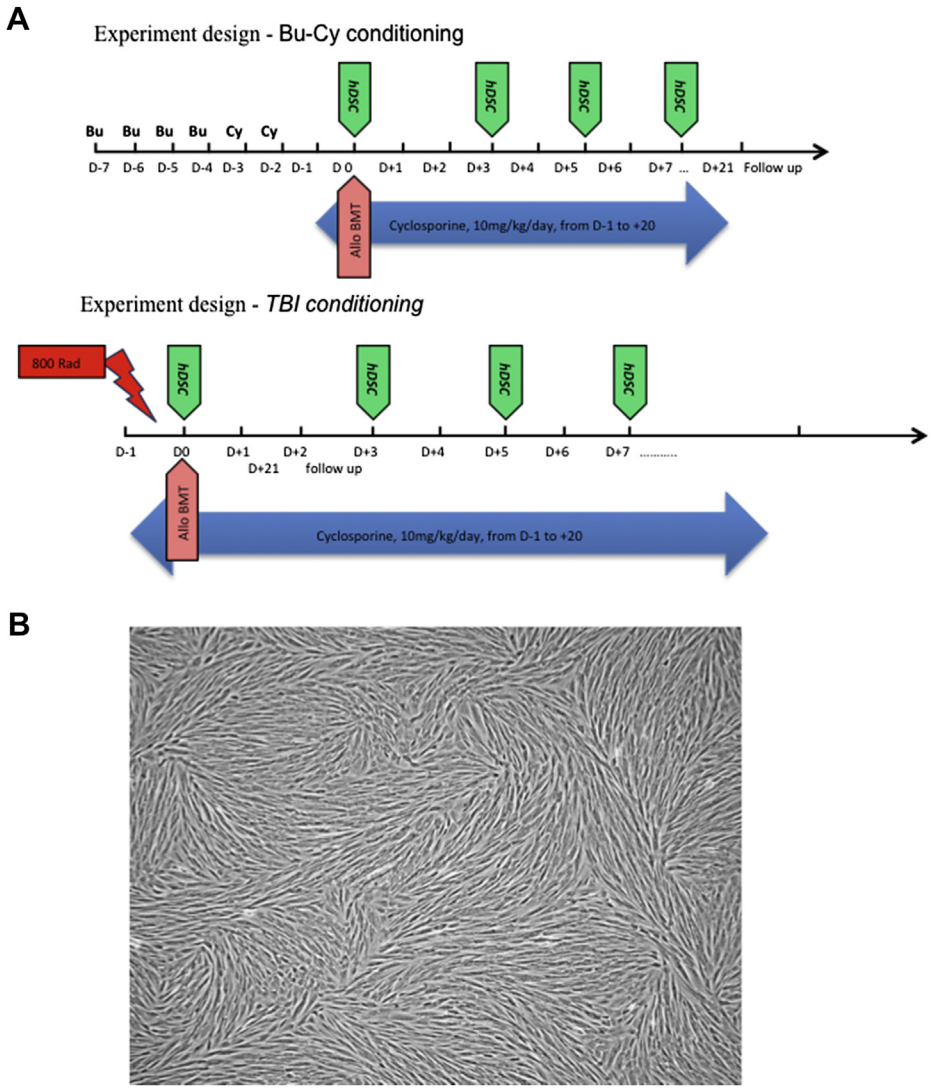

Figure 1. Experiment design and morphology of *h*DSCs. (A) *In vivo* study and experimental groups. BM transplantation and GVHD induction in mice, as well as *h*DSC infusion schedule in GVHD model. (B) Primary cultures of *h*DSCs at passage 3. Magnification ×4.

introduced a protocol for generation of large quantities of decidual stromal cells (DSCs) with significant immunosuppressive properties from fetal membrane layers of the placenta [15,16]. Compared with stromal cells isolated from BM or adipose tissue, DSCs are easily accessible without any invasive procedures. There are few or no ethical considerations because the placenta is normally discarded after delivery.

We used human DSCs (*h*DSCs) successfully for the treatment of steroid-resistant acute GVHD [17]. The preliminary results were promising, but the therapeutic protocol must be optimized because not all patients respond. The purpose of this experimental study was to investigate the underlying mechanisms of DSCs and to optimize the use of DSCs *in vitro* in MLC and after mitogenic stimulation in a well-known mouse model of acute GVHD [18].

Methods

Preparation of DSCs

Isolation and preparation of *h*DSCs has already been described [17]. Briefly, human term placentas were obtained from healthy mothers during elective cesarean section, after we had obtained informed consent. The fetal membranes were carefully dissected from the placenta, washed several times, cut into small pieces and digested with trypsin/ethylene diamine tetra-acetic acid (EDTA) (Thermo Fisher Scientific) by use of a series of incubations and washes. Trypsin-digested material (cell