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Immunosuppressive properties of mesenchymal stromal cell cultures derived from the limbus of human and rabbit corneas

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) cultivated from the corneal limbus (L-MSCs) provide a potential source of cells for corneal repair. In the present study, we investigated the immunosuppressive properties of human L-MSCs and putative rabbit L-MSCs to develop an allogeneic therapy and animal model of L-MSC transplantation. *Methods.* MSC-like cultures were established from the limbal stroma of human and rabbit (New Zealand white) corneas using either serum-supplemented medium or a commercial serum-free MSC medium (MesenCult-XF Culture Kit; Stem Cell Technologies, Melbourne, Australia). L-MSC phenotype was examined by flow cytometry. The immunosuppressive properties of L-MSC cultures were assessed using mixed leukocyte reactions. L-MSC cultures were also tested for their ability to support colony formation by primary limbal epithelial (LE) cells. *Results.* Human L-MSC cultures were typically $CD34^-$, $CD45^-$ and HLA-DR^- and $CD73^+$, $CD90^+$, $CD105^+$ and HLA-ABC^+. High levels (>80%) of CD146 expression were observed for L-MSC cultures grown in serum-supplemented medium but not cultures grown in MesenCult-XF (approximately 1%). Rabbit L-MSCs were approximately 95% positive for major histocompatibility complex class I and expressed lower levels of major histocompatibility complex class II (approximately 10%), CD45 (approximately 20%), CD105 (approximately 60%) and CD90 (<10%). Human L-MSCs and rabbit L-MSCs suppressed human T-cell proliferation by up to 75%. Conversely, L-MSCs from either species stimulated a 2-fold to 3-fold increase in LE cell colony formation. *Conclusions.* L-MSCs display immunosuppressive qualities in addition to their established non-immunogenic profile and stimulate LE cell growth *in vitro* across species boundaries. These results support the potential use of allogeneic L-MSCs in the treatment of corneal disorders and suggest that the rabbit would provide a useful pre-clinical model.

Key Words: *cell therapy, corneal limbus, immunosuppression, mesenchymal stromal cells*

Introduction

The corneal limbus is a narrow transitional zone of tissue located between the cornea and surrounding scleral tissue. Epithelial progenitor cells for replenishing the human corneal epithelium are concentrated within the basal layer of the limbal epithelium (1). This knowledge has been exploited to provide an effective cellular therapy for reconstructing the ocular surface (2–4). More recently, the progenitor cell potential of cells cultivated from the limbal stroma has been studied (5–12). One useful model for studying the biology of these putative limbal stromal progenitors has been to draw comparisons with mesenchymal stromal cells (MSCs) found in cultures established from other tissues such as bone marrow.

Polisetty *et al.* (11) first proposed the concept of a limbal mesenchymal stromal cell (L-MSC) after discovering that cultures established from the human limbal stroma displayed a similar profile of cell surface antigens to bone marrow-derived MSCs. Subsequent studies by the same investigators led to the conclusion that L-MSCs might contribute to maintenance of the limbal epithelial (LE) stem cell niche (10). Data from other studies (6,12,13) support the "niche stromal cell" hypothesis by demonstrating that cultures established from limbal stroma provide

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an effective feeder layer for the *ex vivo* expansion of LE cells. The limbal stroma has also been proposed as a source of progenitor cells for repairing the corneal stroma (8).

An important consideration for therapies based on the use of L-MSCs is to determine a suitable source of these cells. Under ideal conditions, the source of cultures of L-MSCs might be a patient's own healthy tissue. Although the experience of growing limbal epithelium from patient biopsy specimens demonstrates that the required amount of tissue can be safely removed from healthy patient eyes (14), allogeneic donor tissue would offer significant advantages as a source of pre-expanded and banked L-MSCs that could be used for the treatment of patients with conditions affecting either one or both eyes. However, this treatment model would depend on the donor L-MSCs displaying low immunogenicity. To this end, although donor limbal tissue transplants display a high rate of immunologic rejection (15), L-MSCs cultured in standard serum-supplemented growth medium, similar to bone marrow-derived MSCs, display a low immunogenic profile by virtue of low levels of human leukocyte antigen (HLA)-DR expression (5,7,11). However, more recent studies by our own group suggest that optimal growth of L-MSCs is achieved using the MesenCult-XF Culture Kit (Stem Cell Technologies, Melbourne, Australia) (6), and HLA-DR expression under these conditions remains unknown. More recently, Garfias *et al.* (7) reported that soluble factors secreted by L-MSCs suppress T-cell proliferation by 30%. Presumably, this immunosuppressive property might also be observed when donor L-MSCs are placed in direct contact with a patient's immune cells, but this study has yet to be performed.

In the present study, we aim to extend the field of L-MSC research in three critical ways. First, we build on our earlier studies of the effect of culture conditions on L-MSC phenotype (6) by examining the expression of HLAs when these cells are grown in either standard serum-supplemented growth medium or using the MesenCult-XF Culture Kit. Second, we explore *in vitro* the potential existence of a cell with equivalent "stem cell niche properties" to human L-MSCs in cultures established from the limbal stroma of New Zealand white (NZW) rabbits, the established pre-clinical model for cultured LE cell transplants (4,16–20). Third, we explore the immunosuppressive properties of both human L-MSCs and their putative rabbit cell equivalents when cultured in direct contact with immune cells isolated from two mis-matched human donors. In doing so, we seek to establish the feasibility of allogeneic L-MSC therapies and to propose the NZW rabbit as a potential pre-clinical model of L-MSC transplantation.

Methods

Establishment of cultures from human and rabbit limbal stroma

Human corneal tissue was obtained with ethics committee approval and donor consent from the Queensland Eye Bank, Brisbane, Australia. Whole rabbit eyes (adult NZW) were retrieved from euthanized animals via a tissue sharing agreement with the Medical Engineering Research Facility at the Queensland University of Technology. Before digestion, tissue was dissected down to a 1.5–2 mm diameter across the limbal transition between transparent cornea and white sclera. The tissue was washed in three changes of phosphate-buffered saline (PBS; Life Technologies, Melbourne, Australia) and digested with 0.25% dispase (Life Technologies) for 1 h at 37°C to assist removal of LE cells. The LE cells were cultured for subsequent use in colony formation assays with stromal cells (see further on). The remaining limbal stroma was digested in 1 mg/mL collagenase type I for 48 h in Dulbecco's modified Eagle's medium nutrient mixture F-12 (DMEM/F-12; Life Technologies). The dissociated stromal cells were washed and initially seeded at a density of 5×10^3 cells/mL where possible under culture conditions as described previously (6). The culture medium for both species consisted of DMEM/F-12 medium supplemented with GlutaMax (Life Technologies) and 10% fetal bovine serum (FBS; HyClone Thermo Scientific, Melbourne, Australia). For simplicity, this first medium is subsequently referred to as 10% FBS medium and for human L-MSCs was compared with the MesenCult-XF Culture Kit, a serum-free culture system designed for cultivation of human-derived MSCs. Both media were additionally supplemented with 1% penicillin/streptomycin solution (Life Technologies).

Flow cytometry

For analysis of human L-MSCs, we used mouse IgG1 κ isotype control phycoerythrin (PE) (MOPC-21), mouse IgG2b κ isotype control PE (27-35), rat IgG2a κ isotype control PE (R35-95), and the following mouse or rat anti-human antibodies: CD29 (fluorescein isothiocyanate [FITC], TS2/16, IgG1 κ), CD34 (FITC, 581/CD34, IgG1 κ), CD44 (Pacific Blue, 581/CD34, IgG1 κ), CD45 (PE-cyanine dye Cy7, HI30, IgG1 κ), CD73 (allophycocyanin [APC], AD2, IgG1 κ), CD90 (APC, 5E10, IgG1 κ), CD105 (PE, SN6, IgG1 κ), CD141 (PE, 1A4, IgG1 κ), CD146 (FITC, P1H12, IgG1 κ), HLA-ABC (purified, G46-2.6, IgG1 κ) and HLA-DR (purified, 46-6, IgG2a κ). For analysis of rabbit L-MSCs, we