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Features of synergism between mesenchymal stem cells and immunosuppressive drugs in a murine heart transplantation model

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) can be used for immunomodulation therapy after solid organ transplantation. Here, we focus on the immunoregulatory potential of combination therapies of MSCs and classic pharmacotherapy to mediate acceptance of solid organ grafts.
Methods: To determine which drugs influence the immunosuppressive effect of MSCs, we assessed the interaction of MSCs and common clinical immunosuppresants (MMF, sirolimus [Srl], and ciclosporin A [CiA]) in a parent-into-F1 cell transfer model. In this model, the transfer of parental strain T cells into semi-allogeneic F1 recipients induces a graft-versus-host reaction (GvHR). Re-isolated CFSE-labelled T lymphocytes were analyzed by flow cytometry. These findings were compared to a fully allogeneic heart transplantation model.
Results: We found that MSC treatment alone had no significant effect on allograft survival of heterotopic heart grafts. However, MSCs combined with short-term mycophenolate mofetil (MMF) significantly prolonged graft survival. Quantitative analysis of three different MSC – drug combinations in the F1 model revealed, that only the MSC–MMF combination led to a super-additive immunosuppressive effect. We also investigated the effect of MMF and CiA on IFNγ production of stimulated lymphocytes and found that MMF left the expression of IFNγ unaffected, whereas CiA completely abolished the production of IFNγ.
Conclusion: Our data show that the type of concurrent immunosuppression strongly influences the immunosuppressive effect of MSC, most likely through differential secretion of IFNγ. A regimen combining MSCs and MMF was most immunosuppressive.

1. Introduction

Long-term acceptance of solid organ grafts can be achieved with reasonable clinical success using standard pharmacological immunosuppression [1,2]. However, clinical allograft acceptance comes at a price, with the unwanted side effects of life-long drug-based immunosuppression significantly reducing the overall well-being of transplant patients [3]. To overcome this shortcoming of immunosuppressive pharmacotherapy, cellular immunotherapy has been investigated as a promising alternative [4–7]. To modulate anti-donor reactivity in favour of graft acceptance, bone-marrow–derived leukocytes, mostly of donor-origin, have been used [5,8–11]. Although some progress has been made in this area, the need for toxic preconditioning before bone marrow transfer has hampered the broad use of hematopoietic stem cells in the field of organ transplantation.

One alternative to hematopoietic progenitors are bone-marrow-derived mesenchymal stem cells (MSCs). They represent an independent mesodermal stem cell lineage within the bone marrow compartment [12–14] and can easily be harvested and cultured from adult bone marrow and other organs of essentially any mammal [15]. The well-described ability of MSCs to suppress T-cell proliferation in culture has inspired investigations assessing the use of MSCs as immunotherapeutics [12,16]. To date, most immunological studies have applied MSCs to patients with graft-versus-host disease (GvHD) [17,18]. The effectiveness of MSCs in controlling GvHD has prompted us and others to compare the potential of MSCs (combined with short-term, reduced-intensity immunosuppression) to achieve graft acceptance of solid organ allografts to the results obtained in the GvHD studies. Current experimental studies suggest that MSCs can, under certain conditions, prolong allograft survival [19–22] together with immunomodulatory drugs. Understanding the interaction between MSC and drugs in vivo is particularly important for the clinical setting. Specifically, MSC function has been shown to depend on the choice of concurrent immunosuppressants [23].

Abbreviations: CFSE, Carboxy Fluoresceindiacetate Succinimidyl Ester; CiA, Ciclosporin A; GvHD, Graft versus Host Disease; GvHR, Graft versus Host Reaction; MMF, Mycophenolate Mofetil; MSC, Mesenchymal Stem Cell; Srl, Sirolimus.

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In this study, we sought to determine the effect of MSCs on T-cell proliferation in the presence of different immunosuppressive drugs using a fully allogeneic vascularized heart transplantation model and a murine parent-to-F1 adoptive lymphocyte transfer model. In the latter model, the transfer of homozygous parental-strain T cells into non-irradiated semi-allogeneic F1 recipients induces a graft-versus-host reaction (GvHR). It is therefore suitable to visualize alloproliferation ("in vivo MLR").

We show that different immunosuppressive drugs significantly alter the tolerogenic effectiveness of MSCs and that the type of concurrent immunosuppression is crucial for MSC combination therapies in vivo.

2. Materials and methods

2.1. Animals

Six-week-old sex-matched C57BL/6 (B6), C3H, and C57BL/6×C3H F1 (C3B6) mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Animals were housed under pathogen-free conditions in our institute's dedicated animal facility. All animal experiments were carried out in accordance with the regional and regulations of the Upper Palatinate, Germany.

2.2. Expansion and cultivation of MSCs

MSCs were generated from murine bone marrow. Six-week-old mice (B6, C3H, C3B6) were sacrificed by cervical dislocation. Femurs were carefully cleaned of adherent soft tissue, epiphyses were removed under sterile conditions, and the marrow was harvested by inserting a 20-gauge syringe needle into one end of the bone and flushing it with DMEM (Gibco, Invitrogen Germany) supplemented with 20% fetal calf serum (FCS) (HyClone, ThermoScientific, Barrington, USA), 20% horse serum, 100 µg/ml penicillin–streptomycin (Gibco) and 2 mM L-glutamine. The marrow suspension was then inoculated to culture flasks. After 24 h, 48 h, and 72 h, non-adherent cells were removed by replacing the medium. Cells were cultured in a monolayer at 37 °C and 5% CO_2. When primary cultures reached approximately 80% confluence, cells were detached with 0.025% trypsin containing 0.02% EDTA for 5 minutes at 37 °C. Trypsinization was stopped by adding FCS-containing medium. Cells resulting from the initial process of replating were designated first-passage cells. Nutrient medium was subsequently replaced every third day. To

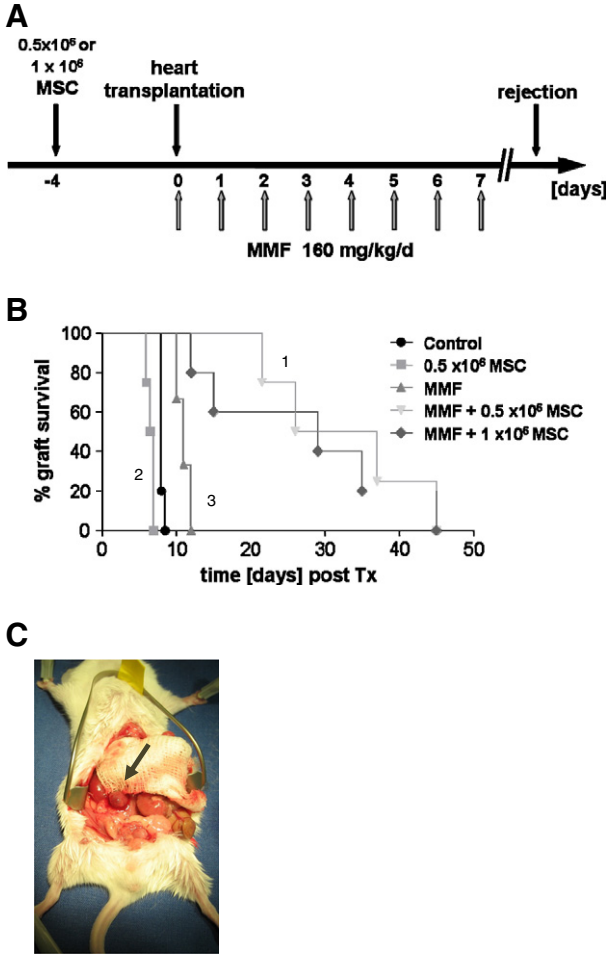

Fig. 1. Prolongation of allograft survival in a murine allogeneic heart transplantation model. (A) Model overview: Allogeneic B6 hearts were transplanted into C3H recipients. Donor-type MSCs were injected 4 days prior to the operation. MMF was given at the indicated dose of 160 mg/kg/d for 7 days. (B) Controls rejected acutely (d 8.1 ± 0.22), MMF slightly prolonged graft survival (d11.0 ± 1.0). Significant prolongation of allograft survival was achieved by a combination regimen of donor-derived MSCs and MMF (d32.4 ± 10.6 for 0.5×10^6 MSC; d27.2 ± 13.79 for 1.0×10^6 MSC). $n=5$ for control group, $n=4$ for MSC group, $n=3$ for MMF only group; $n=4$ and $n=5$ for combination groups (0.5×10^6 and 1.0×10^6 MSCs). p-values for survival against control as indicated: (1) $p=0.0035$, (2) $p=0{,}004$ and (3) $p=0{,}0082$. (C) Photograph of an intraabdominally transplanted murine heart graft. Arrow indicates the graft.

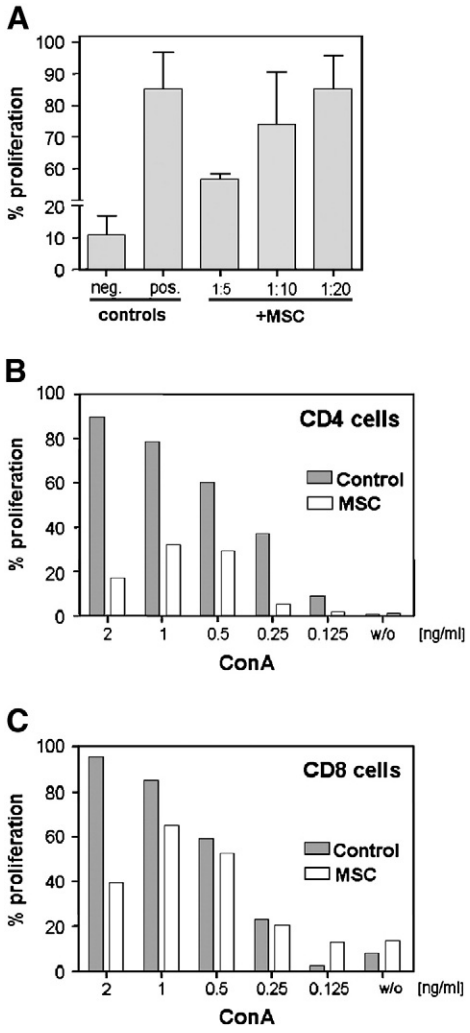

Fig. 2. MSC-mediated suppression of T-cell proliferation in vitro. (A) B6 responder lymphocytes were stimulated by allogeneic, irradiated, CD90-depleted C3H splenocytes in an MLR. Syngeneic B6 cells were used as a negative control. (B + C) B6-derived CD4 positive (B) or CD8 positive (C) lymphocytes were stimulated with increasing doses of ConA. Stimulation was suppressed with equal numbers of C3H MSCs at a ratio of 1:5. $n=3$ experiments were performed. A representative experiment is shown.