BIOLOGY

Canine Bone Marrow-Derived Mesenchymal Stromal Cells Suppress Alloreactive Lymphocyte Proliferation in Vitro but Fail to Enhance Engraftment in Canine Bone Marrow Transplantation

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Stable mixed hematopoietic chimerism has been consistently established in dogs who were mildly immunosuppressed by 200 cGy of total body irradiation (TBI) before undergoing dog leukocyte antigen (DLA)-identical bone marrow (BM) transplantation and who received a brief course of immunosuppression with mycophenolate mofetil (28 days) and cyclosporine (35 days) after transplantation. However, when TBI was reduced from 200 to 100 cGy, grafts were nearly uniformly rejected within 3-12 weeks. Here, we asked whether stable engraftment could be accomplished after a suboptimal dose of 100 cGy TBI with host immunosuppression enhanced by donor-derived mesenchymal stromal cells (MSCs) given after transplantation. MSCs were cultured from BM cells and evaluated in vitro for antigen expression. They showed profound immunosuppressive properties in mixed lymphocyte reactions (MLRs) in a cell dose–dependent manner not restricted by DLA. MSC and lymphocyte contact was not required, indicating that immunosuppression was mediated by soluble factors. Prostaglandin E2 was increased in culture supernatant when MSCs were cocultured in MLRs. The addition of indomethacin restored lymphocyte proliferation in cultures containing MSCs. MSCs expressed CD10, CD13, CD29, CD44, CD73/SH-3, CD90/Thy-1, and CD106/VCAM-1. For in vivo studies, MSCs were injected on the day of BM grafting and on day 35, the day of discontinuation of posttransplantation cyclosporine. MSCs derived from the respective BM donors failed to avert BM graft rejection in 4 dogs who received DLA-identical grafts after nonmyeloablative conditioning with 100 cGy TBI in a time course not significantly different from that of control dogs not given MSCs. Although the MSCs displayed in vitro characteristics similar to those reported for MSCs from other species, their immunosuppressive qualities failed to sustain stable BM engraftment in vivo in this canine model.

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INTRODUCTION

We have reported consistent and sustained engraftment in dogs given a small, sublethal dose of 200 cGy total body irradiation (TBI) before, and immunosuppression consisting of mycophenolate mofetil (MMF) and cyclosporine (CsA) for 28 and 35 days, respectively, after dog leukocyte antigen (DLA)-identical hematopoietic cell transplantation (HCT) [1]. However, when TBI was reduced from 200 to 100 cGy, grafts were nearly uniformly rejected within 20 weeks, even when other immunosuppressive drugs were added to the posttransplantation immunosuppression [2]. Very similar results were seen when rapamycin

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was substituted for MMF in this model [3]. The most likely role of pretransplantation TBI was host immunosuppression, not creation of bone marrow (BM) space, because dogs conditioned with 450 cGy irradiation to the cervical, thoracic, and upper abdominal lymph node chain, in lieu of 200 cGy TBI, engrafted [4]. Accordingly, other pretransplantation immunosuppression has been evaluated along with 100 cGy TBI conditioning, and successful stable engraftment of DLA-identical BM was achieved by blocking host T cell costimulation with cytotoxic T lymphocyte antigen 4 immunoglobulin fusion protein (CTLA4-Ig) or antibody against CD154 along with donor lymphocytes [5,6]. Here, we used the DLA-identical BM graft model to determine whether the immunomodulatory effects of BM donor-derived mesenchymal stromal cells (MSCs) could be substituted for T cell costimulatory blockade in ensuring sustained hematopoietic engraftment.

MSCs have been described as multipotent nonhematopoietic progenitor cells that can differentiate into mature BM stromal cells, osteoblasts, adipocytes, chondrocytes, fibrous tissue, neuroectoderm, and visceral mesoderm [7-9]. They grow in culture as adherent cells with spindle-shaped fibroblastic morphology and typically do not express hematopoietic markers on their surface, but do express adhesion molecules, growth factors and cytokines, and integrins [10]. Cytokines and growth factors produced by MSCs have been implicated in aspects of hematopoiesis. Pertinent to the present study, MSCs display immunomodulatory effects. Human and mouse MSCs suppress in vitro proliferation of lymphocytes induced by alloantigens or mitogens [11-13], prolong skin and cardiac allograft survival in mice [14], and are thought to be useful for enhancing hematopoietic engraftment [15] and treating graft-versus-host disease (GVHD) in human patients [16,17]. The mechanisms underlying these effects of MSCs have not been clearly identified, although most studies have reported an involvement of soluble factors [18].

Ex vivo expanded gene-marked MSCs have been previously evaluated in dogs for their ability to localize in the BM [19]. In the present study, we phenotypically characterized canine MSCs and demonstrated their ability to suppress mixed leukocyte reactions (MLRs). Despite their profound in vitro immunosuppressive activity, BM donor-derived MSCs failed to show in vivo immunosuppressive properties in dogs, as demonstrated by the dogs' inability to sustain stable BM engraftment after low-dose TBI exposure.

MATERIALS AND METHODS

Laboratory Animals

The Institutional Animal Care and Use Committee at the Fred Hutchinson Cancer Research Center, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, approved this study. All dogs were enrolled in a veterinary preventive medicine program as described previously [1]. Cells from 10 DLA-mismatched and unrelated dog pairs (20 dogs) were used for the MLR studies. Four DLA-identical littermate pairs, aged 8-12 months (median, 10 months), were used for BM transplantation. DLA identity was determined by matching for highly polymorphic DLA class 1– and class 2–associated microsatellite markers [20]. In addition, the specific DLA-DRB1 allele identity was confirmed by direct sequencing [21].

BM–Derived Stromal Cell and Cell Lines

BM (25 mL) was obtained from the humeri of donors by aspiration. This sample was hemolyzed with sterile ammonium chloride lysing solution (155 mM ammonium chloride, 10 mM sodium bicarbonate, and 0.1 mM EDTA) in a 37°C water bath for 5 minutes. After centrifugation at 800 rpm for 5 minutes, the resulting pellet was resuspended in 5 mL of Iscove's Modified Dulbecco's Medium (IMDM) plus 10% fetal bovine serum (FBS). BM red blood cell lyses have been shown to be more efficient than Ficoll separation for isolating MSCs [22]. BM cells were plated at a density of 2×10^7 cells per 75-m^2 culture flask (Costar, Cambridge, MA) in 20 mL of IMDM containing 10% FBS and 1% penicillin/streptomycin (100 U/mL). Extra cells were frozen in medium containing 10% DMSO. Nonadherent cells were removed after 72 hours of culture with a change of medium. Subsequently, half of the medium was changed twice a week. After 2-3 weeks, cultured cells were detached using 0.05% trypsin EDTA, washed by centrifugation, and expanded to three 75-cm^2 flasks. After reaching confluency (2-3 weeks), cells were detached using 0.05% trypsin EDTA and tested for MSC function in vitro. Frozen marrow cells were thawed and washed in IMDM, and treated in the same manner as fresh BM cells.

Canine Fibroblasts

The canine fibroblast cell line (A-72) was purchased from American Type Culture Collection (Manassas, VA). In addition, canine primary skin fibroblasts were obtained from skin punch biopsy, cut into several small pieces, and washed in phosphate-buffered saline. Fibroblasts were transferred into 6-well, 25-cm^2 culture flasks (Costar) and cultured in RPMI medium containing 20% FBS and 1% penicillin/streptomycin (100 U/mL). Nonadherent cells were removed after 72 hours of culture by replacing the medium. Subsequently, these cells were cultured in the same manner as MSCs.