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Research Paper

Immuno Modulatory Potential of Bone Marrow Derived Mesenchymal Cells Against a Biological Graft

Veterinary

KEYWORDS : Mesenchymal cells, fish swim bladder, immunomodulation

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ABSTRACT

In the present study bone marrow derived mesenchymal cells of rat (R-BMSCs) were seeded over fish swim bladder (FSB) matrix. This cell- matrix construct was implanted in 20×20mm2 size full thickness skin defect created on rat model. Humoral and cell mediated immune response against this biological graft was tested through ELISA and Lymphocyte Stimulation Test (LST) respectively. Results were compared with that of control model, where fish swim bladder alone, without R-BMCs was used as biological graft. The immune response elicited by test model was significantly (P<0.05) negligible when compared to control model, emphasizing the immuno modulatory potential of bone marrow derived mesenchymal cells.

INTRODUCTION

Recently there has been an explosive advance in the knowledge of mesenchymal cells (MSCs) for their use as donor cells for regenerative therapies. One such incidence is a series of observations indicating that MSCs are immune-privileged, able to survive and differentiate in immunocompatibility-mismatched allogeneic or even xenogeneic transplant recipients. In fact, a number of workers have recently reported that MSCs may have a unique immunological property capable of inducing tolerance in immunocompetent allotransplants or even xenotransplant recipients. Use of MSCs as immunosuppressant agents in autoimmune diseases has been proposed and successfully tested in animal models. Immunomodulation property of mesenchymal cells can be exploited to ameliorate graft rejection hence to facilitae taking of graft (Leblanc et al., 2003).

Mesenchymal cells have the capacity to regulate the immune response by suppressing T and B lymphocyte proliferation in a non–major histocompatibility complex–restricted manner. The mechanisms of such immunotolerance have been the subject of intense study and three interrelated candidate mechanisms are emerging. MSCs appear to evade rejection by (a) being hypoimmunogenic, (b) modulating T-cell phenotype and (c) immunosuppressing the local environment. Finally, another level at which MSCs may modulate immune responses is through the inhibition of B-cell proliferation, as well as their chemotactic behavior and antibody production. Taken together, numerous studies provide strong evidence that MSCs are able to modulate the function of different immune cells in-vitro through several interrelated mechanisms (Atoui and Chiu, 2012).

Materials and methods

Collection and isolation of bone marrow derived mesenchymal cells from rat

Adult male Wistar rats (Rattus norvegicus) were euthanized as per the American Veterinary Medical Association Guidelines on Euthanasia (Beaver et al, 2001). Bone marrow aspirate was collected from femur bone under sterile condition. The material collected was washed with HBSS containing antibiotic and centrifuged at 1500 rpm for 15 min. To separate the mononuclear fraction of the bone marrow, the bone marrow sample was treated with histopaque as per manufactures instruction. After centrifugation, an interface formed between the plasma and the red blood cells, containing the mononuclear cells was selected and resuspended in complete growth medium consisted of Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal Bovine Serum (FBS) (Sigma, USA), antimycotic antibiotic (100 IU/ml). The re-suspended cells were seeded in to a T-25 culture flask and incubated at 37°C in humidified atmosphere with 5% CO_2. The non adherent cells were removed by the 3rd day of incubation and the media was changed with fresh growth media in order to propagate the plastic adherent MSCs. The medium was refreshed every 3 days. After attaining 90 % confluency, the adherent cells were subjected to 1st passaging. Adherent cells were detached from flask by enzymatic method using 0.25% trypsin- EDTA (Hyclone) and repeated the procedure.

Developing Cell matrix Construct

Swim bladders of the fresh water fish (Labeo rohita) were collected. Immediately after collection, the swim bladders were kept in cold normal saline solution containing 0.02% EDTA and antibiotic (Amikacin 1mg/ml). The tissues were washed with sterile normal saline.. The FSB were cut in to 20×20 mm^2 size pieces and treated with a low concentration ethanol for sterilization. Stored at -20^0C till use.

The third passage cells were seeded over the fish swim bladder matrix placed in separate wells of a 6-well cell culture plate. The cell suspension where delivered to the scaffold at a desired concentration (5 x 10^5 cells/10ul). After 10 min scaffolds where flipped over to the other side and same quantity of cells where administered. We preferred six well plates precoated with 2% agarose to prevent cells from attatching or migrating to the culture dish. (Cohen et al, 2006). This cell –matrix construct was then kept in a CO_2 incubator maintained at 37^0C in a humidified atmosphere of 5% CO_2. Morphological assessment of cells seeded matrix was done by scanning electron microscopy at frequent intervals up to day seven to confirm attachment and proliferation of cells over matrix.

In- vitro evaluation of immuno modulatory potential of BMSCs

Experiment was conducted in adult healthy wistar rats of either sex, with two different groups of 8 animals each. Group I was maintained as control group and group II as test group. The immuno modulatory potential of BMSCs was tested by implantation R-BMSCs seeded FSB matrix, over the surgically created 20×20 mm^2 size full thickness skin defect on the dorsal thoracic area of the rats of test group in comparison to control group, where skin defect of the same size and site was implanted with non cell seeded FSB matrix.

Evaluation was done on the basis of humoral and cell mediated immune response. Evaluation of the humoral immune response was done by indirect ELISA. The free protein contents of FSB matrix was estimated and expressed in mg/ml using bovine serum albumin (BSA) as a standard (Lowry et al, 1951). Serum samples were collected at days 0, 21, 28. The hyper immune serum (HIS) was raised in adult rat to check the host immune response against the antigen prepared from fish swim bladder. The immunization dose at the rate of 200 µg/rat was given on day 0, 14 and 21. The hyper immune serum (HIS) was extracted from the blood this rat was used as positive control in ELISA, and sera samples of day zero as negative control. The intensity of color reaction was recorded at 492 ηm using an automatic ELISA reader. The cell mediated immune response was assessed by LST. For this spleen was collected aseptically from the implanted animals of the two groups on day 45 postoperatively and splenocyte culture was performed (Kruisbeek et al, 2004).

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Results

The method used for isolation and culture of R-BMSCs was found suitable. Bone marrow mononuclear cell fraction contains heterogeneous cell population and RBMSCs were isolated on the basis of plastic adherence property. The non-adherent cells were removed on 3rd day after culture and adherent cells grew as fibroblast-like cells by day 5, which further formed symmetric colonies and attained about 90% confluent monolayer by day 12 of primary culture (Fig. 1). Morphological assessment of the matrix by SEM of day 7 post seeding revealed a sufficient amount of proliferation of R-BMSCs over acellular FSB (Fig.2).

With regard to ELISA results a significant (P<0.05) increase in antibody response (on days 21 and 28 post implantation) was observed in group I when compared to immune response of group II. But there was no significant difference (P>0.05) in immune response between group I and positive control (hyper immune serum-HIS). Similarly no significant immune response between group II and negative control (zero day serum sample-before implantation). The result is depicted in graphically (Fig.3a). The results of LST of both the two groups, in comparison with stimulation index (SI) values of Con A and PHA are presented in Fig. 3b. Group I showed a significant (P<0.05) amount of stimulation when compared to group II.

Fig.1: Plated BMSCs reached about 90% confluency by day 12

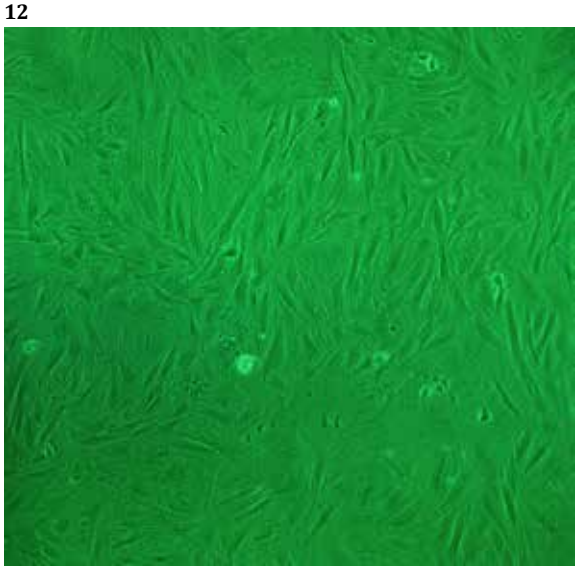


Fig.2: Attatchment and proliferation of RBMSc over acellular FSB by day seven (SEM 5um , x30000)

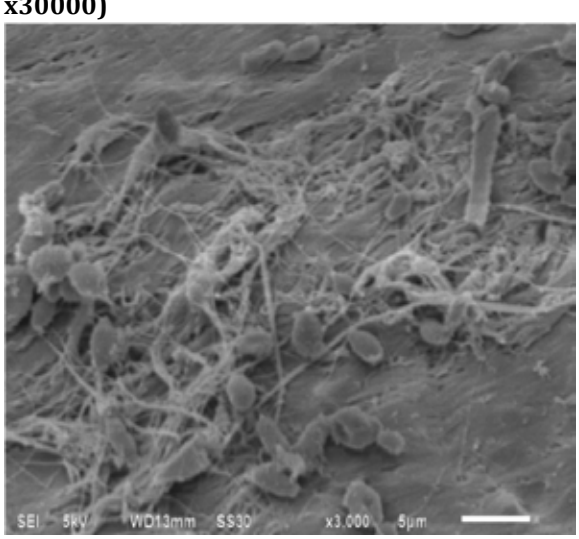
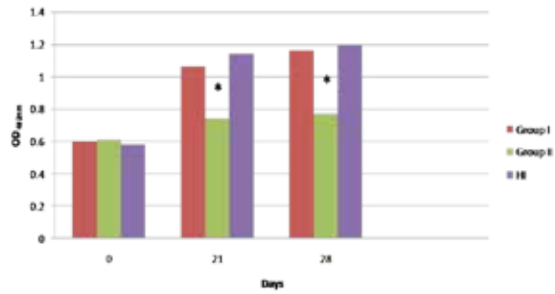
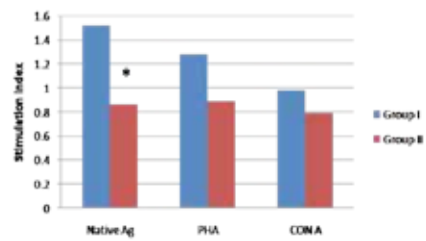


Fig.3a.



Mean+SE absorbance at 492nm wavelength ELISA reading to compare immune response between groups*differ significantly (P<0.05) from group I.

Fig.3b



Mean+SE stimulation index values to compare cell mediated immune response in both the two groups. *differ significantly (P<0.05) from group I.

Discussion

The histocompatibility antigens present on the transplanting cells is responsible for graft rejection in cellular graft (Gulati and Cole, 1984). Proteins of the extra cellular matrix may also play a minor role in eliciting immune response. Even the group II also have the same biological material as graft as in group I, surprisingly they elicited a significantly negligible immune response when compared to group I. This must be due to the ability of bone marrow derived mesenchymal cells to suppress the immune response as per the reports like adult bone-marrow-derived mesenchymal cells are immunosuppressive and prolong the rejection of mismatched skin grafts in animals (Blanc et al, 2004).

A number of surface and intracellular molecules are required to induce a proper immune response or to induce immune suppression (Bassi et al., 2011; Dai et al., 2011). Adhesion molecule and the MHC antigen are involved in the interaction with immune cells, mainly T cells, co-stimulatory molecules and Fas ligand/ Fas receptor interaction are important for T cell activation and effectors function (Mielcarek et al., 2011).

Three broad mechanisms contribute to the immuno modulatory propery of mesenchymal cells. Firstly, mesenchymal cells are hypo immunogenic, often lacking MHC-II and costimulatory molecule expression. Secondly, these cells prevent T cell responses indirectly through modulation of dendritic cells and directly by disrupting NK as well as CD8+ and CD4+ T cell function. Thirdly, mesenchymal stem cells induce a suppressive local microenvironment through the production of prostaglandins and interleukin-10 as well as by the expression of indoleamine 2, 3,-dioxygenase, which depletes the local milieu of tryptophan (Ryan et al., 2005).

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