Available online at www.sciencedirect.com
ScienceDirect
Immunobiology 214 (2009) 179–186
Immunobiology
www.elsevier.de/imbio

# The immunologic properties of undifferentiated and osteogenic differentiated mouse mesenchymal stem cells and its potential application in bone regeneration

Xiaoling Zhang[a], Tingting Tang[a], Qin Shi[b], Julio C. Fernandes[b], Kerong Dai[a,*]

[a]*Joint Orthopaedic Laboratory of Institute of Health Sciences and Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine and Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 225 South Chongqing Road, Shanghai, PR China*
[b]*Orthopaedics Research Laboratory, Research Centre, Sacré-Coeur Hospital, University of Montreal, 5400 Gouin Blvd.West, Montreal, Quebec, Canada H4J 1C5*

Received 23 January 2008; received in revised form 10 July 2008; accepted 16 July 2008

## Abstract

The concept of using mesenchymal stem cells (MSCs) in bone repair has progressively evolved, and the goal of cell-mediated therapy is to prolong the natural physiological abilities of healing, or substitute them, when these are lacking, failing, or progressing too slowly. The future application of MSCs in human therapies depends on the establishment of preclinical studies with other mammals, such as mouse. Surprisingly, the immunologic properties of murine MSCs remain poorly documented. In the present study, flow cytometry revealed that undifferentiated murine MSCs and osteogenic cells differentiated from MSCs (DOCs) express major histocompatibility complex (MHC) class I (H-$2^b$), but not class II (I-$A^b$). After exposure to interferon-gamma (IFN-$\gamma$) for 48 h, MHC class II and costimulatory molecules (B7-1 and B7-2) on the cell surface showed evident up-regulation. Undifferentiated MSCs and DOCs proved to be poor stimulators of T cell proliferation, eliciting alloreactive lymphocyte proliferative responses as low-allogenic stimulators. Initial results show that the expression of MHC class I, MHC class II, B7-1 and B7-2 was similar on human bone morphogenetic protein 2 (BMP2)-expressing recombinant adenoviral vector (AdBMP2) transduced MSCs (30 MOI) when compared with non-transduced cells. However, AdBMP2 gene transfected MSCs elicited significant stimulatory responses. The findings will be important for studying the *in vivo* behaviour and the fate of MSCs after grafting in mouse pathology models in bone regeneration.


***Keywords:*** Bone regeneration; Mesenchymal stem cells (MSCs); Mouse; Osteogenic differentiated; Undifferentiated

*Abbreviations:* AdBMP2, BMP2-expressing recombinant adenoviral vector; AdLacZ, adenoviral vector carrying the *Escherichia coli* LacZ gene encoding galactosidase; BMP2, human bone morphogenetic protein 2; Cpm, counts per minute; DOCs, osteogenic cells differentiated from MSCs; FITC, fluorescein isothiocyanate; IFN-$\gamma$, interferon $\gamma$; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; MOI, multiplicity of infection; MSCs, mesenchymal stem cells; PE, phycoerythrin; SD, standard deviation.

*Corresponding author. Tel./fax: +86 21 63855434.
*E-mail address:* krdai@sibs.ac.cn (K. Dai).

0171-2985/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2008.07.030

## Introduction

The marrow stroma is a heterogeneous mixture of cells including adipocytes, endothelial cells, reticular cells, fibroblastic cells, and smooth muscle cells, which provide growth factors, cytokines, chemokines, and a physical matrix. It has been well established that the stroma contains progenitors that can give rise to bone, cartilage, adipose tissue, and tendon, as well as smooth-, skeletal-, and cardiac muscle, early progenitors of neural cells and a phenotype that supports hematopoietic differentiation (Rombouts and Ploemacher, 2003; Prockop, 1997). As these cells are of mesenchymal origin, their common precursors are referred to as mesenchymal stem cells or marrow stromal cells (MSCs) (Prockop, 1997; Caplan, 1991). In the 1970s, Friedenstein was the first to report that MSCs also possess osteogenic potential. He initially exploited the propensity of these cells to adhere to tissue culture plastic as a means to isolate them from bone marrow (Friedenstein et al., 1974). Since then, many groups have confirmed the osteogenic potential of MSCs and realized that MSCs represent an ideal stem cell source for cell and gene therapy applications because of their easy purification and amplification, and their multipotency (Deans and Moseley, 2000).

MSCs demonstrate extensive proliferative capacity *in vitro* and retain the ability to differentiate into several mesenchymal lineages, this suggests that these cells may be used to regenerate tissues, such as intervertebral disc cartilage (Crevensten et al., 2004), bone (Arinzeh et al., 2003; Chamberlain et al., 2004), and articular cartilage at knee joints (Barry, 2003). It is also noteworthy that MSCs are ideal carriers of genes into the tissues of interest for gene therapy applications (Baksh et al., 2004). Its clinical application for tissue regeneration could be most readily achieved with an allogeneic product, which is a large number of cells derived from a single donor that could be used in multiple recipients. However, a potential limitation to this 'universal donor' concept is the rejection of donor cells by the recipient's immune system. Moreover, the current parameters for a successful transplantation of MSCs are still unclear. To clarify different aspects of MSC biology and develop MSC applications in human diseases treatment, isolating MSCs from different species and conducting extensive preclinical studies are thus required. In this study, we isolated MSCs from mouse, the popular mammalian physiological and pathophysiological model, and initiated work to study the immunologic properties of undifferentiated MSCs and osteogenic cells differentiated from MSCs (DOCs). The findings will be important for studying the *in vivo* behaviour and the fate of MSCs after grafting in mouse pathology models in bone regeneration.

Several approaches to introduce growth factors into MSCs to promote their osteoblastic differentiation have been examined. Viral transduction, particularly the use of adenovirus-mediated gene transfer, can generate stable cell clones with high efficiency and low-cell mortality, thus making it a popular option in gene therapy. Recombinant bone morphogenetic proteins (BMPs) are the latest emerging therapeutic agents for bone regeneration. These molecules have the unique ability to stimulate the differentiation of MSCs to chondrocytes and osteoblasts. Our findings demonstrate that MSCs infected with an adenovirus vector containing BMP2 gene (AdBMP2) have been successfully used to promote osteogenesis and repair critical-sized bone defects in large animals (Dai et al., 2005). Here, we first present immunologic properties of AdBMP2 transfected mouse MSCs, this will prompt the evaluation of this vector, as well as the cell and gene therapy applications of MSCs in bone regeneration.

## Methods

### Mice and primary cultures of MSCs

BALB/c mice (H-$2^d$, I-$A^d$) and C57BL/6 mice (H-$2^b$, I-$A^b$) were obtained from the Laboratory Animal Center of Shanghai Institute for Biological Sciences (Shanghai, China, Certificate number SCXK 2003-0003). Mice used in all experiments were 6–8 weeks old. MSCs were obtained from the femurs and tibias of male C57BL/6 mice as described by Krampera et al. (2003). Handling of the animals was in accordance with policies of Shanghai Jiao Tong University School of Medicine and the National Institute of Health. Mouse MSCs were analyzed by flow cytometry for expression of cell surface antigens CD44, CD45, CD90, and CD105 (Eliopoulos et al., 2005; Romieu-Mourez et al., 2007).

### Osteoblastic differentiation of MSCs in osteoinductive medium

Osteoblastic differentiation was induced by culturing confluent mouse MSCs for 3 weeks in inducing medium as previously described (Qu et al., 1998). The inducing medium was a complete medium supplemented with $10^{-8}$ M dexamethasone (Sigma, St, Louis, MO, USA), 50 μg/ml ascorbic acid (Merck, Darmstadt, Germany), and 10 mM sodium $\beta$-glycerophosphate (Fluka Chemie, Buchs, Switzerland). After cells were maintained in inducing medium for a week, cells were fixed for 2 min in ice-cold methanol before labelling for alkaline phosphatase activity according to Reyes's protocol (Reyes et al., 2001). Osteoblastic differentiated MSCs also were used for flow cytometric analysis and allogenic mixed lymphocyte reaction (MLR).