International Immunopharmacology 64 (2018) 364–371

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Immunomodulatory effects of rhesus monkey bone marrow-derived mesenchymal stem cells in serum-free conditions

Fei Liu[a,1], Yuanmin Li[a,1], Lin Bai[b], Zhen Yang[b], Guangneng Liao[c], Younan Chen[a], Yanrong Lu[a], Jingqiu Cheng[a,*], Jie Zhang[a,*]

[a] Key Laboratory of Transplant Engineering and Immunology, NHFPC, Regenerative Medicine Research Center, West China Hospital, Sichuan University, Chengdu, PR China
[b] Core Facility of West China Hospital, Sichuan University, Chengdu, PR China
[c] Animal Lab Center, West China Hospital, Sichuan University, Chengdu, PR China

ARTICLE INFO

Keywords:
Mesenchymal stem cells
Bone marrow
Cell culture
Immunomodulation

ABSTRACT

Mesenchymal stem cells (MSCs) have generated tremendous interest for treating various diseases due to their self-renewal and differentiation capacities. Many studies have demonstrated the immunoregulatory capability of MSCs; however, most of these studies were conducted with fetal bovine serum (FBS), which has an uncertain composition. In this study, we established a serum-free, xeno-free, completely chemically defined medium for the proliferation and expansion of rhesus monkey bone marrow (BM)-derived MSCs (rBMSCs) in vitro. The growth kinetics, characteristics, immunophenotype, and immunosuppressive abilities of rBMSCs grown in serum-free media (SFM) were evaluated and compared with those of cells grown in serum-containing media (SCM). Moreover, we employed RNA sequencing to evaluate the expression pattern of genes related to immune responses in both culture conditions. Compared to cells grown in SCM, rBMSCs grown in SFM exhibited better biological characteristics regarding cell proliferation and immunosuppressive abilities. Cells from both media types exhibited similar immunophenotypic expression patterns for CD29, CD34, CD45, HLA-DR, CD73, CD90, and CD105. Gene Ontology (GO) terms, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and Gene Set Enrichment Analysis (GSEA) revealed that CXCL8 was downregulated by 4.1 fold in SFM-cultured rBMSCs compared with those in SCM. Furthermore, the mixed lymphocyte culture revealed that the proliferation activity and the expression levels of inflammatory factors of peripheral blood mononuclear cells (PBMCs) were significantly decreased after the addition of the CXCL8 neutralizing antibody, which was related to the elevated immunosuppressive abilities of SFM-suspended rBMSCs. These results suggest a possible cell culture method as well as immunoregulatory mechanisms for clinical cell therapies requiring nonanimal-derived components.

1. Introduction

Mesenchymal stem cells (MSCs) are self-renewing, multipotent progenitor cells that have been isolated from many tissues [1]; bone marrow (BM) is the most common source for both research and clinical use of these cells [2] due to the ease of collection and the relatively high MSC frequency [1×10^4 to 1×10^5 BM-mononuclear cells (MNCs)] [3]. Moreover, MSCs have been considered promising candidates for tissue generation and cell therapies, which have elicited beneficial effects in a variety of animal disease models and human clinical experiments [4].

The gold standard for MSC culturing and ex vivo expansion involves the use of animal-derived reagents. However, the unknown components of animal sera can mitigate the potential risk of transmitting unknown mycoplasma, viruses, prions or unidentified zoonotic agents to cultures [5]. In addition, human autologous and allogeneic supplements, including platelet derivatives, platelet lysate and platelet-released factors and sera, have been assessed in clinical studies, but ethical issues have led to widespread concerns regarding the use of these supplements [6].

Emerging evidence suggests that cells grown in serum-free media (SFM) exhibit better performance regarding cell proliferation, viability, yield and consistency than do cells grown in SCM [7]. Currently,

* Corresponding authors at: Key Laboratory of Transplant Engineering and Immunology, NHFPC, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, PR China.
E-mail addresses: jqcheng@scu.edu.cn (J. Cheng), zjie@scu.edu.cn (J. Zhang).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.intimp.2018.09.012
Received 1 March 2018; Received in revised form 5 September 2018; Accepted 9 September 2018

various serum-free cell culture media are commercially available, but the composition of these commercially produced media has not been fully disclosed. More importantly, whether the chemical composition of these media is fully defined is unclear; this lack of clarity is concerning because certain components could counteract the desired clinical effects [8]. Therefore, xeno-free, chemically defined media represent a great development prospect for critical cell cultures.

MSCs reportedly participate in the regulation of several immune-related disorders [9], such as graft-versus-host disease (GvHD) [10]. Likewise, many studies have shown that MSCs play an important role in innate and adaptive immunity through the suppression of immune cells, including neutrophils, dendritic cells, B lymphocytes and T lymphocytes, macrophages, and natural killer cells [11,12]. The mechanisms underlying the immunomodulatory properties of MSCs are likely mediated by cytokines and cell contact-dependent mechanisms in response to those immune cells [13].

In this study, we used both SFM and SCM for the culture of rhesus monkey BM-derived MSCs (rBMSCs) and compared the immunomodulatory properties of cells after exposure to these media by coculturing the cells with allogeneic human peripheral blood mononuclear cells (PBMCs). Furthermore, the differential expression of genes related to immune responses was assessed with high-throughput sequencing.

2. Methods

2.1. Serum-free culture medium formulation and gelatin coating of tissue culture vessels

Given the uncertainty of the composition of fetal bovine serum (FBS), a new chemically defined serum free medium (SFM) was developed in this study, based on previous reports. Briefly, the SFM used in this study comprised 19 human recombinant proteins and synthetic components, including hormones, growth factors, attachment factors and additional nutrients (Table 1).

For the gelatin coating, a 0.1% gelatin solution was added into T25 tissue culture flasks (Nunc, Thermo Fisher, USA) for at least 30 min at room temperature to completely cover the culture surface. Then, excess gelatin solution was aspirated, and the remainder was allowed to evaporate by leaving the vessel sitting open in the hood for ≤30 min before seeding cells.

Table 1
Components of serum-free medium.

Components	Company
MEM α, no nucleosides	Life Technologies
Insulin-like growth factor (IGF)	Novoprotein
Transforming growth factor beta (TGF-β)	Novoprotein
Platelet-derived growth factor (PDGF)	Peprotech
Fibroblast growth factor (FGF)	Novoprotein
Epidermal growth factor (EGF)	Novoprotein
Sodium bicarbonate	Sigma Aldrich
Glutamine solution	Millipore
HEPS	Gibco
1,4-Diaminobutane	Millipore
Progesterone	Millipore
Dexamethasone	Sigma Aldrich
Hydrocortisone	Sigma Aldrich
Lipid concentration	Life Technologies
Fetuin	Millipore
L-Ascorbic acid	Sigma Aldrich
Insulin-transferrin-selenium	Life Technologies
Albumin	Sigma Aldrich
Serotonin	Sigma Aldrich
Folic acid	Sigma Aldrich

2.2. Isolation and expansion of rBMSCs and PBMCs

MSCs were harvested from the bone marrow of eight 3-to-5-year-old rhesus monkeys as previously described [14]. All animal procedures conformed to the requirements of the Animal Welfare Act, and protocols were approved by the ethics committee at Sichuan University. Briefly, the mononuclear cell fraction was enriched by gradient centrifugation, suspended in Alpha Minimum Essential Medium (α-MEM, Life Sciences, USA) supplemented with 15% FBS (Life Sciences, USA) and 1% penicillin/streptomycin (Life Sciences, USA), and cultured at 37 °C in an incubator containing 5% CO_2. After 7 to 9 days, non-adherent cells were removed using Tryple® Express (Life Sciences, USA). The medium was changed every 3 to 4 days until the cells reached approximately 80% confluence. Then, the first passage cells were cultured in SCM and were then either continually cultured in SCM or cultured in SFM created by our group (Supplementary Table 1, Supplementary Fig. 1).

2.3. Morphology analysis

Cells at passage 3 cultured in either SCM or SFM were fixed with 4% paraformaldehyde for 30 min, stained with 0.5% crystal violet in ethanol and then washed three times with water before imaging to identify the morphology of rBMSCs. The mean cell density was measured by Image-Pro Plus (Version 6.0.0.260, USA).

2.4. Differentiation potential

To assess the differentiation potential of rBMSCs cultured in SCM and SFM, we performed tri-lineage differentiation of rBMSCs into adipocytes, osteocytes and chondrocytes as previously described [15].

2.5. Proliferation assay

The proliferation curves were determined by culturing the cells to 80% confluence, then harvesting and counting rBMSCs at the indicated times. A total of 1×10^5 cells at passage 3 and 5 were seeded into 6-well plates in either SCM or SFM, and the cells were then counted daily for 7 days using a hemocytometer. Cell proliferation activity was detected by a EdU assay kit (Ribobio, Guangzhou, China) and Cell Counting Kit-8 (CCK8) (Dojindo, Kumamoto, Japan) following the manufacturer's instructions.

2.6. Flow cytometry analysis

For the analysis of immunophenotypic expression, cells (2×10^5) at passage 3 cultured in SCM or SFM were trypsinized, rinsed twice with phosphate-buffered saline (PBS), and incubated at room temperature for 30 min with Allophycocyanin (APC)-conjugated anti-HLA-DR (eBiosciences, USA), CD73 (Abcam, USA), phycoerythrin (PE)-conjugated anti-CD29 (BD Biosciences, USA), CD34 (BD Biosciences, San Jose, CA) and CD105 (eBiosciences, San Diego, USA), phycoerythrin-Cy5 (PE-Cy5)-conjugated anti-CD45 (BD Biosciences, USA), and fluorescein isothiocyanate (FITC)-conjugated anti-CD90 (Abcam, USA). Flow cytometry was performed using a BD FACSAria system (BD Biosciences, USA).

2.7. Gene expression profiling

To assess the gene expression profiles of cells cultured with the two different types of media, we cultured rBMSCs from eight healthy rhesus macaques (SCM, n = 3; SFM, n = 5) to passage 3; then, the cells were washed three times with PBS prior to RNA isolation with TRIzol® Reagent. RNA-Seq library construction and RNA high-throughput sequencing were performed by Beijing Genomic Institution (BGI, Shenzhen, China) on a BGISEQ-500 high-throughput sequencer. RNA-