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Membrane complement regulatory protein reduces the damage of transplanting autologous bone marrow mesenchymal stem cells by suppressing the activation of complement

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ABSTRACT

There are few studies on the interaction of transplanting autologous bone marrow mesenchymal stem cells (BMSCs) and complement. In order to further explore the effect of complement on BMSCs, BMSCs were obtained from bone marrow of 20 cases clinical patients, and then experimented in vitro. The cytotoxicity of complement on the mesenchymal stem cells in autologous human serum (AHS) was measured by Europium cytotoxicity assay. The complement membrane attack complex (MAC) deposited on the membrane surface was detected by flow cytometry. Finally, the cytotoxicity on BMSCs was measured after mCRPs overexpression or knockdown. We found that more than 90% of cells derived from bone marrow were identified to be mesenchymal stem cells through detection of cell membrane surface markers by flow cytometry. BMSCs harvested from the 20 patients all had cytotoxicity after incubated with AHS, and the cytotoxicity was significant higher than that incubated with complement inactivated autologous human serum (iAHS). Complement attack complex (MAC) could be detected on the BMSCs incubated with AHS, which implied the complement activation. We also found that mCRPs CD55 and CD59 overexpressions can resist the cytotoxicity induced by complement activation, while mCRPs CD55 and CD59 knockdown can enhance the cytotoxicity. Thus, the results indicated that mCRPs could effectively protect BMSCs from attacking by complement by suppressing the activation of complement.

Introduction

Bone mesenchymal stem cells (BMSCs) are a subpopulation of bone marrow stem cells and are able to differentiate into adipocytes, cartilage cells, bone cells, islet-like cells, and hepaticcells in vitro [1]. BMSCs play an important role in supporting the development of fetal, bone and adult haematopoiesis, as well as in bone repair and skeletal regeneration therapy [2]. Adult BMSCs possess a remarkably diverse array of immunosuppressive characteristics, it has been suggested that allogeneic BMSCs could be used as agents of immune deviation in conditions such as graft versus host disease (GVHD) or autoimmune diseases [3]. However, it has also been reported that the third and fourth clinical trial failures in 12 months for BMSCs therapy [4]. One possible explanation is that BMSCs could still be identified and damaged by the immune system of host after its transplantation.

The complement system plays a complex role in the pathogenesis of autoimmune diseases [5]. On the one hand, complement activation and the ensuing inflammatory tissue damage is detrimental and represents an effector phase reaction in many autoantibody-mediated immune disorders such as lupus [5,6]. On the other hand, complement is recognized to be important in tolerance induction and/or in the disposal of apoptotic cells and clearance of immune complexes [7,8]. The complement system is comprised of three different cascades: the classical, the alternative, and the lectin complement pathway; all of which converge on the formation of the C3 convertase to propagate the complement cascade [9]. It has been reported that mice deficient in the central component of complement system C3 had significantly lower GVHD-related mortality/morbidity and C3 modulated Th1/Th17 polarization in mouse GVHD [10], indicated that a deficiency of complement system that contribute to resistance to GVHD-related damages. Our previous studies have found that BMSCs have a cellular damage following incubation with autologous serum, and the formation of complement membrane attack complex (MAC)

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that induced by complement activation is detected [11], suggested that the complement can identify autologous BMSCs and attack it.

Complement system plays an important role in host defense. However, if not properly regulated, activated complement can also cause significant damage to host tissues. Membrane complement regulatory proteins (mCRPs) anchored on the cell surface function to protect host tissues from bystander injury when complement is activated. In humans, mCRP include decay-accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), complement receptor 1 (CR1, CD35) and CD59 [12,13]. These proteins differ in their mechanism of action and in the way in which they attach to the cell surface. DAF and MCP both act at the level of the C3 convertases; DAF inhibits the activation of C3 and C5 by preventing the formation of new and accelerating the decay of preformed C3 and C5 convertases [14]. MCP regulates C3 activation by functioning as a cofactor protein for factor I-mediated cleavage of C3b [15]. CR1 has both DAF and MCP activities. Finally, CD59 interacts with the terminal complement pathway components C8 and C9 directly regulating the formation of the cytolytic MAC [16,17].

The aim of the present study is to investigate the role of mCRPs in the damage of transplanting autologous bone marrow mesenchymal stem cells. According to the previous reports, we hypothesized that mCRPs may act as a complement activity inhibitor which contributes to the reduction of the damage of transplanting autologous bone marrow mesenchymal stem cells.

Materials and methods

Chemicals

Ficoll separating liquid was obtained from General Electric Healthcare (GE Healthcare, USA); DMEM culture medium was obtained from Life Technologies Corporation (Life Technologies, USA); Fetal bovine serum was purchased from Thermo Fisher Scientific (Invitrogen, USA); pCDNA3.1/FUT-6 was purchased from Shanghai Tai Leng biology company (Shanghai Tai Leng biology company, China); CD73-PE, CD90-FITC, CD105-PE, CD34-PE, and CD45-FITC were purchased from Becton Dickinson Biosciences GmbH (BD Biosciences GmbH, German); Europium reagent box was purchased from Abnova (Abnova, Taiwan); Antibodies CD46, CD55, CD59, and C5b-9 were purchased from Abcam (Abcam, UK).

Isolation of BMSCs

Routine skin preparation, disinfection, and drape in the iliac crest area, then extract 15 ml bone marrow liquid. The extracted bone marrow liquid was slowly injected into 15 ml Ficoll separation solution, then centrifugation for 15 min (1500 r/min), and draw the milky liquid layer into another tube. The milky liquid was washed by centrifugation (1200 r/min) in phosphate buffer saline (PBS) for 2 times. The resultant cell precipitate was resuspended in culture medium and transferred to cell culture flask. Changing half medium firstly after 48 h, the medium was then changed every 2–3 days. When cells were fused with 80%–90%, passaged by 1: 2. The experimental protocol were approved by the Ethics Committee of Hubei province. Bone marrow samples were taken from 20 patients who underwent surgical treatment for lower extremity fractures (12 cases of tibial fractures, 8 cases of femoral fractures), undertaken with the patient's consent before the operation and signed informed consent.

Identification of BMSCs

The expressions of CD34, CD45, CD73, CD90, and CD105 were detected by flow cytometry (BD FACSCalibur Company, USA) to identify BMSCs. Cells for flow cytometry were trypsinized, then collected and transferred into flow tubes and precipitated by centrifugation for 5 min (1000 r/min). The cells were washed by cold PBS for 3 times, then precipitated by centrifugation for 5 min (1000 r/min). The cells concentration was adjusted to 1×10^5/ml by using PBS. Antibodies CD34, CD45, CD73, CD90, and CD105 were added to the cell suspensions, and reaction without light for 15 min after mixing, then precipitated by centrifugation for 5 min (1000 r/min). The clear supernatant extract was removed. The cells were washed by cold PBS for 3 times, then incubated for 15 min with goat anti-mouse IgG-FITC/PE, and reaction without light for 15 min after mixing. The cells were washed by cold PBS for 3 times, then mixed in 100 μl 1% paraformaldehyde, and detected by flow cytometry.

Cytotoxic effects of complement on BMSCs in vitro

The complement cytotoxicity on the mesenchymal stem cells in autologous serum was measured by Europium cytotoxicity assay. 1 ml (1×10^5/ml) BMSCs were incubated with Europium at 37 °C for 30 min, then washed by PBS for 3 times. The labeled cells were incubated with 10% autologous human serum (AHS) or inactivated autologous human serum (iAHS, autologous human serum incubate in water at 56 °C for 30 min to lose the activity of heat sensitive complement in the serum) at 37 °C for 30 min The supernatant fluid were collected for detecting with a fluorescence spectrophotometer, the stimulated luminescence is 485 nm, and the emission light is 538 nm.

Detection of complement MAC on the membrane of BMSCs

Cells were divided into 3 groups: BMSCs group, BMSCs+ AHS group, BMSCs+ iAHS group. Cells were digested with EDTA-free trypsin, then collected to flow tubes and precipitated by centrifugation for 5 min (1000r/min). The clear supernatant extract was removed. The cells were washed by cold PBS for 3 times, then incubated for 15 min with goat anti-mouse IgG-FITC, and reaction without light for 15 min after mixing. The cells were washed by cold PBS for 3 times, then mixed in 100 μl 1% paraformaldehyde, and detected by flow cytometry.

mCRP plasmid electroporation and pSuper-neo plasmid silencing

BMSCs cultivation 2–3 days later, cell monolayers fusion reached 70%-80%. Cells were digested with EDTA-free trypsin, and precipitated by centrifugation for 5 min (1000r/min). The clear supernatant extract was removed. The cells were washed by cold PBS for 3 times, then mixed with PcDNA3.1-CD46, PcDNA3.1-CD55, and PcDNA3.1-CD59 plasmid or pSuper-neo-CD46, pSuper-neo-CD55, and pSuper-neo-CD59 (provided by Tongji Hospital Affiliated to Huazhong University of Science and Technology, China) silencing plasmid to a final concentration of 20 μg/ml. Move the mixture into electroporation container for electric shock operation in accordance with predetermined conditions (250 V, 100 Hz). After the completion of electroporation, cells suspension was inoculated in a preheating medium, then placed in the incubator for culturing after mixing. The transfection efficiency was detected after 12 h of culturing. The expressions of CD46, CD55 and CD59 before and after transfection were detected by flow cytometry.

Statistical analysis

Data were expressed as means ± SD values. Statistical analysis was performed by using SPSS software. The one-way ANOVA followed by a post hoc multiple comparison test was used to

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