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Rat adipose tissue-derived stem cells attenuate peritoneal injuries in rat zymosan-induced peritonitis accompanied by complement activation

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Abstract

Background aims. In patients receiving peritoneal dialysis, fungal or yeast peritonitis has a poor prognosis. In rat peritoneum with mechanical scraping, severe peritonitis can be induced by zymosan, a component of yeast (Zy/scraping peritonitis). Administration of rat adipose tissue-derived stromal cells (ASCs) potentially can improve several tissue injuries. The present study investigated whether rat ASCs could improve peritoneal inflammation in Zy/scraping peritonitis. *Methods.* Rat ASCs were injected intraperitoneally on a daily basis in rats with Zy/scraping peritonitis. *Results.* Peritoneal inflammation accompanied by accumulation of inflammatory cells and complement deposition was suppressed by day 5 after injection of rat ASCs. The peritoneal mesothelial layer in Zy/scraping peritonitis with rat ASC treatment was restored compared with the peritoneal mesothelial layer without rat ASC treatment. Injected rat ASCs co-existed with mesothelial cells in the sub-peritoneal layer. *In vitro* assays showed increased cellular proliferation of rat mesothelial cells combined with rat ASCs by co-culture assays, confirming that fluid factors from rat ASCs might play some role in facilitating the recovery of rat mesothelial cells. Hepatocyte growth factor was released from rat ASCs, and administration of recombinant hepatocyte growth factor increased rat mesothelial cell proliferation. *Conclusions.* Because the peritoneal mesothelium shows strong expression of membrane complement regulators such as Crry, CD55 and CD59, restoration of the mesothelial cell layer by rat ASCs might prevent deposition of complement activation products and ameliorate peritoneal injuries. This study suggests the therapeutic possibilities of intraperitoneal rat ASC injection to suppress peritoneal inflammation by restoring the mesothelial layer and decreasing complement activation in fungal or yeast peritonitis.

Key Words: *adipose-derived stromal cells, complement, membrane complement regulators, peritoneal dialysis, peritonitis*

Introduction

Peritoneal dialysis (PD) is an important renal replacement therapy. There are >196,000 patients with end-stage renal disease currently receiving PD therapy around the world (1). However, many patients must be withdrawn from PD therapy for various reasons (2,3). Impairment of peritoneal function is one of the main reasons for withdrawal from PD therapy. Peritoneal impairment is caused by the use of non-biocompatible PD fluid such as acid-base PD fluid and long-term exposure of the peritoneum to PD fluid. Peritonitis is another important and serious complication leading to withdrawal from PD therapy (3,4). Both conditions are associated with peritoneal tissue injuries and may be related to the development of lethal encapsulating peritoneal sclerosis (5). Such problems must be resolved to improve the prognosis of PD therapy in the future. In particular, fungal or yeast peritonitis is a serious infection in patients on PD (6) because fungal infection is known to be associated with a poor prognosis of peritonitis and can lead to the development of encapsulating peritoneal sclerosis (7,8). We previously reported that zymosan, a component of the yeast cell wall, can induce severe peritoneal inflammation in association with complement activation (9). Zymosan activates the complement activation system through the alternative pathway (10). We also reported that dysregulation of the complement activation system could be one factor associated with the development and augmented severity of

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peritoneal injuries (9,11,12). Targeting the complement activation system might be one approach to control peritoneal injuries.

Studies using embryonic or pluripotent stem cells are valuable for analyzing pathologies, restoring injuries involving hematopoietic cells, neurons, muscle or cardiovascular tissue and developing new therapeutic agents (13–17). As the stem cells of mesenchymal tissues, mesenchymal stromal cells have shown great potential to repair tissue injuries in various animal disease models as alternatives to conventional therapies (18,19). Adipose tissue-derived stromal cells (ASCs) have an added advantage in that the cells can be harvested more easily and less invasively than bone marrow-derived mesenchymal stromal cells (20) and reportedly display immunomodulatory properties (21,22).

Using a rat peritonitis model induced by administration of zymosan after scraping the peritoneum (Zy/scraping peritonitis) (9), we showed that the initial inflammation in severe peritoneal injury was related to complement activation and that peritoneal inflammation of this peritonitis was enhanced by complement activation, supporting the poor prognosis of fungal PD peritonitis. In the present study, we investigated a suppressive effect of rat ASCs in peritoneal injuries, targeting regulation of the complement activation system in the rat Zy/scraping peritonitis model.

Methods

Animals

Male Sprague-Dawley rats weighing approximately 250 g (Japan SLC, Hamamatsu, Japan) were used. All animal experiments described here were carried out in accordance with the Animal Experimentation Guide of Nagoya University School of Medicine.

Reagents and antibodies

Zymosan A was purchased from Sigma-Aldrich (St Louis, MO, USA). Dianeal NPD-4 1.5% (Baxter, Tokyo, Japan) was used as a 1.5% neutral PD fluid (pH approximately 6.4). To investigate the distribution of rat membrane complement regulators (CRegs), anti-rat Crry (monoclonal antibody [mAb] TLD-1C11) was purchased from Hycult Biotechnology (Uden, the Netherlands), and anti-rat CD55 (mAb RDIII7) and anti-rat CD59 (mAb 6D1), which were kindly donated by Prof B. P. Morgan (Cardiff University), were characterized as described (23–25). Fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse immunoglobulin G (IgG) was purchased from MP Biomedicals (Santa Ana, CA, USA). To observe C3b and C5b–9 (membrane attack complex) deposition, we used FITC-rabbit anti-rat C3 (MP Biomedicals) and mouse anti-rat C9 (clone 2A1; Hycult Biotechnology), respectively, followed by incubation with FITC-goat anti-mouse IgG (MP Biomedicals). Naphthol AS-D chloroacetate ($C_{20}H_{16}ClNO_3$), N,N-dimethylformamide and Fast Blue BB Salt hemi(zinc chloride) salt ($C_{17}H_{18}N_3O_3Cl \cdot 1/2\ ZnCl_2$) were purchased from Sigma-Aldrich for an esterase reaction to detect neutrophils. To observe ED1-positive cells, mouse anti-rat monocyte (clone ED1) was purchased from BMA Biomedicals (Augst, Switzerland). To recognize mesothelial cells along the peritoneal surface, we used monoclonal mouse anti-human cytokeratin, which was cross-reactive against rat (Dako, Glostrup, Denmark).

Preparation and characterizations of rat ASCs and primary cell cultures of rat mesothelial cells

Rat ASCs were obtained from inguinal adipose tissue from male Sprague-Dawley rats and cultured in low serum culture medium according to previous reports (26,27). Briefly, in the cell culture of rat ASCs, the basal medium was a 3:2 mixture of Dulbecco's modified Eagle's medium (Nissui Pharmaceutical, Tokyo, Japan) and MCDB 201 medium (Sigma-Aldrich), supplemented with 1 mg/mL linoleic acid-albumin (Sigma-Aldrich), ITS liquid media supplement (100×) as 1:100 (v:v) (Sigma-Aldrich), 0.1 mmol/L ascorbic acid phosphate ester magnesium salt (Wako Pure Chemical Industries, Osaka, Japan), 50 U/mL penicillin and 50 μg/mL streptomycin (Gibco Life Technologies, Grand Island, NY, USA). Cells were cultured in culture medium containing 2% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT, USA) and 10 ng/mL human fibroblast growth factor 2 (PeproTech, Rocky Hill, NJ, USA).

Primary culture of mesothelial cells was obtained according to our previous report (11). Briefly, dissected omentum from a Sprague-Dawley rat was digested in 10 mL of 0.25% trypsin with 1 mmol/L ethylenediamine tetraacetic acid (Gibco Life Technologies) for 30 min at 37°C, followed by incubation for 1 h in fresh 0.25% trypsin with 1 mmol/L ethylenediamine tetraacetic acid at 37°C. The residual omentum fragment was removed from the cell suspension. M199 medium (Invitrogen, Carlsbad, CA, USA) with 10% FBS and a mixture of 50 U/mL penicillin and 50 μg/mL streptomycin (Gibco Life Technologies) as 1:100 (v:v) was added into the cell suspension and centrifuged at 1000 rpm for 5 min. The cell pellet was re-suspended in 4 mL of M199 medium with 10% FBS, plated into type 1 collagen-coated 6-cm dishes (IWAKI; Asahi Glass Co., Tokyo, Japan) and incubated at 37°C for 4 days. Characterization