Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Fluid shear stress suppresses ICAM-1-mediated transendothelial migration of leukocytes in coculture model

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ARTICLE INFO

Article history:
Received 16 May 2018
Accepted 27 May 2018
Available online xxx

Keywords:
Coculture system
Atherosclerosis
Endothelial–smooth muscle cell interaction
Adhesion molecules

ABSTRACT

The adhesion and migration of leukocytes to arterial endothelial cells (ECs), one of the indicators of early atherogenesis, is believed to be correlated with the blood flow conditions and interactions between vascular cells including vascular smooth muscle cells (SMCs). In this study, we investigated the effect of fluid shear stress on the transendothelial migration of leukocytes in a coculture model (CM) composed of human umbilical ECs and SMCs, a layer of collagen type I, and a porous membrane. Following exposure to a fluid shear stress of 1.5 Pa for 24 h, human mononuclear leukocytes were seeded on the EC surface and cultured for 1 h. Leukocytes migrating across the EC layer were observed by confocal laser scanning microscopy. The number of migrating leukocytes in the statically cultured CM was significantly larger than that in the static EC monoculture model. The exposure to the shear stress significantly decreased the leukocyte migration induced by the coculture condition. In the static CM, fluorescence staining and Western blotting showed a higher expression of intercellular adhesion molecule-1 (ICAM-1) of ECs. These results indicate that SMC-derived bioactive soluble factors may stimulate the ICAM-1 expression of cocultured ECs, possibly leading to leukocyte migration into the subendothelial space.

1. Introduction

The effects of fluid shear stress on the functions of vascular endothelial cells have been investigated because the formation of atherosclerosis tends to localize at the bifurcation and curved sites of arteries, which are expected to be predominated by a low shear stress, the flow separation from the vessel wall, and complex flow patterns [1,2]. The adhesion of blood mononuclear leukocytes to the endothelial cells of arteries is one of the indicators of early atherogenesis [3,4]. Leukocytes adhere to the endothelium and migrate into the vessel walls mediated by adhesion proteins expressed on the endothelial cell surface such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Till date, in vitro experiments using endothelial cells in a monoculture have revealed that the expression of adhesion proteins is not only affected by exogenous cytokines such as tumor necrosis factor-α (TNF-α) and interleukine-1β (IL-1β) but also by the shear stress acting on endothelial cells [5–7]. Concurrently, previous studies have also reported that smooth muscle cells interact with endothelial cells and have an effect on the properties of leukocyte adhesion to endothelial cells. For example, Rainger and Nash showed that endothelial cells cocultured with smooth muscle cells were sensitized to respond to TNF-α, and they changed the E-selectin expression used for leukocyte capture [8]. Chiu et al. demonstrated that a coculture with smooth muscle cells induced the endothelial cell gene expression of VCAM-1 and ICAM-1, whereas shear stress inhibited the coculture-induced gene expression of these adhesion proteins [9].

These previous studies used a coculture system in which endothelial cells and smooth muscle cells were cocultured on the opposite sides of a porous membrane. Because there was no space in their coculture systems to allow leukocytes to migrate, it was difficult to observe the transendothelial migration of leukocytes. Several studies, on the other hand, have cocultured endothelial cells and smooth muscle cells separated by a collagen layer and assessed the migration of leukocytes [10,11]. Moreover, it was

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https://doi.org/10.1016/j.bbrc.2018.05.182
0006-291X/

shown that the number of leukocytes that migrated into the collagen layer under the coculture condition increased compared with that under a monoculture condition [10]. However, the coculture systems used by these studies were not appropriate for the flow-exposure experiment, possibly owing to the lack of sufficient mechanical stiffness to endure the fluid shear stress conditions. Hence, the effect of shear stress on leukocyte migration under a coculture condition has not been completely elucidated so far.

We have studied the cellular interaction between endothelial cells and smooth muscle cells using a coculture model that is suitable for the fluid-flow exposure experiment at a physiological level of the shear stress [12–14]. In this study, we evaluated the adhesion and migration of leukocytes using the cocultured model subjected to a fluid shear stress. We observed that leukocytes migrated to the collagen layer separating the endothelial and smooth muscle cells in the model.

2. Materials and methods

2.1. Cell culture

Human endothelial cells and smooth muscle cells were isolated from umbilical cords using enzymatic digesting methods, as previously described [12,14]. They were grown in gelatin-coated culture dishes in Medium 199 (M199) (Invitrogen, USA) containing 20% fatal bovine serum, 1 unit/mL penicillin–streptomycin (Invitrogen), and 100 ng/mL human basic fibroblast growth factor (bFGF, Austral Biological, USA). Cells with 2–8 passages were used for the experiments.

2.2. Construction of coculture model

The endothelial–smooth muscle cells coculture model was constructed as previously described [12,13]. Smooth muscle cells were cultured in dishes until they attained confluence. Collagen gels were prepared by mixing 5 mg/mL bovine skin collagen type I (Koken, Japan), 10 × M199, and reconstruction buffer (0.05 M NaOH, 0.2 M HEPES, and 0.26 M $NaHCO_3$). The mixture was poured into a dish, and immediately after, a porous membrane (pore diameter: 5 μm) was placed on the collagen solution. The mixture was allowed to polymerize for at least 1 h at 37 °C, and endothelial cells were then seeded on the membrane (Fig. 1). The coculture models were cultured for at least 48 h prior to the experiments. A model constructed without smooth muscle cells, termed as the monoculture model, was also used in the experiments (Fig. 1).

2.3. Flow-exposure experiment

The flow circuit and flow chamber have been previously described [12,14]. The cocultured dish was incorporated into a parallel-plate flow chamber and exposed to a steady wall shear stress of 1.5 Pa, which could be assumed to be an average magnitude for the physiological shear stress, for 24 h at 37 °C in 95% air/5% CO_2 atmosphere.

2.4. Leukocyte adhesion and migration assay

Mononuclear leukocytes were isolated from human peripheral blood by density gradient centrifugation with Lymphoprep™ (Axis-shield, Norway). To aid visualization, the leukocytes were incubated with a culture medium containing 10 μM of calcein-AM (Molecular Probes, USA) for 90 min, which did not affect the adhesion [15]. The study protocols were officially approved by the Ethics Committee for Clinical Research with Human Subjects of Tohoku University.

After the flow-exposure experiments, the coculture model was incubated statically for 1 h with a medium containing mononuclear leukocytes (2.0×10^5 cells/cm^2), allowing the leukocytes to bind to the surface of the endothelial monolayer. The model was then gently washed thrice with phosphate-buffered saline (PBS) to remove the unadhered leukocytes. After the model was fixed with 10% formalin, the cells in the model were stained with 150 nM rhodamine–phalloidin (Molecular Probes, USA). Sequential fluorescent images of the model along the z-axis were captured at an interval of 1 μm using a confocal laser scanning microscope (Olympus, Japan).

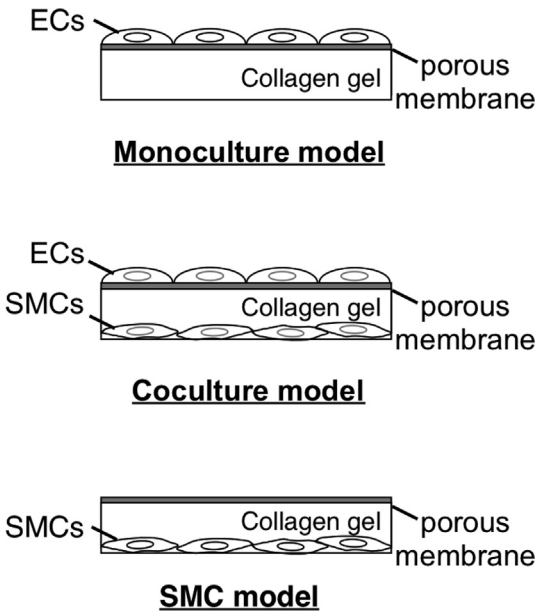

Fig. 1. Schematic of the experimental models used. The coculture model is composed of endothelial cells (ECs) cultured on a porous membrane and smooth muscle cells (SMCs), and a collagen layer. The monoculture model and SMC model do not include SMCs and ECs, respectively.

2.5. Immunofluorescent staining

After exposure to the shear stress, the coculture model was incubated for 1 h at room temperature (RT) with mouse monoclonal antibody directed to ICAM-1 (R&D systems, USA). Subsequently, the model was rinsed twice with PBS and incubated for 45 min at RT with FITC-conjugated anti-mouse IgG. Fluorescent images of ICAM-1 were captured by fluorescent microscopy (Olympus).

2.6. Western blotting

In the models, the endothelial cells were washed thrice with ice-cold PBS and lysed in ice-cold triton/NP-40 lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate, 1% NP-40, 1% SDS, 100 μM sodium orthovanadete, 1 μM phenylmethylsulfonyl, and 1% protease inhibitor cocktail). The cell lysates were subjected to SDS-PAGE, transferred to polyvinylidene difluoride membrane, and stained with a primary antibody against ICAM-1 (R&D systems). An alkaline phosphatase (AP)-conjugated secondary antibody (Chemicon International, USA) was detected by an amplified AP immuno-blot kit (Bio-Rad, USA). The band intensity was quantified from scanned membrane images using ImageJ software (National Institutes of Health, USA).

Please cite this article in press as: N. Sakamoto, et al., Fluid shear stress suppresses ICAM-1-mediated transendothelial migration of leukocytes in coculture model, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.182