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Developmental endothelial locus-1 prevents development of peritoneal adhesions in mice

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ABSTRACT

Postoperative peritoneal adhesions, fibrous bands formed in the peritoneal cavity following surgery, represent a common, challenging and costly problem faced by surgeons and patients, for which effective therapeutic options are lacking. Since aberrant inflammation is one of the key mechanisms underlying peritoneal adhesion formation, here we set out to study the role of developmental endothelial locus-1 (Del-1), which has been recently identified as an endogenous inhibitor of inflammation, in the formation of postoperative peritoneal adhesions using a mouse model of peritoneal adhesions induced by ischemic buttons. Del-1-deficient mice had a higher incidence of adhesions, and their adhesions had higher quality and tenacity scores. Del-1 deficiency also led to enhanced inflammation mediators and collagen production. Finally, Del-1 supplementation decreased the incidence and severity of postoperative peritoneal adhesions. Taken together, these results indicate a protective role for Del-1 in postoperative peritoneal adhesion formation.

1. Introduction

Postoperative peritoneal adhesions (PPA) are fibrous bands formed between the normally separated organs, the omentum, and the abdominal wall after peritoneal or pelvic surgery. PPA can cause high morbidity and have a poor prognosis, and an incidence of greater than 50% [1–7]. Adhesion formation can cause serious postoperative complications, including intestinal obstruction, female infertility, and chronic abdominal and pelvic pain [6,8–10].

The peritoneal membrane is a serous membrane that consists of a monolayer of mesothelial cells and a thin underlying layer of connective tissue. It lines the abdominal wall and most of the visceral organs to support and protect them [11]. Surgical trauma can induce an inflammatory response and increase exudation of fibrin onto the damaged peritoneum [11,12]. Fibrin exudation can cover the nude peritoneum to prevent further damage but also may cause the peritoneal tissue to adhere to adjacent organs. If this fibrinous exudate is degraded via a process called fibrinolysis within 2–3 days after surgery, normal peritoneal healing will occur. If not, the accumulated fibrinous exudate will develop into a fibrin-rich matrix and irreversibly form permanent adhesions as early as 7 days after surgery [9,13]. Surgical injury also causes leukocytes to accumulate in the traumatized tissues. Proper inflammatory responses contribute to the normal restitution of peritoneum, but, excessive inflammation may lead to adhesion formation [6,11,12,14–16]. Inflammatory cells-derived cytokines can lead to impaired fibrinolysis by upregulating the expression of plasminogen activator inhibitor-1 (PAI-1), an inhibitor of plasmin which

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serves as a main fibrinolytic enzyme to degrade fibrin [6,11,16]. Besides proinflammatory cytokines, inflammatory cells also secrete chemokines and growth factors to induce the activation, differentiation, proliferation, and migration of fibroblasts, leading to increased production of ECM (extracellular matrix) components [6,11,12,14,15,17]. Activated fibroblasts can invade into the incompletely degraded fibrinous matrix, resulting in a mature adhesive band [18,19].

Numerous attempts have been made to prevent the formation of PA, but with limited success [19–22]. One of the reasons for this may be our incomplete understanding of the pathogenesis of PPA [5]. To develop more effective preventive and therapeutic approaches, a full understanding of the underlying molecular and cellular mechanisms is needed.

Developmental endothelial locus-1 (Del-1) is an endogenous anti-inflammatory factor that can inhibit the lymphocyte function-associated antigen-1 (LFA-1), an adhesion molecule that promotes leukocyte adhesion to endothelial cells [23–30]. Del-1 is normally expressed by endothelial cells to regulate the inflammatory response. Previous studies demonstrated that Del-1 protects against a variety of inflammation-related diseases [24–29]. Del-1-deficient (Del-$1^{-/-}$) mice have also been shown to be more susceptible to bleomycin-induced pulmonary fibrosis [31]. Similar to fibrosis, PPA is a disease featuring enhanced ECM deposition [6,13,32]. We thus hypothesized that Del-1 plays a protective role in PPA formation. Here, we investigated the role of Del-1 in the development of PPA using a PA model induced by ischemic buttons.

2. Materials and methods

2.1. PPA mouse model

C57BL/6J WT male mice were purchased from Orient-Bio (Korea) and housed under specific pathogen-free (SPF) conditions. Del-1 gene-deficient mice on a C57BL/6 background were a kind gift from Prof. T. Chavakis (Dresden University, Germany) and were maintained under SPF condition. Animal studies were approved by the Asan Institute for Life Sciences Institutional Animal Care and Use committee (Project No: 2016-14-189). PPA were mimicked as described previously [5,33,34]. Details are described in the Supplementary Material. For Del-1 supplementation in Del-$1^{-/-}$ mice, 50 μg Del-1-Fc protein (Y-Biologics, Daejon, Korea) was dissolved in 500 μl of PBS and administered intraperitoneally once just after closing the abdomen. Adhesions were evaluated on POD 7 according to the grading standards (Supplementary Table 1). Details are described in the Supplementary Material.

2.2. Masson's trichrome staining

Peritoneal ischemic buttons and normal peritoneum sections were prepared as described in the Supplementary Material. A Masson's trichrome staining kit (HT15; Sigma-Aldrich) was used according to the manufacturer's instructions, with slight modifications. Details are described in the Supplementary Material.

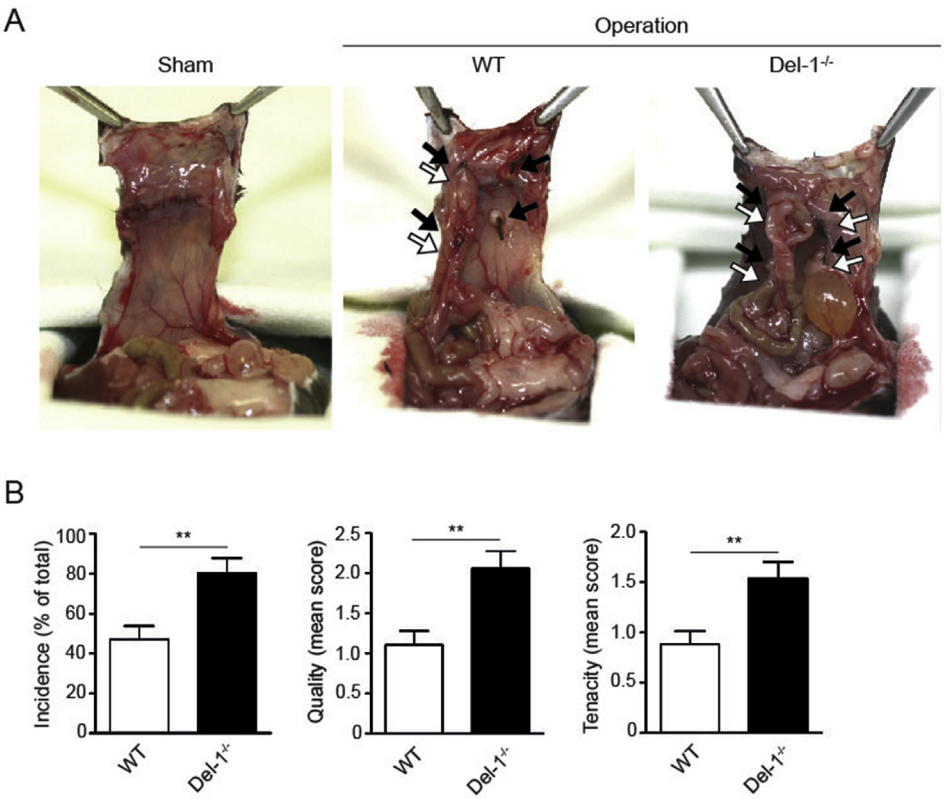

Fig. 1. Del-1-deficient mice show increased peritoneal adhesion formation after surgery. PPA was induced using ischemic buttons in WT and Del-$1^{-/-}$ mice. PPA were scored on POD 7. (A) Representative images of PPA, in which white arrows indicate PA and black arrows indicate ischemic buttons. (B) Bar graphs comparing the incidence, quality, and tenacity of PA in WT and Del-$1^{-/-}$ mice on POD 7. Data are the means ± SEM (n = 14–17/group). For quality and tenacity, **p ≦ 0.01 by Student's t-test. For incidence, **p ≦ 0.01 by Mann-Whitney U test.

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