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Impact of repeated abdominal surgery on wound healing and myeloid cell dynamics

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ABSTRACT

Background: Even though wound dehiscence is a surgical complication, under certain medical conditions, repetition of the laparotomy (LT) (relaparotomy) can become inevitable. In addition to the risks associated with this surgical operation, relaparotomy can interfere with the tissue healing and contribute to the development of chronic wounds.
Methods: In an experimental relaparotomy wounding model, this study investigated the impact of repeated surgery on wound healing and on the immune cells of myeloid origin.
Results: The first repeat of the LT triggered fibrosis and marginally interfered with the wound healing; however, the second operation completely abrogated the healing process. Splenomegaly was observed as an indicator of the chronic inflammation and the systemic effect of repeated laparotomies. In the blood stream, the spleen, and the liver, these repeated surgeries exhibited a major impact on the $CD11b^{+}Ly6C^{+}Ly6G^{-}$ monocytes. On the other hand, especially, whespecially the second relaparotomy resulted in a massive purging of neutrophil granulocytes into the circulation. These $CD11b^{+}Ly6C^{+}Ly6G^{+}$ neutrophils that were disseminated on repeated abdominal laparotomies had a proinflammatory character that positively influenced T cell proliferation and displayed a high capacity for production of reactive oxygen species.
Conclusions: The repetition of abdominal LT not only interferes with the wound healing but also contributes to the development of imperfectly healing wounds which have systemic impact on immune compartments.

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Introduction

Following abdominal surgery, a repeat laparotomy (relaparotomy) can be required due to the urgencies such as internal bleeding, hematoma, abscess, and disruption of anastomoses.[1,2] Alternatively, relaparotomy can be planned for the removal of mesenteric vascular occlusions or in cases with ongoing peritonitis.[3,4] Although increasing the risk of morbidity and mortality, multiple relaparotomies are performed when clinical deterioration is observed.[5-7] Expectedly, tissue repair mechanisms and immune system are critically affected following the recurrent surgical interventions.[8,9]

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Wound healing is a dynamic process which is influenced by many intrinsic and extrinsic factors.[10,11] Essentially, inflammatory signals produced subsequent to the injury have a significant role in the course of tissue healing. During the inflammatory phase, pathogenic organisms and debris are cleared, wound bed is sealed by clot formation, and tissue repair mechanisms are simultaneously triggered.[10,12] Immune cells that are recruited into the site of inflammation regulate the proliferation and maturation of stromal and parenchymal cells that reestablish and maintain homeostasis.[13-15]

Nevertheless, tissue healing can be hindered by the presence of infections, foreign bodies, and ischemia that would lead to ulcers.[16,17] While the surgical operations are performed under antiseptic conditions with sterile equipment, exposure to microorganisms is almost inevitable.[17] In addition, activation of the immune system can also be stimulated even by the suture material recognized as a foreign body.[18] Constant breakdown of the tissue has been considered as another reason for the chronic inflammation.[19] As a consequence, the tissue healing becomes dysregulated, and deposition of connective tissue and fibrosis are observed.[15,20] Therefore, reinterventions following the abdominal surgery within the same period of hospitalization would augment inflammatory reactions and interfere with the regular wound healing process.[1-7]

Neutrophils, which are identified with Ly6G and Ly6C markers in mice, are the first immune cells to infiltrate the wounded area to form the initial line of defense and trigger the repair.[21-23] They are responsible of debridement, regulation of inflammation, and recruitment of other leukocytes, for example, monocytes/macrophages, into the inflamed sites.[22] These myeloid cells not only protect the host from invading microorganisms but also maintain tissue remodeling and sustain angiogenesis.[23,24] On the other hand, prolonged presence of the neutrophils in the wound area has been associated with the delay in repair and may even result in a bystander tissue destruction basically through the secretion of proteases and reactive molecules.[22,25,26]

Even though the local actors of wound healing are frequently studied, their systemic effect and distribution are not very well known. The present study aims to explore whether the chronic inflammation triggered by repeated laparotomies could influence the systemic distribution, amount, and function of the myeloid cells in different compartments. Here, undesirable effects of the relaparotomy on wound healing and on the dynamics of myeloid cell production and distribution were demonstrated.

Materials and methods

Animals and experimental model

Inbred BALB/c mice, female, 6-8 weeks old, (Kobay As., Ankara, Turkey) were housed under standard conditions in a cage cabinet ventilated with high efficiency particulate air-filtered air. Female mice were preferred because male are more prone to aggression, and fighting may negatively affect postoperative period. The experimental setup is summarized in Figure 1A. The mice ($n = 28$) underwent laparotomy (LT) on day 0. A combination of 150 mg/kg ketamine (Ketalar 5%; Pfizer, New York, NY) and 5 mg/kg xylazine (Alfazyne 2%; Bayer, Leverkusen, Germany) was intraperitoneally administered as an anesthetic regime. Following disinfection of the skin (Povidone-iodine 10%; KİMPA, Istanbul, Turkey), the animals were transferred under a laminar flow cabinet where the air is drawn through a high efficiency particulate air filter to create a particle-free working environment. The skin and the muscular abdominal wall were cut by a mid-line LT (~2 cm) using sterile surgical instruments. Then, both the muscle and skin layers were closed with slowly absorbable sutures (4-0, Vicryl, Ethicon, Norderstedt, Germany). Post-LT, one group of mice was sacrificed on day 2 ($n = 7$), and another group was sacrificed on day 7 ($n = 7$) (Suppl. Fig. 1A). In addition, on day 7 post-LT, the sutured mid-line incision of the remaining 14 mice was reopened, and these mice underwent a second LT (repeat laparotomy 1, re-LT1). After 7 days re-LT1 (i.e., 14 days post-LT), another group of mice ($n = 7$) was sacrificed. Finally, the abdomen of the last remaining seven mice was again opened for a third time to establish a repeat laparotomy 2 (re-LT2) group. These last animals were killed a week after re-LT2 (i.e., 21 days post-LT) (Fig. 1A). A control (no surgery) group ($n = 5$) was also used. Health parameters were followed daily, and body weight was measured biweekly.

During the experiment period, no drainage of pus or no microbial colonization were observed. After initial recovery from the operation distress, no behavioral change was noted in the mice. In addition, the body weights did not significantly differ among the groups; expectedly, a slight loss in weight was detected in the mice that underwent third abdominal LT (Suppl. Fig. 2B). Collectively, the health conditions of all mice were not significantly hampered by the surgical procedures.

In the conduct of all, experiments were carried out in accordance with the ARRIVE guidelines and the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The project was approved by the Hacettepe University Institutional Animal Care and Use Committee (approval no: 2015/30-02) before its commencement.

Immunophenotyping and cell sorting

Cell suspensions were prepared by mechanical agitation from the spleen and liver tissues and were filtered through a 40 μm mesh. These suspensions and the blood samples were separated by Histopaque-1119 (Sigma, Steinheim, Germany) 1.119 g/mL density gradient centrifugation (25 min, 400 × g at 25°C, without brake). Following gradient centrifugation, approximately 148×10^3 cells/mg, 40×10^5 cells/mg, and 65×10^3 cells/μL could be collected over the 1.119 phase of liver, spleen, and blood, respectively. Gr-1 marker is found on mouse myeloid cells together with high levels of CD11b expression.[27,28] Monocytic and granulocytic subtypes can also be recognized by labeling the isoforms of Gr-1, Ly6C and Ly6G. All three surface molecules (CD11b, Ly6C, and Ly6G) are expressed by Gr-1-high neutrophil granulocytes, whereas monocytes are Gr-1-low and they only express the Ly6C.[27] The cells were labeled with monoclonal antibodies against CD11b (M1/70), Gr-1 (RB6-8C5), Ly6C (HK1.4), and Ly6G (1A8) (BioLegend, San Diego, CA). The percentage of positive cells was

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