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The Role of Pro-inflammatory and Anti-inflammatory Interleukins in the Repair of Experimental Soft Tissue Injuries Complicated by Textile Foreign Bodies

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ABSTRACT

The healing of wounds resulting from the use of military weapons can be complicated by the presence of foreign bodies. This work aimed to study the effect of textile foreign bodies on the production of interleukins during the repair process. 60 rats were divided into 4 groups: intact (IG), control (CG), and two experimental (EG1, EG2). In CG, wounds were sutured without implantation of foreign bodies. A uniform consisting of 100% cotton was used as foreign bodies for EG1, a uniform consisting of 65% cotton and 35% polyester - for EG2. A violation of the dynamics of production of interleukins was registered. The values of IL-1β and IL-6 in EG1 after 60 days exceeded the values in CG 2.07 times and 2.92 times, and in EG2 - 1.37 times and 2.35 times, respectively ($p<0, 05$). The content in the experimental groups of IL-4 increased on the 30th day, and IL-10 - on the 60th day, but was lower than in the CG. The presence of textile foreign bodies in wounds created unfavorable for healing, which disrupted the production of interleukins. High levels of IL-1β and IL-6 throughout the experiment in animals with textile implants confirmed the prevalence of pro-inflammatory mechanisms, which significantly slowed down the process of damage repair.

Keywords
Wound healing; Foreign bodies; Interleukins; Inflammation; Regeneration

Introduction

The healing of chronic wounds remains a topical issue in physiology. The process of transition of acute wounds to chronic ones occurs under the influence of both local and systemic factors. One of the local factors is foreign bodies that get into the wound when injured, including injuries sustained as a result of the use of military weapons. The direct penetrating action of the wounding projectile and the vortex flow leads to the penetration of fragments of military projectiles, various hard materials, and clothing into the wounds of the soft tissues (Yongqiang et al., 2020). Fragments of textiles often remain *in situ*, as they can be difficult to detect or remove, contribute to the development of inflammation, which can lead to a long-term non-healing chronic wound (Lovely et al., 2018).

Wound healing takes place in four stages: hemostasis, inflammation, proliferation, and remodeling. Damage repair processes have been characterized histologically, but a complete understanding of the biochemical and cellular events that control normal and especially pathological wound healing is lacking (Bodnár et al.,2018; Princeet al., 2020). It is known that interleukins play an important role in the regulation of wound healing processes. After damage, IL-1β is one of the first to be synthesized; it induces the synthesis of other pro-inflammatory cytokines such as TNF-α and IL-6, low molecular weight mediators of inflammation (nitric oxide and prostaglandins), chemokines that attract neutrophils to the inflammatory zone; also activates T- and B-lymphocytes, stimulates hematopoiesis, phagocytosis, increases the permeability of the

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vascular wall (Nasonov& Eliseev, 2016; Dinarello,2011). IL-6 is formed in the area of traumatic or infectious injury and stimulates the synthesis of a wide range of acute-phase proteins by hepatocytes, hematopoiesis, differentiation of T- and B-lymphocytes (Tanaka et al., 2014). An important feature of IL-6 is its effect on the transition of the phase of early inflammation to the later, the so-called "switching" of leukocytes. This occurs by reducing the recruitment of neutrophils, recruiting monocytes, and differentiating them into macrophages (Narazaki& Kishimoto, 2018). After the cessation of the action of the damaging factor, cleansing the wound from necrotic masses, the production of pro-inflammatory interleukins decreases, and the secretion of anti-inflammatory interleukins is activated. IL-10 and IL-4 inhibit the synthesis of proinflammatory cytokines, the cytotoxic activity of T-cells, macrophages, regulates the proliferation and apoptosis of B-lymphocytes, fibroblasts, epithelial and endothelial cells (Steen-Louws et al., 2019). With normal healing of acute wounds, the synthesis of pro-inflammatory and anti-inflammatory cytokines is strictly coordinated and ensures the sequence and duration of the repair stages, and leads to complete healing in the optimal time. In chronic wounds, due to the action of local or systemic damaging factors, this coordination is impaired and the healing process takes a very long time or becomes impossible. A significant amount of work has been carried out to study the role of IL-1β, IL-6, IL-4, IL-10 in various kinds of chronic wounds (Beidler et al., 2009;Matsuura et al., 2019; Pavlov et al., 2020). However, the authors failed to find similar studies for soft tissue injuries complicated by foreign textile bodies.This work aimed to study the role of pro-inflammatory IL-1β and IL-6, as well as anti-inflammatory IL-4 and IL-10 in the healing of experimental soft tissue wounds complicated by textile foreign bodies.

Methods

Permission of the Ethics and Bioethics Commission of the Kharkiv Medical Academy of Postgraduate Education dated 11/12/2019 was obtained for the study.

This study was performed on 60 male Wistar rats aged 5 months and weighing 240 ± 30 grams. Laboratory animals were kept in the vivarium of the Kharkiv Medical Academy of Postgraduate Education. The conditions of detention complied with international regulations and included a natural light regime, optimal temperature (20-22 °C), a standard diet, free access to water and food (Guide for the Care and Use of Laboratory Animals, 2011). The animal cages were of sufficient size with natural bedding and ventilation. Experimental work was carried out by international requirements for the treatment of the animal (Directive 2010/63/EU, 2010; European Convention, 1986).

By randomization, the rats were divided into 4 groups. The intact group (IG) for determination of the normal interleukins' content had 6 healthy rats.

Injury modeling was carried out in 3 groups of 18 animals each. Animals' anesthesia was performed with Zoletil (tiletamine hydrochloride and zolazepam hydrochloride) intramuscularly (10 mg/kg of animal body weight). The experiment was started after the onset of the surgical stage of anesthesia. The animals underwent a layered soft tissue incision of the right thigh posterior surface 1.0 cm long with a surgical scalpel No. 11 with a partial section of the muscles. In the control group (CG), wounds were sutured without foreign bodies' implantation. Experimental group 1 (EG1) animals were implanted with uniforms fragments of 100% cotton (camouflage form "Dubok"). Uniform fragments consisting of 65% cotton and 35% polyester (material 3403) were implanted into experimental group 2 (EG2) animals. The size of the implanted fragments was 0.5 × 0.5 cm. Wound suturing was carried out in layers with an atraumatic needle with 4/0 polypropylene suture, single-row interrupted surgical sutures. All

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surgical operations were performed in a sterile environment in compliance with the rules of asepsis and antisepsis. Removal of laboratory animals was carried out with the design of the study in equal groups of 18 animals (6 from each group) on the 7th, 15th, 30th day after modeling the injuries with implantation of textile foreign bodies. Euthanasia was performed by an overdose of anesthesia.

Blood for research was taken from the heart. The cytokines content in serum was determined by ELISA using IL-1β, IL-6, IL-4, IL-10 (Vector-Best) kits.

Statistical processing of the results was performed using Statistica 6.0 (StatSoft Inc., USA) statistical analysis package. To describe the results obtained, the data were presented as M ± SE, where M is the arithmetic mean, SE is the standard error of the arithmetic mean. The significance of differences between groups (statistical significance) was determined using the non-parametric Kruskal-Wallis ANOVA test for independent samples. Differences were considered statistically significant at $p < 0.05$. For histograms used in histological and cytokine, examinations were plotted by GraphPad Prism 8 software (GraphPad Software, USA).

Results

Analysis of the studies showed that the highest content of the proinflammatory cytokines IL-1β and IL-6 in CG animals was on the 15th day. On the 30th day, these indicators decreased and by the end of the experiment did not differ significantly from IG. In the experimental groups, the content of IL-1β was also maximal on the 15th day, but then it decreased not so significantly, exceeding the values in CG by 2.07 times for EG1 and 1.37 times for EG2 ($p < 0.05$) on the 60th day (fig. 1A). The level of IL-6 in EG1 was the highest on the 30th day, in EG2 - on the 15th, but also at the end of the experiment, it was 2.92 times higher in EG1 and 2.35 times higher than in CG ($p < 0.05$)(fig. 1B).

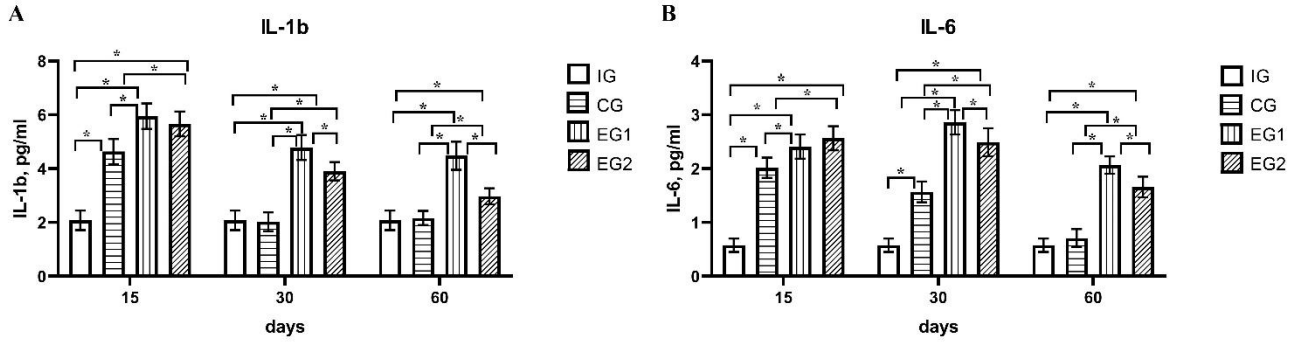

Fig. 1. The content of pro-inflammatory interleukins at different times of the experiment: a) IL-1β, b) IL-6 (* - $p < 0.05$).

The dynamics of anti-inflammatory cytokine production in all groups were multidirectional. It should be noted a tendency to an increase in IL-4 on the 30th day and IL-10 on the 60th day in the experimental groups, however, their values during these periods were lower than the maximum values in CG ($p < 0.05$) (fig. 2).

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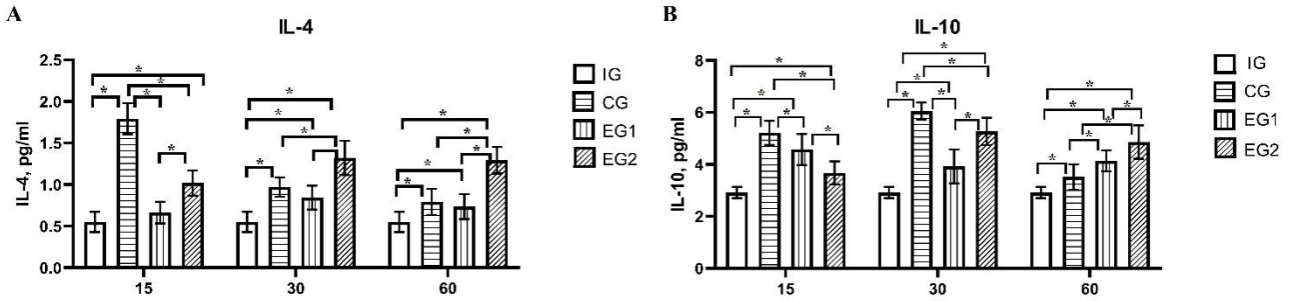

Fig. 2. The content of anti-inflammatory interleukins at different times of the experiment: a) IL-4, b) IL-10 (* - $p < 0.05$).

Discussions (Times New Roman, bold, 12)

The data of our study indicate the important role of disorders in the system of pro-inflammatory and anti-inflammatory cytokines in the healing of wounds complicated by textile foreign bodies. High levels of IL-1β and IL-6 in the blood of animals from the experimental groups could maintain the inflammatory process in the foreign body region for a long time (Beidler et al., 2009; Krzystek-Korpacka et al., 2019). As is known, most chronic wounds tend to persistently increase levels of proinflammatory cytokines. In our study, the long-term synthesis of pro-inflammatory interleukins was probably associated with the presence of foreign bodies. Fragments of textiles, due to their sorption abilities, could retain wound fluid containing decay products of destroyed soft tissues, hemorrhages, and dead leukocytes. Studies of fluids from chronic wounds show increased levels of enzymes, antioxidants, matrix metalloproteinases, which can destroy the extracellular matrix and growth factors (Barrientos et al., 2008; Bodnár et al., 2018). In addition, during muscle movement, the textile implant could injure the young granulation tissue filling the wound defect. All these signals from the damaged area contributed to the long-term production of IL-1β and IL-6 in animals of the experimental groups. Also, our study showed a violation of the dynamics of the secretion of anti-inflammatory IL-4 and IL-10 (in comparison with CG). Their increased level in the long-term healing period is characteristic of various kinds of chronic, long-term healing injuries and, possibly, is a compensatory process (Matsuura et al., 2019; Prince et al., 2020). Thus, in chronic wounds complicated by textile foreign bodies, a vicious circle of positive feedback is created between local factors promoting inflammation and the systemic response of the body in the form of production of IL-1β, IL-6, IL-4, IL-10.

Due to the disturbance of the processes of pro-inflammatory and anti-inflammatory cytokine secretion, strictly coordinated in the norm, the phase of inflammation prevailed for a long time in the repair of injuries in animals of the experimental groups. Histological examination showed a significant increase in the number of polymorphonuclear leukocytes in the foreign body area at all periods compared to the control group (Pavlov et al., 2021). In this case, the maturation of granulation tissue, that is, the phase of remodeling occurred later. More pronounced signs of inflammation and a deterioration in the quality of healing were observed in animals in EG1. This was probably due to the 100% cotton content of the implanted textile fragments. Surgical studies show that natural suture material (silk, cotton) has pronounced sorption properties, which causes aseptic inflammation up to the formation of necrosis (Javed et al, 2012; Dragovic et al, 2020). In contrast, synthetic filaments are more inert and produce a minimal cellular response. The textile

implants in EG2 contained a significant proportion of synthetic fibers (35%), as a result of which, apparently, the inflammatory processes were less intense, and the healing took place somewhat faster.

Conclusion

The presence of textile foreign bodies in experimental soft tissue wounds created conditions unfavorable for healing at the site of injury, which disrupted the production of both pro-inflammatory IL-1β and IL-6 and anti-inflammatory IL-4 and IL-10. The high levels of IL-1β and IL-6 recorded throughout the experiment in animals with textile implants confirm the prevalence of pro-inflammatory mechanisms involving a large number of immunocompetent cells, which significantly slowed down the process of damage repair..

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Conflict of interest

The authors have no conflicts of interest to declare.

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