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Response of circulating immune cells to major gunshot injury, haemorrhage, and acute surgery

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Summary

Purpose: The purpose of this study was to use an established porcine model to investigate the effects on immune function of severe gunshot injury.
Methods: Twelve pigs sustained two standardised rounds, one through right femur and one through left upper abdomen. First aid treatment and acute surgery was started immediately. Blood samples were drawn before shooting and after 75 min. Circulating neutrophils were isolated and reactive oxygen species (ROS) measured. Serum levels of tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-10 were determined at 0, 75 min, as well as 2 h after incubation with 1 μg/ml endotoxin in an ex vivo whole blood model.
Results: TNF-α, IL-1β, and IL-6 significantly increased at 75 min. ROS in circulating granulocytes tended to increase (NS). Incubation with endotoxin led to a more than 100-fold increase of TNF-α pre-trauma, compared to a three-fold increase post-trauma ($p < 0.0001$ between groups). A similar pattern was obtained for IL-1β, and IL-6. IL-10 was below detection in all samples. The granulocytes maintained their ability to react to the protein kinase C activator phorbol myristate acetate (PMA) after trauma.
Conclusion: Severe gunshot injury and peritraumatic stress rapidly activate circulating immune cells, but reduce their capacity to react to a subsequent challenge to endotoxin.

Introduction

Multiple organ failure is one of the leading causes of secondary death after major trauma.[8] Despite intensive research interest and a vastly increased insight in triggering mechanisms, no decisive breakthrough of either prophylaxis or treatment has

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materialised.[21] Major trauma with extensive destruction of tissues exposes the organism to an overwhelming inflammatory load that may easily exceed its clearing capacity.[24,28] It is generally believed that a minority of multi-traumatised patients react with an immediate and exaggerated autodestructive inflammatory response so severe that sequential organ dysfunction and early demise result. A number of those who recuperate from the initial insult will later succumb due to delayed immunosuppression and increased susceptibility to infection.[24,26]

Following major trauma, a considerable load of living microorganisms and bacterial products may enter the circulation.[18,25] Abdominal gunshot wounds penetrating the intestines inevitably lead to extensive soiling of the peritoneal cavity. Furthermore, shocked polytraumatised victims often develop dysfunction of the gastrointestinal mucosal barrier even without direct injury, and are later equally threatened by gut-derived virulent microbes and their toxins.[30] Indeed, bullet wounds in any location may introduce a considerable load of microorganisms into the body.[7] A moderate activation of the immune system is mandatory in order to eliminate these foreign invaders, and a smooth course of events hangs upon a complex and finely adjusted interplay between immune cells. Many aspects governing post-injury inflammation and anti-inflammation are still incompletely understood,[1] but release of proximal pro-inflammatory cytokines like tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) occupies a key position.[9,22]

It has long been known that animals pre-exposed to a low dose of bacterial endotoxin, react to a subsequent lethal injection with reduced mortality.[19] Diminished serum levels of TNF-α after re-exposure are a hallmark of endotoxin tolerance or hyporeactivity.[34] Later investigations suggest cross-reactivity with other exogenous stimuli, including trauma and surgical stress.[4,10,17] It is not clear whether this represents a beneficial adaptive response, or rather exposes the victims to an increased risk of developing sepsis and multiple organ dysfunction. Moreover, the initial injury may even represent a trigger for an enhanced response to a second hit.[16,20]

In an established and standardised porcine model of gunshot injury, we wanted to study the early influence of major trauma/haemorrhage/acute surgery on the activation of the inflammatory machinery. As an extension of the in vivo investigation, lipopolysaccharide (LPS) was added to an ex vivo whole blood model to simulate post-traumatic intrusion of endotoxin into the circulation.

Methods

Animal preparation

The study was conducted as part of a course in traumatology and war surgery, arranged by the Norwegian Armed Forces and the University of Oslo, as described previously.[12] Norwegian landrace pigs ($n = 12$) weighing 41.6 ± 1.6 kg were used for the study. The animals were handled according to the Animal Welfare Act and statutes from the Norwegian Ministry of Agriculture. To avoid any form of suffering they were fully anaesthetised at the start of the experiment and remained anaesthetised to the end. Medetomidin hydrochloride (CliniPharm, Switzerland) 0.06 mg/kg and tiletamin/zolazepam (Boehringer Ingelheim, Germany) 3 mg/kg intramuscularly were used for premedication, followed by butorphanol tartrate (American Home Products Corporation, USA) 0.2 mg/kg as an analgesic. After induction of anaesthesia, the pigs were orally intubated and hand ventilated with room air. As an additional precaution to assure complete freedom of pain, lidocaine 2% was given epidurally in the lumbosacral region. The dose was adjusted to body length. Adequacy of analgesia was tested with forceps pressure interdigitally. An ear vein cannula was used for infusion of fluids and injection of drugs. After sterile cut-down of the left groin, the femoral artery was isolated and cannulated with a Secalon-T subclavian catheter (Viggo, Denmark) for haemodynamic monitoring and blood sampling. Anaesthesia was continued with a mixture of fluothane, oxygen, and air.

Experimental procedure

While fully anaesthetised the animals were transported to a firing range. They were exposed to a standardised trauma: one rifle shot from a distance of 25 m hitting the right thigh, and one pistol shot from short distance against the upper abdomen. The entrance points were marked in advance. To avoid immediate demise care was taken to avoid direct injury to liver, spleen or large abdominal blood vessels. After shooting, first aid treatment was immediately started, including dressing of wounds, compression of external bleeding, control of ventilation, and intravenous fluid resuscitation. The pigs were then transported to a nearby field hospital where initial life saving surgery was performed by trained surgeons. Fluid was infused as needed to compensate for blood loss and to maintain a systolic blood pressure at or above 90 mmHg. Normal saline (0.9% NaCl) and Dextran 70/NaCl were used for resuscitation. Blood transfusion or blood substitutes