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Phagocytizing activity of PMN from severe trauma patients in different post-traumatic phases during the 10-days post-injury course☆

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ABSTRACT

Objective: Phagocytizing leukocytes (granulocytes and monocytes) play a fundamental role in immunological defense against pathogens and clearance of cellular debris after tissue injury due to trauma. According to the "two-hit hypothesis", phagocytes become primed due to/after trauma. Subsequently, a secondary stimulus may lead to their exaggerated response. This immune dysfunction can result in serious infectious complications, also depending on trauma injury pattern. Here, we investigated the phagocytizing capacity of leukocytes, and its correlation to trauma injury pattern.

Material/methods: Peripheral whole blood was taken daily from 29 severely injured trauma patients (TP, Injury Severity Score, ISS ≥ 28) for ten days (1–10) following admission to the emergency department (ED). Sixteen healthy volunteers served as controls (HV). Samples were incubated with opsonized *Staphylococcus aureus* labelled with pHrodo fluorescent reagent and the percentage of phagocytizing activity was assessed by flow cytometry. Abbreviated Injury Scales (AIS) ≥ 3 of head, chest and extremities were used for injury pattern analysis.

Results: Overall distribution of active phagocytes (out of 100% phagocytizing leukocytes) in TP included granulocytes with 28.6 ± 1.5% and monocytes with 59.3 ± 1.9% at ED, and was comparable to HV (31.5 ± 1.6% granulocytes and 60.1 ± 1.6% monocytes). The percentage of phagocytizing granulocytes increased significantly after D2 (39.1 ± 1.2%), while the percentage of phagocytizing monocytes (52.0 ± 1.2%, $p < 0.05$) decreased after D2. These changes persisted during the whole time course. Phagocytizing activity of granulocytes (27.9 ± 2.8%) and monocytes (55.2 ± 3.3%) was significantly decreased at ED compared to HV (42.4 ± 4.1% and 78.1 ± 3.1%, respectively). After D2 up to D10, phagocytizing activity was significantly enhanced in granulocytes. Phagocytizing activity of monocytes remained decreased on D1 and has risen continuously during the ten days time course to values comparable to HV. No significant differences in phagocytosis could be associated to certain injury pattern.

Conclusions: Our data demonstrate that the increasing percentage of phagocytizing granulocytes may indicate their enhanced mobilization out of bone marrow persisting until post-injury day 10. Furthermore, an initially decreased phagocytizing activity of granulocytes is strongly increased in the 10-days post-injury course. The altered activity of phagocytes due to injury could not be linked to any trauma injury pattern, and emerged rather as a general characteristic of phagocytes after severe trauma.

Abbreviations: AIS, abbreviated injury scale; CD, cluster of differentiation; CRP, C-reactive protein; D, day; Ctrl, control; DAMP, damage-associated molecular pattern; ED, emergency department; EDTA, ethylenediaminetetra-acetic acid; G-CSF, granulocyte-colony stimulating factor; HLA-DR, human leukocyte antigen-DR; HV, healthy volunteers; ICU, intensive care unit; IL, interleukin; ISS, injury severity score; LPS, lipopolysaccharide; MHC II, major histocompatibility complex; PAMP, pathogen-associated molecular pattern; PCT, procalcitonin; PLT, platelets; PMNL, polymorphonuclear leukocytes; PRR, pattern recognition receptors; SIRS, systemic inflammatory response syndrome; ROS, reactive oxygen species; TP, trauma patients.

☆ Parts of this study have been presented as meeting abstracts.

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1. Introduction

Trauma is (9%) one of the main causes of death worldwide, and its incidence is expected to rise significantly over the next years (Mathers et al., 2009; Peden and Hyder, 2002). Early post-injury death occurs primarily due to injuries of the central nervous system and/or haemorrhagic shock after significant blood loss. Late post-injury mortality is often caused by secondary complications, such as sepsis, acute respiratory distress syndrome (ARDS) or multiple organ dysfunction syndrome (MODS) (Osborn et al., 2004; Pfeifer et al., 2009; Probst et al., 2009a; Sauaia et al., 1995; van Wessem and Leenen, 2014).

As the so-called "first hit" severe trauma triggers an uncontrolled systemic inflammatory response syndrome (SIRS) due to tissue injury, which is primarily mediated by the innate immunity. Immediately after trauma both, endogenous damage-associated molecular patterns (DAMPs) *e.g.* heat shock proteins, high-mobility group box 1 (HMGB1) or defensins as well as pathogen-associated molecular patterns (PAMPs) activate phagocytes, which are the effector cells of the innate immune response (granulocytes and monocytes) (Giannoudis et al., 2010; Lenz et al., 2007; Seong and Matzinger, 2004; Wutzler et al., 2013; Zhang et al., 2010). Their activation induces the production and release of inflammatory cytokines, chemokines, but also the recruitment of immune cells to the infection site as well as the mobilization of young neutrophils (granulocytes) from the bone marrow (Nacionales et al., 2015; Riley and Rupert, 2015; Wutzler et al., 2013). SIRS is also characterized by an increased activity of polymorphonuclear neutrophils (PMNs) (Bhatia et al., 2005; Lenz et al., 2007). Phagocytosis by monocytes and PMNs plays a fundamental role in immunological defense strategies against microorganisms and/or pathogens, but also in clearance of cell components mostly resulting from cell death.

Thus, with regard to the "two-hit" hypothesis, trauma constitutes the "first hit" depending on injury severity and injury pattern. Trauma is assumed to initially prime the immune system, including phagocytes for subsequent "second hits" such as post-injury surgery or secondary exposure to pathogens (Gebhard and Huber-Lang, 2008; Pape et al., 2001; Visser et al., 2011; Wutzler et al., 2013). Subsequently, a "second hit" may augment the pro-inflammatory immune response and result in detrimental effects (Pape et al., 2001; Wutzler et al., 2013). Previous studies have identified an altered phagocytizing function of monocytes and neutrophils in several diseases (Engelich et al., 2001; Halle et al., 2015; Morris et al., 2005). With regard to clinical infectious complications *e.g.* sepsis, divergent results on phagocytizing activity of circulating neutrophils and monocytes have been reported (Hirsh et al., 2001; Spittler et al., 2000; Taneja et al., 2008; Xu et al., 2012). A reduced phagocytizing activity of neutrophils in the first 24 h of sepsis has been a negative predictor for survival (Danikas et al., 2008). Spittler et al. have shown that septic patients with increased phagocytizing rates of monocytes had worse outcome (Spittler et al., 2000). On the other hand, reduced phagocytizing activity of monocytes and neutrophils has been reported in post-traumatic or post-operative sepsis (Hirsh et al., 2001). Phagocytosis of PMNs after severe tissue injury has been increased at first, third and fourteenth day after trauma compared to controls (Pap et al., 2006). Xu et al. have identified genes for reduced phagocytosis of monocytes from patients with CARS, but no differences between healthy volunteers and patients with SIRS (Xu et al., 2012).

It is known that next to these inflammatory changes, both, the development of post-traumatic complications as well as the subsequent outcome depend on the injury pattern, trauma mechanism and injury severity (Rixen et al., 2001; Wutzler et al., 2013). Hence, trauma patients with head injuries, penetrating trauma and severe injuries show higher mortality rates (Baum et al., 2015; Rixen et al., 2001; Rowell et al., 2011). Severe traumatic brain injury has been associated with reduced inflammatory response (Lustenberger et al., 2016).

Thus, this study was performed in order to analyze the phagocytizing behaviour of monocytes and granulocytes in different post-traumatic phases during the ten post-injury days. Furthermore, the relevance of the trauma injury pattern for their phagocytizing capacity was determined.

2. Patients and methods

2.1. Ethics

This study was performed in the University Hospital Frankfurt of the Goethe-University with institutional ethics committee approval (312/10), in accordance with the Declaration of Helsinki and following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE)-guidelines (von Elm et al., 2008). All healthy volunteers as well as all enrolled patients have signed the written informed consent form themselves or written informed consent was obtained from the nominated legally authorized representative on the behalf of participants in accordance with ethical standards.

2.2. Study setting and population

Severely injured traumatized patients (TP) with an Injury Severity Score (ISS) $\geq$28 between 18 and 80 years were included at admittance to the emergency department (ED) of the University Hospital Frankfurt. Inclusion criteria consisted of a history of acute blunt or penetrating trauma. Patients with known pre-existing immunological disorders, HIV and Hepatitis, immune-suppressive and anti- coagulant medication, concomitant acute myocardial infarction, burns, thromboembolic events and/or lethal injury (24 h mortality) were excluded, resulting in a cohort of 29 patients fulfilling all criteria for the study. Sixteen healthy volunteers served as control.

2.3. Study protocol

Based on the Abbreviated Injury Scale (AIS) the Injury Severity Score (ISS) was determined (Baker et al., 1974). The ISS was calculated as the sum of the squares of the highest AIS code in each of the three most severely injured AIS body regions. All trauma patients were treated in the ED according to the Advanced Trauma Life Support (ATLS) standard and the polytrauma guidelines (Marzi, 2012). The diagnosis of pneumonia was given by pulmonary infiltrates on chest X-ray and at least positive blood culture or bronchial alveolar lavage and/or sputum culture (Bauer et al., 2005). Sepsis was defined by fullfilling the criteria for SIRS and a proven source of infection (Calandra et al., 2005). SIRS was existent by at least two of the following criteria: temperature > 38 °C or <36 °C; heart rate > 90 beats per minute; respiratory rate > 20 breaths per minute or arterial carbon dioxide tension (PaCO2) < 32 mmHg; and white blood cell count > 12.000 cells/mm^3 or <4000 cells/mm^3, or with >10% immature (band) forms.

2.4. Blood sampling

Upon arrival at the ED vital parameters were recorded. Blood samples were obtained from TP on admittance to the ED, and daily for 10 days following trauma. The first blood samples were obtained as early as possible together with the routine bloodwork after admission of the patient to the ED in ethylene-diaminetetraacetic acid (EDTA) tubes (Sarstedt, Nürmbrecht, Germany) and kept at