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Early Complementopathy after Multiple Injuries in Humans

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Abstract

After severe tissue injury, innate immunity mounts a robust systemic inflammatory response. However, little is known about the immediate impact of multiple trauma on early complement function in humans. In the present study we hypothesized that multiple trauma results in immediate activation, consumption and dysfunction of the complement cascade and that the resulting severe “complementopathy” may be associated with morbidity and mortality.

Therefore a prospective multicenter study with 25 healthy volunteers and 40 polytrauma patients (mean injury severity score [ISS] = 30.3 ± 2.9) was performed. After polytrauma serum was collected as early as possible at the scene, upon admission to the emergency room and 4, 12, 24, 120 and 240 hours post trauma and analysed for the complement profile. Complement hemolytic activity (CH-50) was massively reduced within the first 24 h after injury, recovered only 5 days after trauma and discriminated between lethal and non-lethal 28-day outcome. Serum levels of the complement activation products C3a and C5a were significantly elevated throughout the entire observation period and correlated with the severity of traumatic brain injury and survival. The soluble terminal complement complex SC5b-9 and mannose-binding lectin (MBL) showed a biphasic response after trauma. Key fluid phase inhibitors of complement, such as C4b-binding protein (C4BP) and factor I, were significantly diminished early after trauma.

The present data indicate an almost synchronically rapid activation and dysfunction of complement suggesting a trauma-induced “complementopathy” early after injury. These events may participate to the impairment of the innate immune response observed after severe trauma.

Keywords

multiple injuries; complement; C5a; C3a; MAC; neutrophils

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Introduction

The complement system represents the first line of defence against microorganisms and tissue damage by sensing, targeting and clearing pathogen- and danger-associated molecules (1,2). Complement activation products, such as C3a, C5a and the membrane attack complex (MAC) play a crucial role in the body's "danger management". However, there is a relative paucity of data on complement activation early after severe trauma. The few studies available merely describe posttraumatic systemic depletion of C3 and C5 with enhanced C3a/C3 ratios, in patients developing posttraumatic respiratory failure or multiple-organ dysfunction (3,4). The anaphylatoxin C5a evokes all classical signs of inflammation and primes the cellular immune defence (5). However, while beneficial in adequate amounts, excessive production of C5a paralyzes bactericidal neutrophil functions (6) by inducing intra- and intercellular danger signalling defects (7). C3a also exhibits multiple proinflammatory functions and exerts direct antimicrobial effects by disruption of bacterial plasma membranes (8). Further complement-mediated antimicrobial effects are provided by the MAC, which assembles a "pore" into damaged or infected cell membranes. Elevated levels of MAC in injured patients have previously been demonstrated (4). However, when excessively generated during systemic inflammation or following trauma, the MAC may become a sudden instrument of harm, targeting not only foreign organisms, but also host tissue. Therefore, several intrinsic complement inhibitors, such as C4b-binding protein (C4BP) and factor I, tightly control complement activation.

Clinically, early death after trauma is often caused by primary brain injury or extensive blood loss, while late mortality frequently develops due to the subsequent overshooting systemic inflammatory response and its associated immune- and multi-organ dysfunction (9). Experimental studies mimicking blunt chest trauma (10) or hemorrhagic shock (11,12) have suggested an important role of complement in the respective pathophysiology, but little is known about the immediate changes of complement or its fluid phase regulatory proteins after multiple trauma in humans. Therefore, the present complement analysis was performed in sera obtained from polytrauma patients as a function of time and compared to a healthy volunteer cohort. Sera were obtained immediately after severe trauma at the scene (mean 25 min after impact) and up to 240 h post trauma. We hypothesized that multiple injuries will induce rapid complement activation and consumption. In addition, we sought to associate the extent of trauma-induced complement changes with mortality of polytraumatized patients.

Material and Methods

Study Design

This was a dual-center, prospective, cohort study of multiple-injured patients approved by the Independent Ethics Committee of the University of Ulm (Approval # 117/97, 125/02). For the trauma cohort, 40 polytrauma patients admitted to the University Hospital Ulm or the Military Hospital Ulm (both trauma level I centers) via the medical helicopter rescue service were included over a time period of 26 months. Patients less than 18 years old or enrolled in other studies were excluded. Whole blood was drawn from trauma patients directly at the scene by the helicopter doctor, after admission to the emergency room, 4, 12, 24, 120 and 240 hours after admission and immediately put on ice. Serum was then separated from clotted blood and stored at -80°C in a freezer until analysis. The prospective data collection included patient demographics, the injury time, arrival time of the emergency doctor at the scene, injury mechanism and severity, injury pattern, initial injury severity score (ISS) and the Glasgow Coma Scale (GCS), prehospital fluid administration, time of admission to the ER, and hospitalization time. Patients were followed until hospital

discharge or death. The control cohort was represented by 25 healthy adult volunteers (mean age: 28 ± 3 years, 19 males, 6 females).

Characteristics of the Trauma Patients

The 40 polytrauma patients (36 males and 4 females, mean age: 31 ± 2 y, ranging from 18 to 72 y) were injured by vehicle accidents as a major trauma mechanism (22/40 car, 14/40 bicycle), two by high energy falls and two by others. Trauma patients displayed a mean ISS of 30.3 ± 2.9 and a mean Glasgow Coma Scale (GCS) at the scene of 9.1 ± 0.8 . The injury pattern was rather heterogeneous as reflected by the number of the injured organ systems involved (n/40) with their corresponding Abbreviated Injury Scale (AIS): 16/40 head = 3.3 ± 1.1 ; 24/40 thorax = 3.3 ± 0.3 ; 15/40 abdomen = 3.6 ± 1.4 ; 30/40 extremities = 3.2 ± 0.1 ; in 8 cases a spine injury was present. The overall mortality rate of the 28 d observation period was 8/40 (20%). The cause of death was in 5/8 cases the multiple injury pattern, whereas in two cases an extreme thoracic trauma (AIS thorax = 6) and in one case an abdominal trauma was the leading fatal cause (AIS abdomen = 5). The overall ISS of the non-surviving patients was 58.2 ± 8.2 (in detail: AIS head = 4.3 ± 1.1 ; AIS thorax = 4.8 ± 0.5 ; AIS abdomen = 3.8 ± 0.6 ; AIS extremities = 4.0 ± 1.0), the mean GCS of the deceased patients was 4.2 ± 0.8 and the mean time period after trauma till decease was $79 \text{ h} \pm 48 \text{ h}$, among those five patients did not survive the first day (ISS 61.4 ± 8.6). The i.v. fluid administration within the first hour after trauma included colloidal fluids $1936 \pm 196 \text{ ml}$ and saline $1400 \pm 124 \text{ ml}$. Sera were drawn from trauma patients directly at the scene (mean $25.1 \pm 1.4 \text{ min}$ after trauma), after admission to the emergency room (mean $71 \pm 5 \text{ min}$ after trauma), and 4, 12, 24, 120 and 240 hours after admission. The control cohort was represented by 25 healthy volunteers (mean age: 28 ± 3 years, 19 males, 6 females).

Reagents and Chemicals

Unless otherwise specified, reagents were purchased from Invitrogen (Grand Island, NY) and chemicals were purchased from Sigma-Aldrich Corporation (St. Louis, MO).

CH-50 Assay

The function of the complement system was assessed by measurement of the hemolytic serum activity. Briefly, sensitized sheep red blood cells (Colorado Serum Company, Denver, CO) were exposed at 37°C for 60 min to serial dilutions of serum samples in Tris-buffered saline (TBS), pH 7.35. The complement reaction was stopped by addition of 1 ml ice cold TBS (with 0.05% gelatin) followed by a centrifugation step ($2500 \times g$, 5 min). Absorbance of the supernatant fluid was determined at 541 nm and serum concentration inducing 50% of hemolysis (CH-50) was determined.

Measurement of C3a, C5a, and SC5b-9 by ELISA

The concentration of C3a and C5a was determined by ELISA analysis (Quidel Corporation, San Diego, CA and DRG Instruments GmbH, Marburg, Germany, respectively) according to the instructions of the manufacturer.

Soluble terminal complement complex SC5b-9 in serum was determined by the SC5b-9 ELISA kit (Quidel Corporation, San Diego, CA). To assess the maximal serum capacity to generate the SC5b-9, sera were *ex vivo* activated with zymosan (10 mg/ml serum) for 30 min at 37°C . After a centrifugation step, the supernatant fluids were diluted 1:3,000 and analyzed by the SC5b-9 ELISA kit. All ELISA assays were performed strictly according to the manufacturer's instructions.

Measurement of Mannose-Binding Lectin (MBL)

Serum levels of mannose-binding lectin (MBL) were measured as previously described in detail (13) by a low volume, mannan-based fluorochrome-linked immunoassay (FLISA) using an Odyssey and Aeries infrared imaging system (Li Cor Biosciences, Lincoln, Nebr). Briefly, MBL-dependent binding to mannan-coated plates was quantified to the level of C3 convertases in a single assay using a human MBL-deficient serum and a quality controlled purified MBL protein as an internal standard (Staten Serum Institute, Copenhagen, Denmark). This FLISA was capable to quantitatively assess the functional status of MBL.

Measurement of C4BP total, C4BP- β , and factor I by ELISA

The plates were coated overnight at 4°C with 5 μ g/mL of rabbit antibodies PK9008 or PK9205 (both generated in house) diluted in 75 mM sodium carbonate for C4BP and factor I detection, respectively, as described previously (14,15). After quenching for 1 h with 50 mM Tris-HCl, 0.15 M NaCl, 0.1% Tween, pH 7.5 (washing buffer) supplemented with 3% fish gelatin, the plates were incubated for 1 h with the serum. For detection of total C4BP and β -chain-positive C4BP, MK-104 (for detection of C4BP α -chain (C4BP total)) (15) and MK-2B (for detection of C4BP β -chain (C4BP- β)) (16) antibodies were used. Factor I was detected by mouse anti-human factor I (MRCOX21) at concentration of 5 μ g/mL and incubated for 1 h as described previously (17).

Goat anti-mouse antibody, conjugated with horseradish peroxidase (Dako Sweden AB, Stockholm, Sweden), was added and incubated for 1 h. The plates were developed using OPD (Dako Sweden AB, Stockholm, Sweden) as a substrate, and the absorbance at 490 nm was measured spectrophotometrically. The plates were washed four times after each antibody incubation. The antibodies were a kind gift of Prof. B. Dahlbäck (Lund University).

Protein Assay

For quantification of total protein in sera, a BCA Protein Assay Kit (Pierce, Rockford, IL) was used according to the manufacturer's instructions.

Statistical Analysis

All values were expressed as mean $\pm$ SEM. Data sets were analyzed with one-way ANOVA or one-way ANOVA on ranks where applicable; differences in the mean values among experimental groups were then compared using the Student-Newman-Keuls Test or Dunn's Test, respectively. For correlation analyses Pearson correlation coefficient was calculated inclusive the 95% confidence limits. Results were considered statistically significant when $P < 0.05$.

Results

Changes of complement hemolytic activity after trauma

Trauma-induced changes in complement function were assessed by hemolytic serum activity. As shown in Figure 1A, hemolytic complement activity was significantly reduced in trauma sera obtained at the scene. Four hours after trauma, complement hemolytic activity was virtually abolished. Five days post trauma CH-50 values gradually returned to ranges observed in healthy volunteers, and were found to be significantly elevated 10 d after trauma (Figure 1A). Figure 1B illustrates typical CH-50 titration curves in sera from healthy volunteers (mean 1:160). Hemolytic activity of sera obtained from polytrauma patients surviving at the scene revealed a significant reduction of complement-mediated hemolysis (mean 1:90), represented by a left-shift of the CH-50 curve. Scene non-survivors and ER

survivors displayed a further reduction of the mean CH-50 values. The lowest CH-50 values overall were observed in patients succumbing to their injuries in the ER (1:20).

Robust posttraumatic generation of the anaphylatoxin C3a

Serum concentrations of C3a were determined as a function of time after trauma. A robust increase of serum C3a levels was found at the scene (n=36), and C3a concentrations remained significantly elevated up to 10 d after injury (Figure 2A). To avoid misinterpretation of the determined anaphylatoxin concentrations by dilution effects of the early fluid reconstitution, data were correlated to the measured serum protein levels, as previously described (25). When C3a serum concentrations were evaluated for their association with the severity of traumatic brain injury, a negative correlation between the GCS score and C3a levels measured in the ER was determined (Pearson correlation coefficient: $r=-0.41$, $p=0.03$; 95% confidence limit: -0.67 till -0.05) (Figure 2B). Similar results were found when the GCS score was compared with C3a serum levels at the scene (Pearson correlation coefficient: $r=-0.68$, $p<0.001$; 95% confidence limit: -0.83 till -0.49; data not shown). Significantly higher serum concentrations of C3a were measured during the ER period in patients who later succumbed to multiple trauma (n=7) (Figure 2C). A weak, yet statistically insignificant correlation was found between ISS and C3a concentrations (data not shown).

Immediate production of C5a after multiple injuries

In the present analysis, significantly enhanced serum concentrations of C5a were detected at the scene. While levels remained elevated throughout the entire observation period, a second peak was noted >120 h after trauma (Figure 3A), when samples were compared to healthy volunteer sera. There was a positive correlation between C5a serum levels early after trauma and the extent of traumatic brain injury (represented by GCS) (Pearson correlation coefficient $r=-0.53$; $p<0.01$, 95% confidence limit: -0.74 to -0.24) (Figure 3B). However, no significant correlation was found between the GCS score and C5a concentrations measured in the ER. To some extent, C5a levels in sera obtained at the scene very early discriminated between later survivors and non-survivors of trauma (Figure 3C).

Early decrease in systemic mannose-binding lectin (MBL)

As depicted in Figure 4, within the first 24 h after multiple injuries there was a significant drop in MBL serum levels with a nadir at 12 h post trauma, when virtually no MBL was detected. Beyond 5 days, the MBL levels in trauma survivors were rising again more than 3-fold in comparison to the healthy control subgroup.

Formation of the soluble terminal complement complex SC5b-9 after multiple injuries

Untreated serum of polytrauma patients exhibited a biphasic SC5b-9 response after polytrauma. A robust increase of soluble C5b-9 was found immediately after trauma (at the scene), mirroring the decreased early posttraumatic complement haemolytic function. Between 4 – 24 h post injury, only trace amounts of SC5b-9 were observed, comparable to serum levels detected in healthy volunteers (Figure 5A). Beyond 5 d after injury, serum levels of soluble C5b-9 exhibited a second peak. Serum concentrations of SC5b-9 failed to correlate with the ISS and failed to discriminate between survivors and non-survivors. *Ex vivo* activation of serum with zymosan was performed to evaluate the remaining capacity of serum to assemble SC5b-9 after polytrauma in comparison to healthy volunteers. As depicted in Figure 5B, zymosan-activation of volunteer sera resulted in a robust SC5b-9 generation while the capacity of polytrauma sera to generate SC5b-9 was significantly reduced immediately after injury in comparison to healthy volunteers. However, during the first 24 h after injury, serum capacity for SC5b-9 formation showed some inconsistencies

but was mainly reduced by at least one third in comparison to the normal serum capacity. Zymosan-activated serum obtained 5 or 10 d after injury revealed a completely restored SC5b-9 production (Figure 5B).

Changes of fluid phase complement inhibitors in serum after multiple trauma

The complement cascade and its activation are tightly controlled by various fluid phase complement inhibitors. Thus, the most potent soluble inhibitors were investigated to determine if early posttraumatic complement activation was due to a dysfunctional complement inhibition. At the scene, serum concentrations of C4BP and factor I were both significantly decreased when compared to levels observed in healthy volunteers (Figure 6A-C). The serum protein levels of C4BP total, C4BP- β , as well as factor I were reduced by more than 50% 4 h after trauma and restored only 5 d after trauma. Beyond 5 d, serum concentrations of all studied complement inhibitors were increased in comparison to levels seen in healthy volunteers.

Discussion

Severe trauma is known to massively challenge various host defense systems. Among the proteolytic systems (18), the complement system has been implicated to sense, translate and clear danger signals from damaged tissues (1,2). If the “danger clearance” by the innate immune system is overburdened, a dysfunctional immune response may result and culminate in a systemic inflammatory response and even multi-organ dysfunction (9,19). A vast amount of trauma studies have investigated the danger response and therapeutic consequences after admission to the hospital, but only a limited number of studies have focused on the immediate immunological changes occurring within the initial “golden hour” after trauma (4,19-22).

The present study provides evidence of complement activation and consumption instantly after severe trauma. Early global complement dysfunction occurred, reflected by significantly reduced CH-50 values within the first hour post trauma, which was somehow associated with an early death after trauma (Figure 1). The observed rapid complement dysfunction was paralleled by instant serum increases of C3a, C5a and SC5b-9, all of which were already enhanced at the scene. Since any volume reconstitution therapy has a significant impact on the interpretation of biochemical data (23), all values of the complement activation products were adjusted to the serum protein levels to prevent misinterpretation by dilution effects, except for the CH-50 values to avoid assay interferences. However, it cannot be excluded, that the application of blood products (fresh frozen plasma, red blood cells) or massive transfusions of saline or hydroxyethyl starch have some influence on the complement status of patients.

Present findings are strongly supported by a recent study describing complement activation via the alternative pathway in over 200 patients with major (and often single) trauma and much lower median ISS (ISS=17, range 9-26) (20).

Several previous studies suggested that increased concentrations of C3a in serum or bronchoalveolar fluids within the first 24 h after trauma might predict the posttraumatic development of adult respiratory distress syndrome (ARDS) (21,24-26) or multiple organ failure (MOF) (24,27). However, despite detection of increased serum anaphylatoxin concentrations post trauma, clinical studies failed to confirm C3a or other complement fragments as specific markers for ARDS or MOF (28,29). In the present study, no correlation between initial lung tissue injury (determined by the lung Abbreviated-Injury-Scale [AIS]) and observed serum C3a levels was found. In contrast, C3a (Figure 2B) and C5a (Figure 3B) serum concentrations were associated with the severity of traumatic brain

injury (Figure 2B) as assessed by the GCS during the ER phase or at scene, respectively. Subgroup analysis of the two appearing populations at very low GCS (< 6) in Figure 3B did reveal an enhanced mortality ratio of 4/7 in the upper group versus 1/7 in the lower group. It is tempting to speculate, that low initial GCS in combination with high C5a serum levels could be associated with adverse outcome. These findings are supported by experimental data illustrating significantly enhanced anaphylatoxin serum concentrations up to 7 days after traumatic brain injury (30). However, association-cause relationships between complement activation products and GCS cannot be drawn so far, based on a lack of experimental and clinical data.

More than two decades ago, it has been shown that multiple trauma may result in chemotactic desensitization of neutrophils to C5a/C5adesarg (25). In present study, an immediate serum peak of C5a was found at the scene (Figure 3), which was associated with an early peak of C5a-dependent chemotactic serum activity for normal neutrophils (data not shown). Once generated, C5a is well known to be capable of inducing all classical signs of inflammation. However, previous studies found clear evidence of C5 depletion after extensive injuries (31) but failed to detect elevated C5a concentrations in plasma (32). Technical detection limits in the past, different time points of blood sampling (i.e. well after hospital admission) or binding of the majority of C5a to the ubiquitous abundantly expressed C5aR may account for these differences. Nevertheless, the exact mechanisms of C5a generation during the early trauma response remain to be determined. It is unlikely that the alternative pathway of complement activation is solely responsible for the observed instant peak. Activation and subsequent depletion of the MBL-dependent portion of the lectin-pathway might also take place during the early course after trauma as suggested by present data (Figure 4), and supported by recent findings that demonstrated a correlation between decreasing plasma MBL levels and increasing injury severity (20). However, other proteolytic systems, such as extensively activated coagulation factors, may provide a direct cleavage of C3 and C5 and help to generate C3a and C5a directly after major trauma (20,33,34). Furthermore trauma-induced ischemia might contribute to early complement activation by ischemia specific neo-antigen expression leading to classical complement activation (35). Thus, trauma-induced ischemia and the activation of catalytically active serine protease systems (such as the coagulation cascade) may extensively cross-talk with the complement system and amplify the generation of complement activation products, which vice versa may contribute to further tissue damage.

Upon assembly on the cell surface, the MAC lyses invading, infected or damaged cells and thereby plays an important role in host defense. Its soluble form, SC5b-9, has previously been considered as a non-active form of MAC. This notion has recently been challenged by a publication demonstrating that SC5b-9 contributes to inflammation by increasing vascular permeability (36). Our present data provide evidence for a biphasic SC5b-9 response with a first peak immediately after trauma, followed by a return to almost background levels 4-12 hours later, and a second peak beyond 5 days post injury. Especially hypoperfusion as mirrored by enhanced base deficit values has been shown to correlate with enhanced SC5b-9 generation (20), which could contribute to the early peak in SC5b-9 until a sufficient perfusion status can be achieved after 4-12 hours. In contrast to the SC5b-9 response the serum levels of C3a and C5a remained elevated during the whole observation period after trauma. This might be partially due to down-regulation of complement-regulatory proteins on various cascade levels (37). In addition, the described “serine protease system” crossroads between the complement and coagulation system (33,34) may lead to direct generation of the anaphylatoxins C3a and C5a but not to the same extent of MAC, resulting in the different posttraumatic activation kinetics of C3a, C5a, MAC and SC5b-9, respectively. Furthermore, neutrophils and probably other cell types may internalize their

anaphylatoxin receptors upon trauma stimulation allowing sustained levels of C3a and C5a. However this is still rather speculative and warrants further investigation.

In accordance with our findings, an increase of circulating MAC has been described 90 min after multiple trauma (3). Interestingly, these changes were described exclusively in patients sustaining thoracic injuries (3). In contrast, in the present polytrauma cohort, 60% of patients suffered thoracic injuries, but circulating MAC levels failed to correlate with the lung AIS (data not shown). However, there is some evidence of dysfunctional MAC generation after trauma as robust *ex vivo* stimulation of complement by zymosan resulted in a reduced maximal production of MAC.

Our study has some shortcomings. First, a much larger number of patients will have to be enrolled to determine with ample statistical power, whether complement activation products represent valuable predictors for trauma severity or outcome. Serum levels of complement anaphylatoxins may closely reflect the inflammatory state of the patient and may then aid the decision-making for clinical management of this challenging patient population. Second, we have followed both patient populations for 10 d after injury. Changes in complement activation, consumption and dysfunction may occur even after 240 h and therefore may not have been detected. Large prospective and randomized studies are needed to address these pressing issues.

In present study, we furthermore sought to determine, whether the proposed trauma-induced complement changes were paralleled by dysfunction of key fluid phase complement inhibitors, such as C4BP and factor I. Serum levels of both C4BP isoforms and factor I were significantly reduced early after trauma, indicating a dysregulation of intrinsic complement regulators immediately after severe trauma. C4BP has recently been shown to directly interact with components of the extracellular matrix (38), which are abundantly released after severe tissue trauma. Thus, multiple trauma may lead not only to extensive activation of proteolytic systems (21,39) but also to disturbance of the “delicate balance” between complement activation and inhibition (40). However, there was no correlation between the reduced levels of fluid phase inhibitory proteins and the outcome of polytrauma patients. In this regard, we have recently found a significant dysregulation of membrane-bound key complement-regulatory proteins on leukocytes from multiple-injured patients (37). Together with the exogenous complement activation pathways (e.g. clotting and the fibrinolysis cascade), the simultaneous existence of complement activation, depletion and dysfunction in association with a significant disturbance of complement inhibitors (37) could be termed as a post-traumatic “complementopathy”, in analogy to the post-traumatic “coagulopathy” (41) with activation, depletion and dysfunction of coagulation factors and their regulators (31,42).

Conclusion

In summary, the present data provide evidence of a “complementopathy” in polytrauma patients immediately after the trauma impact, which may affect the post-traumatic immunosuppression and therefore the outcome of the clinical course. Since the complement system seems to play a decisive role in the host’s molecular “danger management” after severe trauma, we call for future clinical studies to evaluate whether immunomodulation of the trauma-induced complement response may represent a promising future therapeutic strategy following multiple injuries.

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Figure 1.

(A) Complement function after multiple-injury as assessed by hemolytic serum activity (CH-50) determined from either healthy volunteers ($n=25$) or polytraumatized patients ($n=40$) with a mean ISS of 30.3 ± 2.9 at several time points after injury. * $P<0.05$, ** $P<0.01$ between trauma group and healthy volunteers. (B) Comparison of complement hemolytic serum activity between healthy volunteers, survivors and non-survivors at the scene and at admission to the emergency room (ER).

Figure 2.

(A) Generation of C3a as a function of time after polytrauma. * $P < 0.05$, ** $P < 0.01$ between trauma group and healthy volunteers. (B) Inverse correlation between serum concentration of C3a in the emergency room (ER) and initial severity of traumatic brain injury as assessed by the Glasgow Coma Scale (GCS). (C) Subgroup analysis of serum C3a concentrations at the scene or at admission to the emergency room (ER) obtained from healthy volunteers, survivors and non-survivors.

Figure 3.

(A) Trauma-induced generation of the anaphylatoxin C5a in serum of polytraumatized patients (n=40) versus healthy controls (n=25). * $P < 0.05$, ** $P < 0.01$ versus healthy volunteers. (B) Inverse correlation of C5a serum concentration at the scene (25.1 ± 1.4 min after injury) and initial GCS reflecting primary traumatic brain injury. (C) Differences in C5a concentrations in serum obtained from polytrauma survivors and non-survivors early after injury.

Figure 4. Serum MBL levels as a function of time after polytrauma in similar samples. * $P < 0.05$, ** $P < 0.01$ between trauma group and healthy volunteers.

Figure 5.

(A) Concentration of the soluble terminal complement complex C5b-9 (SC5b-9) in otherwise non-activated serum from healthy volunteers or polytraumatized patients at different time points after injury. (B) Measurement of SC5b-9 formation in *ex vivo* zymosan (10 mg/ml)-activated serum obtained before from either healthy controls or multiple-injured patients at various time-points after trauma. * $P < 0.05$, ** $P < 0.01$ versus healthy volunteers.

Figure 6. Assessment of fluid phase complement inhibitors early after trauma. Measurement of (A) C4BP-total, (B) C4BP-β serum concentrations from healthy volunteers or multiple-injured patients as a function of time after trauma. (C) Measurement of factor I concentrations in serum of healthy volunteers vs. multiple-injured patients at several time points post trauma. * $P < 0.05$, ** $P < 0.01$ between trauma group and healthy volunteers.