Sepsis and SIRS are affections with major alterations in inflammatory activity. The impact of prostaglandins (PG) and leukotrienes (LT) produced from white blood cells (WBC) in this context is not completely understood. Thirty nine patients with sepsis or SIRS were investigated in comparison to 10 healthy controls. WBC were collected and separately exposed to arachidonic acid (AA) or to nothing else. After centrifugation, the generated PGE$_2$ and LTC$_{4}$ with or without stimulation were measured in the supernatant. LT-levels were significantly higher during sepsis/SIRS than in controls whereas PG-levels of patients were decreased to those of controls in basic condition. The relation between the level with and without stimulation showed a significant higher ratio in PG in contrast to LTs. The survivor’s ratio in LT levels was significantly higher than that of non-survivors, which did not differ from controls. Generation of LT from WBC is enhanced during sepsis/SIRS, but LT generation after stimulation only in survivors but not in non-survivors. This inability of WBC to generate LT during sepsis in non-survivors could be predictive regarding the outcome of sepsis/SIRS and may be part of the “immunoparalysis” seen during sepsis in association with bad outcome.

Key words: eicosanoids, prostaglandins, leukotrienes, Systemic Inflammatory Response Syndrome (SIRS), sepsis, inflammatory response

INTRODUCTION

Sepsis, a life-threatening disorder that arises through the body’s response to infection, remains a major challenge in medicine. It represents the leading cause of death in critically ill patients in Europe and the United States and its incidence is increasing worldwide (1).
Although many data have been accumulated to understand the underlying pathogenetic mechanisms in bacterial-host interactions and the consequent inflammatory response, there is still no concept covering all peculiarities. Initially, spreading of bacteria has been blamed for the generalizing systemic inflammation while nowadays the suggestion of an uncontrolled regulation of the inflammatory response is taking over (2). A consensus conference defined sepsis as the “systemic inflammatory response syndrome (SIRS)” that occurs during infection (3). Uncontrolled liberation of numerous proinflammatory mediators certainly seems to play a key role in the pathogenesis of sepsis and SIRS, sometimes described as a cytokine storm (4). TNF-α and IL-6 are the first to increase to extreme high levels only minutes after exposition with endotoxin or after trauma. Although these cytokines are considered to be culprits, they also may have beneficial effects in sepsis. In this context, numerous trials of agents that block the inflammatory cascade failed to demonstrate any benefit in patients with sepsis. One reason for the failure of anti-inflammatory strategies in patients with sepsis may be a change in the syndrome over time. As sepsis persists, there is a shift toward an anti-inflammatory immunosuppressive state (5). This Compensatory Anti-inflammatory Response Syndrome (CARS) is associated with a kind of “immunoparalysis” (6), anergy (7), and even death of immune cells, characterized with an inability to clear infection and a predisposition to nosocomial infections.

Besides cytokines eicosanoids have also been suggested to play a key role in pathophysiology of sepsis and SIRS (8). Proinflammatory cytokines, including IL-1, TNF-α and IL-6 also trigger the release of prostaglandins, leukotrienes and PAF from various cell types (2). The eicosanoids PG and LT are well known to cause inflammation, pain and shift of thrombocytes. With regards to these eicosanoids mostly levels in the blood have been explored but an analysis of cellular synthesis and metabolism of prostaglandins and leukotrienes is missing.

Especially the functional “capacity of cells” to produce eicosanoids seems to play a major role in the complexity of sepsis (9).

To address these questions, we quantified the concentration of prostaglandin E₂ (PGE₂) and peptide-leukotriene synthesis and release in vitro by peripheral blood cells at base line and upon experimentally induction by addition of exogenous substrate. This enabled us to study the formation and metabolism of these arachidonic acid derived substances.

MATERIAL AND METHODS

Study population

Thirty-nine patients (eight female, mean 74.91 years and 31 males, mean 65.83 years) suffering from SIRS or sepsis in accordance with the consensus criteria (3) and Apache II-Score > 20 (10) were investigated. Patients taking drugs interfering with the metabolism of eicosanoids (e.g. high-dose corticosteroids, NSAIDs, cytokines, leukotriene-antagonists) were excluded as well as those
close to death, missing chances to survive or those with a history of intolerance to COX-inhibitors. Three packs of thrombocytes or erythrocytes were allowed at maximum.

Ten healthy individuals (3 female, mean 38.7 years, 7 males, mean 37.7 years) served as controls. No regular medication other than oral contraceptives was taken.

The study was performed in accordance with the ethical standards of the Helsinki Declaration 1975 (revised 1983). The protocol of the study was approved by Institutional Ethical Committee of Erlangen-Nuremberg University. Patients gave their written informed consent before participating in this study.

**Functional-Eicosanoid Testing using living peripheral blood cells**

Venous blood (5ml) was collected using S-monovette® (Sarstedt, Hannover, Germany), including heparin to prevent clotting. All samples were anonymous. Peripheral blood cells were processed as previously published (11) with slight modification using LiPiDoC®-SIRS (SIAT, Bad Essen, Germany). Briefly, collected cells were diluted in vitro using cell incubation buffer. Thereafter they were incubated with diluents, arachidonic acid, or acetylsalicylic acid at room temperature for 20 minutes. Reaction was stopped by storing the samples at -20°C for up to six weeks until further processing for analysis of eicosanoids using competitive enzyme-immunoassays (EIA). For control experiments parallel samples were prepared and processed as described. But before storage at -20°C samples were centrifuged (900g, 4°C, 7min.), supernatant was collected in separate tubes. Integrity of centrifuged cells was checked microscopically by trypan-blue exclusion test and live-dead test. The live-dead test was performed according to the instructions of the manufacture (Molecular Probes, Göttingen, Germany). There were neither conspicuous results concerning cell morphology nor cell integrity comparing blood samples stored immediately upon incubation and those of separate supernatant. The same was true for measurement of eicosanoids measured in supernatant of the samples.

**Measurement of eicosanoids**

The stored samples were defrosted and centrifuged (900g, 4°C, 7 min). Thereafter, aliquots of the supernatants were transferred to of highly specific and sensitive competitive PGE₂- or pLT- EIA plates (SPI-bio, Paris, France / SIAT, Bad Essen, Germany), which was specifically produced and validated for use in combination with LiPiDoc®- kits. The LiPiDoc®- kits were performed according to instructions of the manufacture. In brief, standards and samples were analysed in duplicates in parallel for PGE₂ and pLT. Results are presented as mean±SEM in pg/ml, as ratio by dividing the levels of the different group by the level of the controls or as stimulation index by dividing the level upon stimulation by the basic level within the group.

**Statistical analysis**

Values are presented as the mean. Statistical analysis was performed by t-Test of Satterthwaite. The significance level was at least p<0.05.

**RESULTS**

**Septic patients vs. healthy controls**

The Eicosanoid-pattern of patients suffering from sepsis/SIRS was different from the pattern of healthy individuals (healthy individuals set as 100%). This
was obvious regarding the absolute values of prostaglandins and leukotrienes. Especially the lower basic levels of PG (70.7% from 100%; 93.5 pg/ml in sepsis to 131.9 pg/ml in controls) in patients with SIRS or sepsis were significant (p<0.001). However, the elevation of PG levels upon stimulation with arachidonic acid was not significantly different from healthy controls (120.6% from 100%, 167.5 pg/ml in sepsis after AA to 138.9 pg/ml in controls after AA). In contrast basic LT of patients during SIRS or sepsis were elevated (146.3% to 100%; 180.1 pg/ml in sepsis to 114.9 pg/ml in controls, p<0.001) in relation to healthy individuals as well as the levels upon stimulation (170.9% to 100%; 247.9 pg/ml in sepsis after AA to 129.9 pg/ml in controls after AA, p<0.001) These findings are illustrated in Fig. 1. By normalization to the controls these findings are clarified (Fig. 2).

To focus on the development of eicosanoid levels under stimulation, the relation between the level under stimulation and the basic level was calculated basing upon the absolute values. A significantly higher ratio in PG (1.71±0.85, compared to 1.31±0.54; p<0.05) was found in contrast to a non significant change in LTs levels (Fig. 3).

**Survivors vs. non-survivors in septic patients**

Basic PG levels and PG levels upon stimulation as well as basic LT levels and LT levels upon stimulation showed no difference between survivors and non-

![Fig. 1. Absolute amount of PG and LT in patients and controls](image-url)
survivors. When the stimulation index was calculated a similar elevated PG ratio in survivors compared to non-survivors was found. However, a significant

Fig. 2. Ratio of quantity during sepsis to controls concerning Prostaglandin E₂ (PGE₂) and Leukotrienes (LT) before and after stimulation with arachidonic acid (AA)

Fig. 3. Stimulation index calculated by basic and AA-induced synthesis of PG and LT from WBC of patients during sepsis/SIRS and controls
difference could be found in the LT ratio: the survivor’s ratio showed to be significantly higher (1.52±0.66 compared to 1.22±0.26, p<0.05) than in controls in contrast to the non-survivor group, which does not differ from the controls (Fig. 4).

**DISCUSSION**

In our study in septic patients compared to healthy controls we found a different pattern of eicosanoid levels and the capability of white blood cells of these patients to respond to stimulation with AA in eicosanoid production. PG basic levels in sepsis were significantly decreased but isolated white blood cells were able to respond to stimulation with AA. In contrast, LT levels were already elevated but white blood cells were able to increase LT production after stimulation with AA only in patients which survived the septic state but not in the non-surviving group.

Lipid metabolism in sepsis is known to be excessive (450% compared to normal) like the sepsis metabolism in general (12). This is in consens with our results revealing an increased stimulation of PG in isolated WBC. Therefore eicosanoids seem to play a key role in the hyperinflammatory state in sepsis. This is demonstrated by a huge metaanalysis studying 18 randomised controlled studies (13) showing a shorter ICU duration and less infectious complications in
critical ill patients using γ-3-FA (fatty acid) enriched infusion with its significantly less biological active metabolites and subsequent reduced cytokine (TNF, IL-1β, II-2, II-6) production (14). However, other clinical trials performed to test the hypothesis that blocking eicosanoid synthesis with ibuprofen can improve survival in sepsis did not show any survival benefit (15, 16). In deed, even former investigations showed that prostaglandins PGE₁, PGE₂ and PGI₂ administration may even have some beneficial effects in response to sepsis (17).

One reason for the failure of these “anti-inflammatory” strategies in patients with sepsis may be a change in the syndrome over time. As sepsis persists, there is a shift toward an anti-inflammatory immunosuppressive state (2). This Compensatory Anti-inflammatory Response Syndrome (CARS) is associated with “immunoparalysis” (18, 19), anergy (9), and even death of immune cells, characterized with an inability to clear infection and a predisposition to nosocomial infections.

The lower basic levels of PG and most importantly the inability of the septic WBC of the non-surviving patients to further increase LT production may be part of an already existing hypoinflammation ongoing in sepsis/SIRS. Low cellular energy state as mentioned by Morlion (9) may be one explanation for this decreased synthesis capability under stimulation by AA in these cells of the non-survivors.

In addition LTB₄ up-regulates components of the immune response, such as the production of IL-6 by human monocytes (8), modulates leukocytes and supports adhesion to the endothelium (2). Septic patients present suppressed neutrophil chemotactic response to leukotriene B₄ stimuli compared with healthy controls (20). In consens with our results Morlion and coworkers (9) found that the survivors of sepsis showed significant higher levels in synthesis capacity than the non-survivors or healthy individuals, which did not differ. Activated leukocytes migrate from bloodstream to inflammatory tissues (21) and the change of surface markers leads to reduced HLA-DR expression (22) causing monocytes from patients with severe infections to lose their capacity to mount a pro-inflammatory response after stimulation with bacterial products compared to healthy subjects. Further studies are necessary to clarify these interpretations, especially with regard to the time frame in the septic disease.

The amount of WBC in the surviving group (15.58*10⁶/ml) compared to the non-survivors (15.54*10⁶/ml) show no difference as well as the platelets (220.10*10⁶/ml to 216.53*10⁶/ml).

Potential factors which increase the generation of LT like COX-inhibitors or dialysis are not responsible since both groups were treated in the same way. This means that all known confounding factors which have been taken into account are excluded in our study.

Most interesting is a significant difference between surviving patients and non-survivors regarding LT but not to PG. So it has to be speculated that a peculiar mechanism or an overridden regulation is accountable for this
phenomenon. Fumeaux’s postulation of a dysregulation of leukocyte activities as genesis of sepsis (23) may be right and functional eicosanoid testing like performed in our study might turn out as a tool to find out people at risk for a worse outcome. Whether this is indicative as a marker for the bad outcome or whether this even causes the bad outcome remains unanswered.

In conclusion, the generation of LT from WBC seems to be enhanced during sepsis/SIRS, but LT generation after stimulation only in survivors but not in non-survivors. This inability of WBC to further enhance LT production during the course of sepsis in non-survivors could be explained as part of the “immunoparalysis” seen during sepsis in association with bad outcome. The functional analysis of LT and PG during sepsis/SIRS might turn out to be a suitable approach for the estimation of the future course of the disease. Further studies have to show whether surviving patients after a long period still render this peculiar feature. This would enable to detect individuals at risk to experience sepsis/SIRS.

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