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Phospholipase A in Acute Lung Injury after Trauma and Sepsis: Its Relation to the Inflammatory Mediators PMN-Elastase, C3a, and Neopterin

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Summary. Inflammatory mediators involved in the pathogenesis of the adult respiratory distress syndrome (ARDS) are products of the humoral cascade systems like the complement cascade and substances released from neutrophil granulocytes and macrophages like proteases, O₂-radicals and arachidonate products. Phospholipase A₂ (PLA) was shown by Vadas et al. to be correlated with circulatory shock in the sepsis syndrome, the probably most important underlying disease of ARDS. In a clinical study in 48 patients at risk for ARDS after trauma and sepsis we found plasma PLA elevated (52 ± 5 U/l) in sepsis, with a positive correlation to the complement split product C3a ($r=0,42$, $p<0,01$) and neopterin ($r=0,49$, $p<0,05$), which serves as a marker of macrophage stimulation. Elastase- α 1PI and C3a showed higher plasma levels in patients with ARDS compared with non-ARDS patients, whereas the neopterin and PLA concentrations were not different with regard to ARDS. The relation between PLA and neopterin shown in the study is consistent with the possibility of macrophages being a source of the plasma PLA, as reported in experimental studies.

Key words: Phospholipase A – Lung injury – PMN-elastase – C3a – Neopterin

Introduction

Polymorphonuclear neutophilic leukocytes (PMNs) play an important part in the pathogenesis of acute lung injury (ARDS), especially in the case of its systemic etiology like trauma and sepsis [14, 19]. Such serious events are followed by widespread activation of the humoral cascade systems including the coagulation cascade, the fibrinolytic system, and the kallikrein-kinin system. There is also evi-

dence of a direct stimulation of the complement cascade on the alternative pathway [18]. Activated PMNs aggregate in the small vessels of the lung damaging the endothelial barrier by liberating toxic lysosomal substances (proteases, O₂ radicals, and arachidonate products). This barrier destruction is followed by a protein-rich interstitial and intra-alveolar lung edema, which is a characteristic of the adult respiratory distress syndrome. Similarly macrophages from the tissues as well as from the circulation may be activated and induced to liberate proteolytic or oxidative substances [3, 16]. Phospholipase A (PLA) may be involved in the pathogenetic process by generating eicosanoids. Vadas et al. have reported a correlation of circulating phospholipase A₂ levels with septic shock. In addition, a higher ARDS incidence has been postulated at high PLA values [20]. Therefore in the case of the underlying diseases of ARDS, such as trauma and sepsis, there should be a correlation of plasma PLA to inflammatory mediators associated with trauma and sepsis, including elastase from PMNs, complement split products (C3a), and activation of macrophages indicated by neopterin.

Material and Methods

In 48 patients suffering from trauma and sepsis we measured the levels of PMN-elastase (elastase- α 1PI) and C3a every 6 h and 12 h respectively, over a period of up to 14 days. Phospholipase A was measured in 35 patients and neopterin in 16 patients on a 12 h basis. The trauma group consisted of 21 patients, the sepsis group of 27 patients. Phospholipase A activity was measured in serum by a modification of the method published by Hoffmann et al. [6], using the commercially available Nefa-Test (Waco Chemicals, Neuss) and lyophi-

lized substrate from (gift) the Boehringer Research Laboratories (Tutzing). PMN-elastase was measured in citrate plasma as a complex with its physiological inhibitor alpha1-protease inhibitor (El- α 1PI) by an ELISA test system from Merck, Darmstadt. C3a (EDTA plasma) and neopterin levels (serum) were evaluated by double-antibody radioimmunoassays (J^{125}) from Amersham, Braunschweig, and Henning, Berlin, respectively. The normal ranges of the inflammatory mediators are assumed to be lower than 150 ng/ml for elastase, 50–100 ng/ml for C3a, below 10–20 nmol/l for neopterin, and 10–20 U/l for phospholipase A. The blood was carefully drawn from an indwelling central venous catheter (with 10 ml blood rejected first), centrifuged immediately, and plasma or serum was frozen at -70°C until assay.

Sepsis was defined by the occurrence of the sepsis syndrome with at least two of its unspecific signs: leukocytosis $>12,000$ or $<4,000$, thrombocyte counts $<100,000$ or a fall of more than 40%, body temperature $\geq 38.5^{\circ}\text{C}$, and signs of shock. The proof of a bacterial infection by positive blood culture or an infectious focus (abscesses, etc.) was mandatory [9]. All patients in the trauma group had a polytrauma with an injury of the abdominal or thoracic cavity or multiple bone fractures. The polytrauma score (PTS [12]) was greater than 15 with an average value of 32.

ARDS was defined as acute lung injury, occurring after the typical triggering events (trauma, sepsis), showing a marked decrease in paO_2 ($\text{paO}_2 < 50 \text{ mm Hg}$ at $\text{FiO}_2 0.21$ or $\text{paO}_2 < 75 \text{ mm Hg}$ at $\text{FiO}_2 > 0.4$ and $\text{PEEP} > 5 \text{ cmH}_2\text{O}$) and bilateral pulmonary infiltrates or signs of lung edema in the chest X-ray [1, 13]. Excluding criteria were the prior existence of lung disease and pulmonary edema caused by congestion (pulmonary capillary wedge pressure $> 18 \text{ mm Hg}$).

The period of day 1 to 4 was assumed to be representative of the early time after admission and was used to compare the patient groups. Statistics were performed taking the Mann-Whitney U-test for unpaired samples using the SPSS/PS⁺ computer program.

Results

There was a markable difference in the mean values and the time course of PLA between trauma and sepsis (Fig. 1). On admission the plasma values in the trauma group were only slightly elevated to a level of $19 \pm 3 \text{ U/l}$ (mean \pm SEM), compared with $52 \pm 5 \text{ U/l}$ (mean \pm SEM) in the sepsis group. In the course of the disease, posttraumatic values ascended to about 50 U/l coincident with the occurrence of infectious complications, whereas in the sepsis group the concentrations declined, but remained elevated for the period investigated. In contrast, plasma concentrations of PMN-elastase (El- α 1PI) were highly elevated both in trauma and sepsis ($486 \pm 28 \text{ ng/ml}$ (mean \pm SEM) and $539 \pm 38 \text{ ng/ml}$ (mean \pm SEM) respectively; Fig. 2).

The time course of the plasma values of the complement split product C3a was different in trauma and in sepsis. It was similar to that of PLA, with lower mean concentrations at the beginning after trauma ($430 \pm 30 \text{ ng/ml}$, mean \pm SEM) and high values in sepsis ($919 \pm 58 \text{ ng/ml}$, mean \pm SEM; Fig. 3).

Comparing PLA with other mediators on a simultaneous time protocol basis, gives no correlation with El- α 1PI in trauma and only moderate correlation with El- α 1PI in sepsis ($r = 0.42$, $P < 0.05$). The correlation of PLA to C3a shows similar levels in the case of trauma ($r = 0.35$, $P < 0.05$) and sepsis ($r = 0.42$, $P < 0.01$; Fig. 4).

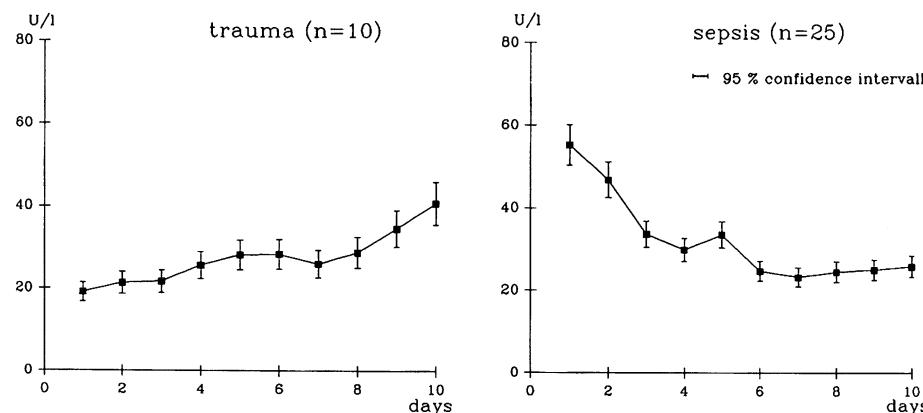


Fig. 1. Time courses of serum PLA up to 10 days after trauma (left) and sepsis (right). Values are given as mean \pm 95% confidence interval of the mean (see text)

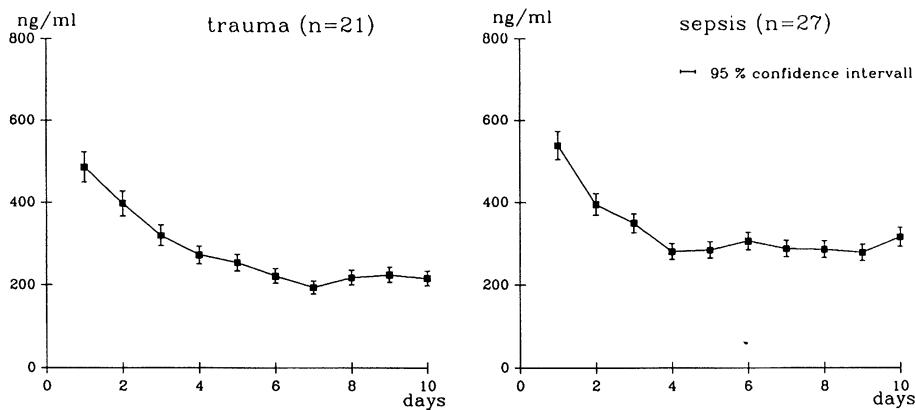


Fig. 2. Time courses of plasma concentrations of PMN-elastase (El- α 1PI) after trauma (left) and sepsis (right). Mean \pm 95% confidence interval, see text

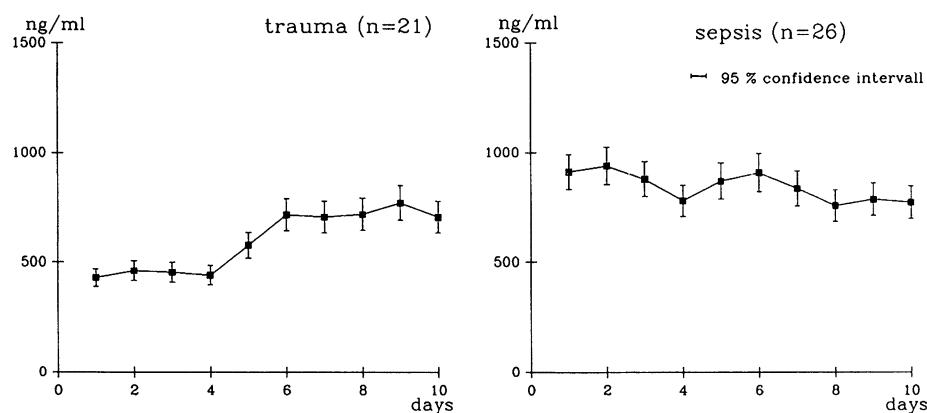


Fig. 3. Time courses of plasma C3a after trauma (left) and sepsis (right). Mean \pm 95% confidence interval, see text

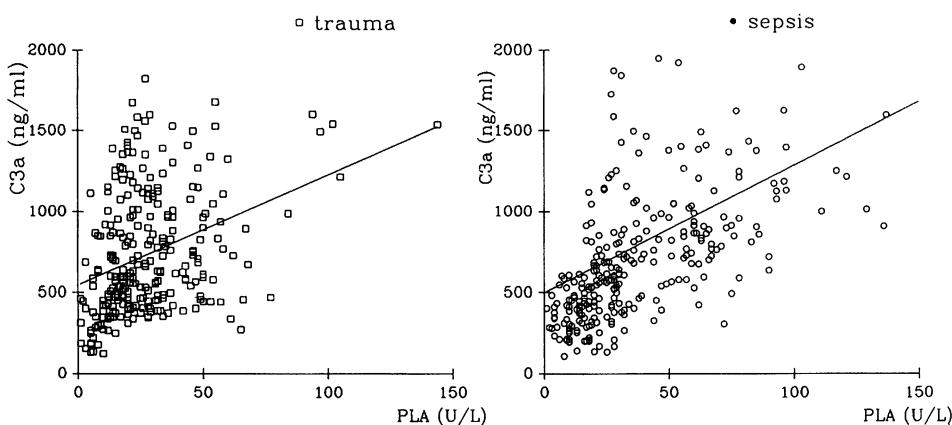


Fig. 4. Scatterplots of PLA vs C3a in the case of trauma (left) and sepsis (right); see text

Neopterin was not found to be elevated immediately after trauma (10 ± 2 nmol/l, mean \pm SEM), whereas in patients with sepsis there were remarkably high levels of this marker (102 ± 21 nmol/l, mean \pm SEM), with maximal values above 300 nmol/l. The correlation between neopterin and PLA was stronger than that of PLA to the other inflammatory mediators (trauma: $r = 0.52$, $P < 0.01$; sepsis: $r = 0.49$, $P < 0.05$; Fig. 5).

During the early period of the disease, PLA values showed no significant difference in patients with and without ARDS, irrespectively whether they were in the trauma or sepsis group: trauma ARDS 22 ± 3 U/l vs non-ARDS 21 ± 2 U/l; sepsis ARDS 39 ± 3 U/l vs non ARDS 41 ± 3 U/l (mean \pm SEM; Fig. 6). The neopterin levels in plasma did not differ between ARDS and non-ARDS, either (data not shown). In contrast, the levels of

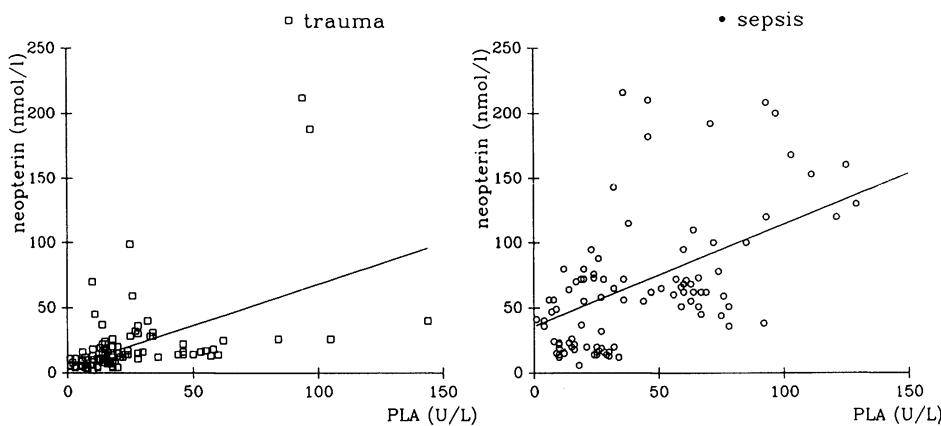


Fig. 5. Scatterplots of PLA vs neopterin in the case of trauma (left) and sepsis (right); see text

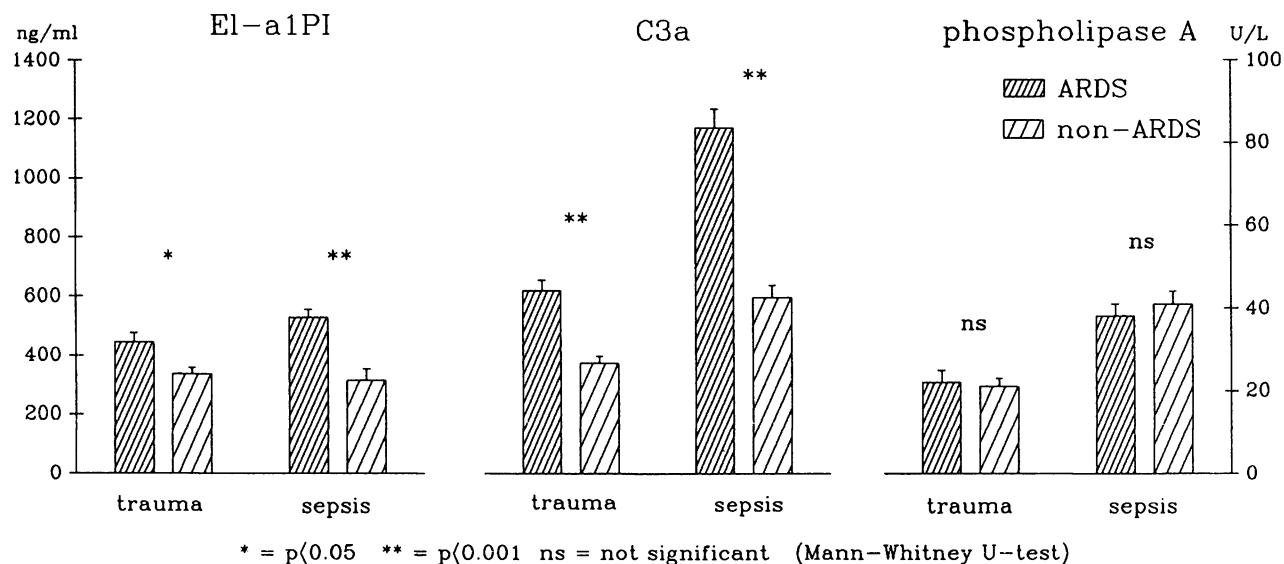


Fig. 6. Bar diagrams of elastase- α 1PI, C3a, and phospholipase A after trauma and sepsis with and without ARDS. Each bar represents the mean \pm SEM of the early period. Narrow hatched, ARDS; wide hatched, non-ARDS (see text)

El- α 1PI and C3a were found to be higher in patients with ARDS compared with the non-ARDS group (separated for trauma and sepsis): El- α 1PI 528 ± 28 ng/ml vs 315 ± 19 ng/ml in the sepsis group and 444 ± 33 ng/ml vs 338 ± 20 ng/ml in the trauma group. The levels of C3a were $1,172 \pm 63$ ng/ml vs 598 ± 39 ng/ml in the septic patients and 619 ± 37 ng/ml vs 374 ± 22 ng/ml in the traumatized patients (all values are given for ARDS vs non-ARDS groups; mean \pm SEM; Fig. 6).

Discussion

Although all the inflammatory mediators investigated are probably involved in the pathogenesis of ARDS, this involvement occurs in different ways and on different levels of the inflammatory process

[10, 15]. The activation of the cascade systems leads to a degranulation of polymorphonuclear neutrophils, reacting also on the humoral systems in further protein degradation (coagulation, fibrinolysis, complement). Early ARDS after trauma seems to be based more on microvascular alterations of the lung by coagulation disorders and microthrombi [8]. In patients with sepsis, however, a generalized inflammatory process involves all cascade systems, stimulating the complement cascade via both the classic and the alternative pathways [16, 23].

In this study we found elevated levels of elastase-alpha1PI in both groups, indicating PMN degranulation due both to tissue damage and to infection (Fig. 2). Since the PLA levels were not elevated early after trauma, there was no correlation with the concentrations of elastase- α 1PI in trauma. In sepsis a borderline correlation could be

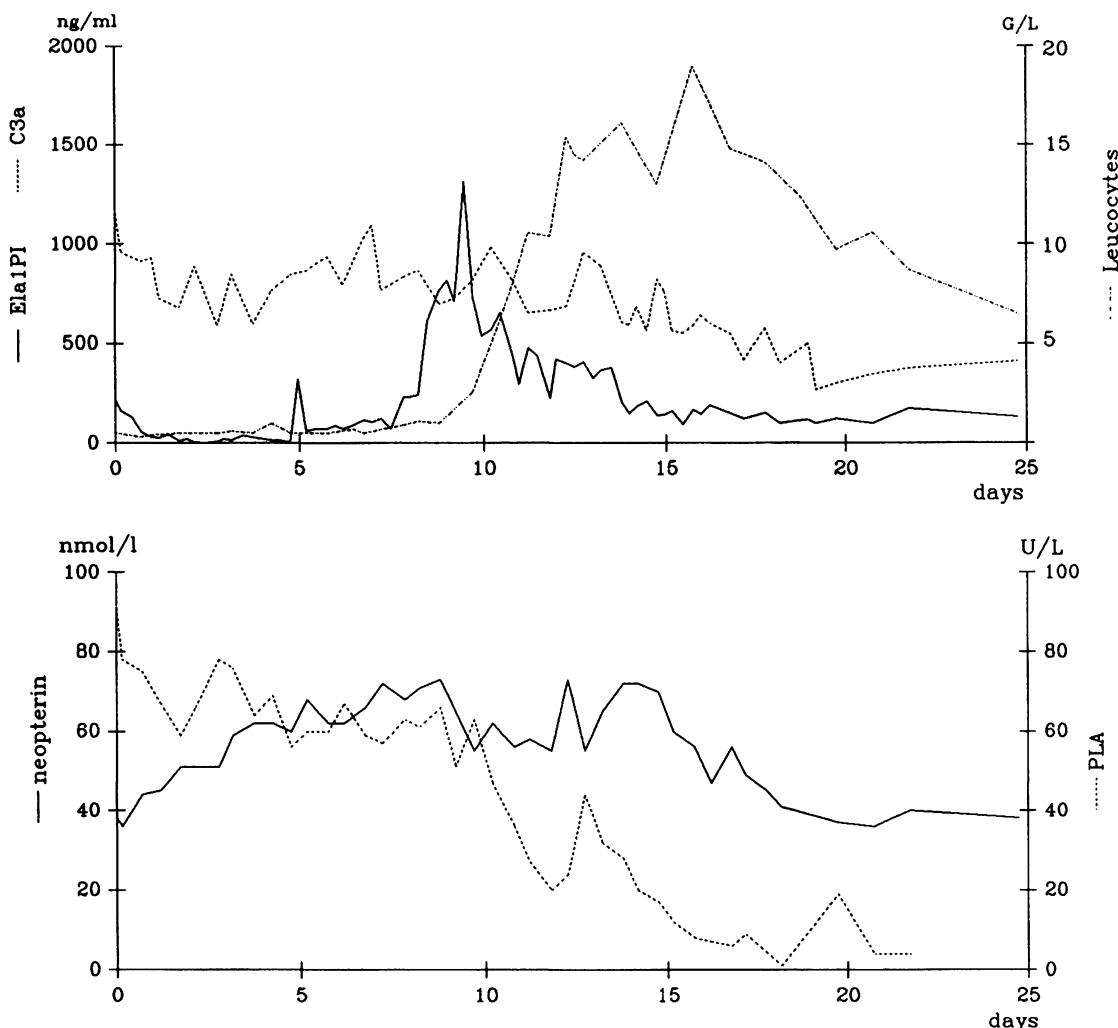


Fig. 7. Time courses of El- α 1PI, C3a, leukocyte counts, PLA, and neopterin levels of a patient suffering from sepsis and toxic agranulocytosis (because of self-medication with chloramphenicol). At the time of leukopenia ($< 1.0 \text{ G/l}$) the plasma levels of El- α 1PI were markedly lowered, although the patient showed clinical signs of the sepsis syndrome. Both PLA and neopterin levels were elevated at this time. The neopterin levels paralleled the PLA values with a time lag of about 3–4 days. During bone marrow recovery El- α 1PI levels rose to high values (sixfold above normal). At convalescence the levels of all the inflammatory mediators decreased

shown, although special cases of sepsis indicate that there was no relation to PMN degranulation (Fig. 7). PLA paralleled the complement activation measured by C3a. Regarding the underlying diseases, especially trauma, the mean time courses of PLA and C3a were similar (Fig. 3). High plasma values of C3a were found in the presence of low or moderately raised PLA concentrations, particularly in sepsis. However, the reversal could not be seen, indicating that complement activation is not necessarily followed by increased PLA levels in plasma (Fig. 4).

In a retrospective study Vadas et al. found higher PLA values in the serum of patients with septic ARDS compared with septic patients without

out signs of severe ARDS [20]. Recently the same authors published a prospective study, showing a strong correlation between PLA₂ levels and magnitude and duration of the circulatory derangement in septic shock [22]. In contrast our results showed no significant relation between the serum PLA levels with the manifestation of ARDS. Since there is a high incidence of acute lung injury in patients with sepsis, especially associated with Gram-negative septic shock [9], very high plasma concentrations could be measured in these patients. However, in patients with posttraumatic ARDS there was no increase of PLA (Fig. 6).

The interpretation of the data lacks information on the source of the PLA measured. As the

PLA in plasma is not of pancreatic origin [5, 22], the enzyme may be liberated from a great number of cells from the tissues or from the circulation. Some experimental data on interleukin 1 or endotoxin stimulation in animals [2, 21] as well as the findings of Sipka [17] show the possibility of macrophages being the source of the enzyme measured in the plasma of patients with major infections. This corresponds with the correlation of PLA activity and neopterin levels in our study (Fig. 5). Neopterin is a product of the tetrahydrobiopterin pathway, liberated by macrophages after an interferon-gamma stimulus [7, 11]. Although the substance has no known biological activity, it serves as a marker of the stimulation of macrophages. Like PLA, the levels of neopterin were elevated only in infection, but not after trauma. Furthermore, in patients with various forms of leukemia, as shown by Hiefinger [4], plasma PLA concentration was highly elevated only in a case of monocytic leukemia.

In the individual time courses of patients suffering from sepsis the relation of PLA and neopterin was more distinct. In Fig. 7 time courses of El- α 1PI, C3a, neopterin, and PLA levels are shown in a patient suffering from a perityphilitic abscess and sepsis in the presence of toxic agranulocytosis. Whereas at the time of leukopenia El- α 1PI concentrations were markedly lowered, both PLA and neopterin levels were elevated, the latter following the PLA concentrations in plasma by a time lag of 3 to 4 days.

In our study the plasma concentrations of PLA were not related to the occurrence of acute lung injury. Plasma values of PLA did correlate with the course of underlying diseases, i.e., sepsis, but not with their respiratory manifestations. An improved understanding of the characteristics and the origin of the PLA will be necessary to clarify the enzyme's pathogenic potential. Our data concerning the relation between neopterin and PLA measured in plasma of patients with sepsis syndrome are consistent with experimental data showing an origin from macrophages.

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