

Types 1 and 2 Plasminogen Activator Inhibitor and Tumor Necrosis Factor Alpha in Patients with Sepsis*

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Summary

We have determined the plasma concentrations of types 1 and 2 of plasminogen activator inhibitor (PAI-1 and PAI-2), tumor necrosis factor (TNF- α) and endotoxin in 47 patients with bacterial infection (22 patients presented with positive blood cultures). Results were compared with those observed in 30 healthy subjects. There was a significant increase in PAI-1 and TNF- α in patients as compared to controls ($p < 0.0001$), whereas no differences for PAI-2 were observed. PAI-1 and TNF- α were significantly higher in 18 patients with gram-negative bacteremia as compared to all other patients ($p < 0.0001$). However, no correlation between the analyzed parameters and either endotoxin or clinical outcome was observed. We conclude that there is an increase of PAI-1 and TNF- α in patients with sepsis, which is not related to the endotoxin concentration. Our results suggest that PAI-1, but not PAI-2, is the main plasminogen activator inhibitor in human sepsis.

Introduction

Fibrin and microthrombi are commonly detected in many organs of patients with sepsis (1). The plasminogen activator-plasmin system is the major defence against fibrin deposition on vessel walls (2). The activation process as well as the action of plasmin are regulated by inhibitors. In recent years at least four immunologic distinct molecules with plasminogen activator inhibitor (PAI) activity have been identified (for review see ref. 3). Two of such inhibitors are now well characterized: endothelial-type (PAI-1) and the placenta-type (PAI-2). PAI-1 is a single-chain polypeptide of M_r 50,000 present in plasma, platelets and in the conditioned medium of endothelial cells (4). High PAI-1 levels have been found in different clinical conditions related to thrombotic phenomena (5, 6) as well as in human sepsis (7, 8). PAI-2 exists in two molecular forms. One is of M_r 48,000 and is the dominant form in placenta (9); the other is of M_r 70,000 mainly present in pregnancy plasma (10). Both forms are present in cultures of monocytes (11). PAI-2 appears in plasma during the first trimester of pregnancy and increases in the course of gestation (12). Its physiopathological role in thrombotic disease is less well established, although it has recently been observed that human monocytes/macrophages can release PAI-2 upon endotoxin stimulation (13).

Tumor necrosis factor (TNF- α), a polypeptide derived from mononuclear phagocyte cells, is being recognized as an important mediator in a variety of processes (14) and it is thought to play a major role in mediating the effects of endotoxin in bacterial sepsis

(15). A role for TNF- α on fibrinolysis via the induction of PAI has also been suggested (16, 17).

This study was undertaken to investigate the relationship among endotoxin, TNF- α and types 1 and 2 PAI in patients with sepsis, in order to know whether a decreased fibrinolytic activity may contribute to the fibrin deposition within blood vessels, a typical finding in human septicemia.

Patients and Methods

Patients

Forty-seven patients with local and disseminated bacterial infections were studied. There were 32 males, 15 females and the mean age was 49 ± 18 years (range 16–72 years). The diagnosis of septicemia was established in 22 patients based on the typical clinical picture, repeated temperature over 38.5°C and positive blood cultures. The remaining 25 patients presented with a localized site of infection and negative blood cultures. A control group consisted of 30 age-matched healthy subjects. Blood from the antecubital vein was collected into 0.1 vol trisodium citrate (final concentration 0.011 M) and immediately cooled on ice. All samples were taken from patients in the morning before antibiotic therapy was started. Platelet poor plasma was obtained by centrifugation for 15 min at $2,500 \times g$ and 4°C and stored at -70°C until use.

Methods

Endotoxin concentration was determined by using a limulus lysate chromogenic peptide substrate (Coatest endotoxin, Kabi Diagnostica, Sweden).

PAI-1 activity was determined by adding t-PA to diluted plasma and the measurement of residual t-PA as previously described (18). The detection limit is 0.5 U/ml.

Human PAI-2 antigen was determined in plasma samples by using the commercially available enzyme immunoassay TintelizeTM PAI-2 (Biopool, Umeå, Sweden) based on the ELISA described by Lecander and Astedt (10). Briefly, 20 μl of $1/3$ plasma sample dilution or 20 μl of standards (from 0–100 ng/ml) were added to micro-test wells pre-coated with 100 μl solution of mouse monoclonal anti-human PAI-2 antibody. The plate was incubated for 2 h at 25°C on a microtest plate shaker to which 50 μl of a second goat polyclonal anti-PAI-2 antibody conjugated to peroxidase were added. The plate was incubated for 1 h at 25°C and washed with PBS-EDTA-Tween 20 buffer. Finally 200 μl of orthophenylene diamine were added to the plate, the reaction was stopped with 50 μl of a 3 mol/l H_2SO_4 solution and the absorbance at 492 nm was recorded. The detection limit of the assay is 5 ng/ml.

TNF- α was assayed in plasma samples by immunoradiometric assay using the IRE-MEDGENIX-TNF- α -IRMA kit (Belgium). Anti-TNF- α coated tubes were incubated with 200 μl of either plasma or standards (0 to 5,000 pg/ml in Tris. HCl buffer). Assay tubes were subsequently incubated with 50 μl anti-TNF- α ^{125I} in phosphate-buffered for 16 h at room temperature. Tubes were washed with phosphate-buffered saline containing 0.05% Tween 20 and the remaining radioactivity was determined in a gamma counter for 60 s. Radioactivity bound to the tube reflects the antigen concentration. The standard curve is prepared by plotting cpm against the standard concentrations. TNF- α concentration of samples can be obtained by interpolation of cpm values. This assay has already been validated for human plasma (19). The detection limit is 5 pg/ml.

* Dedicated to Professor M. Verstraete on the occasion of his 65th birthday.

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The data were evaluated using the Mann-Whitney test for comparison of means and SD.

Results

Forty-seven patients with local and disseminated bacterial infection were studied. Mean plasma endotoxin concentration in patients was 1.4 ± 2.3 ng/ml (not detectable in controls). The typical pattern of disseminated intravascular coagulation (DIC) was observed in 2 patients. Fig. 1 shows the distribution of plasma PAI-1 activity and the concentrations of PAI-2 and TNF- α in the studied groups. There was a markedly significant increase ($p < 0.0001$) in the plasma PAI-1 activity (6.6 ± 6.9 U/ml) and TNF- α levels (78 ± 111 pg/ml) in patients as compared to controls (0.8 ± 0.7 U/ml and 7.9 ± 4.5 pg/ml respectively). Both in patients and controls PAI-2 levels were below detection limit in the majority of cases and thus no statistically significant differences were observed.

Twenty-nine patients showed either negative blood cultures or gram-positive bacteremia and in 18 patients bacteriological studies identified gram-negative bacteria. The PAI-1 activity and TNF- α concentrations in these patient groups are shown in Table 1. There was a significant increase ($p < 0.0001$) in both PAI-1 and TNF- α in patients with gram-negative bacteremia as compared to all other patients. However, when patients were arbitrarily divided according to endotoxin concentration (lower or higher than 1,000 pg/ml) such differences were not observed (Table 2). High PAI-1 activity was detected in the two patients with DIC (6.3 and 36.2 U/ml) whereas TNF- α values were elevated in 1 patient (28.1 and 7.5 pg/ml respectively).

No correlation between endotoxin, PAI-1 and TNF- α could be established. Neither PAI-1 nor TNF- α discriminated patients with the development of respiratory distress syndrome, shock, renal failure or overall mortality (data not shown).

Table 1 Plasma levels of PAI-1 and TNF- α in relation to blood cultures

	Negative blood cultures or gram-positive bacteremia (n = 29)	Positive blood cultures with gram-negative bacteremia (n = 18)	p value
PAI-1 (U/ml)	3.29 ± 6.30	12.26 ± 3.70	0.0001
TNF- α (pg/ml)	35.99 ± 37.99	145.68 ± 148.61	0.0001

Table 2 Plasma levels of PAI-1 and TNF- α in relation to plasma endotoxin concentration

	Endotoxin < 1,000 pg/ml (n = 26)	Endotoxin > 1,000 pg/ml (n = 18)	p value
PAI-1 (U/ml)	6.19 ± 5.28	7.15 ± 8.52	NS
TNF- α (pg/ml)	83.34 ± 133.12	71.38 ± 72.57	NS

Discussion

This study shows that in a group of patients with local infection and sepsis the plasma PAI-1 activity and TNF- α levels are significantly elevated whereas no differences were observed in PAI-2 concentrations. We had previously shown high PAI-1 activity in patients with bacterial infection (8). Our present result confirms that report and indicates that PAI-2 levels in the control group and in patients with either local or disseminated bacterial infection, with or without associated DIC, were in general below the detection limit.

In recent reports elevated PAI activities were observed in patients suffering from septicaemia (7, 8, 20). It has also been demonstrated that high serum PAI-1 levels may serve as a prognostic marker for survival in septic shock (21). Recently, Suffredini et al. studied the changes of the fibrinolytic system

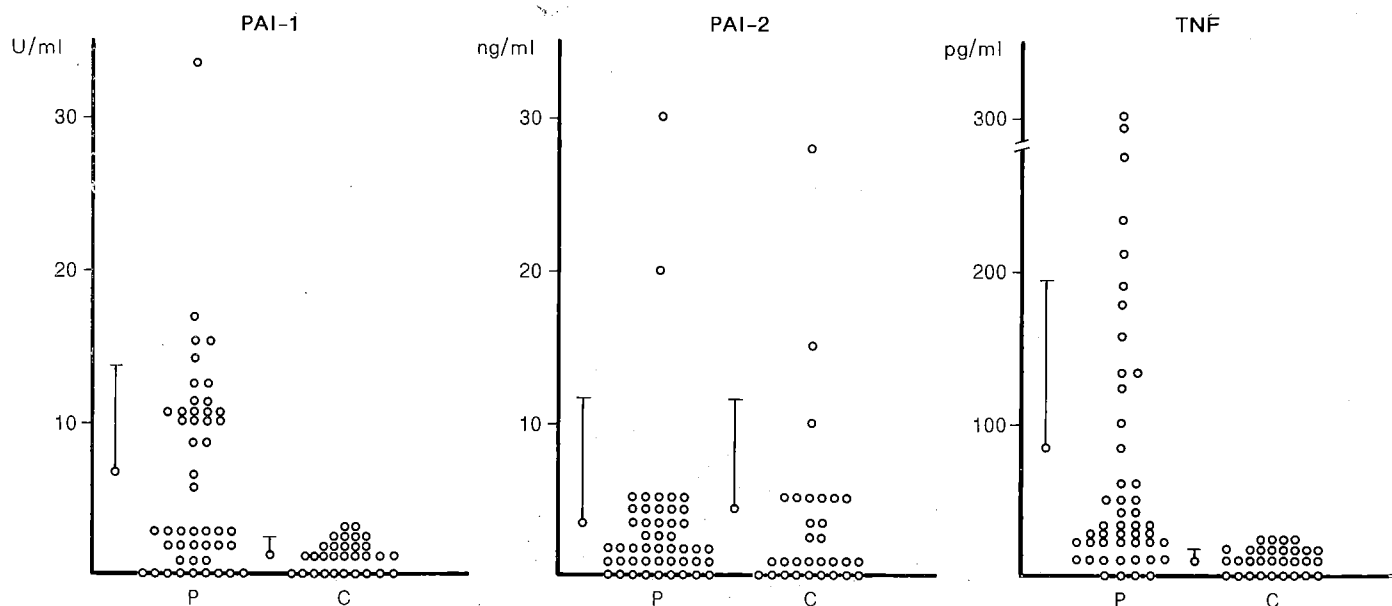


Fig. 1 Distribution of PAI-1 activity, PAI-2 antigen and TNF- α in patients (P) and controls (C). The mean + SD is also shown. Significant differences for PAI-1 and TNF- α ($p < 0.0001$) whereas no differences for PAI-2 were observed

after a single dose of endotoxin to normal subjects. They showed that the decrease in fibrinolytic activity was mainly due to the appearance of PAI-1 activity (22). Endotoxin seems to be a potent stimulus for the release of PAI-1 since as shown in this report higher levels were observed in sepsis by gram-negative bacteria as compared with either gram-positive sepsis or negative blood cultures. The increase of PAI-1 may contribute to the endotoxin-induced reduction of the fibrinolytic activity and the fibrin deposition within blood vessels, which is in agreement with experimental observations (23, 24).

Little is known about the possible role of PAI-2 in human sepsis. It has been experimentally shown that human peripheral blood cells respond to lipopolysaccharide with increased release of an inhibitor which shows specificity towards the urokinase type plasminogen activator and antigenic similarity to type 2 but not type 1 plasminogen activator inhibitor (13). However, few studies have measured PAI-2 levels in clinical plasma samples. High antigen values have been observed in pregnant women (12) but it is not generally detected in the non-pregnant state (25). In this study PAI-2 concentrations in the control group and in septic patients were in general below the detection limit. Our results, in apparent contradiction with the *in vitro* cellular response of this inhibitor upon endotoxin stimulation (13), suggest that the *in vivo* antifibrinolytic activities in response to endotoxin (23, 24, 26), partially responsible of fibrin deposits in human tissues, are not related to an increase of PAI-2.

Using a sensitive immunoradiometric assay we have shown a significant increase of TNF- α in patients with bacterial infection. These findings are consistent with those of previous investigators in patients with gram-negative bacteremia, meningococcal disease and parasitic infections, as well as in normal volunteers after endotoxin administration (27-32). Whether this *in vivo* effect is directly due to endotoxin, or to an indirect pathway after lipopolysaccharide stimulation of macrophage cell lineage involving other monokines as interleukin-1, is not yet known. Although no correlation between endotoxin, TNF- α and PAI-1 could be established it is interesting to notice that both endotoxin and TNF- α can induce endothelial cell secretion of PAI-1 (16, 17, 33). Consequently, the observed increase of PAI-1 and TNF- α in the group of gram-negative bacteremia patients could indicate a pathophysiological relationship between them during the *in vivo* events in gram-negative infection.

The lack of correlation between endotoxin and either PAI-1 or TNF- α is not surprising. Firstly, because *in vivo* experimental and clinical studies have demonstrated that a minimum dose of endotoxin is able to induce a marked inhibitor response (7, 22). On the other hand, the recent demonstration of a transient raise in TNF- α concentration in response to endotoxin (31), even though the endotoxin-induced abnormalities persist for several hours, could indicate that hospitalized patients with sepsis may have passed the peak of elaboration of TNF- α (34). Finally, we were unable to demonstrate any relationship between TNF- α levels and outcome or severity of illness in patients with sepsis, confirming another report (32).

In conclusion, this study shows that there is an increase of PAI-1 and TNF- α in patients with sepsis which is not related to the endotoxin concentration. Our results strongly suggest that PAI-1 is the main plasminogen activator inhibitor in human sepsis, whereas PAI-2 seems to play no significant role. These findings support recent *in vivo* data indicating that the control of PAI-1 and TNF- α generation in response to endotoxin (35-37) may represent a therapeutic approach for DIC associated with human sepsis.

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