Tissue factor pathway inhibitor does not influence inflammatory pathways during human endotoxemia


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Activation of coagulation induces a proinflammatory response in in vitro and animal experiments. Inhibition of the tissue factor–dependent pathway of coagulation inhibits cytokine release and prevents death in gram-negative sepsis models in primates. This study investigated the influence of blocking the coagulation system by tissue factor pathway inhibitor (TFPI) on endotoxin-induced inflammatory responses in healthy humans. Eight men were studied in a double-blind, randomized, placebo-controlled cross-over study. They received a bolus intravenous injection of 4 ng/kg of endotoxin, followed by a 6-h continuous infusion of either TFPI (0.2 mg/kg/h after a bolus of 0.05 mg/kg) or placebo. Endotoxin-induced activation of coagulation was prevented completely by TFPI. In contrast, TFPI did not influence leukocyte activation, chemokine release, endothelial cell activation, or the acute phase response. Thus, complete prevention of coagulation activation by TFPI does not influence activation of inflammatory pathways during human endotoxemia.
was studied on 2 occasions 6 weeks apart. The subjects fasted overnight before endotoxin administration. At 7:00 A.M. 2 iv canulas were inserted, 1 for endotoxin administration and blood collection, the other for infusion of TFPI or placebo. Endotoxin (Escherichia coli lipopolysaccharide [LPS], lot G; United States Pharmacopeia Convention) was administered at 9:00 A.M. as a bolus iv injection at 4 ng/kg of body weight. TFPI (recombinant human TFPI/SC-59735; Chiron) was given immediately after endotoxin injection as a bolus of 0.05 mg/kg of body weight, followed by a continuous 6-h infusion of 0.2 mg/kg/h. In control experiments, the solution used for diluting TFPI was given as a placebo.

Blood collection. Blood was obtained from an iv canula 20 min before endotoxin administration and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 h thereafter. Blood for coagulation assays was collected in siliconized vacutainer tubes (Becton Dickinson) containing 0.105 M sodium citrate; the ratio of anticoagulant to blood was 1:9 (vol/vol). Blood for all other assays was collected in K3-EDTA–containing tubes. Leukocyte counts and differentials were assessed by Stekker analyzer (STKS; Coulter). All blood samples, except those for determination of leukocyte counts and differentials, were centrifuged at 1600 g for 15 min at 4°C, and plasma was stored at −20°C until assays were done.

Assays. The plasma concentrations of prothrombin fragment F1+2 and thrombin-antithrombin complexes (TAT) were measured by ELISA (Beringwerke), as were chemokine concentrations. Monocyte chemotactic protein (MCP)-1 was measured by using purified monoclonal mouse anti–human MCP-1 as coating antibody, biotinylated affinity purified goat IgG anti–human MCP-1 as detecting antibody, and recombinant human MCP-1 as standard (all from PharMingen) as standard. For determination of macrophage inflammatory protein (MIP)-1β levels, purified monoclonal mouse anti–human MIP-1β was used as coating antibody, biotinylated affinity purified goat IgG anti–human MIP-1β as detecting antibody, and recombinant human MIP-1β as standard (all from R&D Systems). Detection limits of the assays were 8.2 pg/mL (MCP-1) and 15.6 pg/mL (MIP-1β). IL-8 was measured by ELISA, according to the manufacturer’s instructions (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). Antigenic levels of von Willebrand factor (vWF) [7] and sE-selectin [8] were measured by ELISA. LPS–binding protein (LBP) was measured by ELISA by use of polyclonal rabbit anti–human LBP as capturing antibody, biotinylated anti-CRP monoclonal antibody as detecting antibody, and CRP (Beringwerke) as standard. Plasma C-reactive protein (CRP) levels were determined by ELISA with polyclonal rabbit anti–human CRP as capturing antibody, biotinylated anti-CRP monoclonal antibody as detecting antibody, and CRP (Beringwerke) as standard. Concentrations of elastase-α1–antitrypsin complex (elastase) were determined by RIA [10].

Statistical analysis. Data are mean ± SEM. Differences in results between TFPI and control experiments were tested by repeated measurement analysis of variance. P < .05 was considered to be significant.

Results

Activation of coagulation. As reported elsewhere [6], administration of endotoxin resulted in the activation of coagulation, as reflected by increased levels of prothrombin activation fragment F1+2 and TAT. TFPI given at the dose studied in

Figure 1. Mean ± SE neutrophilic granulocytes and plasma concentrations of elastase-α1–antitrypsin (elastase) complexes, von Willebrand factor (vWF), and sE-selectin, after endotoxin administration and infusion of tissue factor pathway inhibitor (TFPI; ◦) or placebo (△). P values (NS, not significant) indicate no difference between TFPI and placebo experiments.
the present investigation completely prevented the increase in both markers of thrombin generation.

**Leukocyte activation.** Endotoxin injection elicited activation of neutrophilic granulocytes, as reflected by a biphasic change in neutrophil counts involving an initial neutropenia (1 h, 0.84 ± 0.1 × 10^9/L), followed by neutrophilia (12 h, 10.4 ± 1.3 × 10^9/L) and systemic release of elastase. The plasma concentration of elastase peaked after 4 h, increasing from 44 ± 7 to 244 ± 52 ng/mL. None of these endotoxin-induced changes was influenced by TFPI (figure 1).

**Endothelial cell response.** Endotoxin elicited endothelial cell activation, as indicated by increases in the plasma concentrations of vWF (from 77% ± 9% to 364% ± 59% after 5 h; P < .05) and sE-selectin (from 73 ± 18 to 457 ± 49 ng/mL after 6 h; P < .05). TFPI infusion did not influence this endothelial cell response (figure 1).

**Chemokines.** MIP-1β levels rapidly increased after endotoxin injection (from 31 ± 5 to 9479 ± 537 pg/mL after 2 h; P < .05). In addition, IL-8 and MCP-1 levels increased, reaching maximum values 3 h after endotoxin administration (from 5 ± 1 to 1539 ± 235 pg/mL and from 205 ± 49 to 75,923 ± 6370 pg/mL, respectively; both P < .05). Chemokine release induced by endotoxin was not influenced by TFPI (figure 2).

**Acute phase response.** CRP levels increased from 0.7 ± 0.2 to 60.2 ± 9.0 µg/mL at 24 h after endotoxin and from 0.4 ± 0.1 µg/mL to 65.5 ± 5.8 after endotoxin and TFPI (P = not significant [NS]). The increase in LBP levels after endotoxin also was not influenced by TFPI (from 16.1 ± 2.0 to 50.4 ± 5.4 µg/mL and from 14.2 ± 1.5 to 42.1 ± 3.0 µg/mL, respectively; P = NS).

**Discussion**

Activation of the TF/VIIa pathway is considered to be crucial for initiation of the coagulation system during bacteremia and endotoxemia. Under physiologic conditions, TF cannot be detected on the luminal surface of the vascular endothelium and is found in very low quantities on circulating blood cells. However, during infection and after stimulation with endotoxin or tumor necrosis factor (TNF), TF is rapidly induced on blood mononuclear cells and on vascular endothelium [1]. Elimination of TF activity in bacteremic primates results in complete inhibition of coagulation activation [3, 5, 11, 12]. In accordance, we found recently that infusion of TFPI caused a dose-dependent reduction of the procoagulant response to endotoxin in healthy humans in vivo [6]. The main conclusion of the present study is that TFPI infused at a dose that prevented endotoxin-induced thrombin generation did not influence other proinflammatory effects of endotoxin, including leukocyte activation, endothelial cell activation, chemokine release, and the acute phase protein response. These data suggest that low-dose endotoxin elicits systemic inflammation by a coagulation-independent mechanism.

Our findings contrast with in vitro and animal experiments that suggested that the activation of coagulation contributes to various proinflammatory pathways. IL-6 and IL-8 production by monocytes and endothelial cells is stimulated during ex vivo clotting of human blood [13]. Several coagulation factors may contribute to this inflammatory response. Thrombin stimulates the release of cytokines, such as IL-1, IL-6, IL-8, and TNF. The thrombin-induced cytokine response by monocytes and endothelial cells likely is mediated by thrombin catalytic activity and can be prevented by hirudin [1]. Exposure of cultured human endothelial cells to factor Xa stimulated the production of IL-6, IL-8, and MCP-1 and the expression of the adhesion molecules sE-selectin, intercellular adhesion molecule–1, and vascular cell adhesion molecule–1 by a mechanism independent of thrombin [14]. Activated protein C, an endogenous anticoagulant, has several effects on the inflammatory response, such as down-regulation of TNF production by macrophages, blocking neutrophil activation, inhibiting sE-selectin–mediated cell adhesion, and up-regulating IL-6 and IL-8 production [15].

The role of the TF/VIIa-dependent pathway of coagulation
on inflammatory responses in vivo has been studied in lethal sepsis models in nonhuman primates. In these models, blocking TF/VIIa by TFPI or active site-degraded factor VIIa both inhibited thrombin generation and IL-6 and IL-8 release and prevented organ failure and death [1, 3]. There are several possible explanations for why our findings differ from those in primate models of sepsis. If one assumes that thrombin contributes to inflammatory responses, it can be hypothesized that the amount of thrombin generated after low-dose endotoxin administration is not sufficient to stimulate inflammatory responses. The much higher amounts of thrombin formed during lethal sepsis, on the other hand, could contribute to the IL-6 and IL-8 response. Alternatively, it is possible that cytokines are produced by different cell types during low-grade endotoxemia and severe sepsis. The prolonged IL-6 and IL-8 response that is found during sepsis in primates [3] could be produced by endothelial cells, which predominantly produce these 2 cytokines after stimulation [1, 14], whereas the more transient increase in cytokines observed after low-dose endotoxin injection could be attributed to monocytes. Thus, TFPI may attenuate the cytokine response by endothelial cells with a much smaller effect on endotoxin-induced cytokine production by monocytes.

Yet another possible explanation is that neither thrombin nor other coagulation factors, but organ failure and ischemia, contributes to the inflammatory response during sepsis. If so, anticoagulation would be able to attenuate this inflammatory response during severe bacteremia by preventing disseminated intravascular coagulation leading to organ failure. Endotoxin administration in healthy humans, on the other hand, does not induce organ failure, which may explain why anticoagulation does not influence signs of inflammation in this model. Finally, it can be hypothesized that TF has direct proinflammatory effects independent of activation of coagulation [2]. If so, it is possible that our endotoxin model does not induce enough TF expression to contribute to inflammatory changes.

The present study, unlike previous animal experiments, shows no influence of TFPI on endotoxin-induced inflammatory pathways in humans. More studies are required to determine the exact role of activation of coagulation on inflammatory pathways during sepsis and endotoxemia.

References