Thrombin and leukocyte recruitment in endotoxemia

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Woodman, Richard C., Diane Teoh, Derrice Payne, and Paul Kubes. Thrombin and leukocyte recruitment in endotoxemia. Am J Physiol Heart Circ Physiol 279: H1338–H1345, 2000.—Because thrombin has been implicated in sepsis, it has been proposed that antithrombin III (AT III) is beneficial due to its anticoagulatory and antiadhesive effects. Using intravital microscopy, we visualized leukocyte-endothelium interactions in postcapillary venules of the feline mesentery exposed to lipopolysaccharide (LPS). At a concentration of AT III that blocks leukocyte adhesion in postischemic mesentery, we found no role for thrombin in LPS-induced rolling, adhesion and emigration, or microvascular dysfunction. Furthermore, AT III did not attenuate leukocyte-endothelial interactions after tumor necrosis factor-α superfusion of the mesentery. In contrast, fucoidan, a selectin inhibitor, prevented almost all LPS-induced rolling and reduced adhesion, emigration, and microvascular dysfunction. In a model of endotoxemia, leukocyte recruitment into mesentery or lungs was unaffected by AT III. Finally, in a human cell system that mimics the flow conditions in vivo, human neutrophils rolled, adhered, and emigrated similar to the feline postcapillary microvessels, and AT III had no effect on leukocyte recruitment induced by LPS. If AT III has beneficial effects in endotoxemia, it is not due to a direct effect upon leukocyte rolling, adhesion, or emigration in postcapillary venules in vivo.

sepsis; endotoxin; adhesion; antithrombin III

THROMBIN, a SERINE PROTEASE that is released at sites of vascular injury, is a key enzyme in the coagulation pathway and in thrombosis. Aside from these important functions, there is a growing body of evidence to suggest that thrombin may also play a key role in the onset of inflammation. In particular, numerous investigators have demonstrated that thrombin may affect leukocyte infiltration into inflamed tissues, impacting at each stage of the cascade of events that lead to leukocyte recruitment. The initial phase of leukocyte recruitment, leukocyte rolling, is dependent on selectin expression, and thrombin has been shown to induce rapid P-selectin mobilization to the surface of endothelium (within minutes) to induce leukocyte rolling (7, 20, 22). In addition, Kaplaniski et al. (9) have shown an increase in E-selectin expression, and we have documented a very dramatic increase in E-selectin-dependent leukocyte rolling 4 h postthrombin exposure to endothelium (2, 19). The second phase of leukocyte recruitment, firm adhesion, can also be induced by thrombin as a result of rapid endothelial platelet-activating factor production (30) and interleukin (IL)-8 production (9). These chemoattractants found on the surface of endothelium then activate rolling neutrophils so they can adhere to constitutive and synthesized intercellular adhesion molecule-1 (23). Additionally, thrombin can activate the production of tumor necrosis factor-α (TNF-α) and IL-1β to further amplify leukocyte recruitment (1, 11). In conditions like ischemia-reperfusion of mesentery where thrombin is a dominant proinflammatory molecule, antithrombin attenuates selectin-dependent leukocyte rolling and subsequent adhesion and vascular dysfunction (3).

In endotoxemia, there is good evidence that 1) disseminated intravascular coagulation (DIC) and acute thromboembolic events (3, 6, 24) and 2) inappropriate inflammation, particularly neutrophil infiltration into tissues, (10, 14–16, 21) both contribute significantly to sepsis-induced injury of various organs. Although there is excellent evidence that thrombin is increased during sepsis and plays a role in dysregulated coagulation (3, 6, 24, 26), the evidence for its role in inappropriate inflammation and leukocyte adhesion associated with sepsis is insufficient to permit general conclusions. There are a few studies to suggest that antithrombin III (AT III) can reduce sepsis-induced neutrophil recruitment into the lungs (19, 20). However, adhesion may not be the mechanism of leukocyte recruitment in septic lungs (27, 28). A number of investigators have demonstrated that, unlike most other organs, selectin- and integrin-independent leukocyte recruitment occurs in lungs possibly by leukocytes physically trapping in capillaries (13). Moreover, whether the reduced sepsis-induced leukocyte recruitment in lungs with antithrombin therapy is related to its effects on leukocyte-endothelial cell interactions or due to an indirect impact of reduced DIC and thrombosis remains unclear. Elucidating whether antithrombin therapy affects leukocyte recruitment is paramount to understanding how best to intervene in the pathophysiology of sepsis. If adhesive mechanisms are not affected by antithrombin therapy then perhaps...

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tandem antiadhesive and antithrombin therapy may be warranted.

Finally, determining the mechanisms of action of thrombin is also timely from the clinical perspective. Recent results from patients with severe sepsis have revealed a trend with AT III toward a resolution of existing organ failures, a lower incidence of new organ failures, and an overall reduction (23%) in 30-day mortality (5). Although it has been postulated that one mechanism of action is an antiadhesive effect of AT III, as already stated, this conclusion may be premature. Therefore, we systematically tested the hypothesis that antithrombin therapy may interfere at multiple sites of the adhesion cascade and thereby reduce vascular dysfunction associated with sepsis. We used intravital microscopy to visualize leukocyte behavior in the microvasculature after the administration of LPS. Based on AT III data that demonstrated an important role for thrombin in ischemia-reperfusion of the cat mesentery (20), we examined whether identical AT III dose regimens could provide an antiadhesive benefit in local or systemic endotoxemia or in response to TNF-α, a cytokine implicated in endotoxemia. We also assessed microvascular permeability alterations in single microvessels exposed to LPS and AT III. Finally, we examined whether AT III could inhibit lipopolysaccharide (LPS)-induced leukocyte-endothelial cell interactions under flow conditions in vitro in a human system.

METHODS

Intravital microscopic studies. The experimental preparation used in this study is the same as described previously (20, 25). Briefly, cats (1.2–2.4 kg) were fasted for 24 h and initially anesthetized with ketamine hydrochloride (75 mg im). The jugular vein was cannulated, and anesthesia was maintained by the administration of pentobarbital sodium. A tracheotomy was performed to support breathing by artificial ventilation. Systemic arterial pressure was monitored by a pressure transducer (Statham P23A; Gould, Oxnard, CA) connected to a catheter in the left carotid artery. A midline abdominal incision was made, and a segment of small intestine was isolated from the ligament of Treitz to the ileocecal valve. The remainder of the small and large intestine was extirpated. Body temperature was maintained at 37°C using an infrared heat lamp. All exposed tissues were moistened with saline-soaked gauze to prevent evaporation. Heparin sodium (10,000 U; Elkins-Sinn, Cherry Hill, NJ) was administered, and then an arterial circuit was established between the superior mesenteric artery (SMA) and left femoral artery. SMA blood flow was continuously monitored using an electromagnetic flowmeter (Carolina Medical Electronics, King, NC). Blood pressures were continuously recorded with a physiological recorder (Grass Instruments, Quincy, MA).

Cats were placed in a supine position on an adjustable Plexiglas microscope stage, and a segment of mid-jejunum was exteriorized through the abdominal incision. The mesentery was prepared for in vivo microscopic observation as previously described (20, 25). The mesentery was draped over an optically clear viewing pedestal that allowed for translumination of a 3-cm segment of tissue. The temperature of the pedestal was maintained at 37°C with a constant-temperature circulator (model 80; Fisher Scientific, Pittsburgh, PA). The exposed bowel was draped with saline-soaked gauze while the remainder of the mesentery was covered with Saran Wrap (Dow Corning, Midland, MI). The exposed mesentery was suffused with warmed bicarbonate-buffered saline (pH 7.4) that was bubbled with a mixture of 5% CO₂ and 95% N₂. The mesenteric preparation was observed through an intravital microscope (Optiphot-2; Nikon, Mississauga, Canada) with a ×25 objective lens (Wetzlar 1/25/0.35; E. Leitz, Munich, Germany) and a ×10 eyepiece. The image of the microcirculatory bed (×1,400 magnification) was recorded using a video camera (Digital 5100; Panasonic, Osaka, Japan) and a video recorder (NV8950; Panasonic).

Single unbranched mesenteric venules (25–40 μm diameter, 250 μm length) were selected for each study. Venular diameter was measured either on- or off-line using a video caliper (Microcirculation Research Institute, Texas A & M University, College Station, TX). The number of rolling and adherent leukocytes was determined off-line during playback analysis. Rolling leukocytes were defined as leukocytes that moved at a velocity less than that of erythrocytes in a given vessel. The number of rolling leukocytes (flux) was counted using frame-by-frame analysis. To obtain a complete leukocyte rolling velocity profile, the rolling velocity of all leukocytes entering the vessel was measured. A leukocyte was defined as adherent to venular endothelium if it remained stationary for >30 s. Adherent cells were measured at 10-min intervals as described below and were expressed as the number per 100-μm length of venule. Erythrocyte velocity ($V_{rBC}$) was measured using an optical Doppler velocimeter (Microcirculation Research Institute), and mean erythrocyte velocity ($V_{mean}$) was determined as $V_{rBC}$/1.6 (8). Wall shear rate was calculated based on the Newtonian definition: shear rate = ($V_{mean}$/$D_v$) × 8 s⁻¹, where $D_v$ is the venular diameter.

Experimental protocol: in vivo experiments. Baseline measurements of blood pressure, SMA blood flow, $V_{rBC}$, and vessel diameter were obtained. In the first series of experiments, local LPS was delivered by superfusion over the exteriorized mesentery. This prevented any systemic, hemodynamic disturbances and permitted the direct assessment of AT III on leukocyte-endothelial cell interactions. Experiments were carried out in animals that received either a high or low local concentration of LPS (1.0 or 0.1 μg/ml), AT III before a high concentration of LPS (1.0 μg/ml), AT III before a low concentration of LPS (0.1 μg/ml), and AT III after 3 h of low LPS superfusion. In the first series of experiments, the preparation was videotaped for 10 min, and then the mesentery was superfused for 4 h with LPS (1.0 or 0.1 μg/ml). The microvasculature was videotaped for the last 10 min of every hour. In the experimental group, a protocol identical to that above was completed; however, the animals received the intravenous bolus of AT III (250 U/kg; Bayer Canada, Etobicoke, Ontario) 30 min before LPS administration. Another group of animals was given AT III at 3 h of LPS superfusion, and then the mesentery was superfused for 1 h. To ensure that the LPS-induced leukocyte recruitment was indeed selectin dependent, as a positive control, cats received fucoidan, a general selectin inhibitor, at a preestablished concentration (10 mg/kg; see Ref. 12). In the second group of experiments, systemic LPS was administered (1 mg · kg⁻¹ · h⁻¹) at a concentration that caused a slow reduction in blood pressure over 4 h. The mesentery was again observed with and without AT III pretreatment. At the end of the experiment, lung biopsies were taken for determination of neutrophil recruitment into lungs (see Myeloperoxidase assay). The concentration of AT III used in this study was the clinically recommended dosage.
In a separate series of experiments, animals had TNF-α (200 U/ml; Collaborative Biochemicals, Bedford, MA) super fused over the mesentery for 2 h. The microvasculature was monitored for an additional 2 h, with recordings being made for the last 10 min of each hour. Similar to the above experiments, AT III (250 U/kg) was given intravenously 10 min before the exposure of the mesentery to TNF-α.

Cell culture. Human umbilical vein endothelial cells (HUVEC) were harvested from freshly obtained umbilical cords as previously described (20). Briefly, umbilical cord veins were rinsed of formed blood products with warm PBS after which the vein was filled with collagenase (320 U/ml in PBS). After a 20-min incubation period at 37°C, the cords were gently massaged to ensure detachment of endothelial cells from the vessel wall. The digest was collected in centrifuge tubes, and the collagenase activity was inhibited with heat-inactivated FBS, after which the tube was centrifuged (400 g for 10 min). The pellet was resuspended in medium 199 supplemented with 20% FBS and antibiotics but no endothelial cell mitogen. The cells were then seeded in fibronectin-coated T-25 culture flasks and were grown to confluence (2–5 days). Upon confluence, the HUVEC were detached from the flasks with Trypsin-EDTA and were seeded heavily on fibronectin-coated glass coverslips. Only the first three passages of HUVEC were used for all in vitro experiments.

Neutrophil isolation. Human neutrophils were harvested from acetate-citrate-dextrose anticoagulated venous blood collected from healthy donors. All isolation steps were performed. MPO activity was determined using an assay described previously (16) but with the volumes of each reagent modified for use in a 96-well ELISA plate. Change in absorbance at 450 nm over a 90-s period was determined using a kinetic microplate reader (Molecular Devices).

RESULTS

LPS-induced microvascular hemodynamic parameters are unaffected by AT III. Two local concentrations of LPS were used in these studies (1.0 and 0.1 μg/ml of LPS in the superfusate). With the higher concentration of LPS, there was a consistent and profound microvascular response, whereas at the lower concentration of LPS a more variable and often less profound response was noted. Figure 1 summarizes the microvascular hemodynamic effects of 4 h of LPS superfusion (1.0 μg/ml) on the feline mesenteric microvasculature. Venular diameter dropped by only 1–2 μm, but V_{RBC} was reduced by 50%, and shear rates through the postcapillary microvessels were also halved at 4 h of LPS. AT III administration had absolutely no effect on these hemodynamic alterations (Fig. 1). At the lower concentration of LPS, the hemodynamic alterations were more pronounced.
were less dramatic, but again AT III pretreatment at a concentration that completely reversed many microvascular sequelae during reperfusion had no notable effect on LPS-induced hemodynamic alterations (data not shown). It is unlikely that the higher concentration of LPS was affecting the systemic circulation as blood pressure decreased by ~15% throughout the 4-h experimental protocol, an effect not different from untreated animals (data not shown).

LPS-induced leukocyte recruitment is selectin dependent but is unaffected by AT III. Figure 2 demonstrates that leukocyte rolling flux (A), leukocyte adhesion (B), and leukocyte emigration (C) all increased dramatically over the first 4 h of LPS exposure. Leukocyte rolling flux ranged from 30 to 40 cells/min under control conditions, was elevated as early as 60 min, and continued to increase to 170 cells/min within the next 3 h. Although there may have been a slight delay in the number of rolling cells within the first 60 min of LPS in AT III-pretreated animals, by 2, 3, and 4 h of endotoxemia, the number of rolling cells was elevated and was not different from untreated animals. To ensure that the rolling was selectin dependent, fucoidan, a general selectin inhibitor, was added and reduced rolling by >95%. The number of adherent cells progressively increased over 4 h of endotoxemia from ~1 cell/100 μm venule length to 30 cells/100 μm venule length. AT III pretreatment did not alter the leukocyte adhesion profile over the first 4 h of superfusion. Most importantly, the number of emigrated leukocytes in the extravascular space increases from 1 cell/field of view to 80 cells/field of view. AT III did not affect the increase in leukocyte recruitment; >70 cells/field emigrated out of the vasculature at 4 h. The reduction in leukocyte rolling induced by fucoidan resulted in a very significant decrease in adhesion and emigration. Therefore, the increase in leukocyte recruitment was selectin dependent but thrombin independent.

AT III and LPS-induced microvascular permeability alterations. Although the number of adherent and emigrated leukocytes generally dictates the amount of vascular dysfunction, it is conceivable that thrombin could directly affect microvascular permeability during endotoxemia. The data (Fig. 3), however, demonstrate an increase in microvascular permeability that is similar in magnitude regardless of whether AT III pretreatment was given. The increase in FITC-albumin leakage was 8- to 10-fold in the presence or absence of AT III. In the absence of rolling, adhering, and emigrating cells in animals treated with fucoidan, microvascular dysfunction was prevented by 60%, suggesting that leukocyte recruitment was an important contributor to the vascular dysfunction associated with endotoxemia.

The lack of effect of AT III may be related to the high concentration of LPS that was used and the many mediators in addition to thrombin that may have been released. However, at a lower dose of LPS (0.1 μg/ml), where leukocyte rolling, adhesion, emigration, and microvascular dysfunction was less than that observed at the higher dose of LPS, AT III did not reduce the leukocyte recruitment or microvascular dysfunction.
AT III does not reverse the microvascular effects of LPS. In case AT III was being rapidly cleared in this model, another series of experiments was performed in which AT III was added after leukocyte rolling, adhesion, and emigration and after microvascular dysfunction had been initiated with LPS. The results revealed that leukocyte recruitment and microvascular permeability alterations proceeded to occur and were not reversed by a bolus infusion of AT III (Fig. 4); leukocyte rolling, adhesion, emigration, and microvascular dysfunction were not abrogated when AT III (250 U/kg) was administered between 3 and 4 h.

Systemic LPS and AT III. Although AT III was unable to affect selectin-dependent leukocyte-endothelial cell interactions or subsequent leukocyte adhesion and emigration, others have reported some beneficial effects in leukocyte recruitment in lungs (28). When LPS was infused systemically, at 1 mg·kg⁻¹·h⁻¹, the blood pressure decreased by ~35%, intestinal blood flow was reduced by >50%, and microvascular permeability increased approximately fivefold at 4 h (Table 1). With systemic LPS infusion, the number of rolling leukocytes was not increased, but the cells rolled very slowly (data not shown). The number of adherent and emigrated cells was increased (Fig. 5, A and B). Similarly, in the lung, there was a very large increase in neutrophil numbers (Fig. 5C). AT III did not affect the leukocyte rolling, adhesion, and emigration in the mesentery (Fig. 5, A and B) and failed to reduce neutrophil influx in the lung (Fig. 5C). Interestingly, AT III did provide significant benefit with respect to a reduction in blood pressure 4 h after LPS (Table 1). The other hemodynamic parameters were similar with and without AT III (Table 1).

### Table 1. Hemodynamic parameters with systemic endotoxin and AT III

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>LPS</th>
<th>AT III</th>
<th>AT III + LPS</th>
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<td>Blood pressure, mmHg</td>
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<td>75 ± 13*</td>
<td>119 ± 4</td>
<td>109 ± 10†</td>
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<tr>
<td>Intestinal blood flow, ml·min⁻¹·100g⁻¹</td>
<td>63 ± 6</td>
<td>36 ± 5*</td>
<td>75 ± 4</td>
<td>30 ± 4*</td>
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<tr>
<td>Permeability, %</td>
<td>10 ± 1</td>
<td>48 ± 11*</td>
<td>8 ± 2</td>
<td>54 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. LPS, lipopolysaccharide; AT III, antithrombin III. *P < 0.05 relative to respective control or AT III value. †P < 0.05 relative to LPS value.

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Fig. 4. Series of experiments wherein AT III was given 3 h after LPS (0.1 μg/ml). Leukocyte rolling (A), adhesion (B), emigration (C), and microvascular permeability (D) are all presented. For AT III-treated group and untreated group, n = 7.

Fig. 5. Systemic endotoxemia was induced (1.0 mg·kg⁻¹·h⁻¹) for 4 h. Adhesion (A) and emigration (B) of leukocytes in mesentery and neutrophil recruitment in the lungs (C) are presented. †P < 0.05 relative to LPS value.
AT III and LPS-induced neutrophil recruitment in a human in vitro system. Because our feline data are not entirely in agreement with previously published rat data, the issue of species specificity was considered. Therefore, we also examined the effect of AT III in human systems. LPS-treated endothelium for 4 h induced profound neutrophil adhesion and emigration. Because the cells tether and roll for only a brief period before adhering in this system, only the adhesion and emigration data are shown in Fig. 6. These studies reveal that pretreatment of endothelium with AT III (5 U/ml) was unable to reduce human neutrophil accumulation on LPS-treated endothelium. Posttreatment with AT III also had no effect. We believe that this was a sufficient amount of AT III, as exposure of confluent HUVEC monolayers to thrombin (1 U/ml) resulted in a rapid and sustained increase in neutrophil adhesion that was inhibited by >90% if the monolayers were pretreated (Fig. 6) or posttreated with AT III (20).

AT III does not affect TNF-α-induced leukocyte recruitment in vivo. In a small group of animals, the mesentery was exposed to TNF-α (200 U/ml), and the results at 4 h revealed that leukocyte rolling, emigration, and microvascular dysfunction were not different in animals pretreated with AT III. Although there was a 50% decrease in adhesion, as already noted, this did not translate into a reduction in leukocyte emigration or microvascular dysfunction (Fig. 7).

DISCUSSION

Thrombin, the terminal serine protease of the coagulation cascade, is known to cleave fibrinogen and activate platelets, but it has also been reported to induce inappropriate leukocyte recruitment during inflammatory conditions. In this study, we visualized single inflamed postcapillary venules exposed to LPS to assess whether thrombin inhibition would impact leukocyte recruitment and microvascular dysfunction.
associated with endotoxemia. Although some investigators have proposed an antiadhesive role for AT III in sepsis (28), our results clearly demonstrate that neither AT III pretreatment nor posttreatment in a feline model had any effect on LPS-induced selectin-dependent leukocyte rolling, adhesion, emigration, or microvascular dysfunction. Moreover, leukocyte recruitment associated with TNF-α, a key cytokine produced during endotoxemia, was unaffected by AT III. This is in contrast to previously published studies from our laboratory with AT III in feline ischemia-reperfusion injury (20). In that study, AT III almost entirely abrogated reperfusion-induced neutrophil rolling, adhesion, and emigration. Additionally, in an in vitro human system that permits direct visualization of leukocyte-endothelium interactions under flow conditions, there was also no apparent benefit for AT III in attenuating LPS-mediated leukocyte-endothelial interactions.

Although these results are negative, they provide important, timely information with respect to thrombin as a potential therapeutic intervention in sepsis. Inadvertent activation of both the coagulation and inflammatory cascade via LPS will lead to the production of multiple mediators that results in both DIC and inappropriate leukocyte recruitment. Thrombin has been postulated to initiate both the inflammatory and coagulation cascade; however, evidence for the former is limited. Our results do not dismiss a role for thrombin in DIC but strongly argue that AT III alone is unlikely to reduce the inflammatory cascade. This information would be useful since administration of AT III to septic patients revealed marginal improvement in mortality (5) that may be greatly enhanced with appropriate antiadhesive therapy.

Initially, we examined local administration of LPS, which provided the opportunity both in vivo and in vitro to directly assess a role for thrombin on LPS-induced, leukocyte-endothelial cell interactions without confounding factors associated with intravenous administration of LPS. These include overproduction of hepatic-derived cytokines, hemodynamic alterations due to depressed myocardial function, and leukopenia as a result of leukocyte sequestration in the lungs. From this work, our data suggest that the mechanism of action of AT III in sepsis is not as an antiadhesive molecule for LPS. Although a potential complicating factor may be the existence of multiple thrombin receptor subtypes, each mediating different responses (17, 29), blocking thrombin with AT III circumvents this possibility.

Uchiba et al. (28) have postulated that AT III releases prostacyclin from endothelial cells, inhibits leukocyte recruitment/activation, and thereby protects at least the pulmonary vasculature from injury induced by LPS. It is important to note that the adhesion cascade is somewhat different in lung than in other organs, which may explain the discrepancy. In the pulmonary vasculature, unlike other vascular beds, leukocyte recruitment in response to LPS may not be dependent upon selectins and integrins but perhaps occurs due to physical trapping (4, 18). Because leukocyte recruitment in the mesentery is entirely dependent on the selectins and integrins, it is conceivable that nonadhesion molecule-dependent leukocyte recruitment in the lung (neutrophil trapping) is affected by AT III via prostacyclin, an event not seen in the mesentery. However, when we administered LPS systemically, the results revealed that AT III had no effect on LPS-mediated leukocyte recruitment in the pulmonary vasculature, a result different from Uchiba et al. (28). In their rat model of endotoxemia, the neutrophil influx peaked at 90 min and returned to near control levels by 4 h, and AT III reduced leukocyte recruitment by 20% at this optimal early time. It is apparent that this early decrease in neutrophil recruitment in the rat system with AT III did not translate into a similar effect on neutrophil recruitment in feline lungs at 4 h or a decrease in neutrophil recruitment at 4 h in the human in vitro system.

It may be argued that our results are due to insufficient amounts of AT III or that AT III was not efficient at inhibiting the biological effects of thrombin. On the basis of experiments in this study and on an earlier published study, there are at least two reasons to exclude this consideration (20). First, our studies were able to demonstrate that an in vitro concentration of AT III that mimics the concentration used in vivo completely inhibited a large concentration of thrombin-induced leukocyte recruitment. Second, we are fortunate to have a positive control; the concentration of AT III used herein was very effective at inhibiting ischemia and reperfusion-induced leukocyte recruitment in vivo both as a pretreatment and posttreatment regimen. The concentration used in vivo in our animal model was derived from the dose that is efficacious in patients with AT III deficiency and venous thrombosis.

We believe that this series of experiments was warranted, since clinical trials have been initiated with AT III in septic patients, and it has been postulated, but not demonstrated, that there is a role for thrombin at the leukocyte-endothelial cell interface. If indeed AT III proves to be beneficial in sepsis, as has been demonstrated in animal models, these results strongly support a role for thrombin in an adhesive-independent manner, perhaps as an important modulator of cytokine cascades and/or inappropriate platelet activation, but not indiscriminate leukocyte recruitment.

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