Controlled Insect-Sting Challenge in 55 Patients: Correlation Between Activation of Plasminogen and the Development of Anaphylactic Shock

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The pathogenesis of anaphylactic shock is not completely understood. Mast cell degranulation products may stimulate endothelial cells, leading to activation of fibrinolytic and coagulation systems. We investigated the activation of these systems in insect-sting anaphylaxis. Fifty-five patients with a previous insect-sting anaphylactic reaction and 8 volunteers were challenged with an in-hospital sting. Plasma levels of von Willebrand factor (vWF), coagulation, and fibrinolytic parameters were assessed. After the sting challenge, 20 patients developed anaphylactic symptoms, 7 of whom developed hypotension. In only these 7 patients, but not in the volunteers or in the other patients with no or mild anaphylactic symptoms, vWF levels increased from 107% ± 33% (mean ± SD) before, to 235% ± 134% 60 minutes after the onset of clinical symptoms. This increase of vWF was accompanied by an increase of circulating tissue-type plasminogen-activator (tPA) levels from 5 ± 3 μg/L to 50 ± 59 μg/L and of plasminogen-α2-antiplasmin complex (PAP-c) levels from 6 ± 3 nmol/L to 297 ± 225 nmol/L. Both tPA and PAP-c levels peaked 5 minutes after the onset of clinical symptoms. Such increases of tPA and PAP-c were not observed in the volunteers or in the patients who did not develop shock. The increase of tPA and PAP-c levels in the hypotensive patients correlated positively with the degree of mast cell degranulation and inversely with the mean arterial pressure. We conclude that activation of plasminogen may be involved in the pathogenesis of anaphylactic shock induced by insect venom.

Hypotensive reactions during anaphylaxis are related to the release of mast cell mediators, such as histamine and tryptase, in the circulation.14 These mediators may interact with the endothelium and induce vasodilation and increased vascular permeability. In this way, they may contribute to a rapid drop of the blood pressure, one of the main features of a life-threatening, cardiovascular, anaphylactic reaction.13,7 Besides regulating vascular permeability and tone, endothelial cells play a major role in the regulation of the coagulation and fibrinolytic systems.8,10 Hence, alterations in the plasma level of coagulation and fibrinolytic parameters can be expected to occur during anaphylaxis.

In vitro, the fibrinolytic system and the contact system of coagulation can be activated or inactivated by isolated mast cell and basophil mediators.11–14 In animal models of anaphylaxis, activation of both systems has been observed.15,16 Clinical case reports suggest that these two systems are activated during anaphylactic reactions caused by drugs and food additives,17 vascular grafts,18 or radiographic contrast media,19 although this is not consistently found.20,21 Activation of coagulation and fibrinolysis has also been suggested in Hymenoptera venom anaphylaxis. Smith et al4 reported a mild activation of fibrinolysis in the absence of contact system activation in 2 of 14 patients suffering from insect-sting anaphylaxis. Ratnoff and Nose12 observed an “impressive, but not further characterized” anticoagulant effect and a mild degree of fibrinolysis in a single patient suffering from wasp-sting anaphylaxis.

Several studies7,23–25 have shown that many patients with a history of insect-sting anaphylaxis are treated unnecessarily, whereas in some patients treatment is withheld wrongly when the classical selection criteria for venom immunotherapy (ie, specific IgE in plasma or skin tests) are applied. Therefore, in The Netherlands and some other European countries, patients may be selected for venom desensitization by sting challenge under controlled intensive care conditions. However, other investigators and clinicians may feel uncomfortable with this sting-challenge procedure and still use the classical selection criteria for venom immunotherapy. The approach of deliberate sting challenges offered us the opportunity to study prospectively the sequential release of mediators during anaphylaxis. In view of the potential endothelial cell stimulation and the ensuing involvement of fibrinolysis and coagulation in anaphylactic shock reactions, we decided to investigate the activation of both systems in this sting-challenge setting in a group of 55 patients with a history of insect-sting anaphylaxis and in 8 healthy volunteers.

MATERIALS AND METHODS

Subjects. Fifty-five consecutive patients with a history of an anaphylactic reaction to either a yellow-jacket or a honey-bee sting were subjected to a sting-challenge test. Correct insect identification was ensured by patient identification of the doctor's description of the two predominant Hymenoptera in The Netherlands, supplemented by skin tests and determination of specific IgE. Also challenged were 8 healthy volunteers, including 5 individuals who had been stung in the past by an insect of the species in question without experiencing anaphylactic symptoms. All subjects were in good health; none were pregnant; none had cardiac disorders; and none
used a β-blocker, antihistaminic, mast cell stabilizing, or any other drug. Electrocardiogram, chest X-ray, and results of routine blood examination were normal in all of the subjects. The patients were first seen in the outpatient clinic, where detailed oral and written information was given on the protocol of the study entitled “Anaphylactic reactions after insect-sting challenge.” After informed consent, the patients were entered in the study protocol, which had been approved by the medical ethical review board of The Eemland Hospital (Amersfoort, The Netherlands).

**Study protocol.** The provocation procedure with living insects was performed as described elsewhere. In short, before the sting challenge, all subjects were placed on a continuous heart rate monitoring device in the intensive care unit, and an intravenous line was inserted in each arm. A yellow jacket (Vespula germanica) or a honey bee (Apis mellifera) was induced to sting the lower left arm for 30 seconds. After that, the insect was killed. Blood pressure was recorded automatically at 1- to 5-minute intervals. Mean arterial pressure (MAP) was calculated as two-thirds diastolic pressure plus one-third systolic pressure.

Anaphylactic reactions were graded as follows: no reaction, no systemic reaction (ie, symptoms distinct from the sting site); mild reaction, one or more of the following symptoms: generalized urticaria, itching, erythema, edema, gastrointestinal symptoms, or respiratory symptoms; and shock, >15 mm Hg decrease in MAP, compared with prechallenge values, with or without symptoms graded as mild. After the challenge, only the patients with respiratory or cardiovascular symptoms on challenge were started on venom immunotherapy.

**Blood sampling.** The interval between the sting and the onset of clinical symptoms may vary considerably, as already reported, and is confirmed by the present study. To obtain a uniform pattern of data appropriate for statistical analysis, blood was collected before the sting challenge (“pre”) and at 5, 15, and 15 minutes after the moment at which the patient indicated that a reaction had started. Similar blood sampling was performed in subjects who did not show an anaphylactic reaction, starting at 15 minutes after the insect sting. The procedure of blood sampling has been described elsewhere.

However, serial samples after provocation were collected from only 42 subjects, ie, from the 8 volunteers, from all 20 patients with an anaphylactic reaction after provocation, and from the first 14 of 35 patients with no reaction. Because the data of the other 21 nonreacting patients were not expected to provide additional information, postchallenge samples were not collected from these patients. Immediately after collection, the blood samples were put on ice and centrifuged, and the plasma was deep-frozen at −70°C within 15 minutes after collection. All 5 samples of each subject were tested in the same assay procedure.

Plasminogen activator inhibitor-1 (PAI-1) and D-dimers were measured in blood that was collected in 10 mmol EDTA (final concentration). The other parameters were measured in plasma, collected in siliconized tubes containing EDTA and polybrene (final concentrations of 10 mmol/L and 0.05% [wt/vol], respectively) to prevent in vitro activation of coagulation, fibrinolytic, and contact systems.

**Assays.** Specific serum IgE as well as skin thresholds against the venom concerned were determined as described. von Willebrand factor (vWF) was determined as described elsewhere.

and expressed as percentage of normal values. Tissue-type plasminogen activator (tPA) and urokinase PA (uPA) were determined with sandwich-type enzyme-linked immunosorbent assays (ELISAs), as described. Both ELISAs recognize free and complexed tPA and uPA equally well. Results were related to standard curves based on recombinant human tPA (Boehringer, Ingelheim, Germany) or two-chain urokinase (Choay Laboratoire, Paris, France) and expressed in micrograms per liter. Plasminogen-α2-antiplasmin complex (PAP-c) was measured with a radioimmunoassay as described.

**Results** were related to a standard curve that consisted of pooled plasma in which a maximal amount of complexes was generated by the addition of urokinase and expressed in nmol/liter. PAI-1 was measured with a sandwich-type ELISA in which the monoclonal antibody (MoAb) CLB-anti-PAI-1-2C8 was used as catching antibody. Biotinylated polyclonal rabbit antibodies raised against PAI-1 purified from Hep-G2 cells were used as detecting antibodies. The assay was developed with streptavidin-horseradish peroxidase. Results were related to a standard curve of purified PAI-1 and expressed in micrograms per liter. D-dimers were measured with a commercial ELISA according to manufacturer’s instructions (Kabi Diagnostics, Amsterdam, The Netherlands). Results were expressed in micrograms per liter.

Levels of functional α2-antiplasmin were measured with a newly developed assay that resembles the assay described for functional C1-inhibitor. This new test is based on the property of α2-antiplasmin to bind covalently to plasmin. Plasma samples, collected in EDTA-polybrene, were incubated at a final dilution of 1 to 1,000 with plasmin coupled to sepharose beads. After a washing step, the beads were incubated with [125I]-labeled MoAb against α2-antiplasmin to quantitate binding of the α2-antiplasmin to the plasmin-sepharose. Results were compared with those obtained in pooled normal plasma and expressed as percentage of α2-antiplasmin in this pool. Results of this new assay, obtained with plasma samples not collected in EDTA-polybrene, closely correlated with those of a chromogenic assay (n = 60, r = .9). Experiments in which polybrene was added to plasma samples showed that the assay was not influenced by this cationic detergent at the concentrations used for our blood sampling procedure.

**Statistical analysis.** Except for the MAP, none of the parameters appeared to be normally distributed after the sting challenge. In such cases, the data were log-transformed before analysis. Student’s t-tests were used to compare data of two groups. To assess relationships, data from all 20 patients with an anaphylactic reaction were taken together. Correlations were calculated by linear regression analysis, and P values were calculated with Pearson’s product moment correlation coefficients. A P value less than .05 was considered to represent a significant difference.

**RESULTS**

**Clinical course.** Fifty-five patients and 8 volunteers were challenged with an insect sting. The blood sampling procedure was completed in only 34 patients and 8 volunteers (see under Materials and Methods). Characteristics of these 42 subjects are given in Table 1. As in earlier studies, no direct relation was observed between the subject characteristics, insect species, skin tests, insect-specific IgE and IgG (data not shown), epidemiologic, or clinical data of the previous reaction, on the one hand, and the grade of the reaction after the sting challenge, on the other. As observed before, most patients developed no reaction or a less severe reaction after sting challenge compared with the previous reaction. Only a minority developed a reaction of the same grade as before. In the present study, 7 of the 24 patients with a previous shock reaction developed a shock reaction after challenge.

In the 20 patients who showed an anaphylactic reaction after sting challenge, the anaphylactic symptoms started at 1 to 49 minutes (median, 10 min) after the sting. The elapsed time between the sting and the first symptoms of anaphy-
Two patients developed an acute hypotensive reaction after the sting challenge that was probably due to causes other than acute vasodilation. At 25 minutes after the sting challenge, 1 of these 2 patients developed angina pectoris with documented electrocardiographic changes characteristic of ischemia, which was accompanied by a decrease in the blood pressure. Hypotension in this patient was presumably due to acute heart failure, caused by sting-induced mast cell mediators. The other patient suffered from a protracted form of anaphylaxis (itching) and developed hypotension 35 minutes after the onset of symptoms. In this patient, hypotension was accompanied by bradycardia, sweating, and pallor and, in all likelihood, represented a vasovagal collapse.

An antihistaminic drug (clemastine, 1 mg/mL, administered intravenously, maximum of 6 mL) was administered to 4 patients with mild reaction (gastrointestinal and/or respiratory symptoms) and to all 7 patients with anaphylactic shock. These last 7 patients also received fluid replacement (Haemaccel, Behringwerke AG, Wardorf, Germany; intravenously, maximum of 2.5 L) starting the moment that hypotension developed. None of the patients received epinephrine.

All but 2 of the patients with an anaphylactic reaction recovered fully within 4 hours after the sting challenge. However, these 2 patients did not require additional therapy. All 7 patients with an anaphylactic shock were kept overnight in the intensive care unit, but no late reaction occurred. All 55 patients left the hospital in good clinical condition.

### Table 1. Characteristics of the 42 Subjects Challenged With a Living Insect and in Whom the Entire Blood Sampling Procedure Was Completed

<table>
<thead>
<tr>
<th>Group</th>
<th>Volunteers</th>
<th>No</th>
<th>Mild</th>
<th>Shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No.</td>
<td>8</td>
<td>14</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Insect*</td>
<td>5/3</td>
<td>11/3</td>
<td>9/4</td>
<td>4/3</td>
</tr>
<tr>
<td>Male/female</td>
<td>3/5</td>
<td>10/4</td>
<td>7/6</td>
<td>3/4</td>
</tr>
<tr>
<td>Previous reactions†</td>
<td>—</td>
<td>4/10</td>
<td>6/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>33 ± 23</td>
<td>42 ± 14</td>
<td>46 ± 10</td>
<td>43 ± 11</td>
</tr>
<tr>
<td>Specific IgE (RAST)$</td>
<td>0 (0/2)</td>
<td>2 (0/4)</td>
<td>2 (0/4)</td>
<td>2 (0/4)</td>
</tr>
<tr>
<td>Skin threshold (10⁶ µg/ml)$</td>
<td>2 (−2/2)</td>
<td>2 (−3/1)</td>
<td>2 (−5/1)</td>
<td>2 (−7/0)</td>
</tr>
</tbody>
</table>

Abbreviation: RAST, radio allergosorbent test.

* Number of patients challenged with a yellow jacket/honey bee.
† Number of patients with a previous reaction graded as mild/shock, see Materials and Methods.
‡ Mean ± SD.
§ Median (range).

Laxus was significantly shorter in the patients with anaphylactic shock (median 8 minutes; range, 1 to 37) than in those with a mild reaction (median, 11 min; range, 3 to 49; P < .05). The MAP remained unchanged or decreased by less than 15 mm Hg in the volunteers and in the patients with a mild reaction or no reaction. However, in the patients with an anaphylactic shock the MAP decreased from 97 ± 11 mm Hg to 65 ± 19 mm Hg (ie, 63% ± 21% of initial values) at 5 minutes after the first symptoms of anaphylaxis.

### Endothelial activation

Plasma levels of vWF were measured to assess activation of endothelium. Prechallenge levels of vWF were identical in all 4 groups of subjects (for all subjects: 107% ± 33%, mean ± SD; range, 61 to 216; median, 103). No change in vWF level was observed in patients with a mild reaction or no reaction or in any of the volunteers. In contrast, vWF levels increased in all 7 patients, with an anaphylactic shock to 233% ± 134% 60 minutes after the onset of clinical symptoms (Fig 1). In the 3 anaphylactic shock patients with the highest vWF levels, multimers of vWF were measured as described elsewhere. A significant increase of the highest multimers of vWF, up to a molecular weight of 5 × 10⁶ d, was found in only these 3 patients. Thus, evidence of activation of endothelium was found only in the 7 patients with an anaphylactic shock after sting challenge.

### Fibrinolytic parameters

Circulating plasma levels of tPA, uPA, PAP-c, PAI-1, and D-dimers were measured to assess fibrinolytic activation. Prechallenge levels of tPA did not differ among the various groups (5 ± 3 µg/L; range, 3 to 13; median, 3; Fig 2A). Significant changes in tPA levels did not occur in the volunteers or in the patients with no reaction, nor did they occur in all but 1 of the patients with a mild reaction after the sting challenge. In this particular patient with pulmonary distress, tPA levels increased from 10 to 26 µg/L at 15 minutes after the first symptoms. In contrast, tPA levels increased substantially in all but 2 of 7 patients with an anaphylactic shock. The tPA levels in these patients were already significantly elevated 1 minute after the onset of the clinical symptoms and reached peak values of 50 ± 59 µg/L at 5 minutes (range, 5 to 185; median, 30; P < .001 relative to prechallenge values; Fig 3A). The excep-

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**Fig 1.** Plasma levels of vWF after insect-sting challenge. Plasma levels of vWF (percentage of normal), before and after an in-hospital challenge with a yellow-jacket or a honey-bee sting, are given as mean ± SD. The four groups shown comprise 8 volunteers (D), 14 patients without anaphylactic symptoms (C), 13 patients with a mild reaction (B), and 7 patients with an anaphylactic shock (A), as defined under Materials and Methods. Levels before challenge (Pre) and the indicated time-points represent the time elapsed in minutes after the onset of clinical symptoms. The 1-minute point in the nonreacting patients and in the volunteers was arbitrarily taken at 15 minutes after the insect sting. *A significant increase relative to the prechallenge value.
tions were 1 patient with acute heart failure and 1 with a vasovagal collapse (see under Clinical Course). In these 2 patients, tPA levels increased from 4 to 6 and from 4 to 7 μg/L, in both cases 15 minutes after the first symptoms of anaphylaxis. tPA levels differed significantly in the anaphylactic shock group compared with the group with a mild reaction ($P < .01$). Prechallenge levels of uPA were not detectable (<1 μg/L) and remained undetectable in all individuals (data not shown).

Prechallenge plasma levels of PAP-c did not vary among the 4 groups of subjects (for the whole group: 6 ± 3 nmol/L; range, 2 to 13; median, 5; Fig 2B). After sting challenge, PAP-c levels remained the same in the volunteers, in the patients who experienced no reaction, and in all but 1 of the patients with a mild reaction (Fig 3B). In this particular patient with pulmonary distress (the same as the one described having a significant increase in tPA levels), PAP-c levels increased from 4 to 200 nmol/L at 15 minutes after the onset of anaphylactic symptoms. In contrast, PAP-c levels increased substantially in all but 1 of the 7 patients with an anaphylactic shock after the sting, from the first minute of clinical symptoms onward. Peak levels of 297 ± 225 nmol/L were found 5 minutes after the first symptoms (range, 19 to 607; median, 307; $P < .0001$, compared with prechallenge values; Fig 3B). The exception was 1 patient with acute heart failure (see under Clinical Course) in whom PAP-c levels increased only moderately, from 6 to 19 nmol/L, 15 minutes after the first clinical symptoms. PAP-c levels differed significantly in the anaphylactic shock group relative to the group with a mild reaction ($P < .01$).

Using a newly developed assay, we studied functional α2-antiplasmin in polybrene-EDTA samples. Prechallenge levels were normal, ie, 95% ± 11% of pooled normal plasma (range, 77 to 126; median, 95%). Significant decreases in α2-antiplasmin were only observed in patients with shock after challenge. Lowest α2-antiplasmin levels of 51% ± 33% were observed 5 minutes after the development of anaphylaxis for the patients with anaphylactic shock (range, 10 to 85; median, 55%; $P < .05$). In particular, the 2 patients with the highest levels of PAP-c (607 and 528 nmol/L at 5 minutes respectively) developed the lowest levels of α2-antiplasmin (10 and 26% at 5 minutes, respectively).

Prechallenge plasma levels of PAI-1 did not vary among the 4 groups of subjects (mean, 33 ± 28 μg/L; range, 4 to 128; median, 26; data not shown). No significant changes in PAI-1 levels occurred in any of the groups after sting challenge, not even 60 minutes after the onset of anaphylactic symptoms in the patients with a cardiovascular reaction.

Prechallenge plasma levels of D-dimers showed no variation among the 4 groups of subjects (mean, 156 ± 89 μg/L; range, 36 to 445; median, 126; data not shown). No significant changes in D-dimer levels were observed in the volunteers, in the patients with mild or no reaction after sting challenge. However, in the patients with an anaphylactic shock, D-dimer levels increased gradually to 351 ± 149 μg/L at 60 minutes after the first anaphylactic symptoms (range, 186 to 644; median, 293; $P < .05$ relative to prechallenge values).

Correlations between various fibrinolytic parameters were investigated in the 20 patients with anaphylactic symptoms after sting challenge. The correlation between tPA and PAP-c levels at 5 minutes after the onset of anaphylactic symptoms was highly significant ($r = .90; P < .001$). PAI-1 levels did not correlate significantly with any of the fibrinolytic parameters. The correlations between tPA and PAP-c at 5 minutes with corresponding D-dimer levels were not significant, but became significant when related to D-dimer levels at 15 minutes after the onset of anaphylactic symptoms ($r = .58, P < .05$ and $r = .65, P < .05$, respectively). tPA levels correlated significantly with vWF levels at 5 minutes after the first symptoms ($r = .81; P < .001$; Fig 4A). Thus, these data show a marked increase in tPA accompanied by activation of plasminogen in patients experiencing an anaphylactic shock after sting challenge.

Relation of mast cell products to endothelial and fibrinolytic parameters. In an earlier study with insect-sting anaphylactic patients, we found significant increases in plasma levels of both tryptase and histamine that were related to hypotension. Because of the observation of marked plasminogen activation in the patients with anaphylactic shock in the present study, we assessed the relation of released mast cell products to endothelial and fibrinolytic parameters in all 20 patients with anaphylactic symptoms after sting challenge. The correlations between levels of tryptase

![Fig 2. Plasma levels of tPA and PAP-c after insect-sting challenge. Plasma levels of (A) tPA (μg/L) and (B) PAP-c (nmol/L), both before and after an in-hospital challenge with a yellow-jacket or a honey-bee sting, are given on a log-scale as mean ± SD. The four groups shown and the x-axis are as in Fig 1.](image-url)
and histamine, on the one hand, and levels of vWF, tPA, and PAP-c, on the other, were significant (Table 2 and Fig 4B). Levels of tryptase and histamine did not correlate with D-dimer levels or PAI-1 levels (data not shown) at this time point. Thus, the degree of mast cell degranulation correlated with the extent of both endothelium and plasminogen activation in patients with anaphylactic symptoms after sting challenge.

Relation of MAP to activation of endothelium and plasminogen. Hypotension is one of the key features of cardiovascular anaphylactic reactions. We investigated the correlation between the change in MAP values and endothelium or fibrinolytic parameters. The change in MAP values inversely correlated with plasma levels of vWF, tPA, and PAP-c at 5 minutes after the onset of anaphylactic symptoms ($r = .59, P < .05; r = .67, P < .01$; and $r = .74, P < .001$, respectively). The correlation between the change in MAP values and plasma levels of PAP-c at 5 minutes is shown in Fig 5.

### DISCUSSION

In this study we describe that patients with an anaphylactic shock after an insect-sting challenge show a very rapid and substantial activation of plasminogen in plasma, corresponding to complexation of approximately 20% of the total α2-antiplasmin content of plasma. Activation of plasminogen in plasma has been described in various diseases and experimental conditions. To our knowledge, however, an activation comparable with that seen in the patients with anaphylactic shock described here has only been described after thrombolytic therapy.

The marked increase of PAP-c in the patients with shock raised the possibility that this increase reflected in vitro rather than in vivo generation. However, several considerations argue against an in vitro generation. First, fibrin was most likely not present in our plasma samples, which were collected on EDTA and polybrene. Second, all baseline samples and all but one of the values in nonhypotensive subjects and volunteers no mild shock reaction reaction Fig 3. Plasma levels of (A) tPA (µg/L) and (B) PAP-c (nmol/L) in individual patients and volunteers at 5 minutes after the onset of anaphylaxis (in the nonreacting subjects, 19 minutes after the sting) are given on a log-scale. Bars represent mean levels of the groups. The four groups shown are as in Fig 1.

Fig 4. Correlation between log-transformed plasma levels of vWF (%) and levels of (A) tPA (µg/L) and (B) tryptase (IU/L) in 20 patients with anaphylactic symptoms after insect-sting challenge, at 5 minutes after the onset of anaphylaxis.

| Table 2. Correlation Coefficients Between Mast Cell Products and Endothelium and Fibrinolytic Parameters (Log Transformed) in 20 Patients With Insect-Sting Anaphylaxis, All at 5 Minutes After the Onset of Anaphylactic Symptoms |
|-----------------------------|-----------------|-----------------|
|                             | Tryptase        | Histamine       |
| vWF                         | 0.68*           | 0.71*           |
| tPA                         | 0.84†           | 0.83†           |
| PAP-c                       | 0.83†           | 0.82†           |

* $P < .01$.
† $P < .001$. 

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after the challenge had normal PAP-c levels, indicating that the sampling procedure itself did not generate PAP-c in the plasma samples. Third, all samples were deep-frozen within 15 minutes after collection, preventing any catalytic activity thereafter. Fourth, in studies in which plasma samples were collected in a similar way, we did not observe such marked increases in PAP-c levels despite the fact that these samples contained at least similarly elevated tPA levels.10,29,38 Fifth, we performed additional tests on in vitro plasmin formation. After adding 50 μg/L recombinant tPA to normal plasma for a period up to 1 hour at room temperature, we observed an increase up to 35 nmol/L in PAP-c level, which is nearly 10 times less than that observed in our patient samples. This amount of in-vitro-generated PAP-c may even be underestimated, because the recombinant-tPA preparation contained up to 5% of two-chain tPA. Sixth, we observed a significant relation between PAP-c levels at 5 minutes and D-dimer levels at 15 minutes that is consistent with in vivo rather than with in vitro activation of plasminogen. Finally, even when the in-vivo-released high levels of tPA may have induced some plasmin generation in vitro, there is no reason to suppose that this would not have occurred in vivo as well. Thus, although we cannot definitely exclude that some in vitro generation of plasmin may have contributed to the PAP-c levels in some of the plasma samples, we conclude that most plasmin was formed in vivo during anaphylactic shock.

The correlation between tPA and PAP-c levels and the absence of significant changes of uPA levels in the patients suggested that the observed activation of plasminogen was mainly mediated by tPA. The activation of plasminogen was accompanied by a decrease of functional α2-antiplasmin and by activation of endothelial cells, as reflected by an increase in vWF and by an increased multimerization of this protein (data not shown). The increase of vWF was sustained, consistent with its relatively long half-life time in plasma (12 to 24 hours).35 Although some nonhuman mast cell lines have been found to contain tPA,36–41 this has never been reported for human mast cells. Therefore, we assume that the increase of tPA in our patients was, at least for the major part, due to the release by endothelium. Such a release may occur via several mechanisms; it may be secondary to the formation of thrombin or to ischaemia or may result from the interaction of other mediators with the endothelium. We can only speculate with regard to the mechanism underlying the release of tPA in the patients with insect-sting anaphylaxis.

Thrombin is able to induce the release of tPA by endothelial cells in vitro.42 Studies in baboons receiving intravenous injections of factor Xa bound to phospholipid vesicles indicate that in vivo thrombin may also trigger the release of tPA by the endothelium.43 To our knowledge, there are no prospective studies on the coagulation system in a significant number of patients with anaphylactic shock. However, case reports do indicate that activation in some patients may occur because fibrinogen as well as plasminogen levels may be decreased,42 and the prothrombin and activated partial thromboplastin times may be prolonged.2 To establish the formation of thrombin in our patients, we assessed levels of circulating TAT-c. Unfortunately, in all subjects, including the healthy volunteers as well as the patients who did not develop an anaphylactic reaction, levels of these complexes in plasma increased indiscriminately of the clinical reaction (data not shown). This was presumably due to the (unavoidable) use of intravenous catheters. Therefore, the results of the coagulation studies in our patients were seriously flawed. However, a strong activation of coagulation most likely did not occur in any of the patient groups after the challenge, because platelet counts did not decrease after the challenge in any subject (data not shown). Moreover, the course of D-dimer levels also argued against a substantial formation of fibrin. In only the patients with anaphylactic shock was a mild increase up to 351 ± 149 μg/L at 60 minutes observed, whereas in the other patients groups these levels did not change significantly during the observation period. Thus, taken together our observations are not in favor of a strong activation of the coagulation system in the patients with anaphylactic reactions after insect-sting challenge.

Hypoxemia, acidosis, and circulating (nor-)epinephrine may activate endothelial cells to induce the release of tPA.44–46 We previously observed a correlation between (nor-)epinephrine levels and hypotension in patients with insect-sting anaphylaxis.34 The maximal decrease of the mean arterial pressure in our patients occurred at 5 minutes after the onset of clinical symptoms34 and correlated with the increase of tPA (r = −.67, P < .01). Thus, the release of tPA in our patients might have occurred secondarily to the decrease in blood pressure and/or the release of catecholamines. However, the time-relationship of the increase of tPA with the decrease in blood pressure or with the increase in circulating catecholamines did not favor this explanation, because we did not observe a delay between these latter phenomena and the ensuing release of tPA. In addition, in 2 of the 7 patients with shock, hypotension was not primarily due to vasodilation but rather to acute heart failure and vasovagal collapse (see Clinical Course). In these patients, hardly any increase of tPA (up to 7 μg/L) was observed.
indicating that hypotension itself may not induce the release of tPA in vivo.

In addition to the mediators described in this and in other studies,7,8,34 we also measured plasma levels of interleukin-6 and interleukin-8. These levels did not increase in any of the patients, including those who developed an anaphylactic shock after the sting challenge (data not shown). This virtually excluded the possibility that the observed release of tPA and vWF by the endothelium was due to stimulation by cytokines.5 Moreover, the kinetics of the increase of vWF and tPA (occurring within a few minutes after the onset of symptoms) also argued against involvement of cytokines, because the effects of the latter on coagulation and fibrinolysis in vivo take at least 30 minutes.45,47 An additional argument against the involvement of cytokines was the observation that PAI-1 levels did not increase in our patients, which, to our knowledge, has never been described for cytokine-induced activation of the endothelium.

A fourth mechanism to explain a tPA-dependent activation of the fibrinolytic system involves activation of the endothelium by mediators released by degranulating mast cells. Mast cell degranulation products such as histamine are able to induce vasodilation and to enhance vascular permeability1,3,7 and have been shown to mediate the release of tPA.58-60 We previously found that anaphylactic shock due to insect stings is accompanied by a sharp increase in systemically released histamine and tryptase.2 The release of these mast cell mediators correlated with activation of the endothelium as assessed by changes in vWF levels (Fig 4) and also correlated with the increase of tPA and PAP-c (Table 2). Thus, the activation of fibrinolysis, as it occurred in the patients with anaphylactic shock after the insect sting challenge, may have resulted from the direct effects of mast cell degranulation products on the endothelium.

The increase of circulating PAP-c in the patients with shock described in this study is extreme and exceeds that seen during endotoxemia.10,36,37 Similar levels have been reported to occur only during thrombolytic therapy.16 This marked plasminogen activation was apparently not due to the presence of additional plasminogen activators in the insect venom, because in vitro experiments in which purified venom (ALK, Copenhagen, Denmark) was added to human plasma provided no evidence for this (data not shown). Some fibrin may have been formed in the patients, and this may have catalyzed the activation of plasminogen by tPA in our patients.31 Another factor contributing to the plasminogen activation was the absence of an increase in plasma levels of the inhibitor PAI-1 during the observation period. In the 2 patients with the most pronounced anaphylactic shock, additional measurements were made in blood samples collected at various intervals up to 24 hours. Also in these samples, PAI-1 levels did not increase (data not shown). We assume that the difference in PAI-1 levels observed during anaphylaxis compared with that during experimental endotoxemia16,37 are related to the different nature of the stimuli to the endothelium.

The clinical significance of this seemingly inappropriate plasminogen activation to the induction of anaphylactic shock is not known. Histamine is known to induce vasodilation and increased vascular permeability,1,3,7 which may lead to hypotension. Moreover, we observed a significant correlation between the degree of systemically released histamine and the decrease in MAP.2 Therefore, most likely, histamine is the main mediator inducing hypotension in the patients with anaphylactic shock after the insect-sting challenge. However, in individual patients with anaphylactic symptoms other than hypotension, levels of released histamine partly overlapped those observed in hypotensive patients.5 Thus, histamine is presumably not the only mediator that affects the endothelium and induces hypotension during an anaphylactic reaction. In the present study, we show that plasminogen is markedly activated during anaphylactic shock, which paralleled both the degree and the kinetics of hypotension during the anaphylactic reaction (Figs 2B and 5). In vitro, plasmin has been shown to be able to cleave biologically active peptides from precursor proteins, for example, from complement components (C3a, C5a, and C2-kinin).51,52 Therefore, we suggest that activation of plasminogen, in addition to degranulation of mast cells, may be involved in the pathogenesis of anaphylactic shock.

ACKNOWLEDGMENT

We thank A. Eerenberg, M. Freen, and Dr Ph.G. de Groot for technical support; A.M. van der Linden-Bröcker, Dr H. Barrowclough, and I. Seeger for linguistic review; and Prof Dr J.J. Sixma for critical review of this paper.

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Controlled insect-sting challenge in 55 patients: correlation between activation of plasminogen and the development of anaphylactic shock

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