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## Activation of the Contact System in Insect-Sting Anaphylaxis: Association With the Development of Angioedema and Shock

By Peter-Willem G. van der Linden, C. Erik Hack, Anke J.M. Eerenberg, Albert Struyvenberg, and J. Kees van der Zwan

**A postulated role of the contact system in anaphylactic reactions to insect stings was investigated. During prospective, in-hospital sting challenge, we collected serial blood samples from five normal volunteers and 16 patients with a history of insect-sting anaphylaxis. Activation of the contact system was assessed by measuring plasma levels of factor XIIa-C1-inhibitor and kallikrein-C1-inhibitor complexes as well as those of cleaved high molecular weight kininogen (HK). In addition, antigenic levels of (pre)kallikrein, factor XII, and HK were measured. No significant changes in contact system parameters were observed in any of the five volunteers or the four patients who did not develop an anaphylactic reaction after sting challenge. In contrast, significant changes in contact system parameters**

**occurred in 7 of the 12 patients with anaphylactic symptoms after challenge. Peak levels of either C1-inhibitor complex were found 5 minutes after the onset of anaphylactic symptoms. The increase in C1-inhibitor was most pronounced in the 4 patients with angioedema, 2 of which also developed shock. However, activation of HK was observed in all four patients with angioedema, the two patients with shock but no angioedema, as well as in 1 of the remaining 6 patients with anaphylactic symptoms other than angioedema or shock. Thus, activation products of the contact system may be involved in the pathogenesis of angioedema and shock in insect-sting anaphylaxis.**

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**A**NAPHYLACTIC reactions to insect stings may differ substantially in their clinical presentation, ranging from skin or gastrointestinal to pulmonary or cardiovascular symptoms.<sup>1,2</sup> It is generally accepted that mast-cell mediators, eg, histamine, prostaglandins, or leucotrienes, mediate most of the symptoms.<sup>3-5</sup> Supplementary to mast-cell mediators, angioedema may be induced by kinins<sup>6-8</sup> as well as by complement-activation products.<sup>9,10</sup> Anaphylactic shock, on the other hand, may be caused by the additional cardiovascular effects of activation products of the fibrinolytic<sup>11-12a</sup> and the complement system,<sup>10,13-16</sup> or to endothelial factors.<sup>17</sup> Thus, different inflammatory mediators might be involved in the pathogenesis of the various anaphylactic symptoms.

The contact system of coagulation consists of the proteins (pre)kallikrein, factor XII, and high molecular weight kininogen (HK). During activation of this system, the nonapeptide bradykinin is generated, which might increase vasopermeability and induce vasodilation.<sup>6,7,17-24</sup> Several investigators have suggested a relation between anaphylaxis and activation of the contact system.<sup>25-34</sup> In vitro, some purified mast-cell mediators are able to activate (pre)kallikrein

and factor XII,<sup>25</sup> whereas tryptase, the predominant mast-cell neutral protease, is able to inactivate HK (reviewed by Schwartz<sup>26</sup>). Other in vitro experiments with human cell lines<sup>27,28</sup> as well as animal studies<sup>29</sup> have indicated that contact activation may play a role in the pathogenesis of allergy. Moreover, clinical case reports have also provided evidence for a role of the contact system in allergic reactions, eg, nasal allergy<sup>30</sup> or skin reactions to pollen extracts,<sup>31</sup> and in anaphylactic reactions to radio contrast media<sup>32</sup> or to vascular grafts.<sup>33</sup> Smith et al<sup>34</sup> showed a decrease in HK but not in (pre)kallikrein or factor XII in 2 of 14 patients with insect-sting anaphylaxis. On the other hand, Ratnoff<sup>1</sup> found decreased levels of both HK and (pre)kallikrein but not of factor XII in one patient suffering from wasp-sting anaphylactic shock. More conclusive proof for activation of the contact system in insect-sting anaphylactic patients would be provided by the demonstration of increased plasma levels of activation products, such as complexes of C1-inhibitor with kallikrein and factor XIIa.<sup>35,36</sup>

Results of several studies<sup>16,37-41</sup> have shown that a sting-challenge procedure might give better selection of patients who are eligible for venom immunotherapy than insect-specific IgE and skin tests do. Therefore, in The Netherlands and a number of other European countries, some patients are selected for venom desensitization by sting challenge under controlled intensive-care conditions, while investigators and clinicians who feel uncomfortable with this sting-challenge procedure continue to use the classical criteria for venom hypersensitivity.<sup>42</sup> The sting-challenge approach offered us the opportunity to study prospectively the sequential release of mediators during anaphylaxis. In previous studies, we observed a relation of activation of mast cells,<sup>40</sup> plasminogen,<sup>12a</sup> and complement<sup>16</sup> to the severity of insect-sting anaphylactic shock. In the present study, a possible role of the contact system in insect-sting anaphylactic reactions was prospectively investigated by assessing changes in plasma levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor, cleaved HK, as well as in those of (pre)kallikrein, factor XII, and HK. The results were analyzed in relation to the clinical course, including the development of angioedema and anaphylactic shock.

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## MATERIALS AND METHODS

**Subjects.** Sixteen 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. Five healthy volunteers were also challenged, including three individuals who had been stung in the past by an insect of the species in question without developing anaphylactic symptoms. All subjects were in good health, none was pregnant or had a cardiac disorder, and none of them used a  $\beta$ -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 out-patient clinic, where detailed oral and written information was given on the protocol used for the study "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.

The subjects in this study on contact activation were part of a larger group we studied with respect to the release of mast-cell mediators,<sup>40</sup> complement activation,<sup>16</sup> cardiovascular mediators,<sup>41</sup> and plasminogen activation.<sup>12a</sup> Serial blood samples were obtained in tubes suitable for contact-system analysis in only the last 42 of the total group of 69 subjects. Because all subjects had two indwelling intravenous (IV) catheters, which may activate the contact system and thus blur the activation caused by insect-sting challenge, we decided to exclude prospectively any subject with a prechallenge level of kallikrein-C1-inhibitor exceeding  $300 \times 10^{-5}$  U/mL, which is above the 95th percentile of kallikrein-C1-inhibitor in plasma samples obtained in our laboratory from normal blood donors (median 60; range 50 to  $490 \times 10^{-5}$  U/mL<sup>36</sup>).

**Study protocol.** The provocation procedure using live insects was performed as described elsewhere.<sup>12a,16,40,41</sup> In short, before the sting challenge, all subjects were placed on a continuous heart rate monitoring device in the intensive care unit and an IV line was inserted in each arm. A yellow jacket of the species *Vespula germanica* or a honey-bee of the species *Apis mellifera* was induced to sting the lower left arm for 30 seconds, after which the insect was killed. Blood pressure was recorded automatically at 1-minute intervals in unstable and at 5-minute intervals in stable conditions. Mean arterial pressure was calculated as two-thirds diastolic pressure plus one-third systolic pressure.

Reactions were defined as: no reaction = no systemic reaction; anaphylactic reaction = one or more of the following generalized symptoms: urticaria, itching, erythema, angioedema, gastrointestinal symptoms, respiratory symptoms, or shock. Erythema, urticaria, and angioedema were defined as generalized reactions in areas not directly adjacent to the sting site. They were assessed by both the patient and one of the investigators (P.W.G. vd L.). Angioedema was defined as edema of the face, tongue, extremities, or genitalia.<sup>8</sup> Shock was defined as more than 15 mm Hg decrease in mean arterial pressure, compared with prechallenge values, and necessitating treatment. After the challenge, only the patients with respiratory or cardiovascular symptoms on challenge were started on venom immunotherapy. Insect determination was performed as described elsewhere.<sup>16,40,41</sup>

**Blood-sampling procedure.** Blood was collected in siliconized Vacutainer tubes (Becton Dickinson, Plymouth, U.K.) to which EDTA (10 mmol/L, final concentration) and Polybrene (0.05%, wt/vol, final concentration; Janssen Chimica, Beerse, Belgium) had been added to prevent in vitro activation of the contact system.<sup>35</sup> The mixture was centrifuged for 10 minutes at 1,300g within 20 minutes after sampling and the plasma samples were stored in aliquots at  $-70^{\circ}\text{C}$  until tested.

The interval between the sting and the onset of clinical symptoms may vary considerably, as described elsewhere.<sup>16,39,40</sup> To obtain a

uniform pattern of data, appropriate for statistical analysis, blood samples were collected before sting challenge ("pre") and 1, 5, 15, and 60 minutes after the moment at which the patient reported the onset of a reaction. Starting 15 minutes after the insect sting, similar blood sampling was performed in nonreacting subjects.

**Assays.** Activation of the contact system was assessed by measuring plasma levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor.<sup>35,36</sup> These complexes were determined with radio-immunoassays that are able to detect 0.05% activation of (pre)kallikrein or factor XII in plasma.<sup>35,36</sup> Results were expressed in U/mL, 1 U/mL being the maximal amount of C1-inhibitor complexes that can be generated in pooled plasma.<sup>36</sup> Assessment of cleaved HK by immunoblotting assay was performed as described elsewhere.<sup>43</sup> Levels of (pre)kallikrein, factor XII, and HK antigens were determined as described elsewhere.<sup>35,36,43</sup> Results were expressed in percentage of normal pooled human plasma. Ranges of normal values are 71% to 133% for (pre)kallikrein, 25% to 174% for factor XII, and 74% to 136% for HK.<sup>36,43</sup> Levels of functional and of reactive-site cleaved C1-inhibitor were determined as described elsewhere.<sup>44</sup>

**Statistical analysis.** Mann-Whitney rank tests were used to compare data of two groups. Fisher's exact tests were used to compare proportional numbers of patients between different groups. For assessment of relationships, data of all 12 patients with an anaphylactic reaction were pooled. Correlations were calculated by linear regression analysis and *P* values with Pearson's product moment correlation coefficients. A *P* value less than .05 was considered to represent a significant difference.

## RESULTS

**Clinical course.** Forty-two subjects, including 8 volunteers, entered the study and were challenged with an insect sting. However, 21 of them, including 3 volunteers, showed levels of kallikrein-C1-inhibitor exceeding  $300 \times 10^{-5}$  U/mL after insertion of two IV catheters. These subjects were excluded from further analysis (see Materials and Methods). There was no difference in grade of reaction between the excluded and the included subjects in this study. Four of the remaining 16 patients and all five volunteers developed only a local swelling at the site of the sting but no anaphylactic symptoms. The patient characteristics and the clinical symptoms of the remaining 12 patients who did develop an anaphylactic reaction after sting challenge, are given in Table 1. In these 12 patients, the anaphylactic symptoms started at 1 to 40 minutes (median: 10 minutes) after the sting. The time elapsed between the sting and the first symptoms of anaphylaxis was shorter in the patients with anaphylactic shock (median: 8 minutes) than in those with a mild reaction (median: 11 minutes), but this difference was not significant. As in earlier studies,<sup>16,39,41</sup> no relation was observed between either the subject characteristics, insect species, skin tests, insect-specific IgE and IgG, epidemiologic or clinical data of the previous reaction on the one hand and the clinical symptoms after the sting challenge on the other (data not shown).

An antihistaminic drug (clemastine, 1 mg/mL, IV, maximum dose 6 mL) and fluid replacement (Haemaccel, Behringwerke AG, Wardorf, Germany; IV, maximum of 2.5 L) was administered only to the four patients with anaphylactic shock, starting when hypotension developed. None of the patients required additional therapy, ie, epi-

**Table 1. Characteristics and Symptoms in 12 Patients With Insect-Sting Anaphylaxis**

| Patient (no.) | Age (yr) | Sex | Insect (hb/yj) | Anaphylactic Symptoms                                                                                                                                          |
|---------------|----------|-----|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1             | 47       | F   | yj             | Erythema, urticaria                                                                                                                                            |
| 2             | 48       | F   | hb             | Itching, anxiety                                                                                                                                               |
| 3             | 50       | F   | yj             | Erythema, itching, urticaria                                                                                                                                   |
| 4             | 31       | F   | yj             | Erythema, itching, urticaria                                                                                                                                   |
| 5             | 65       | M   | yj             | Erythema, itching, urticaria, angioedema (face)                                                                                                                |
| 6             | 48       | M   | yj             | Erythema, itching                                                                                                                                              |
| 7             | 26       | M   | hb             | Erythema, itching                                                                                                                                              |
| 8             | 50       | M   | hb             | Itching, erythema, urticaria, anxiety, angioedema (face, tongue), nausea                                                                                       |
| 9             | 55       | F   | yj             | Itching, angina pectoris, nausea, shock (MAP-21%)*                                                                                                             |
| 10            | 41       | M   | hb             | Erythema, itching, anxiety, hoarseness, shock, (MAP-51%)*                                                                                                      |
| 11            | 66       | F   | yj             | Erythema, itching, angioedema (face, tongue), nausea, arrhythmias, shock (MAP-27%)*                                                                            |
| 12            | 59       | F   | yj             | Erythema, urticaria, itching, anxiety, angioedema (face, tongue, both extremities), stomach pain, vomiting, shortness of breath, arrhythmias, shock (MAP-65%)* |

Abbreviations: hb, honey bee; yj, yellow jacket.

\* Maximum change in mean arterial pressure (MAP) relative to prechallenge value.

nephrene. All subjects left the hospital in good clinical condition.

**Plasma levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor.** Prechallenge levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor (mean  $\pm$  SD) were identical in all subject groups. No change in these levels was observed in either the volunteers or the patients who showed no reaction after sting challenge (Fig 1; Table 2). In patients with anaphylactic symptoms after sting challenge, kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor levels increased with marked individual differences. Analysis of these changes in the various subgroups showed that the increases in kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor levels were significant in the shock group ( $P < .05$ ) and in the group with angioedema ( $P < .005$ ), but not in the group with generalized urticaria (Fig 1; Table 2).

A second method to assess the association between contact activation and the clinical symptoms was studying the proportion of patients in each group, in whom C1-inhibitor complexes after the challenge exceeded the arbitrary level of 200% of initial values. In this way, contact activation occurred in 9 of the 12 patients with anaphylactic symptoms, including all four patients with angioedema. This contrasted with a similar increase of C1-inhibitor complexes in only 1 of the 9 nonreacting subjects ( $P < .01$ ). Elevated C1-inhibitor complexes ( $>200\%$ ) at two successive time points, indicating a sustained activation of the contact system, were strongly associated with the development of angio-

edema ( $P < .002$ ). This was found in all four patients with angioedema but in none of the other nine anaphylactic patients and in none of the nine nonreacting subjects.

**Plasma levels of cleaved HK.** Cleaved HK was assessed with an immunoblot assay<sup>43</sup> in plasma samples taken before and at 15 and 60 minutes after the challenge. Increased levels were found in only two patients who developed shock, one of them with angioedema as well (patients I and IV in Fig 2). In one other patient with both shock and angioedema, cleavage of HK could not be assessed because antigenic levels were dramatically decreased (patient III in Fig 2).

**Plasma levels of (pre)kallikrein, factor XII, and HK antigens.** Plasma samples were collected on Polybrene to prevent in vitro activation of the contact system.<sup>35</sup> This procedure precluded the use of functional assays for contact factors. Therefore, in addition to measuring specific contact activation products (see above), we assessed antigenic levels of contact system proteins.

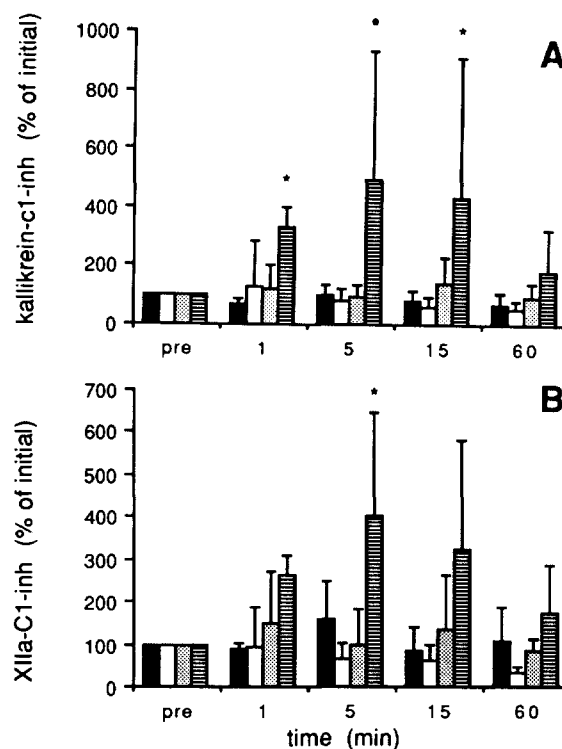


**Fig 1. Contact activation in groups of subjects after insect-sting challenge.** Plasma levels of (A) kallikrein-C1-inhibitor and (B) factor XIIa-C1-inhibitor, before and after an in-hospital challenge with a yellow jacket or a honey-bee sting, expressed as mean  $\pm$  SD (percent of initial values), are shown for five volunteers (■), four patients without anaphylactic symptoms (□), eight patients with an anaphylactic reaction without angioedema (▨), and four patients with an anaphylactic reaction including angioedema (▩), as defined under Materials and Methods. "Pre": levels before challenge; 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. Asterisk indicate a significant increase relative to the prechallenge value.

**Table 2. Contact Activation in Various Groups of Subjects After Insect-Sting Challenge**

| Subject       | No. | Initial Levels of C1-Inhibitor-Complex (10 <sup>-5</sup> U/mL)* |             | Levels of C1-Inhibitor-Complex at 5 min (10 <sup>-5</sup> U/mL)* |             |
|---------------|-----|-----------------------------------------------------------------|-------------|------------------------------------------------------------------|-------------|
|               |     | Kallikrein                                                      | Factor XIIa | Kallikrein                                                       | Factor XIIa |
| Volunteer     | 5   | 175 ± 85                                                        | 16 ± 5      | 171 ± 108                                                        | 26 ± 20     |
| No reaction   | 4   | 194 ± 112                                                       | 22 ± 13     | 163 ± 101                                                        | 11 ± 8      |
| Reaction      |     |                                                                 |             |                                                                  |             |
| All           | 12  | 168 ± 73                                                        | 22 ± 13     | 301 ± 301†                                                       | 35 ± 37†    |
| Shock         | 4   | 186 ± 81                                                        | 34 ± 14     | 542 ± 404‡                                                       | 67 ± 47‡    |
| No shock      | 8   | 159 ± 67                                                        | 15 ± 7      | 180 ± 104                                                        | 18 ± 12     |
| Urticaria     | 6   | 142 ± 54                                                        | 12 ± 5      | 352 ± 355†                                                       | 32 ± 36†    |
| No urticaria  | 6   | 194 ± 80                                                        | 31 ± 12     | 249 ± 224                                                        | 37 ± 37     |
| Angioedema    | 4   | 170 ± 68                                                        | 22 ± 17     | 634 ± 318§                                                       | 71 ± 43§    |
| No angioedema | 8   | 167 ± 75                                                        | 21 ± 11     | 134 ± 46                                                         | 16 ± 10     |

\* Mean ± SD

† No statistically significant difference compared with the other relevant groups.

‡  $P < .05$  compared with nonshock patients and to prechallenge values.§  $P < .005$  compared with nonangioedema patients and to prechallenge values.

Prechallenge levels of (pre)kallikrein, factor XII, and HK antigens were identical in all subjects (for all subjects: 96% ± 18%, range 78 to 124, median 92; and 103% ± 32%, range 52 to 161, median 105; and 95% ± 10%, range 78 to 110, median 95, respectively). Table 3 shows the prechallenge levels as well as levels at 15 minutes after the onset of clinical symptoms, the time point of the nadir of these antigenic levels. Factor XII levels did not change significantly in any subject after sting challenge. Only in the four patients with angioedema did (pre)kallikrein levels decrease significantly, 15 minutes after the onset of symptoms (78% ± 18%,  $P < .05$ ; Table 3). No significant changes in (pre)kallikrein levels occurred in any of the other (sub)groups.

Levels of HK decreased by more than 15% of prechallenge values in only six patients: in all four angioedema patients with or without shock, in one patient with shock but no angioedema, and in one patient with urticaria. One of the patients with both shock and angioedema showed a dramatic drop in HK: HK levels dropped from 88% before challenge to less than 1%, 15 minutes after the onset of clinical symptoms (patient III in Fig 2). In one of the patients with shock but no angioedema, HK levels dropped by only 12%, but increased levels of cleaved HK were observed (patient 1, Fig 2). Thus, activation of HK, as reflected by decreased levels of HK antigen and/or increased levels of cleaved HK, occurred in 7 of the 12 patients with anaphylaxis after sting challenge, including the six patients with angioedema and/or shock.

**Plasma levels of C1-inhibitor.** Prechallenge levels of functional C1-inhibitor were  $2.4 \pm 0.2 \mu\text{mol/L}$ . These levels did not change significantly in any of the subjects after challenge.

**Relation between contact-activation parameters and mean arterial pressure, mast-cell mediators, or von Willebrand factor (vWF).** Hypotension is one of the salient features of a cardiovascular anaphylactic reaction. We investigated the correlation between changes in kallikrein-C1-inhibitor or factor XIIa-C1-inhibitor levels and changes in mean arterial pressure values. Plasma levels of kallikrein-C1-inhibitor were only marginally related to the decrease in mean arterial pressure ( $r = .61$ ,  $P < .05$ ). We do not think that this statistically significant correlation was clinically significant, because it depended on a single data point. Factor XIIa-C1-inhibitor did not show a statistically significant correlation with the decrease in mean arterial pressure.

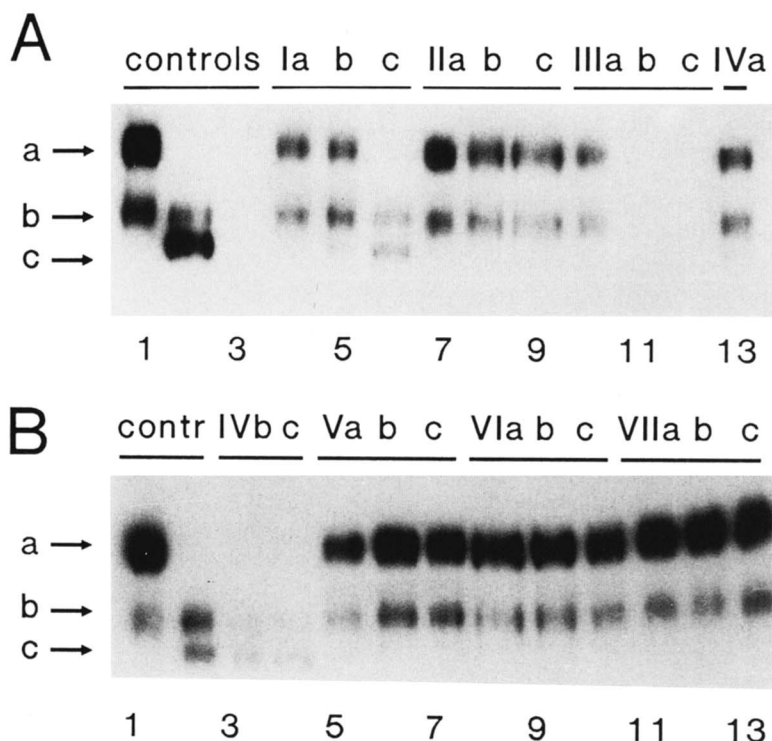


**Fig 2. Immunoblot analysis of HK in samples from patients after insect-sting challenge.** Plasma samples were electroforested under nonreducing conditions, blotted on nitrocellulose sheets, and incubated with <sup>125</sup>I-anti-HK antibodies (see Materials and Methods). (A) lane 1: normal plasma; lane 2: dextran-sulphate activated plasma (prepared by incubating normal plasma with an equal volume of dextran-sulphate [50  $\mu\text{g/ml}$  in phosphate-buffered saline] for 20 minutes at 37°C); lane 3: HK-deficient plasma. (B) lane 1: normal plasma; lane 2: dextran-sulphate-activated plasma. The other lanes contain plasma samples from several patients (indicated with roman numbers) before (a) and at 15 minutes (b) and 60 minutes (c) after the onset of clinical symptoms after sting challenge. Arrows indicate bands with M, 130,000 (a); 110,000 (b); and 95,000 (c).

**Table 3. Contact System Parameters in Various Groups of Subjects After Insect-Sting Challenge**

| Subject       | No. | Initial Levels (% normal)* of |            |          | Levels (% normal)* at 15 Minutes of |            |          |
|---------------|-----|-------------------------------|------------|----------|-------------------------------------|------------|----------|
|               |     | PK                            | Factor XII | HK       | PK                                  | Factor XII | HK       |
| Volunteer     | 5   | 93 ± 20                       | 103 ± 26   | 96 ± 5   | 96 ± 14                             | 108 ± 32   | 97 ± 5   |
| No reaction   | 4   | 98 ± 13                       | 87 ± 59    | 94 ± 8   | 84 ± 20                             | 82 ± 53    | 94 ± 7   |
| Reaction      |     |                               |            |          |                                     |            |          |
| All           | 12  | 92 ± 19                       | 112 ± 30   | 93 ± 13  | 82 ± 18†                            | 91 ± 18†   | 79 ± 27† |
| Shock         | 4   | 85 ± 15                       | 114 ± 15   | 86 ± 5   | 70 ± 10†                            | 81 ± 17†   | 54 ± 31§ |
| No shock      | 8   | 95 ± 20                       | 111 ± 35   | 97 ± 14  | 91 ± 18                             | 97 ± 17    | 92 ± 13  |
| Urticaria     | 6   | 104 ± 15                      | 96 ± 17    | 100 ± 12 | 84 ± 13†                            | 82 ± 17†   | 80 ± 37† |
| No urticaria  | 6   | 79 ± 15                       | 128 ± 31   | 87 ± 11  | 81 ± 22                             | 101 ± 14   | 77 ± 11  |
| Angioedema    | 4   | 108 ± 18                      | 103 ± 15   | 98 ± 10  | 78 ± 18†                            | 81 ± 17†   | 64 ± 38‡ |
| No angioedema | 8   | 84 ± 14                       | 116 ± 34   | 91 ± 14  | 84 ± 18                             | 97 ± 16    | 86 ± 15  |

Abbreviations: PK, (pre)kallikrein antigen; HK, high molecular weight kininogen.

\* Mean ± SD

† No statistically significant difference compared with the other relevant groups.

‡  $P < .05$  compared with nonangioedema or nonshock patients and with prechallenge values.§  $P < .002$  compared with nonshock patients and with prechallenge values.

In earlier studies done in insect-sting anaphylactic patients, we found significant increases in plasma levels of the mast-cell products tryptase and histamine,<sup>40</sup> as well as of vWF, a parameter of endothelial activation.<sup>12a</sup> These increases proved to be related to the clinical severity of anaphylaxis (eg, hypotension). We analyzed the changes in plasma levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor in relation to those of tryptase, histamine, and vWF. Plasma levels of either C1-inhibitor complex correlated significantly with plasma levels of tryptase ( $P < .01$ ), histamine ( $P < .01$ ), and vWF ( $P < .05$ ; Fig 3).

## DISCUSSION

Anaphylactic shock in rats caused by proteins<sup>29</sup> and in the human by radio contrast media<sup>32</sup> or vascular grafts<sup>33</sup> has been shown to be accompanied by some degree of contact activation. In insect-sting anaphylaxis, activation of the contact system has been reported incidentally. In one patient with shock and angioedema, HK and prekallikrein levels dropped, whereas factor XII levels did not change, 14 hours after the sting.<sup>11</sup> Two other patients with shock after an insect sting had decreased HK levels, but unchanged (pre)kallikrein and factor XII levels, 1 hour after the onset of symptoms.<sup>34</sup>

Consistent with these case reports, we observed in the present study a decrease in antigenic levels of (pre)kallikrein and HK in patients who developed angioedema and/or shock after sting challenge. These parameters did not decrease in any of the subjects who did not develop an anaphylactic reaction after challenge. This decrease in (pre)kallikrein and HK antigens was probably due to the rapid clearance of these activation products from the circulation,<sup>44</sup> as it was associated with the generation of contact activation products, such as kallikrein-C1-inhibitor, factor XIIa-C1-inhibitor, and cleaved HK. Thus, our finding indicate that activation of the contact system may occur during anaphylactic reactions after insect-sting challenge.

It is generally accepted that bradykinin, one of the activa-

tion products of the contact system, is responsible for the induction of most of the clinical symptoms after activation of this system.<sup>6-8,17-24</sup> However, bradykinin is rapidly degraded in plasma.<sup>7,45-48</sup> Thus, although the determination of plasma levels of bradykinin might be the preferred method to assess contact activation in our patients, it is not a practical approach. Several alternative procedures to assess contact activation have been described, eg, determination of cleaved HK<sup>49</sup> or kallikrein- $\alpha$ 2-macroglobulin complexes.<sup>50</sup> In our study, contact activation was assessed by measuring plasma levels of cleaved HK, kallikrein-C1-inhibitor, and factor XIIa-C1-inhibitor. These C1-inhibitor complexes were quantitated with radioimmunoassays that can detect activation of 0.05% of plasma (pre)kallikrein or factor XII in vitro.<sup>35,36</sup> The half-life time of clearance of both C1-inhibitor complexes from the circulation is approximately 50 minutes in septic patients<sup>36</sup> and 32 to 47 minutes in rats.<sup>44</sup> This rapid clearance may explain why, in contrast to the patients described here, no significant elevations of both C1-inhibitor complexes were found in patients with sepsis. Blood in these septic patients was collected at 6-hour intervals,<sup>36</sup> whereas it was collected within a period of 1 hour in our anaphylactic patients.

A sustained activation of the contact system in our patients was investigated by assessing levels of C1-inhibitor complexes that exceeded 200% of prechallenge values in at least two subsequent blood samples. This sustained activation was observed in all four patients with angioedema, including two patients with shock, but not in any of the other subjects, including two patients with shock but no angioedema. This suggests that sustained activation of the contact system is associated with the development of angioedema after sting challenge. In one of the two patients with shock but no angioedema, however, HK levels dropped by more than 15% of prechallenge values. In the other patient, cleaved HK was found (patient I; Fig 2). Although the reason for this discrepancy between activation of HK and generation of C1-inhibitor complexes is not clear, these results imply involvement of the contact system in the develop-

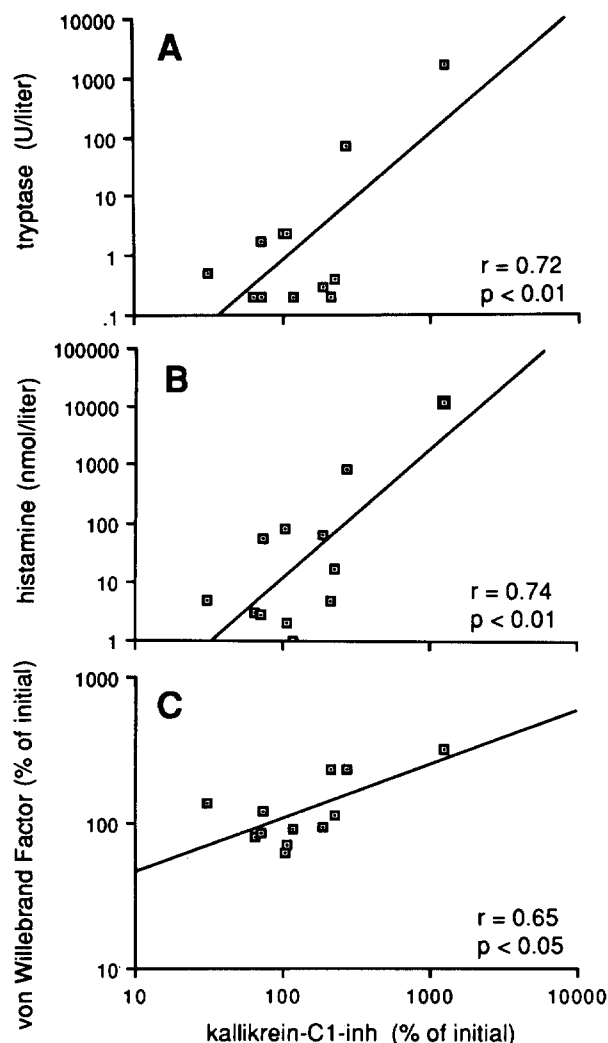


**Fig 3.** Relation of contact activation to activation of mast cells and endothelium. The change in kallikrein-C1-inhibitor levels (percent of initial levels) 5 minutes after the onset of clinical symptoms, is plotted against (A) tryptase levels (U/liter), (B) histamine (nmol/L), and (C) vWF (percent of initial levels) for all 12 patients with anaphylactic symptoms after sting challenge.

ment of anaphylactic shock as well as that of angioedema in insect-sting anaphylaxis.

In one patient, HK dropped to less than 2% of prechallenge values within 5 minutes after the onset of clinical symptoms (patient III; Fig 2). We are not aware of data on the clearance of cleaved HK in vivo. If this rapid and pronounced decrease of HK was caused by kallikrein-mediated cleavage, it would imply that cleaved HK is cleared with a half-life of less than 2 minutes. However, in two other patients cleaved HK was observed in plasma samples collected at 15 and at 60 minutes after the onset of clinical symptoms (I and IV; Fig 2), indicating a much slower clearance. These apparent differences in clearance of cleaved HK may be caused by individual variances or by proteolytic cleavage of HK by proteinases other than kallikrein, eg, tryptase.<sup>26</sup>

Five of the 12 patients with anaphylactic symptoms after

sting-challenge did not show activation of the contact system, according to sustained elevations of C1-inhibitor complexes and cleaved HK. Thus, in these patients systemic activation of the contact system did not occur. However, it cannot be excluded that local activation of the contact system, not detectable in plasma, might have contributed to the pathogenesis of anaphylactic symptoms as has been shown for allergic reactions in the nose<sup>30</sup> and the skin.<sup>31</sup>

Patients with attacks of hereditary angioedema (genetic deficiency of C1-inhibitor) have increased plasma levels of contact-activation products and depressed (pre)kallikrein and HK levels<sup>8,50-52</sup> in the absence of hypotension.<sup>53</sup> The reason why hypotension does not occur in acute attacks of hereditary angioedema is not known. An explanation might be that the changes in contact system parameters in this disease reflect local rather than systemic activation. Similarly, one might postulate that the elevation of the levels of kallikrein-C1-inhibitor and factor XIIa-C1-inhibitor in the plasma of our patients with angioedema but no shock, was also caused by local rather than systemic activation of the contact system.<sup>54,55</sup> The possibility that in our patients angioedema developed as a consequence of an acute acquired C1-inhibitor deficiency was excluded because levels of functional C1-inhibitor did not significantly decrease during the observation period. In previous reports, we showed complement activation as well as plasminogen activation in patients with shock after sting challenge.<sup>12a,16</sup> Thus, our observations further emphasize the resemblance between angioedema caused by insect stings and that caused by hereditary deficiency of C1-inhibitor. In the latter condition, complement activation and plasminogen activation occur simultaneously during attacks of angioedema.<sup>8,9,50-53</sup> In a review article, Orfan and Kolski<sup>56</sup> discussed the involvement of a C2-kinin, a fragment generated from C2b by plasmin,<sup>9</sup> in the pathogenesis of angioedema. Further investigations are needed to establish the role of a C2-kinin in mediating insect-sting-induced angioedema.

The contact system is involved in many physiologic and pathologic processes, eg, blood coagulation, fibrinolysis, complement activation, and activation of the renin-angiotensin system.<sup>6-9,15,54-58</sup> It presumably plays a role in inflammation, in septic shock, in the toxic side effects of interleukin-2 therapy, and in the development of angioedema caused by a genetic deficiency of C1-inhibitor.<sup>6,7,36,43,52,53,56</sup> Some of these mediator systems have been shown to be activated during anaphylactic reactions, ie, the complement,<sup>10,13-16</sup> angiotensin,<sup>6,7,15</sup> and fibrinolytic systems.<sup>11,34</sup> Thus, the combined effects of activation products of different mediator systems, each acting systemically or locally, may be responsible for the divergence of clinical symptoms of (insect-sting) anaphylaxis.

The mechanism underlying contact activation after insect-sting challenge in our patients is not known. Although tryptase, the predominant neutral protease of mast cells, may inactivate HK without inducing contact activation,<sup>26</sup> other mast-cell mediators (heparin, chondroitin sulphate E) are able to activate factor XII and (pre)kallikrein in vitro.<sup>25</sup> Furthermore, activated basophils<sup>27</sup> and lung cells (probably mast cells)<sup>28</sup> may release a kallikrein-like substance. There-

fore, a possible route of contact-system activation could be direct stimulation by mast-cell or basophil mediators. The observed correlations between kallikrein-C1-inhibitor and factor XII-C1-inhibitor and both tryptase and histamine (Fig 3) support this hypothesis.

In conclusion, we have shown that the development of angioedema in insect-sting anaphylactic patients is accompanied by activation of the contact system. Therefore, contact-activation products may be involved in the pathogenesis of angioedema in anaphylaxis. In individual patients, these products might possibly contribute to the pathogenesis of anaphylactic shock.

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