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Fibrin formation during ongoing cutaneous allergic reactions: Comparison of responses to antigen and codeine

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Background: *Fibrin formation, assessed by fibrinopeptide A levels, was evaluated over a 5-hour period at skin chamber sites challenged continuously with pollen antigen or codeine in 10 reactive individuals.*
Methods: *The levels of fibrinopeptide A at antigen sites were compared with those at sites challenged with buffer diluent alone or with codeine for the first 3 hours, followed by antigen challenge during the subsequent 2 hours.*
Results: *Findings showed: (1) fibrinopeptide A levels were higher at antigen challenge sites than at codeine challenge sites by the third hour, with these levels at both sites greater than those at buffer sites; (2) antigen challenge of the previous codeine sites during the third to fifth hours led to a further increase in fibrinopeptide A levels; (3) fibrinopeptide A levels correlated with chamber fluid immunoglobulin G levels but not with chamber fluid histamine levels.*
Conclusions: *Because antigen and codeine both activate mast cells prominently, these findings suggest that other factors play a role in the persistent fibrin formation at allergic skin reaction sites. Because antigen activates both basophils and mast cells and codeine only activates mast cells, we conclude that both basophils and mast cells contribute to the persistent fibrin formation at sites of allergic reactions. (J ALLERGY CLIN IMMUNOL 1993;91:956-60.)*

Key words: *Coagulation, fibrin, fibrinopeptide A, antigen, codeine, skin chamber, late phase responses, mast cells, basophils, histamine, IgG*

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Abbreviations used
Ig: Immunoglobulin
SEM: Standard error of the mean

Activation of the coagulation pathway results in the local production of many substances that could affect allergic reactions. The production of such vasoactive mediators as bradykinin and the formation of fibrin are two important consequences of activation of this pathway. The effects of bradykinin, a vasoactive mediator, could promote increased leakage of substances through the blood vessels. Fibrin formation could serve to aggregate fluids locally at the site of inflammation and thus contribute to the prolonged indurated reaction (late-phase response) observed at the sites of antigen injection in the skin. In fact, earlier histopathologic studies have suggested that fibrin formation is pathognomonic for an antigen-induced IgE-mediated late phase response.[1]

In previous studies we have found increased activation of the plasma Hageman factor and kallikrein systems and increased formation of fibrin sites of ongoing cutaneous allergic reactions.[2, 3] We have previously demonstrated persistent histamine release at sites of prolonged antigen exposure in the skin of reactive subjects.[4] We have shown that this persistent release of histamine was probably secondary to basophil activation, since it was not accompanied by tryptase release and did not occur after prolonged exposure to codeine, which activates mast cells but not basophils.[5] To determine whether basophil activation could be playing a role in fibrin formation, we compared fibrin formation at cutaneous sites after prolonged exposure to antigen or codeine, using the observation that fibrinopeptide A levels in the chamber fluid can serve as as marker for fibrin formation.[3] Fibrinopeptide A is a 16-amino acid peptide with a molecular weight of 1535 d. It is cleaved from the A α chains of fibrinogen by thrombin. The action of thrombin produces a fibrin monomer and two molecules each of fibrinopeptide A and fibrinopeptide B. The concentrations of fibrinopeptide A can thus be directly related to the activation of fibrinogen and the amount of fibrin monomer produced.

METHODS

Subjects

Ten subjects with skin reactivity to $\leq$10 PNU/ml of grass or ragweed pollen antigen (Greer Extracts, Lenoir, N.C.) underwent skin chamber challenge. All subjects manifested indurated responses in the skin 6 to 8 hours after intradermal injection of 10 PNU/ml of ragweed or grass pollen antigen. None had an indurated response at the same time interval after intradermal injection of 1 mg/ml of codeine.

Skin chamber challenge

Four skin chambers were placed over skin sites at which epidermis had been removed after the formation of blisters by gentle heat and suction as previously described.[6] To remove the products produced at the site after generation of the blister, all sites were washed with buffer (phosphate-buffered saline) twice for 15 minutes each, and the fluid was removed and discarded. Each subject underwent the following exposures at each of the four sites:

Site 1, 100 PNU/ml of antigen hourly for the first 3 hours and then 100 PNU/ml through the fourth and fifth hours
Site 2, buffer hourly for the first 3 hours and then through the fourth to fifth hours
Site 3, codeine 1 mg/ml, hourly for the first 3 hours and then through the fourth to fifth hours
Site 4, codeine 1 mg/ml, hourly for the first 3 hours and then antigen 100 PNU/ml, through the fourth and fifth hours

After each period of incubation, the chamber fluids were removed completely and then replaced with fluids as listed above.[3] No heparin was used in any of the chamber fluids because fibrin formation cannot be measured in the presence of heparin.[3]

The chamber fluids were stored at -70° C until they were analyzed for histamine, IgG, and fibrinopeptide A.

Assay of skin chamber fluids

Histamine assay. Histamine was measured in two ways. In chamber fluids not containing codeine a radioenzymatic assay was used as previously described and detected histamine levels as low as 2 ng/ml.[7] Since codeine interferes with the radioenzymatic activity for histamine, samples containing codeine were analyzed by a radioimmunoassay (AMHAC, Westbrook, Maine) as previously reported and detected levels as low as 1 ng/ml.[3]

IgG assay. IgG was measured in chamber fluids by radial diffusion assay, with the use of low-level immunodiffusion plates (Kallstead, Sanoffi Diagnostics, Chaksa, Maine) and could detect levels as low as 20 mg/dl.

Fibrinopeptide A. Fibrinopeptide A levels were determined by a radioimmunoassay kit (Caltane Corp., Manhasset, N.Y.) that detected concentrations as low as 0.1 ng/ml.[3] We had previously determined that there was no interference in the assay by pollen extracts in phosphate-buffered saline and that varying doses of synthetic fibrinopeptide A added to saline solution, buffer, or antigen site chamber fluids were detected in a linear dose-response curve that was parallel to the curves with the standards and diluents provided in the commercial kit.[3]

Statistics

All data are presented as mean $\pm$ SEM unless otherwise stated. The values were normally distributed (Kolmogarov-

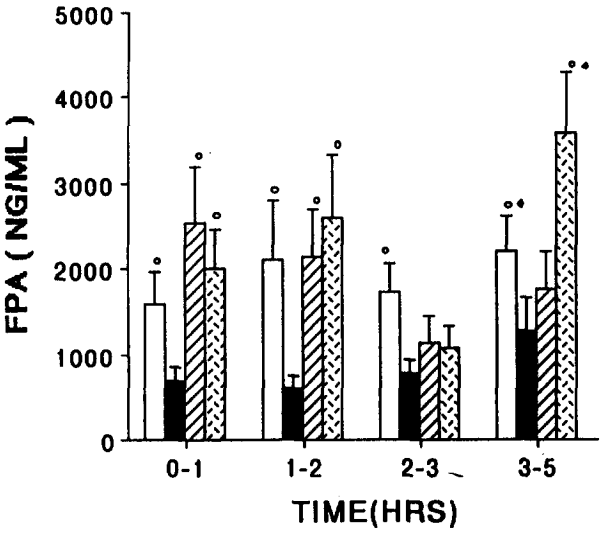

FIG. 1. Fibrinopeptide A levels *(FPA)* are depicted on the ordinate and the time in hours on the abcissa in skin chamber fluids from sites exposed to antigen *(open bar),* buffer *(solid bar),* codeine *(diagonally striped bar),* and codeine sites exposed to antigen in the third to fifth hours *(dashed bar).* The values represent the mean ± SEM. The *circle* denotes values significantly ($p < 0.001$) different from those at the buffer sites and the *diamond* represents values significantly different ($p < 0.05$) from those at buffer and codeine sites.

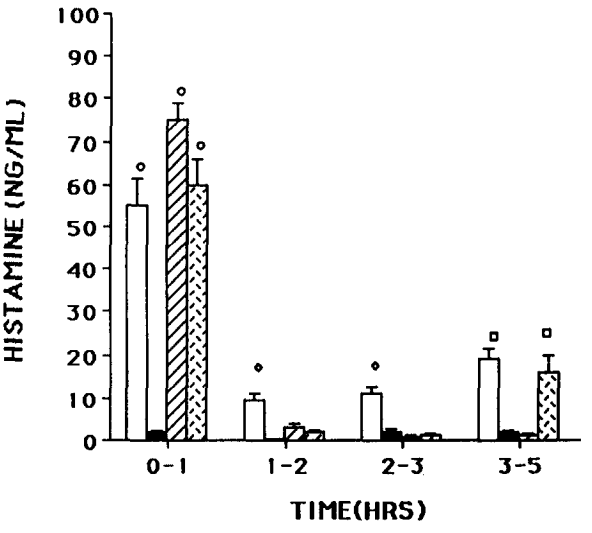

FIG. 2. Histamine levels are depicted on the ordinate and time in hours on the abcissa in skin chamber fluids from sites exposed to antigen *(open bar),* buffer *(solid bar),* codeine *(diagonally striped bar),* and codeine sites exposed to antigen from the third to fifth hours *(dashed bar).* The values represent the mean ± SEM. The *circles* denote values significantly ($p < 0.001$) different from those at both buffer and codeine sites. The *squares* denote values significantly ($p < 0.001$) different from those at buffer and codeine sites that were not exposed to antigen.

Smirnov analysis); therefore, these data were analyzed first by two-way analysis of variance with time and challenge (buffer, antigen, codeine) as the dependent factors. If a significant difference was detected, these data were analyzed by Student's *t* test for paired values to determine statistical significance. Correlations were determined with linear regression analysis.

RESULTS

Fibrinopeptide A levels

Fibrinopeptide A levels in skin chamber fluids over buffer challenge sites were similar and relatively constant (616 to 779 ng/ml) in the hourly collections during the first 3 hours. The 1267 ng/ml mean level in the 2-hour collection in the fourth to fifth hours was approximately twice the mean hourly level (634 ng/ml) in the first 3 hours (Fig. 1).

At the antigen sites skin chamber fibrinopeptide A levels were significantly higher than those at buffer sites throughout the 5-hour period and varied from 1593 ng/ml in the first hour to 2110 ng/ml in the second hour, to 1734 ng/ml in the third hour and to 2205 ng/ml from the third to fifth hours (averaging 1103 ng/ml/hour).

At the codeine site a different pattern was seen. Fibrinopeptide A levels were highest in the first hour (2534 ng/ml) and steadily declined thereafter (2142, 1140, and 879 ng/ml/hr by the third to fifth hours). In fact, at the third hour, the levels of fibrinopeptide A at the codeine sites were not different from those at the buffer sites and were significantly lower than those at the antigen sites.

At the codeine sites that were challenged with antigen in the third to fifth hours, a significant elevation of fibrinopeptide A occurred when compared with the buffer or codeine sites that were not challenged with antigen.

Histamine levels

The histamine levels are depicted in Fig. 2. As expected, the highest levels were seen in the first hour at both the antigen and codeine sites (55 ng/ml and 60 to 75 ng/ml, respectively). The amount of histamine released at the antigen sites was significantly lower than that released at the codeine sites in the first hour. After the first hour, histamine levels at antigen sites ranged from 8.5 to 11 ng/ml/hr throughout the 5-hour challenge period and were significantly greater than the hourly histamine levels at sites challenged with codeine for 5 hours (1 to 3 ng/ml). However, the histamine levels during the fourth to fifth hours at sites exposed to antigen, after 3 hours of codeine challenge, averaged 8 ng/ml/hr, which is similar to

the levels at the antigen site (8.5 ng/ml/hr) and significantly greater than those at the codeine site (1 ng/ml/hr). Buffer site levels of histamine remained at levels of 1 to 2 ng/ml throughout the 5-hour period and were significantly lower than: (1) the antigen site at all time periods; (2) the first-hour codeine sites; (3) the codeine site challenged with antigen from the third to fifth hours.

IgG levels

IgG levels in these chamber fluids are depicted in Fig. 3. IgG levels were minimally elevated during the 5 hours of buffer exposure (37 to 29 mg/dl). The highest IgG levels occurred at the codeine sites in the first hour, with levels at both codeine and antigen sites significantly elevated compared with that at the buffer site during the first and second hours of exposure. The hourly rates at the codeine sites were 176 mg/dl in the first hour and ranged from 50 to 70 mg/dl throughout the subsequent 4-hour period. There was no significant difference in the third to fifth hours between the subsequent codeine site challenged with antigen (67 mg/dl) and the site continuously exposed to codeine (54 mg/dl). IgG levels at the antigen site ranged from 50 to 70 mg/dl in the first 3 hours. By the third to fifth hours IgG levels had decreased to 30 mg/dl/hr at the antigen site, which was identical to that seen at the buffer site during that period.

Correlations

There was not a significant association seen when the chamber fluid levels of histamine and fibrinopeptide A were compared ($r = 0.147$; $p = 0.074$). However, the levels of histamine in the chamber fluids did significantly correlate with the level of IgG ($r = 0.243$; $p = 0.002$), even when buffer sites were excluded ($r = 0.181$; $p = 0.05$). Furthermore, the fibrinopeptide A levels were even more strongly associated with IgG levels in the chamber fluid ($r = 0.366$; $p < 0.001$) even when buffer sites were excluded ($r = 0.311$; $p = 0.001$). According to multiple regression analysis, when fibrinopeptide A levels were compared with both IgG and histamine levels, the correlation was essentially the same as when fibrinopeptide A levels were compared with IgG levels alone ($r = 0.370$; $p < 0.001$).

DISCUSSION

We have demonstrated a difference in the pattern of fibrin formation at sites of antigen and codeine challenge. Even though codeine at 1 mg/ml elicited higher histamine release in the first hour (60 to 75 ng/ml) compared with antigen sites (55 ng/ml) and at least as much fibrinopeptide A in the first hour (2000 to 2500 ng/ml vs 1593 ng/ml) by the third hour, the fibrinopeptide A levels at the codeine sites had fallen significantly below those at the antigen sites (1140 ng/ml vs 1743 ng/ml). Furthermore, challenge with antigen in the third to fifth hours at sites of previous codeine incubation resulted in a significant increase of fibrinopeptide A (3594 ng/ml) compared with sites incubated with codeine alone (1758 ng/ml).

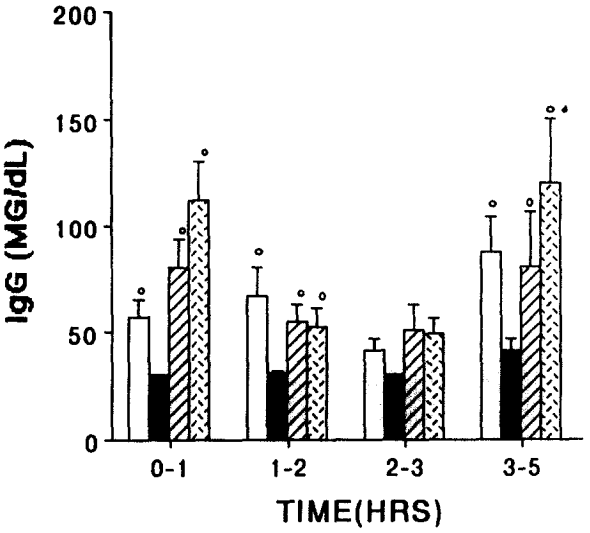

FIG. 3. IgG levels are depicted on the ordinate and time in hours on the abcissa in skin chamber fluids from sites exposed to antigen *(open bar),* buffer *(solid bar),* codeine *(diagonally striped bar),* and codeine sites exposed to antigen from the third to fifth hours *(dashed bar). Circles* denote values significantly ($p < 0.05$) different from those at buffer sites. The *diamond* represents values significantly ($p < 0.05$) different from those at sites exposed to codeine but not antigen.

Although the factors responsible for increased accumulation of fibrinopeptide A at antigen sites have not been determined in this study, several aspects of the data suggest that basophils are involved. First, as in previous studies, continuous exposure to antigen but not codeine resulted in prolonged histamine release (9 to 10 ng/ml/hr vs 1 to 3 ng/ml/hr). Rechallenge of codeine sites with antigen in the third to fifth hours resulted in increased release of histamine (8 ng/ml/hr) compared with that at the codeine site (1 ng/ml/hr). Because codeine activates mast cells and antigen activates both mast cells and basophils, it is reasonable to assume that basophils are responsible for the difference in histamine release that is observed at antigen and codeine sites. Indeed, in our previous studies we had demonstrated that the prolonged release of histamine at sites continuously challenged with antigen is not accompanied by tryptase release, which is consistent with the theory that basophils are the source of this histamine.[5]

Although it would be reasonable to conclude that fibrin accumulation is directly related to histamine release, which results in microvascular leakage and protein deposition in the tissue, this was not borne out by the data. There was no correlation between the amounts of histamine released and the amount of fibrinopeptide detected at the sites. If one considers IgG as a marker of vascular leakage, there was a correlation between IgG and fibrinopeptide levels at these sites, which although significant, was not very striking (r = 0.360 to 0.370). Therefore fibrinopeptide accumulation is more complicated than simple leakage of proteins and may involve either mast cell or basophil activation of plasma substrates to activate the fibrin pathways.[8, 9] This is illustrated by a comparison of the data in this report with that from our previous study, which first demonstrated fibrinopeptide A accumulation at antigen challenge sites.[3] In the current study, using vigorous washes, we managed to reduce the IgG and fibrinopeptide A accumulation at the buffer sites to about half of that in our previous study. In addition, IgG accumulation at the antigen sites was also reduced to about half of that seen in our previous study. Despite this, the amount of fibrinopeptide at antigen sites was similar to that in our previous study. This implies that although plasma leakage is important, other factors must be acting on the plasma substrates to account for the degree of fibrin accumulation in these sites.

In summary, continuous antigen exposure at skin chamber sites results in a more prolonged formation of fibrin than continuous codeine challenge. Furthermore, when sites previously exposed to codeine are exposed to antigen, an increase of fibrin formation occurs. For the reasons described above, we think basophils are responsible, at least in part, for this increased formation of fibrin. Finally, since intradermal antigen administration but not codeine administration results in a late-phase response, we suggest that prolonged fibrin formation at the sites of antigen injection may be related to the development of this response.

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