

THE RELEASE OF HISTAMINE FROM BLOOD CORPUSCLES BY ANTIGEN-ANTIBODY REACTIONS

IV. The Histamine Release from Whole Blood before and after Treatment of Patients Allergic to Animal Dander with Staphylococcus Vaccine

By

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In previous articles we have discussed histamine release from blood corpuscles to serum after incubation with the relevant allergens before and after specific antiallergic treatment (hyposensitization) (3). Moreover, we (4) have demonstrated a certain influence of staphylococcus vaccine on histamine release, when incubating whole blood with pollen extract, in pollen-hypersensitive patients. Histamine release was significantly decreased after vaccine treatment; the symptoms were alleviated also during the pollen season by applying staphylococcus vaccine.

In view of these good results we extended our investigation to include allergens other than pollen, and we studied the clinical effect in greater detail.

MATERIAL AND METHODS

Cases of atopic dermatitis, several of which associated with rhinitis, were selected according to the following principles:

- a) obvious and durative eczematous lesions whose onset occurred during infancy.
- b) positive skin tests with extracts of cat and horse dander.
- c) definite case history of symptoms on contacts with these animals.

The patients were examined and questioned, when the injections were given concerning their symptoms, and at the end of the injection series they were given a questionnaire containing the following questions among others:

1. Has improvement occurred?
2. When did improvement occur after introduction of treatment?
3. Was the improvement lasting?
4. During the course of treatment have you had milder or more severe relapses?
5. Have you tolerated contact with animals to which you are hypersensitive?
6. Have the skin infections been less pronounced or remained the same?
7. Have you had fewer colds than formerly?

Before beginning the vaccine treatment the patients' blood was incubated with an extract of the relevant dander, and the histamine release was determined (I in the tables). New histamine-release investigations were made at certain intervals: After about 2 (II in the tables), 4 (III in the tables), and 24 months (IV in the tables). Water extract, strength 1:100, was used for the incubation of the blood when determining histamine release. The method for determining histamine has been previously described (5).

TABLE I
Histamine in Serum after Incubation of Blood with Cat Dander Extracts or Saline before (I) and During (II-IV) Vaccine Treatment.

Patient	Sex	Histamine concentration in serum ng/ml Saline control				Histamine concentration in serum ng/ml Cat dander extract				Clinical results
		I	II	III	IV	I	II	III	IV	
441108	M	51	24	20	15	128	23	20	27	Good
501107	F	22	15	22	19	33	40	49	31	None
490810	M	24	20	23	23	45	21	34	22	Moderate
381212	F	4	21	16	28	14	62	22	42	Good
460130	F	22	21	25	12	46	21	23	17	Good
490914	M	14	23	26	24	37	43	59	38	Moderate
470723	F	25	19	12		39	23	12		Moderate
520121	F	23	21	12	14	36	34	31	27	Good
530429	F	17	31	29	13	58	45	39	58	Good
420510	F	19	24	20	22	41	38	34	39	None
n		10	10	10	9	10	10	10	9	number of values
$\bar{x}$		22.1	21.9	20.5	18.9	47.7	35.0	32.3	33.4	arithmetic mean
s_x		11.9	4.1	5.7	5.7	30.3	13.4	14.1	12.3	standard deviation
$s_{\bar{x}} = s_x/\sqrt{n}$		3.8	1.3	1.8	1.9	9.6	4.2	4.5	4.1	standard deviation from the mean

The vaccine treatment was given once a week up to the highest concentration, after which injections with the highest dose were administered either every or every second week for just over one year. The vaccine used was bacterial staphylococcus vaccine obtained from the National Bacteriological Laboratory; the highest dose contained 3000 bacteria per ml.

RESULTS

Tables 1-6 present the material, the histamine release, the statistical analysis and the clinical results.

The clinical evaluation (good, moderate, none) was based on the examination of the patients' skin and on their own reports on the intensity of the eczema, the frequency of infections in the skin and the upper respiratory tract, and the reactions on contact with animals.

The results show that histamine release decreased significantly already after treatment for two months. In most of the cat-allergic patients this diminished reactivity persisted, whereas when horse-dander extract was used the decrease in

TABLE 2
Difference (d) in Histamine Concentration in Serum between Cat Dander Extract and Saline Control.

	d(I)	d(II)	d(III)	d(IV)
n	10	10	10	9
$\bar{d}$	25.6	13.1	11.8	14.6
sd	20.2	13.2	11.8	12.7
$s_{\bar{d}} = s_d / \sqrt{n}$	6.39	4.18	3.72	4.22
$t = \bar{d} / s_{\bar{d}}$	4.01	3.14	3.17	3.45
$f = n - 1$	9	9	9	8
p	$0.001 < p < 0.01$	$0.01 < p < 0.05$	$0.01 < p < 0.05$	$0.001 < p < 0.01$

n number of differences

$\bar{d}$ arithmetic mean of differences

sd standard deviation of differences

$s_{\bar{d}}$ standard deviation from the mean of differences

t test statistic (tested hypothesis $\mu = 0$)

f degrees of freedom

p probability used for measuring significance

TABLE

*Difference (d) in Histamine Concentration in Serum between Cat Dander Ex
and during*

Patient	d(I)-d(II)	d(I)-d(III)	d(I)-d(IV)
441108	78	77	65
501107	-14	-16	-1
490810	20	10	22
381212	-31	4	-4
460130	24	26	19
490914	3	-10	9
470723	10	14	
520121	0	-6	0
530429	27	31	-4
420510	8	8	5
n	10	10	9
$\bar{D}$	12.5	13.8	12.3
s_D	29.0	26.7	21.9
$\hat{s}_{\bar{D}} = s_D / \sqrt{n}$	9.16	8.45	7.30
$t = \bar{D} / \hat{s}_{\bar{D}}$	1.36	1.63	1.69
$f = n - 1$	9	9	8
p	$p > 0.05$	$p > 0.05$	$p > 0.05$

histamine release was not so pronounced. This is seen most clearly in the diagrams, where the histamine release before the treatment was applied is designated as 100, and the values on other occasions are correlated with this figure.

In the majority of cases so-called skin titration with extract of cat and horse dander was made. No effect of the vaccine treatment was observed.

DISCUSSION

There are contradicting reports in the literature as to the effectiveness of vaccines in the reduction of the symptoms in asthma (1, 2, 6, 7, 8, 9). As previously, we were able to show that injections of staphylococcus vaccine given to the allergic

tract and Saline Control; Difference (D) between the Differences (d) before Treatment.

$d(I) = \frac{d(II) + d(III) + d(IV)}{3}$	
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73.3	
-10.3	
17.3	
-10.3	
23.0	
0.7	
12.0	
-2.0	
18.0	
7.0	

10	number of differences (D)
12.9	arithmetic mean of differences (D)
24.2	standard deviation of differences (D)
7.66	standard deviation from the mean of differences (D)
1.68	test statistic (tested hypothesis $\mu = 0$)
9	degrees of freedom
$p > 0.05$	probability used for measuring significance

patients improved their condition. In the investigation reported on here, both the intensity of the dermatitis and the reaction of the skin and mucous membranes after contact with cat or horse or both was decreased. We also observed that a number of patients needed to use less ointment for the treatment of their skin, after vaccine treatment was introduced. With the exception of three, all the patients stated that their skin had improved after 3, 4 or 5 injections. The skin improvement can perhaps be explained by the fact that in most cases fewer skin infections and, moreover, fewer infections of the upper respiratory tract were observed. The majority of the patients indicated spontaneously that, in comparison with their former condition, they had had fewer colds, and their skin infections had been entirely absent during treatment. It is

TABLE 4

Histamine in Serum after Incubation of Blood with Horse Dander Extracts or Saline before (I) and During (II-IV) Vaccine Treatment.

Patient	Sex	Histamine concentration in serum ng/ml Saline control				Histamine concentration in serum ng/ml Horse dander extract				Clinical results
		I	II	III	IV	I	II	III	IV	
441108	M	51	24	20	15	76	16	19	22	Good
501107	F	22	15	22	19	27		52	44	None
380914	F	14	15	16	8	27	31	41	23	Good
420608	F	27	10	3	10	39	10	3	10	Good
500718	F	25	23	16	53	53	50	45	93	Moderate
381212	F	4	21	16	28	13	68	26	49	Good
490709	M	16	9	17	17	22	18	35	31	Good
490914	M	14	23	26	24	16	25	35	24	Moderate
470723	F	25	19	12		57	26	18		Moderate
520121	F	23	21	12	14	22	18	21	20	Good
510406	F	20	22	11	10	44	32	28	50	Good
530429	F	17	31	29	13	40	68	48	53	Good
420510	F	19	24	20	22	50	54	56	49	None
n		13	13	13	12	13	12	13	12	number of values
$\bar{x}$		21.3	19.8	16.9	19.4	37.4	34.7	32.8	39.0	arithmetic mean
s_x		10.8	6.1	6.8	12.2	18.4	20.3	15.5	22.3	standard deviation
$s_{\bar{x}} = s_x / \sqrt{n}$		3.0	1.7	1.9	3.5	5.1	5.9	4.3	6.5	standard deviation from the mean

also striking, however, that the patients' reactions against the specific allergen were less pronounced.

The decrease in histamine release during the treatment is difficult to understand. We wish to point out, however, that May et al. (11) found that hyposensitization with an allergen does not only decrease histamine release when incubated with this allergen, but also with other allergens. May et al. (12) pointed out that the diminished sensitivity of the leukocytes to an allergen may be due to the fact that the leukocytes sensitized with multiple antigens become unspecifically desensitized by exposure to concentrations of one of the allergens below the concentration required for demonstrable histamine

TABLE 5

Difference (d) in Histamine Concentration in Serum between Horse Dander Extract and Saline Control.

	d(I)	d(II)	d(III)	d(IV)
n	13	12	13	12
$\bar{d}$	16.1	14.5	15.9	19.6
sd	11.5	17.2	11.7	15.1
$s_{\bar{d}} = s_d / \sqrt{n}$	3.20	4.96	3.24	4.35
$t = \bar{d} / s_{\bar{d}}$	5.02	2.92	4.91	4.50
f = n - 1	12	11	12	11
p	p < 0.001	0.01 < p < 0.05	p < 0.001	p < 0.001

n number of differences

$\bar{d}$ arithmetic mean of differences

sd standard deviation of differences

$s_{\bar{d}}$ standard deviation from the mean of differences

t test statistic (tested hypothesis $\mu = 0$)

f degrees of freedom

p probability used for measuring significance

release. This conclusion is based on the results of the *in vitro* experiments, which both Lichtenstein (10) and they carried out.

It is possible that our patients were allergic to staphylococci although the immediate skin reactions were negative in most of them. Investigations are in progress to ascertain whether it is possible to desensitize leukocytes *in vitro* with staphylococcus vaccine. The effect of the vaccine we have found indicate that an immunologic or unspecific activity can explain the good terapeuctical results. The interferon production following administration of bacterial vaccine (14) may be of some importance.

SUMMARY

Histamine release to plasma after the addition of a specific allergen, before and after serial injections of staphylococcus vaccine has been studied in patients with atopic dermatitis.

TABLE
*Difference (d) in Histamine Concentration in Serum between Horse Dander Ex
 and during*

Patient	d(I)-d(II)	d(I)-d(III)	d(I)-d(IV)
441108	33	26	18
501107		-25	-20
380914	-3	-12	-2
420608	12	12	12
500718	1	-1	-12
381212	-38	-1	-12
490709	-3	-12	-8
490914	0	-7	2
470723	25	26	
520121	2	-10	-7
510406	14	7	-16
530429	-14	4	-17
420510	1	-5	4
n	12	13	12
$\bar{D}$	2.5	0.2	-4.8
s_D	18.2	14.9	11.9
$s_{\bar{D}} = s_D / \sqrt{n}$	5.25	4.12	3.44
$t = \bar{D} / s_{\bar{D}}$	0.48	0.04	-1.41
$f = n - 1$	11	12	11
p	$p > 0.05$	$p > 0.05$	$p > 0.05$

Statistically significant differences, between values before and during treatment, were demonstrated, and good clinical results were obtained.

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tract and Saline Control; Difference (D) between the Differences (d) before Treatment.

$d(I) - \frac{d(II) + d(III) + d(IV)}{3}$	
25.7	
-22.5	
-5.7	
12.0	
-4.0	
-17.0	
-7.7	
-1.7	
25.5	
-5.0	
1.7	
-9.0	
0.0	
13	number of differences (D)
-0.6	arithmetic mean of differences (D)
14.3	standard deviation of differences (D)
3.97	standard deviation from the mean of differences (D)
-0.15	test statistic (tested hypothesis $\mu = 0$)
12	degrees of freedom
$p > 0.05$	probability used for measuring significance

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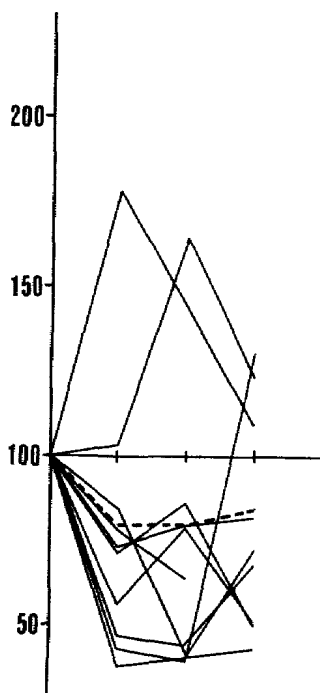


Fig. 1.

The release of Histamine in Serum after incubation of whole Blood with Cat Dander Antigen before (= 100) and during Vaccine treatment. The Lines represent different Patients. The Amount of released Histamine is calculated on the Figures in Table 1. The dashed Line represents the mean Value.

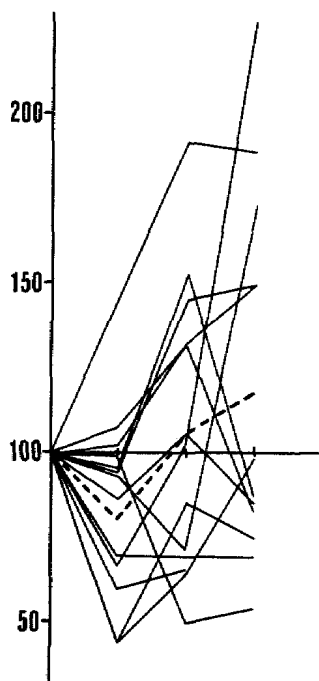


Fig. 2.

The release of Histamine in Serum after incubation of whole Blood with Horse Dander Antigen before (= 100) and during Vaccine treatment. The Lines represent different Patients. The Amount of released Histamine is calculated on the Figures in Table 4. The dashed Line represents the mean Value.

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