

Mite-allergen exposure and spontaneous histamine release in monosensitive and polysensitive mite-allergic patients

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Forty-three patients allergic to mites and suffering mainly from asthma were recruited: 25 were mite-monosensitive and 18 were polysensitive, as determined by skin tests and specific serum IgE determinations with various allergens. *In vitro* spontaneous histamine release (SHR) by washed blood basophils was measured once or several times for each patient. Throughout this study, the mean periods of high and low mite-allergen exposure were defined on the basis of relative indoor humidity and temperature data. For the mite-monosensitive patients, there was a significant increase in mean SHR during the season of high mite-allergen exposure as compared to the months of lower mite-allergen presence ($P < 0.002$). No significant difference between mean SHR values was observed when comparing the monosensitive group during the season of high mite-allergen exposure with polysensitive patients (allergic to mite and pollen) during the period of exposure to both allergens. Differences in mean SHR reported here emphasize the positive relationship between intense allergen exposure and the *in vitro* SHR increase in blood basophils of mite-allergic patients.

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Several publications report changes in spontaneous histamine release (SHR) by human basophils under varying experimental conditions and between groups with different pathologies. May & Remigio (13) observed an increase in SHR from washed blood basophils in patients presenting confirmed allergies to food allergens; these observations were later confirmed by Sampson et al. (18). Basophils from most asthmatic and some atopic subjects spontaneously released histamine in the presence of deuterium oxide, but basophils from nonatopic subjects did not (4). An increase in SHR was also noted in grass pollen-sensitive patients during the pollen season (24) and in some hemodialyzed patients with allergic symptoms (3). Other authors reported that the spontaneous release from the human basophil was Ca^{++} , Mg^{++} , and temperature dependent (2) and needed an energy supply (24).

The results of Akagi & Townley (2) revealed a statistically significant difference between asthmatics and controls in terms of histamine spontaneously released by washed blood basophils, whereas no difference between controls and extrinsic asthmatics was reported in similar experiments by Wahn et al. (22).

In both of the above mentioned studies on asthmatics, few details were given concerning the allergens to which recruited patients were sensitive, or the allergen exposure of the patients at the time of SHR analysis. Our recent findings (24) of the seasonal increase in SHR by basophils in grass pollen-sensitive patients have raised the question of the general influence of pneumallergen exposure upon SHR values obtained in allergics. Since repeated pollen exposure intensifies SHR in a grass pollen-sensitive population, we assumed that this effect would also be observed when allergic patients were continuously exposed to another allergen. With this in mind, we analyzed SHR results obtained throughout the year in mite-sensitive patients primarily showing symptoms of asthma, taking into account the variations in environmental mite-allergen exposure. SHR data obtained during the period of high and low mite-allergen exposure were compared in mite-monosensitive patients, and also between mite-monosensitive and -polysensitive patients during the corresponding periods of high allergen exposure.

Material and methods

Forty-three patients presenting symptoms of asthma with or without rhinitis were recruited in the Consultation d'Allergologie des Hospices Civils de Strasbourg (France) from 1977 to 1980, on the basis of positivity in both cutaneous tests and serum IgE analysis for *Dermatophagoides pteronyssinus* (minimum of class 2 in the RAST assay). Within this group, 25 monosensitive patients displayed positive tests only with mites, and 4 of these subjects presented a RAST class 1 result for cat allergen. Eighteen patients were considered polysensitive since they were positive for mites and had a cumulative RAST class 2 or greater to one or more of the other allergens, including animal dander, fur, mold, and grass-, weed-, or tree pollens.

Medication taken by these patients included drugs currently administered for rhinitis and asthma symptoms (theophylline, antihistamines, sodium cromoglycate, beta-stimulants, or corticoids, alone or in combination); medication was stopped 48 h before the experiment, when possible. Hyposensitization treatment was performed as previously described (14). Characteristics of the patients are summarized in Table 1.

Buffer and reagents. Tris-albumin buffer (Tris-A)

containing 25 mmol/l Tris, pH 7.6, 5 mmol/l KCl, 130 mmol/l NaCl, and human serum albumin at 0.33 mg/ml was used for cell washing. The same buffer supplemented with 0.6 mmol/l Ca^{++} and 1 mmol/l Mg^{++} (Tris-ACM) was used in histamine release experiments.

Histamine release. Ten milliliters of blood was collected on 1 ml EDTA 125 mmol/l, pH 7.4, and 2.2 ml glucose-dextran. The blood suspension sedimented for 40 min, enabling separation of the leukocytes from the bulk of the red blood cells. After centrifugation of the leukocyte-rich suspension, serum was discarded and cells washed twice with 10 ml of Tris-A. All experimental steps were performed at 20°C with centrifugations at 250 g for 10 min. The washed cells were resuspended in 6 ml of Tris-ACM and divided into 1-ml aliquots for triplicate determination of total histamine content and spontaneous histamine release. The cell samples were incubated for 40 min at 37°C with intermittent stirring. After incubation, test-tubes were transferred to 4°C, and the tubes corresponding to spontaneous histamine release were centrifuged; 0.85 ml from the cell suspension for total histamine determination or from the supernatant for SHR determination was transferred to tubes containing 0.1 ml perchloric acid 4 N. After 20 min centrifugation at 4000 g, the su-

Table 1. Characteristics of mite-monosensitive and -polysensitive patients

Patients and period of allergen exposure (day)	N	Age mean (so)	Sex (% male)	Total IgE (iu/ml) mean (so)	Symptoms A: Asthma R: Rhinitis (% total)	Taking drugs (%)	Following immunotherapy (%)
MiteMono ^a							
Low mite-allergen exposure (350–1113)	13	29.5 (9.4)	77	839 (1116)	A: 58 R: 8 A + R: 34	67	73
MiteMono ^a							
High mite-allergen exposure (133–330)	19	30.1 (10.0)	79	781 (973)	A: 67 R: 5 A + R: 28	72	58
	12 ^c	31.0 (11.0)	83	619 (741)	A: 82 R: 0 A + R: 18	73	45
Polysens ^b							
Pollen + mite exposure (95–330)	18	30.9 (11.2)	67	1614 (1497)	A: 50 R: 7 A + R: 43	57	63

^a Monosensitive patients are sensitive only to the mite *Dermatophagoides pteronyssinus* (positivity for cutaneous test and serum specific IgE by RAST).

^b Polysensitive patients are sensitive to *Dermatophagoides pteronyssinus* and present at least a class 2 result in the RAST for one or more mite-unrelated allergens (see Material and methods).

^c Subgroup of patients tested only once.

pernatants were transferred to new tubes for histamine quantification. The histamine content was quantified by an automated fluorometric method (9), and the SHR was expressed as the percentage of total histamine. The mean coefficients of variation for histamine determinations were 6.2% for SHR and 3.5% for total histamine measures.

Total and specific IgE determinations were performed by means of the Phadebas PRIST and RAST method according to the manufacturer's instructions.

Statistics

Student's *t* test and Wilcoxon signed rank order test were performed to compare the mean SHR values of mono- and polysensitive patients during high and low mite-allergen exposure periods, as well as for the study of the interference of hyposensitization on SHR results. For a patient having several SHR determinations during a well-defined period of allergen exposure, the mean value was used in the calculations.

Relative humidity and indoor temperature

Each day during the years from 1977 to 1980, the outdoor temperature and relative outdoor humidity were collected from the annual meteorological data in Strasbourg. A general monthly mean was calculated for these two parameters during the 4-year period, giving rise to the mean outdoor temperature (out T) and the mean relative outdoor humidity (out RH).

For months without indoor heating (June to September) (out T > 13°C), the relative indoor humidity (ind RH) was equal to the relative outdoor humidity (out RH) because of room ventilation. For the same reason, the indoor temperature (ind T) was equal to the outdoor temperature (out T).

For months with indoor heating (out T < 13°C), the relative indoor humidity was calculated from the formula:

$$\text{ind RH} = \text{out RH} \times \text{AH}_{\text{out T}} / \text{AH}_{\text{ind T}}$$

where AH = absolute humidity of the air saturated with water at a given temperature. Diagrams display AH versus temperature. Mean indoor temperature (ind T) during indoor heating months was estimated at 16°C, while mean outdoor temperatures were those already calculated for each month (out T).

Mean monthly data of relative indoor humidity and indoor temperature throughout the year for the period 1977–80 in Strasbourg are presented in Fig. 1A, each mean being attributed to the 15th day of the corresponding month.

Results

All patients were examined at the Hospices Civils de Strasbourg, Service de Pneumologie, between 1977 and 1980.

Table 1 summarizes the main features of the monosensitive and polysensitive mite-allergic patients with respect to sex, age, total serum IgE, symptoms, and medication. No heterogeneity was observed between the groups for the various parameters, except for total serum IgE, where the polysensitive patients presented a higher value (1614 ± 1497 iu/ml) than the monosensitive (annual mean: 805 ± 1017 iu/ml) ($P < 0.03$).

Fig. 1A shows the mean monthly indoor temperature and relative indoor humidity curves for the years 1977 to 1980 in the region of Strasbourg. Taking into account the fact that 50% of indoor RH was the minimum percentage favoring mite breeding, the period from day 103 to day 300 was estimated to be the period of mite multiplication in this area. Earlier work (16) noted that there was a time lag between the beginning of the high mite-breeding period and the establishment of effective mite-allergen-level increase, and that there was a time lag between the end of the high mite-breeding period and the decrease in the mite-allergen level. For this reason the period of effective mite-breeding conditions (days 103–300) was shifted with a lag of 30 days, thus obtaining, for the estimated period of effective high mite-allergen exposure, the period between days 133 and 330 (= season of high mite-allergen level). The period of low level of mite-allergen presence was calculated discarding a 20-day period at each boundary of the season of high mite-allergen level to avoid overlapping of the two seasons: this period was estimated to be between days 350 and 113 (= season of low mite-allergen level).

For polysensitive patients who, in addition to mite sensitivity, were mainly sensitive to various weed, grass, and tree pollens, the pollen season in the region of Strasbourg was defined by daily pollen counts between days 95 and 270. The period of simultaneous presence of grass, tree, and weed pollens and a high level of mite allergens was between days 133 and 270.

Fig. 1B shows the distribution of SHR of monosensitive mite-allergic patients throughout the year. The annual mean SHR was 11.9%, and it appears that most SHR values higher than this mean occurred from day 133 until day 330. These data led to a detailed comparison of the results obtained on the monosensitive patients between the seasons of low and high mite-allergen level.

Two statistical analyses were performed. The first used paired results from patients included once during the season of low mite-allergen level and once

Mite exposure and spontaneous histamine release

Table 2.A Paired spontaneous histamine release results for mite-sensitive patients

	Period of low mite-allergen exposure	Period of high mite-allergen exposure
	6.8	21.9
	3.9	4.3
	2.2	8.2
	3.0	2.9
	10.9	28.9
	10.5	14.0
	3.7	9.2
Mean (sp)	5.9 (3.6)	12.8 (9.5)*

Table 2.B Paired spontaneous histamine release results for healthy controls

	5.0	2.6
	2.8	4.9
	3.3	5.8
	3.3	3.2
	3.2	6.5
	5.2	4.1
Mean (SD)	3.8 (1.0)	4.5 (1.5) ^o

* $P < 0.014$ (Wilcoxon signed rank test).

^o NS.

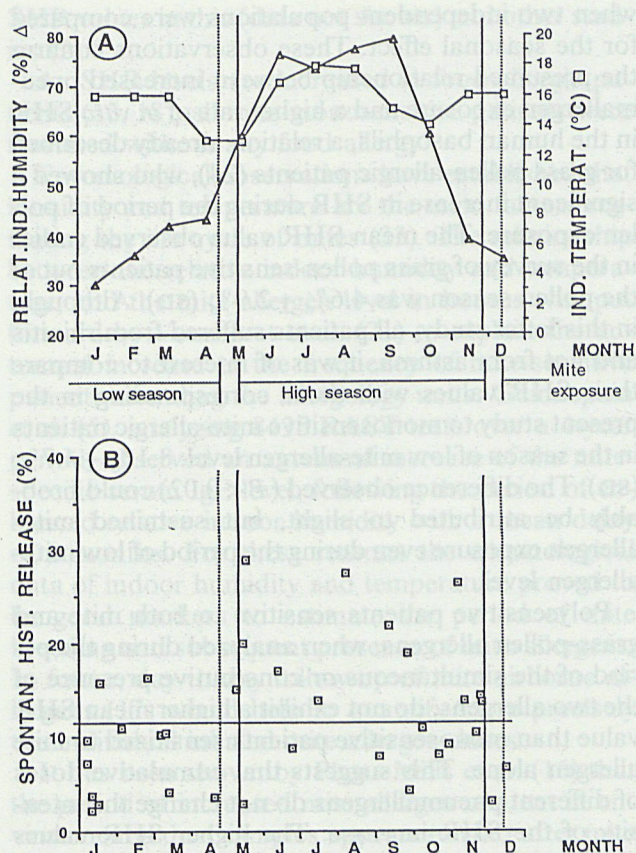


Fig. 1. A. Mean monthly indoor temperature and relative indoor humidity during 1977–80. Vertical solid line shows the limits of the season of high mite-allergen level (days 133–330); vertical dotted line shows the boundaries of the season of low mite-allergen level (days 350–113). B. Spontaneous histamine release values throughout the period 1977–80 for mite-monosensitive patients. The horizontal line indicates the mean of all SHR results.

during the season of high mite-allergen level. Table 2A shows that, with seven such paired results, the difference in mean SHR was significant ($P < 0.01$; Wilcoxon signed rank test) between the two periods. The second analysis compared samples of patients, each of them being tested only once, during the season of high mite-allergen level or during the season of low mite-allergen level. Fig. 2 shows the scatter plot of SHR values observed during low (group 1) and high (group 2) mite-allergen periods, corresponding respectively to 13 and 12 patients. The difference in mean SHR was significant ($P < 0.002$; t test) between the two periods.

In the two tests, there was a significant increase in mean SHR for monosensitive mite-allergic patients during the season of high mite-allergen level. In contrast, no significant difference in mean SHR was observed for paired results from healthy controls studied in and out of the mite-allergen season (Table 2B).

SHR from 18 polysensitive patients (Fig. 2, group 3) was studied during the period of the presence of

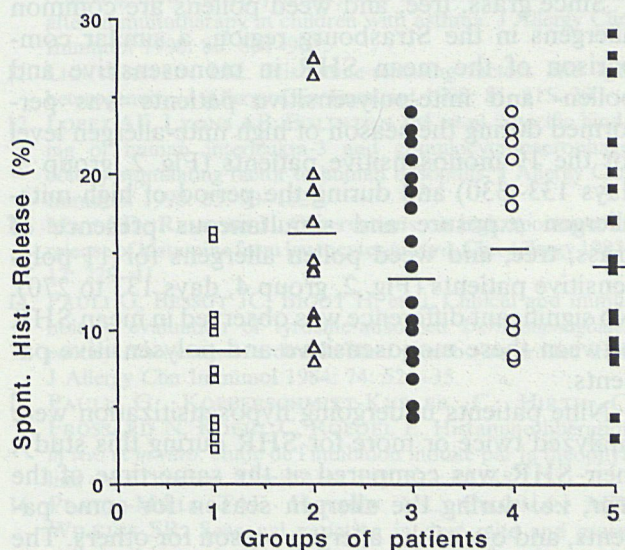


Fig. 2. Spontaneous histamine release for mite-monosensitive patients during the season of low (days 350–133) (group 1) and high (days 133–330) (group 2) mite-allergen level (independent populations). Spontaneous histamine release for polysensitive patients during the season of high mite-allergen level and/or various pollen allergen exposure (days 95–330) (group 3), for polysensitive patients during the period of simultaneous presence of pollen and mite allergens (days 133–270) (group 4), and for all the mite-monosensitive patients during the season of high mite-allergen level (days 133–330) (group 5). For each group, the horizontal bar indicates the corresponding arithmetic mean.

either pollen or mite-allergens (days 95 to 330). The mean SHR of all the monosensitive patients ($n = 19$) analyzed during the season of high mite-allergen level (Fig. 2, group 5, days 133–330) was compared with

Table 3. Paired spontaneous histamine release results with ongoing immunotherapy

Level of immunotherapy	No or < 3 months	> 3 months
	8.6	6.9
	6.9	15.3
	3.8	0.5
	15.0	13.5
	6.0	10.2
	1.9	4.0
	24.5	28.3
	13.6	17.9
	12.4	10.9
Mean (sd)	10.3 (6.9)	11.9 (8.2)*

* $P < 0.087$ (Wilcoxon signed rank test).

the mean SHR of polysensitive patients ($n = 18$) during the season of high mite-allergen level and/or of various pollen species allergen exposure (Fig. 2, group 3, days 95 to 330). There was no significant difference in mean SHR between those monosensitive and polysensitive patients.

Since grass, tree, and weed pollens are common allergens in the Strasbourg region, a similar comparison of the mean SHR in monosensitive and pollen- and mite-polysensitive patients was performed during the season of high mite-allergen level for the 19 monosensitive patients (Fig. 2, group 5, days 133–330) and during the period of high mite-allergen exposure and simultaneous presence of grass, tree, and weed pollen allergens for 11 polysensitive patients (Fig. 2, group 4, days 133 to 270). No significant difference was observed in mean SHR between these monosensitive and polysensitive patients.

Nine patients undergoing hyposensitization were analyzed twice or more for SHR during this study; their SHR was compared at the same time of the year, i.e. during the allergen season for some patients, and out of the allergen season for others. The paired observations were performed at different stages of hyposensitization treatment. At the first SHR analysis the patients had followed either no immunotherapy or a hyposensitization of less than 3 months; at the second analysis the period of immunotherapy was longer than 3 months. With 9 paired results, there was no significant change of mean SHR with immunotherapy (Wilcoxon signed rank test) (Table 3).

Discussion

The present study establishes a significant increase of SHR for monosensitive mite-sensitive patients in the period of maximal mite-allergen exposure. This result was found both when paired observations and

when two independent populations were compared for the seasonal effect. These observations confirm the presumed relationship between increased pneumallergen exposure and a higher rate of *in vitro* SHR in the human basophils, a relation already described for grass pollen-allergic patients (24), who showed a significant increase in SHR during the period of pollen exposure. The mean SHR value observed earlier in the survey of grass pollen-sensitive patients out of the pollen season was $4.6\% \pm 2.9\%$ (SD). Although, in this latter study, all patients suffered from rhinitis and not from asthma, it was of interest to compare their SHR values with those corresponding in the present study to monosensitive mite-allergic patients in the season of low mite-allergen level: $8.1\% \pm 4.7\%$ (SD). The difference observed ($P < 0.02$) could probably be attributed to slight, but sustained mite-allergen exposure even during the period of low mite-allergen level.

Polysensitive patients sensitive to both mite and grass-pollen allergens, when analyzed during the period of the simultaneous or consecutive presence of the two allergens, do not exhibit a higher mean SHR value than monosensitive patients sensitized to mite allergen alone. This suggests that cumulative doses of different pneumallergens do not change the intensity of the SHR increase. The higher SHR values observed in food allergy (13, 18), as compared to pneumallergen sensitivity, could be due to the different route of introduction and different effective quantities of the allergens.

Possible sources of bias which could interfere with SHR results, such as medication, hyposensitization treatment, and total serum IgE levels, were analyzed.

Medication and hyposensitization frequencies were similar for the monosensitive patients in the seasons of high and low mite-allergen level, as well as for the group of polysensitive patients.

The interference of immunotherapy with SHR was checked, as recent work on hyposensitization (8, 10) shows a decrease in basophil responsiveness to histamine-releasing factors in subjects responding well to this treatment. The data obtained here on paired observations, in a well-defined season of allergen exposure, did not show a significant difference in SHR between different stages of immunotherapy. For currently administered medication, the literature (1, 15) has mentioned only the inhibitory effects on SHR or in allergen-induced histamine release. Thus, an increase in drugs taken during the period of allergen exposure would only conceal or lower the increase in SHR observed during this same period.

The difference in total IgE observed between the monosensitive group and the polysensitive patients does not interfere with SHR values reported, as no correlation has been found between IgE levels and

SHR values in this (data not shown) and other studies (2).

As SHR levels are increased in food-allergic patients (13, 18), all mite-monosensitive patients were recruited without any food allergy.

Entomological observations have established that humidity and temperature are the main factors regulating the life cycle of mites (23). The relationship between the relative indoor humidity and temperature, and the mite-allergen level in houses was put forward by Voorhorst et al. (21) to explain the autumnal increase of the frequency of mite-sensitive patients attending the allergology units. Guinnee et al. (5), analyzing 8435 RAST tests over a 4-year period, observed an autumnal increase of the mite-specific serum IgE level, following the period of enhanced relative indoor humidity with a mean delay of 2 months. For these reasons the climatological data of indoor humidity and temperature proved to be good markers to estimate the period of mite breeding and subsequent presence of mite-allergens. In future, day-to-day follow-up of mite airborne allergens (17) would help to quantify more precisely the individual inhaled mite allergen doses.

It has been shown by Platts-Mills et al. (16) that the period of increased mite-allergen content in environmental dust samples appears to start after mite culture conditions are fulfilled and lasts longer than the period of high temperature and relative humidity, as allergen persistence was observed after the peak of live mite numbers. This might explain why, for 2 months after the maximal breeding conditions of August, increased SHR values continued to be observed (Fig. 1).

The present findings suggest strongly that increased SHR is not a true spontaneous phenomenon but an effect of exogenous stimulation by allergens. For this activation of the blood basophils, several hypotheses have been proposed, such as the production of histamine-releasing substances (8, 11, 18, 19) or the priming of basophils by hemopoietic growth factors (7, 12). In conclusion, high SHR values strongly indicate the need to search for a permanently offending allergen.

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