

Skin reactivity, basophil degranulation and IgE levels in ageing

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Summary

Skin tests with histamine, the histamine-liberator, codeine, and various allergens as well as blood basophil degranulation by anti-IgE or anti-IgG4 and total serum IgE levels have been studied in two female populations of different ages (average 23.1 and 73.9 years). All thirty-one patients selected for this study were clinically non-allergic.

We observed a trend towards reduced skin reactions to histamine and codeine in the higher age-group; on the other hand, we could not find any decrease of basophil degranulation as a sign of basophil impairment with age. Likewise no difference in total serum IgE levels have been noticed.

No correlation between skin tests and basophil degranulation was observed; yet patients with isolated, positive skin tests to house dust and/or *Candida albicans* showed a statistically significant reduced blood basophil degranulation by anti-IgE or anti-IgG4.

Introduction

It is a common observation that the incidence of IgE-mediated or IgE-related allergic diseases, such as allergic asthma, rhinitis and atopic eczema, is lower in old rather than young people (Hanneuse *et al.*, 1978; Palma-Carlos, 1967). Some studies have shown that there is an age-associated impairment of cell-mediated immunity and of the IgG or IgM primary antibody response (Pisciotta *et al.*, 1967; Roberts-Thomson *et al.*, 1974). Delespesse *et al.* (1977) observed a reduced serum IgE level, as well as a decrease of IgE antibody production in old people (Orren & Dowdle, 1975); weaker skin reactions to different allergens have also been documented (Barbee *et al.*, 1976). Moreover, some authors described a smaller skin reaction to histamine in elderly patients (Tuft, Heck & Gregory, 1955), but the reports on this point are conflicting (Delespesse *et al.*, 1977; Pupil & Berna, 1976). Little seems to be known about the effects of age on the degranulation of blood basophils; the aim of this paper has been therefore to study

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basophil degranulation and skin reactivity as well as total and specific serum IgE levels in two female populations of different age.

Material and methods

Patients

Two populations of female volunteers of widely different ages were used. Only patients that may definitely be considered as non-atopic (no history of eczema, pollinosis and asthma) have been selected for the study.

The fifteen volunteers of population I, average age $73.9 (\pm 3.2)$ years, were inmates of a nursing home and were free of severe diseases, with the exception of one woman with multiple sclerosis. All sixteen people of population II, average age $23.1 (\pm 1.6)$ years, were healthy young women (medical students).

Methods

Skin tests. All skin tests have been performed on the inner aspect of the forearms.

Intradermal tests. Of the following solutions, 0.02 ml were applied with a 26 gauge needle: (a) Histamine: 0.01% histamine in physiological NaCl solution, sterile filtered; pH 5.9. (b) Codeine: 0.01% and 0.1% codeine phosphate in physiological NaCl, buffered with NaOH to pH 7.25, stabilized with 0.5% phenol and sterile filtered. (c) Housedust: commercially available (Bencard). (d) *Candida albicans*: Concentration: 0.001%, commercially available (Pasteur Institute, Paris):

Prick tests. Grass pollen and *Dermatophagoides farinae*-house dust mite solution have been tested by the prick technique; both solutions are commercially available (Bencard).

The evaluation of intradermal skin tests with histamine and codeine was performed as follows: 20 min after intradermal injection, the weal limit was marked with a ballpoint pen and transferred by scotch-tape to a paper sheet that was xerox-copied. The copied weal was cut and weighed; in order to obtain the corresponding surface in mm² of the weal, multiplication with the mm²/mg factor (0.125) was applied. The flare reaction has not been taken in consideration, due to the frequent difficulty of evaluating the flare limits in the often parchment-like old skin. Previous studies have shown a very close correlation between the sizes of the weal and flare reactions in young people (Berther, Conroy & de Weck, 1980; Voorhorst *et al.*, 1979).

For prick tests to grass pollen, mite allergens and intradermal tests to house dust and *Candida albicans*, recording was done 20 min after testing, according to the following scale:

- = no weal
- + = weal ≤ 5 mm diam.
- ++ = weal ≤ 10 mm diam.
- +++ = weal > 10 mm diam.

Degranulation of blood basophils

Basophil degranulation by various degranulating agents (anti-IgE and anti-IgG4 antibodies) was evaluated by a flow cytometric technique, as described in detail elsewhere (Nakagawa, Stadler & de Weck, 1981b; Nakagawa *et al.*, 1981c).

Briefly 10 ml of venous blood treated with heparin and EDTA was allowed to sediment at room temperature for 60 min with 3 ml of 6% clinical dextran in saline. The

leucocytes were isolated from plasma, residual erythrocytes and platelets by one wash with Tris NH_4Cl * buffer, followed by one wash with Tris A* and resuspended in Tris ACM* to a concentration of approximately 5×10^6 leucocytes per millilitre.

Basophil degranulation was performed in a volume of 0.5 ml, consisting of 0.45 ml cell suspension and 0.05 ml of various concentrations of anti-IgE or anti-IgG4 antibodies in Tris ACM buffer. After incubation at 37°C for 20 min, the cells were washed twice with Tris EDTA* and resuspended in 0.2 ml of the same buffer. Then basophils were stained by adding 0.9 ml of 0.15% alcian blue solution (composed of 39 ml saline, 1 ml glacial acetic acid, 10 ml formaldehyde and 50 ml ethanol) to 0.1 ml of the cell suspension. After a 10-min incubation at room temperature, the samples were applied to a Cytograf (Biophysics, Mod. 6300 A) which yields the percentage of stained cells among the total leucocytes by electronic discrimination. A 1-ml sample with a normal basophil concentration (approximately 2×10^3 per sample) enabled us to count triplicates of percentages of basophils per 10^4 leucocytes, as well as the total number of cells per 0.1 ml volume.

The percentage values of degranulation were calculated according to the formula:

$$\text{Degranulation (\%)} = \frac{\text{Basophil (\%)}_{\text{control}} - \text{Basophil (\%)}_{\text{test}}}{\text{Basophil (\%)}_{\text{control}}} \times 100$$

The maximal degranulation percentage achieved by anti-IgE or anti-IgG4 was the reference for comparisons in this study.

Total and specific IgE

Total and specific IgE were evaluated by PRIST and Phadebas RAST respectively (Pharmacia, Sweden).

The blood samples for the total serum IgE and RAST assays (house dust h_1 , h_2 , h_3 ; mite d_1 , d_2 ; grass pollens g_3 , g_6 , g_8) have been taken simultaneously with those used for the basophil degranulation test.

Statistical analysis

All statistical tests and correlation coefficients were calculated on a Hewlett–Packard calculator by routine methods.

Results

Skin tests

As shown in Table 1, there was no statistically significant difference in the skin reactions to the tested drugs between the young and the old populations. However, a trend to weaker reactions against histamine and codeine in older females could be observed. Despite the absence of clinical allergic symptoms, we obtained some positive intradermal skin reactions to house dust and/or *Candida albicans*. (i.e. in eight different people; Table 2).

* Tris NH_4Cl :	0.015 M Tris and 0.747% NH_4Cl
Tris A:	0.025 Tris, 0.12 M NaCl, 0.005 M KCl and 0.03% human serum albumin, pH 7.6
Tris ACM:	Tris A supplemented with 0.6 mM CaCl_2 and 1 mM MgCl_2
Tris EDTA:	Tris A supplemented with 0.01 M EDTA

Table 1. Weal sizes after intradermal injection of histamine 0.01%, codeine 0.1% and 0.01%

Population	Histamine 0.01% (mm ²)	Codeine 0.1% (mm ²)	Codeine 0.01% (mm ²)
I (n = 15)	165.0 (± 16.0)	138.6 (± 14.9)	104.1 (± 11.3)
II (n = 16)	196.0 (± 10.6)	157.0 (± 9.8)	118.8 (± 11.8)
Significance (P) of I vs II	NS	NS	NS

Table 2. Skin reactions to house dust, *Candida albicans*, grass pollen and mite

Population	House dust (intraderm.)		<i>C. albicans</i> (intraderm.)		Grass pollen (prick test)		Mite (prick test)	
	n	Reaction	n	Reaction	n	Reaction	n	Reaction
I (n = 15)	14	—	10	—	15	—	15	—
	1	++	4	+				
			1†	++				
II (n = 16)	14	—	15	—	15	—	15	—
	2	++	1	+				

† This patient showed a skin reaction both to house dust and *Candida albicans*.

Basophil degranulation

No significant decrease of basophil degranulation induced by anti-IgE or anti-IgG4 has been observed in elderly people. Incubation with antigen solutions has not been performed in this study, since the populations selected were not clinically allergic (Fig. 1).

IgE

No significant differences between young (18.1 ± 3.2 u/ml) and old (17.2 ± 3.8 u/ml) populations could be demonstrated. All RAST assays to house dust, mite and grass pollen allergens have yielded negative results.

Correlation analysis

Skin reactions. The correlation between the skin tests with codeine at the concentration of 0.1% or 0.01% is very high. Both doses seem to be applicable, the 0.1% solution yielding obviously larger weals (Table 3). The correlation coefficient of histamine versus codeine reaches likewise a statistically significant degree (Table 3 and Fig. 2).

Basophil degranulation. We could observe a very high correlation between basophil degranulation induced by anti-IgE and anti-IgG4 (Fig. 3).

Basophil degranulation and skin tests. We did not find any correlation between the sizes of the weal reactions to histamine or codeine and the results of the blood basophil degranulation tests by anti-IgE and anti-IgG4. However, it was evident that females

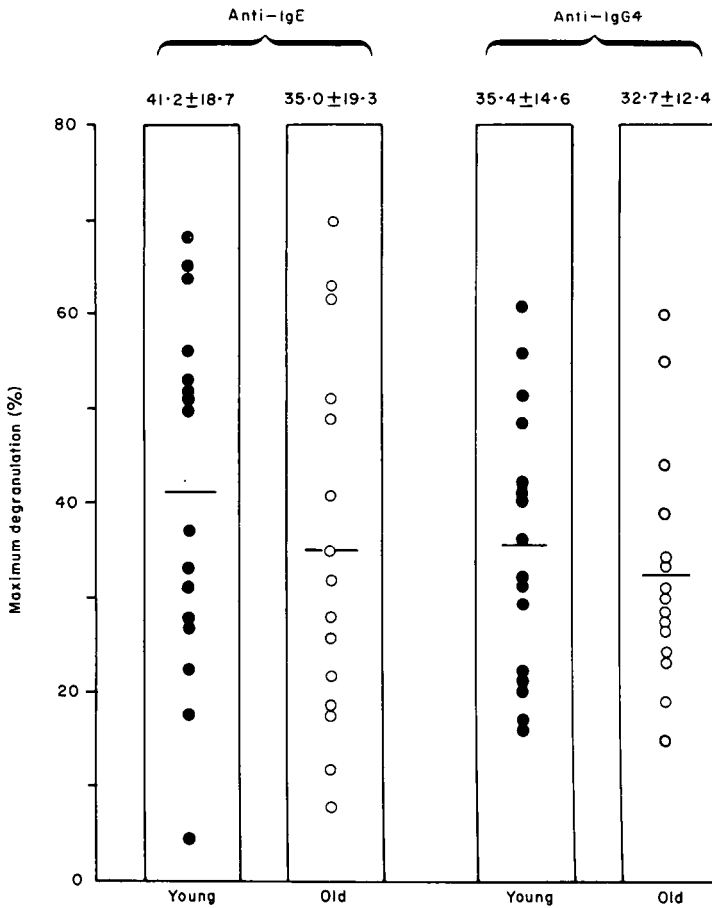


Fig. 1. Maximum percentage of blood basophil degranulation to anti-IgE and anti-IgG4 in old (○) and young (●) females. No statistically significant differences between old and young females could be shown.

Table 3. Linear correlation coefficients in Populations I and II ($n = 31$) between skin tests with codeine 0.01% and 0.1% and histamine 0.01%

Codeine (%) skin test	Skin tests with	
	Histamine 0.01% (<i>r</i>)	Codeine 0.1% (<i>r</i>)
0.1	0.44*	—
0.01	0.49**	0.82***

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

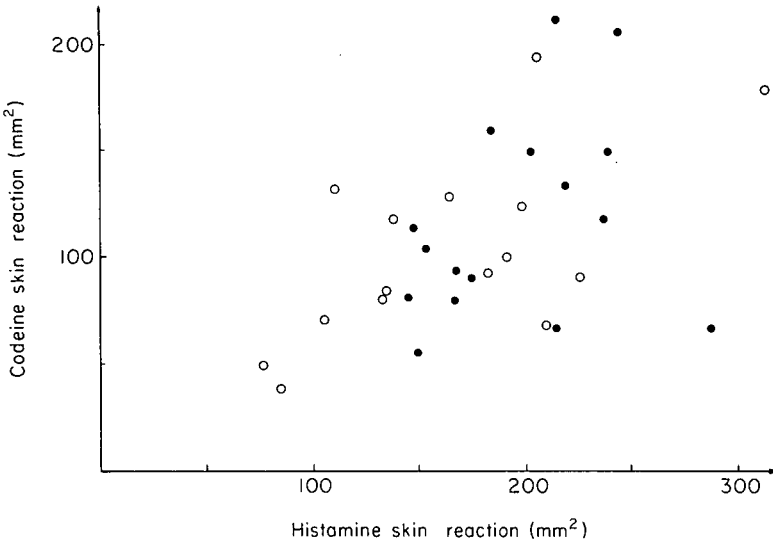


Fig. 2. Correlation of weal reaction to histamine 0.01% and codeine 0.01% in old (○) and young (●) females. The graph shows the tendency of the older females to react less to codeine and histamine; but this did not reach statistical significance.

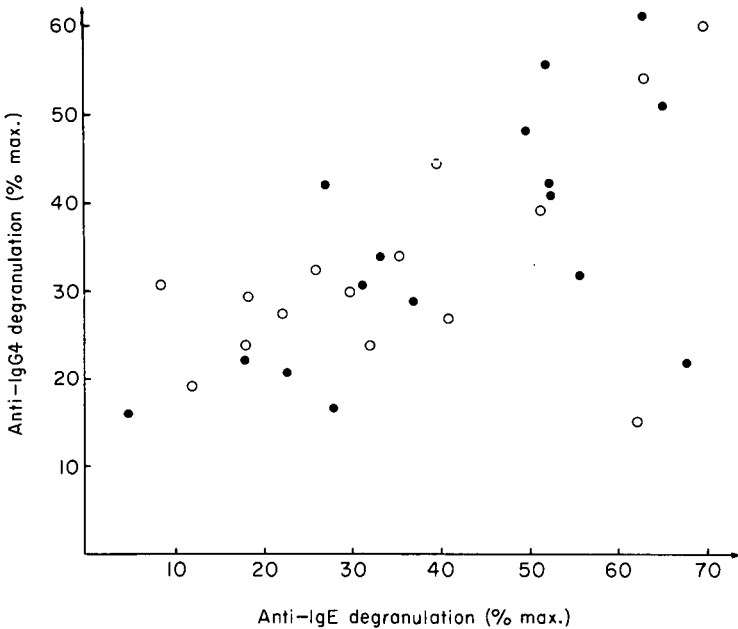


Fig. 3. Correlation between anti-IgE and anti-IgG4 basophil degranulation in old (○) and young (●) females. $n=31$, $r=0.61$, $P<0.001$.

with a positive skin test to house dust and/or *Candida albicans* showed less readiness to basophil degranulation by anti-IgE and anti-IgG4. For degranulation by anti-IgE the difference was statistically significant (Table 4 and Fig. 4).

IgE. No correlation between serum IgE and blood basophil degranulation by anti-IgE or anti-IgG4 could be found (results not shown).

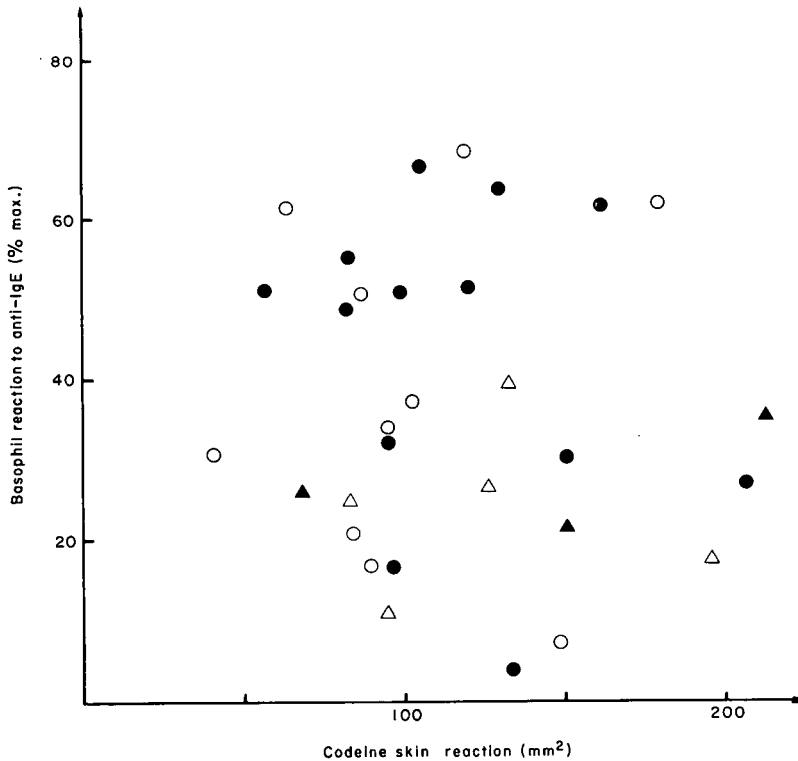


Fig. 4. Anti-IgE basophil degranulation and codeine 0.01% weal sizes in subpopulations of old and young females. ○, Old people without any skin reaction to house dust or *Candida albicans*, $n=10$; △, old people with positive skin tests to house dust and/or *Candida albicans*, $n=5$; ●, young people without any skin reaction to house dust or *Candida albicans*, $n=13$; ▲, young people with positive skin tests to house dust or *Candida albicans*, $n=3$.

Table 4. Maximum percentage of blood basophil degranulation by anti-IgE and anti-IgG4 in clinically non-allergic females with and without positive skin reaction to house dust/*Candida albicans*

Sample groups	<i>n</i>	Maximum degranulation (%)	
		Anti-IgE	Anti-IgG4
(a) Females with positive skin reaction to house dust/ <i>C. albicans</i>	8	26.4 (± 3.3)	28.5 (± 2.7)
(b) Females without positive skin reaction to house dust/ <i>C. albicans</i>	23	42.3 (± 4.4)	35.6 (± 3.1)
Significant difference (<i>P</i>) between (a) and (b)		<0.05	NS

Discussion

No decrease of basophil degranulation induced by anti-IgE or anti-IgG4 could be observed with age. The close correlation between basophil degranulation induced by anti-IgE or anti-IgG4 already observed in a former study (Nakagawa, Moyseyenko & de Weck, 1981a) holds also in older individuals. There is therefore no direct evidence for an altered basophil function with age.

In contrast, we observed a definite trend to smaller weal skin reactions to histamine and to the histamine-liberating drug codeine in the elderly. In the literature, a similar behaviour has been reported (Tuft *et al.*, 1955) but there are conflicting statements by other authors (Delespesse *et al.*, 1977; Pupil & Berna, 1976). A decrease of skin reactivity to the compound 48/80, but not to histamine, has been reported (Pupil & Berna, 1976). It must be recognized that the finding of an impaired reaction to histamine and codeine in elderly skin may be in part an artifact, since it is more difficult to read weal reactions in the often parchment-like skin of old people.

Skin reactivity to histamine correlated very well with the reaction to codeine. This relationship is expected since both reactions depend, either wholly or in part, on the tissue reactivity to histamine. There was, however, no relationship between the sizes of the skin reactions and the basophil reactivity to anti-IgE. Although this observation has been made before (Berther *et al.*, 1980), it is still not clear whether the lack of relationship between skin tests and basophil histamine release is a function of the different challenging agents (codeine *vs* anti-IgE), of the different cell types (mast cells *vs* basophils), or of the different environments (tissue *in vivo* *vs* cell suspension *in vitro*).

Contrary to the evidence presented by other authors (Delespesse *et al.*, 1977; Hanneuse *et al.*, 1978; Orren & Dowdle, 1975), we were unable to demonstrate significantly lower total serum IgE levels in elderly people, although our investigation is based only on females, which should, according to the literature (Delespesse *et al.*, 1977; Orren & Dowdle, 1975), show an even higher decrease of IgE with age. This, however, may easily be explained by the high variance (approx. 18%) observed and the limited number of investigated persons ($n=31$). The lack of correlation between basophil degranulation and total serum IgE is in agreement with previous studies (Conroy, Adkinson & Lichtenstein, 1977; Stahl Skov, Norn & Weeke, 1977).

In the eight individuals (old and young people) with positive skin tests to house dust and/or *Candida albicans*, a significantly lower basophil degranulation by anti-IgE was found; a similar observation has been made in some allergic people with positive skin tests to grass pollens and epithelial allergens from laboratory animals (rat, guinea pig). In the literature we find only anecdotal references to this phenomenon (Bernstein, Vijay & Perelmutter, 1977; Leffert & May, 1975; Stahl Skov *et al.*, 1977); most of the authors have noticed a high correlation between skin test and basophil degranulation to allergens, up to 94% (Burtin, Bloch-Morot & Lebel, 1977; Dry, Leynadier & Luce, 1979; Soifer & Hirsch, 1975; Stahl Skov *et al.*, 1977; Weyer *et al.*, 1978). Whether this observation is really related to a decrease in basophil function, as suggested (Bernstein *et al.*, 1977), or whether in such patients some membrane-bound antibodies blocking the interaction with anti-IgE are involved, would require further study.

Since in centrifuged leucocytes basophils were less numerous in blood samples from allergic than in non-allergics (Garcia *et al.*, 1978), it may also be argued, that the most reactive basophils of allergic patients have already been degranulated and that the remainders are less sensitive to the effect of degranulating agents.

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