Allergy 2001: 56: 436–441
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ALLERGY
ISSN 0105-4538

Short communication

Changes over 13 years in skin reactivity to histamine in cohorts of children aged 9–13 years

Background: Several studies report substantial differences in the prevalence of skin test reactivity to allergens in children from adjacent geographic areas; others report an increased prevalence over time. To find out whether these differences depend on variations in skin reactivity to histamine, we determined the time trend of histamine wheal sizes in successive cohorts of unselected children living in the same area (Viterbo, Italy).
Methods: We conducted three epidemiologic surveys, each including children aged 9 and 13 years. The 1983–7 study investigated 170 children (150 were tested twice); the 1992 study, 158 children; and the 1996 study, 208 children.
Results: In both age groups, the mean diameter of the wheal induced by histamine skin prick tests (10 mg/ml) increased significantly over time (9-year-olds: 3.25 mm in 1983, 4.68 in 1992, and 5.89 in 1996; 13-year-olds: 3.89 mm in 1987, 5.18 in 1992, and 6.50 in 1996) ($P<0.001$ between subsequent studies). The distribution of the wheal diameters for both ages showed a trend to a right shift in the three successive studies ($P<0.001$). The dose-response curves for three histamine concentrations (0.2, 1, and 10 mg/ml) had significantly steeper slopes in 1996 than in 1983–7 ($P<0.001$).
Conclusions: The marked time-related increase in the size of the histamine wheals could help to explain the trend toward an increased prevalence of positive allergen skin test reactions reported during the past years. The causes of increased skin reactivity to histamine remain conjectural.

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Key words: children; epidemiology; histamine wheals; histamine skin reactivity.

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Accepted for publication 4 September 2000

After the limited data of previous reports (1, 2), recent papers suggest, at least in certain geographic areas, an increasing prevalence over time of positive skin test reactions to most of the common allergens tested (including mites, pollens, and molds) among children of the general population (3, 4). Several studies have also shown that the prevalence of positive skin tests differs in adjacent geographic settings (5). Although these findings could depend on differences in the production of specific IgE directed against most of the common allergens, this seems improbable given the genetic basis of IgE production. A more likely hypothesis is that they depend on an increased skin reactivity to the histamine released in the reaction between allergens and surface IgE of mastocytes. This could be demonstrated by providing epidemiologic data showing an increase in skin histamine reactivity over the past few years.

In epidemiologic surveys conducted in 9-year-old (1983, 1992, and 1996) and 13-year-old children (1987, 1992, and 1996), which have, in part, been published elsewhere (4, 6–8), we did skin prick tests with three different histamine concentrations and recorded the diameters of the histamine-induced wheals, always using identical procedures, devices, and field operators, and examining children from the same geographic area.

In this study, to investigate possible changes in skin responsiveness to histamine over time, we reviewed the results of histamine skin test reactivity measured three times during the 13-year period 1983–96 in unselected populations of schoolchildren from the same geographic area.

Material and methods

1983 study

The target population of the initial study consisted of 179 children aged 9 years, including all the fourth-grade students in three small towns in the province of Viterbo near Rome. Informed consent was obtained from the parents of 170 children (87 girls and 83 boys).

1987 study

The 170 families of the children who had given full

consent to the 1983 study were contacted through their junior high school. Eight families had moved to other towns or could not be found. Informed consent was obtained from the parents of 150 children (74 girls and 76 boys).

1992 study

We investigated a group of children randomly drawn from the same schools originally studied in 1983 and 1987. The group comprised three fourth-grade classes (64 children aged 9 years; 33 boys and 31 girls) and four eighth-grade classes (94 children aged 13; 49 boys and 45 girls).

1996 study

The children in this study were randomly drawn from the same schools studied in previous surveys. The group consisted of four fourth-grade classes (89 children aged 9 years; 41 boys and 48 girls) and five eighth-grade classes (119 children aged 13 years; 53 boys and 66 girls).

All the studies were conducted between October and January. For 1 week before testing, all children were asked to refrain from antihistamine medications and from inhaled or oral corticosteroids. Skin prick tests with different concentrations of histamine chloride (10 mg/ml, H_1; 1 mg/ml, H_2; 0.2 mg/ml, H_3) were done in a predefined area on the lower volar side of the left forearm, with a space of at least 2 cm between each prick. These three pricks were the last in a battery of 14 pricks given in a predefined order to each child participating in the three epidemiologic surveys. All histamine dilutions for prick tests to be used in each study were prepared by an expert from our laboratory using standardized procedures in use in our service since before the first epidemiologic campaign (6) and thereafter unchanged. In the 1992 study, the 0.2 mg/ml histamine concentration was not tested. Disposable allergy prickers with a 1-mm tip were used. At 10 min after the skin prick, the mean diameter (the mean of the maximum diameter and its perpendicular) of the histamine-induced wheals was measured with a transparent plexiglass ruler and recorded. All skin prick tests were done by four experienced physicians working in the outpatient service of the II Pediatric Clinic, University of Rome "La Sapienza". Three of the four physicians who did skin prick tests in the 1992 and 1996 studies had also participated in the 1983 and 1987 studies.

The Pepys method for skin prick tests (9) was used throughout the study. The standard instructions for the procedure were to jab the pricker with moderate force through the drops at an angle of 45° to the skin and gently lift. In pilot studies conducted before each of the four campaigns, each field operator did four identical tests with the 10-mg/ml histamine solution on a group of six to eight adult volunteers (normally nurses, students, and colleagues) for a total of 101 subjects examined with four pricks in the four pilot studies. The differences between operators were always nonsignificant. The mean coefficient of variation within each operator's tests was 8.5–20.5%. The coefficient of variation was calculated by dividing the mean of four measures by their standard deviation. In each study, the results were read by a different operator. The interoperator difference in reading performance was 1 mm in 18–13% of readings and >1 mm in 2%. No test yielded $H_1 < H_2$; 3.8% gave $H_1 = H_2$; 1.8%, $H_2 < H_3$; and 11%, $H_2 = H_3$.

All data are expressed as means $\pm$ SE. All data were computed with the software program SPSS. Because data for wheal diameters showed normal distribution, Student's *t*-test for independent samples was used to compare the mean histamine-wheal diameters in the three studies. Kruskal–Wallis one-way analysis of variance was used to compare the distribution of histamine wheals in the three studies. Student's *t*-test for independent samples was used to compare the mean slope of the curves defined in each subject for the wheals induced by the three histamine concentrations used in the two studies 1983 and 1996. The Mann–Whitney U test for nonparametric values and Student's *t*-test yielded identical results.

Results

The mean diameters of the wheals induced by the 10 mg/ml histamine concentration recorded in children aged 9 and 13 years increased significantly over time ($P < 0.001$; Fig. 1A and B). In the 1983–7 studies (the same children tested 4 years apart) and in the 1992 and 1996 studies, histamine elicited a larger mean wheal diameter in children aged 13 than in those aged 9.

The distribution of the wheal diameters elicited in the successive studies by histamine (10 mg/ml) in the 9-year-old and 13-year-old children showed a significant shift of individual values to the right ($P < 0.001$; Fig. 2A and B). Wheal diameters equal to or larger than 6 mm were almost nonexistent in 1983–7, but accounted for about 20–25% in 1992 and about 50% in 1996.

Plotting on a semilog scale the three histamine concentrations against the wheal diameters obtained in the 9-year-old (Fig. 3A) and 13-year-old children (Fig. 3B) yielded far steeper linear dose-response curves for 1996 than for 1983–7 ($P < 0.001$).

Discussion

In the present paper, we describe a time-related increase in the size of the wheals elicited by different concentrations of histamine in three cohorts of 9-year-old

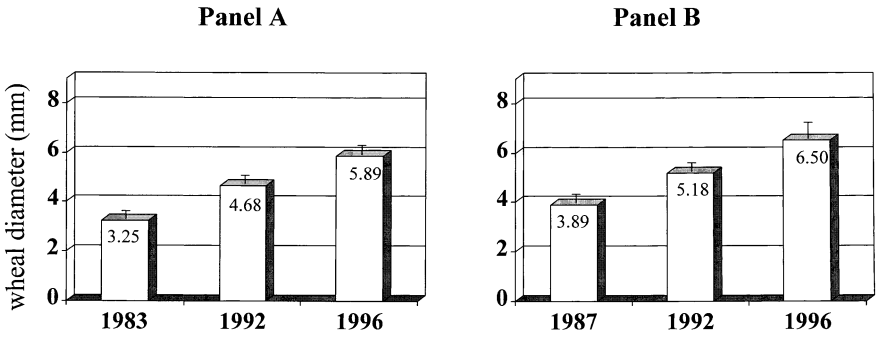

Figure 1. Mean (±SE) diameters of wheals induced by histamine skin prick tests (10 mg/ml) in three surveys conducted in 9-year-old (panel A) and 13-year-old children (panel B). In each panel, all comparisons were statistically significant ($P < 0.001$, by Student's *t*-test).

children tested in 1983, 1992, and 1996, and in three cohorts of 13-year-olds, tested in 1987, 1992, and 1996. In both age groups, we documented a shift in the distribution of the histamine-induced wheal diameter toward larger sizes, with a significant increase in the mean wheal diameter over time. Comparison of the dose-response curves for the two studies that tested the three histamine concentrations yielded significantly steeper dose-response curves in 1996 than in 1983–7.

Methodological problems seem unlikely to have biased our results. The pilot studies conducted before each campaign yielded coefficients of variation similar to or smaller than those reported by others (10–13). Instead of using duplicate tests with a fixed histamine concentration, we used three pricks with different histamine concentrations in each subject. This latter procedure allowed us to check accuracy and also yielded additional meaningful results (dose-response curves).

Because our studies were conducted in the same stable population, with identical materials, methods, and field personnel, we consider several possible confounders unlikely. For example, we can exclude the influence of the many technical factors capable of altering the skin wheal diameters of the histamine skin prick test, including devices (10–14), skin test location, and time of reading (11, 15, 16). A possible limitation of our study is the technique used for allergen prick testing, the Pepys method. When the study began in the 1980s, however, this was the preferred method. To avoid statistical confounding, we therefore continued to use it even in the later studies. Most current studies prefer the allergen pricker method described in the 1989 European Academy of Allergology and Clinical Immunology (EAACI) position paper (21), because, for most investigators, it is more precise.

In adults, although the mean wheal diameter in response to a histamine concentration of 10 mg/ml has been reported to vary widely (between 4 and 10 mm), most reported results range between 4.6 and 6.9 mm (10, 11, 13, 14, 17–19). Age significantly influences the size of histamine-induced wheals: children have smaller

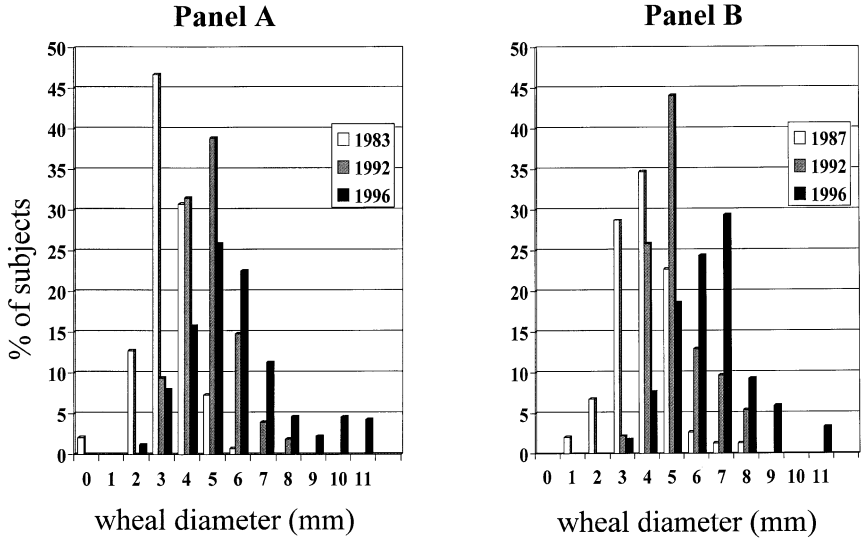

Figure 2. Percent distribution of mean wheal induced by histamine skin prick tests (10 mg/ml) in three different surveys conducted in 9-year-old (panel A) and 13-year-old children (panel B). In both panels, three distributions differ significantly ($P < 0.001$, by Kruskal–Wallis).

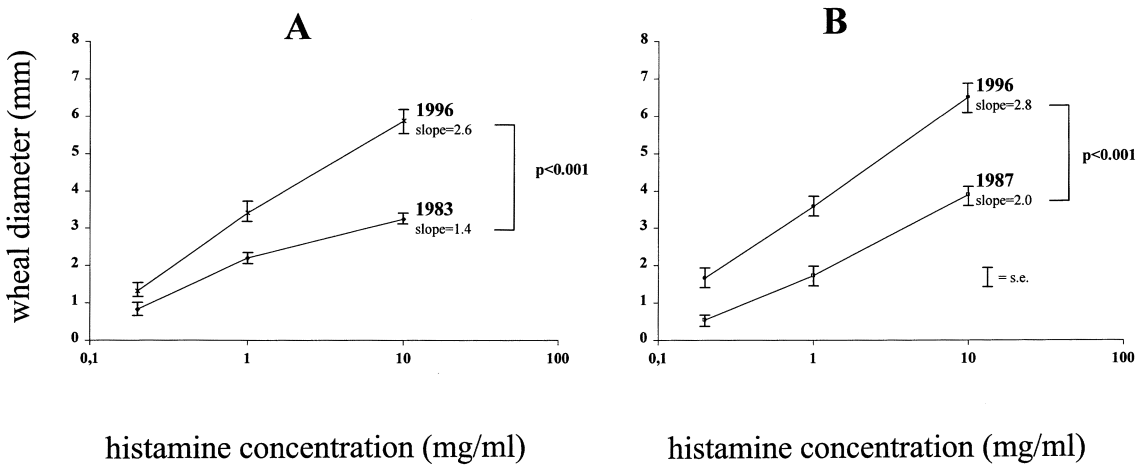

Figure 3. Mean dose-response curves for skin-prick-test reactions induced by histamine (0.2, 1.0, and 10 mg/ml) in 9-year-old children tested in 1983 and 1996 (A) and 13-year-old children tested in 1987 and 1996 (B).

reactions than adults (17, 20) and infants show increasing histamine wheals during their first 24 months of life (17, 18). In all our three studies and for all tested histamine concentrations, we found that 13-year-old children had larger wheals than 9-year-old children. Using a prick test technique similar to ours in a study conducted in 1987, in French children aged 6–14 years, others have reported mean diameters of 3.8–3.9 mm for histamine 10 mg/ml (18). In comparison, our study yielded lower diameters in 1983 (mean 3.25 mm at age 9), identical ones in 1987 (3.89 mm at age 13), and larger ones in 1992 (4.68 and 5.18 mm for the two age groups). Reports providing dose-response curves for histamine wheals have consistently shown a linear correlation between wheal diameter or area dimensions and log concentrations of histamine (16, 20, 21). In studies conducted in adults in the 1980s, the reported slope of these curves was about 2 (1.5-fold increase in the wheal diameter for an increase in the histamine concentration from 1 to 10 mg/ml) (21). Our data yielded a slope for the dose-response curve of 1.4–2.0 in the 1983–7 studies; the slope then increased significantly, reaching values of 2.6–2.8 in 1996 (Fig. 3A and 3B).

Extensive epidemiologic studies failed to disclose larger histamine wheals in atopic than in nonatopic individuals (20, 22). In contrast, another study, investigating responses in manifestly allergic patients and nonallergic subjects, found that although all histamine concentrations tested elicited larger wheals in the manifestly allergic patients, the dose-response curves for normal subjects had significantly steeper slopes (23). Had the differences between our early (1983–7) and later populations (1996) depended on atopy, our 1996 study would have yielded increased histamine wheals but flatter curves. But it did not: the dose curves for 1996 were steeper. This discrepancy suggests that the progressive enhancement of the histamine skin response we observed in recent years is based on mechanisms different from those underlying the skin histamine hyperresponsiveness of manifestly atopic patients.

To our knowledge, no published study has described time-related changes in histamine skin reactivity similar to those we observed (20, 22). Yet Riikjarv et al. noted that Estonian children, who had a lower incidence of positive allergen skin tests than Swedish children, also had smaller histamine wheals (5). Identical findings have been reported in two populations of German children (3). These associations between histamine wheal dimensions and incidence of positive skin prick tests and the changes we observed in histamine skin reactivity could help to explain the rapid increase in the prevalence of positive skin test reactions for many common allergens (1–4). The observation that histamine skin reactivity can be modulated could also account for the differences in positive allergen skin test reactions observed in populations genetically identical, but with different lifestyles (5, 24–26). Our data for children show that for any amount of histamine released by the allergen–IgE–mastocyte interaction, the wheal produced in 1996 was roughly twice as large as the wheal that the same amount of histamine would have produced some 10–15 years before. This change unquestionably means that allergen skin reactions previously read as borderline would now be considered clearly positive.

An important point to remember when interpreting our data is that histamine and allergen dose-response curves are not parallel, the allergen curve being, as a mean, steeper (22). Some individuals might nonetheless have steeper and others flatter allergen dose-response curves (21). Hence our data do not allow us to state whether the observed increase in histamine skin sensitivity can alone explain the increased prevalence of positive allergen skin test reactions.

Many mechanisms could explain an increase in skin sensitivity to histamine: heightened H_1-receptor sensitivity, H_1-induced increased production of vasodilators (28) or other mediators (29, 30), and enhanced action of priming substances (31) or histamine-releasing factors (32). H_1 receptors are also responsible for the stimulation of sensory nerve endings, which, through an antidromic axon reflex, induce the release of substance P and of other mediators. Substance P produces a dose-related wheal-and-flare response in human skin (33, 34). Substance P is mainly cleaved by neural endopeptidase, an enzyme that can be downregulated by irritative stimuli such as cigarette smoke (35). Hence, an increased skin concentration of neuromediators, including substance P, due to enhanced production or reduced cleavage, could substantiate a neurogenic inflammation capable of explaining our results.

In conclusion, our data obtained in the general pediatric population over a 13-year period provide evidence of an increase in the size of histamine-induced wheals, possibly related to increased skin cellular or neurogenic inflammation. We believe that it is important to determine whether in Western countries this is a generalized phenomenon. Studies should also be directed to ascertain the environmental causes of these changes that could influence the rate of positive allergen skin tests and explain why the rates of positive reactions differ in various geographic settings.

Acknowledgments

We thank Dr E. Bonci for the statistical analysis of data, and Drs G. Antognoni, A. Dotta, R. Cutrera, F. Macrì, G. C. Tancredi, G. De Castro, A. Villani, L. Indinnimeo, F. Midulla, I. Milano, R. Rota, M. Pagani, and S. Piro for field collection of epidemiologic data.

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