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Skin reactivity to histamine and to allergens in unselected 9-year-old children living in Poland and Italy

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Several studies have shown a higher prevalence of positive skin-prick tests to airborne allergens in Western than in Eastern European countries. We have recently reported that skin histamine reactivity significantly increased in Italy over the past 15 years. Population differences in skin histamine reactivity could, at least in part, explain the reported differences in positive allergen skin tests. To test this hypothesis we compared histamine skin reactivity and the prevalence of allergen positive skin-prick tests in a sample of Italian and Polish schoolchildren. A total of 336 unselected 9-year-old-schoolchildren (198 in Italy and 138 in Poland) underwent skin-prick tests with three different histamine concentrations (10, 1 and 0.2 mg/ml) and with a panel of common airborne allergens according to the ISAAC protocol, phase two. Mean wheals elicited by skin-prick tests with the three serial concentrations of histamine were significantly larger ($p < 0.001$) and shifted more toward higher values ($p < 0.001$) in Italian than in Polish children. The differences were greater for the intermediate histamine concentration tested (1 mg/ml) than for the highest concentration (10 mg/ml). Skin-prick tests for airborne allergens were more frequently positive in Italian children: wheals ≥ 3 mm induced by any allergen [odds ratio (OR) 1.69; confidence interval (CI) 0.98–2.92] by *Dermatophagoides pteronyssinus* (OR 1.92; CI 0.97–3.80) and by *D. farinae* (OR 3.15; CI 1.16–8.63). Labeling as positive allergen wheal reactions half the size of the 10 mg/ml histamine wheal or larger reduced but did not abolish the Italian–Polish differences. The significantly higher skin histamine reactivity observed in Italian children could help to explain why allergen skin-test reactions differ in the East and West European populations. Moreover, differences in nonallergen-specific factors among populations should be considered in the interpretation of skin test results (e.g. cut-off points). To obtain meaningful results, epidemiological studies of allergies should include serial histamine dilutions.

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Key words: histamine skin reactivity; airborne allergen skin-prick test positivity; Eastern–Western European difference

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Several epidemiological studies have shown a higher prevalence of atopic diseases and of positive skin-prick tests to common airborne allergens in Western than in Eastern European countries both in children (1–4) and in adults (5). Other studies have also demonstrated a time-related increase in the prevalence of positive allergen skin-prick tests within westernized countries (6, 7). This phenomenon has so far been explained by a concomitant increase in Th2 responses to airborne allergens. Indeed, the prevalence of subjects with specific IgE antibodies against airborne allergens has risen in various westernized populations (8–10).

Skin reactions to airborne allergens, however, involve at least two different mechanisms, i.e. the specific interaction between IgE antibodies bound to mast cell membranes and the subse-

quent vasoactive response to the histamine released from mast cells themselves (11). We have recently reported a significant increase in skin reactivity to histamine in serial cohorts of children from the same area (central Italy) (12, 13). Changes in skin reactivity to histamine can deeply affect the positive or negative assessment of allergen skin-prick tests. We therefore hypothesized that differences in the skin response to histamine might help to explain why the prevalence of atopic sensitization differs in Western and Eastern European countries. To test this hypothesis, we examined skin reactivity both to airborne allergens and to serial dilutions of histamine in a large sample of Italian and Polish schoolchildren.

Materials and methods

Study design

The two studies were conducted from October 1998 to January 1999; a total of 336 children aged 9 years were recruited in Italy and Poland and assessed for atopy through skin-prick tests run according to the ISAAC protocol (Module 3.2) (14). The target population in central Italy consisted of all the pupils attending the eight fourth-grade classes at primary schools in three small towns near Viterbo (Ronciglione, Caprarola and Carbognano): of the 207 eligible children, 198 (86 boys, 43%) agreed to participate in the study. Similarly, of the 151 children, all the pupils in the four fourth-grade classes in a small Polish town (Starachowice), 138 (59 boys, 43%) agreed to take part in the study. The two groups of children were drawn from small towns (59,000 inhabitants in Poland and 30,000 in Italy) with picturesque surroundings (a small lake in Italy, forest and rivers in Poland) without undue pollution. Both places are popular tourist areas. Some families are engaged in agriculture; most families live in good, centrally heated houses. The local lifestyle reflects the differences between Poland and Italy. Parents of both populations gave their written informed consent for their children to take part in the study.

Skin-prick tests

For 1 week before testing, all children were asked to refrain from antihistamine medications and from inhaled or oral corticosteroids. When tested, all participants denied using long-acting antihistamine preparations. Skin-prick tests with three different concentrations of histamine chloride (10, 1 and 0.2 mg/ml) and with the respiratory allergens panel suggested by the ISAAC protocol phase 2, were done on the inner and outer sides of the left forearm. The order of the skin tests was as follows (proximal to distal), inner side, 3 cm from the elbow: histamine 10 mg/ml, histamine 0.2 mg/ml, *Dermatophagoides pteronyssinus*, cat hair, mixed grasses (*Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, *Poa pratensis*, *Phleum pratense*, and *Avena elatior*); outer side, 4 cm from the elbow: histamine 1 mg/ml, negative control (50% glycerin in saline), *Dermatophagoides farinae*, *Alternaria alternata*, mixed trees (*Alnus glutinosa, Betula verrucosa* and *Corylus avellana*). The distance between prick tests was 2 cm. A separate lancet was used for each test.

Allergenic extracts (potency 10 HEP/ml), histamine concentrations and the kit of disposable, 1-mm tip metallic lancets used in both countries came from the same batch (ALK-Abello, Horsholm, Denmark).

The skin-prick test was done by firmly directing for 2 s the lancet through the allergen droplet at on angle of 90° to the skin. The skin tests were read 10 min after the skin-test procedure ended, always in the same sequence, starting from the 10 mg/ml histamine pricks. Given the time required for the whole skin testing procedure (about 1 min) and for reading (1–2 min) this procedure allows a mean reading time of 11 min for histamine prick tests and of 12–13 min for allergens (15). The contours of each wheal were outlined with a thin, felt-tipped pen and transferred to the record sheet by means of transparent tape for a permanent record. The size of each wheal was measured as the mean of the longest diameter (a) and the diameter perpendicular to it at its mid-point (b), i.e. $(a + b)/2$. Each diameter was measured to the nearest millimeter. Wheals with a mean diameter < 2 mm were read as 0. Skin prick-test reactions against airborne allergens were assessed as positive by two different criteria: the absolute diameter expressed in millimeters (cut-off 3 mm) and the ratio between the diameters of the allergen wheal and the 10 mg/ml histamine wheal (cut-off 0.5 or more).

Four field operators, two from Italy and two from Poland, and three senior authors, all with previous experience in similar epidemiological studies, met in Poland for 3 days to standardize the procedures. Inter-field operator variability in the results of blocks of eight histamine prick tests performed by each operator on six colleagues and expressed as the coefficient of variation (SD of eight measures divided by their mean) ranged from 7% to 20.5% in each series and from 7% to 14% in the last.

Statistical methods

The Student's two-tailed *t*-test was used to compare the wheals obtained in Italy and Poland and the χ^2 test, odds ratios (ORs) and 95% confidence intervals (CIs) were used to analyze the frequencies of children testing positive in both countries; the distribution of mean wheal diameters was analyzed using the Kruskal–Wallis test. A p-value < 0.05 was considered statistically significant.

Results

Skin-prick tests using the three histamine concentrations (10, 1 and 0.2 mg/ml) yielded significantly larger mean wheal reactions ($p < 0.001$) in Italian than in Polish children (Fig. 1); the intermediate histamine concentration (1 mg/ml) resulted in the largest difference. The frequency distributions of wheal reaction diameters to each histamine concentration were significantly shifted toward higher values in Italian than in Polish children ($p < 0.001$) (Fig. 2: a, b, c). The 1 mg/ml histamine concentration elicited a wheal diameter smaller than 3 mm in almost 90% of the Polish children but in only 14.7% of the Italian children (difference 75.3%, Fig. 2b); whereas the 10 mg/ml histamine concentration elicited a wheal reaction smaller than 3 mm in 5.8% of the Polish children and in 1% of the Italian children (difference of only 4.8%, Fig. 2a).

Italian children had a higher rate of reactions ≥3 mm to at least one airborne allergen (OR 1.69, CI 95% 0.98–2.92), to *D. pteronyssinus* (OR 1.92, CI 95% 0.97–3.80), to *D. farinae* (OR 3.15, CI 95% 1.16–8.63), and to the other allergens tested. When we labeled as positive a wheal reaction half the size of the histamine wheal (10 mg/ml) or larger, Italian children still had a higher rate of positive reactions, but the differences were smaller and always nonsignificant (Table 1a,b).

In neither ethnic group nor in the entire population was a significant difference observed in histamine wheals either for the comparison between boys and girls or for the comparison between children with allergen wheals larger than 2 mm or smaller than 3 mm.

Discussion

We observed that the three histamine concentrations elicited significantly larger wheals and that their size distribution shifted toward larger dimensions in a sample of unselected 9-year-old Italian schoolchildren than in a similar sample of the Polish population. To our knowledge, this is the first full report showing higher skin reactivity to histamine in a Western, than in an Eastern European country.

For the airborne allergens tested, we also found a higher prevalence of positive allergen prick tests in Italian than in Polish schoolchildren: when we used an absolute diameter of 3 mm or more as the criterion for skin-test

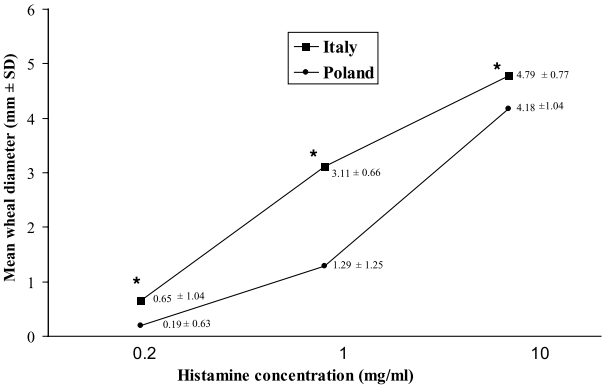

Fig. 1. Dose–response curve for histamine prick wheal diameters (in mm) in 198 Italian and 138 Polish schoolchildren. Values are expressed as means ± SD by the two-tailed Student's *t*-test. *p < 0.001.

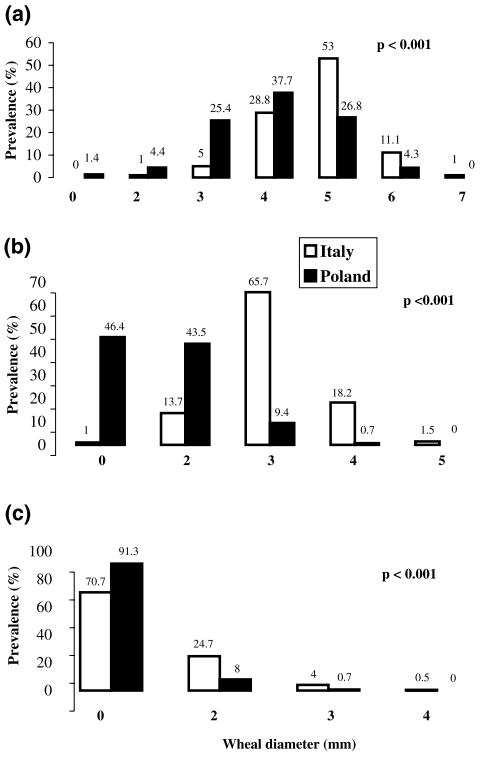

Fig. 2. Distribution of wheal diameters for skin-prick tests with the three histamine concentrations in 198 Italian and 138 Polish schoolchildren (Mann–Whitney *U*-test): (a) 10 mg/ml, (b) 1 mg/ml, (c) 0.2 mg/ml.

Table 1. (a) Wheal diameters 3 mm or larger, (b) wheal diameters half the histamine 10 mg/ml or larger

(a)	Italy (198)	Poland (138)	OR 95% CI
Dermatophagoides pteronyssinus	33 (16.7%)	13 (9.4%)	1.92 (0.97–3.80)
Dermatophagoides farinae	21 (10.6%)	5 (3.6%)	3.15 (1.16–8.63)
Cat	11 (5.6%)	5 (3.6%)	1.56 (0.53–4.59)
Alternaria tenuis	3 (1.5%)	1 (0.7%)	2.10 (0.22–20.41)
Mixed grasses	23 (11.6%)	11 (8.7%)	1.52 (0.72–3.18)
Mixed trees	8 (4%)	3 (2.2%)	1.9 (0.49–7.21)
Children with positive skin test reaction to at least one of the tested allergens	50 (25.2%)	23 (16.7%)	1.69 (0.98–2.92)

(b)	Italy (198)	Poland (138)	OR 95% CI
Dermatophagoides pteronyssinus	37 (18.7%)	17 (12.3%)	1.63 (0.88–3.04)
Dermatophagoides farinae	24 (12.1%)	13 (9.4%)	1.32 (0.65–2.70)
Cat	11 (5.6%)	8 (5.8%)	0.95 (0.37–2.43)
Alternaria tenuis	6 (3.0%)	5 (3.6%)	0.83 (0.25–2.78)
Mixed grasses	24 (12.1%)	12 (8.7%)	1.45 (0.70–3.00)
Mixed trees	11 (5.6%)	7 (5.1%)	1.10 (0.42–2.90)
Children with positive skin test reaction to at least one of the tested allergens	54 (27.3%)	29 (21%)	1.41 (0.84–2.36)

reactivity the difference approached statistical significance. This result confirms and extends the already long list of similar observations from other studies using similar methods to compare Western and Eastern European populations (2–7).

During the preliminary stages of our study, we conducted a series of tests and procedures designed to minimize problems arising from experimental methods that could have biased our results. Both in Poland and in Italy we examined unselected children comparable for age and sex and living in small towns of similar size. In both countries, children were tested at the same time of the year, out of the local pollen season; skin tests were done at the same time of day; the indoor temperature was monitored and only minor differences were observed. The lancet used, the batch of allergens and the histamine preparations were also identical. Field operators in both groups were chosen from among those with extensive experience in similar epidemiological studies. Procedures were standardized according to a cross-over protocol during a 3-day pilot study in Poland. Inter-field-operator variability in measuring the mean histamine wheal diameter was well below 20%, independently from the histamine concentration. We followed the ISAAC protocol, phase 2, module 3-2, which is specifically intended to allow comparisons between centers. We modified only the number of tested histamine concentrations (three instead of one) and the time of reading (starting 10 min after the end of procedures to allow a more precise reading of histamine pricks, which peak at 10–11 min) (15) but the two centers strictly followed an identical procedure. Given these premises, we consider bias from experimental methods unlikely as a cause of the differences we observed.

On the other hand, others have already noted 'unexplained' differences in histamine-induced wheals in Eastern and Western European pediatric populations: indeed, smaller histamine wheals have been observed in Estonian than in Swedish schoolchildren (4) and in schoolchildren from East than from West Germany (MA Riikjärv et al. personal communication) (4). Furthermore, Bråbäck et al. (3) found that histamine at 10 mg/ml elicited a mean wheal reaction of 6.2 mm in Swedish children, but of only 5.5 mm in Polish schoolchildren (age 10–12 years). These investigators attributed this result to 'possible minor differences in technique or to a slightly lower reactivity to allergens'. We believe that our result depends on true differences in skin reactivity to histamine, suggesting that skin reactivity to histamine differs in populations. Indeed, we have recently reported evidence of a temporal trend to an increase in serial studies among Italian schoolchildren (12).

The reasons for the observed geographic or temporal differences in skin reactivity to histamine remain unclear. Once injected in the skin, histamine elicits the characteristic 'triple response' (red spot, flare and wheal, Lewis 1927) that derives from stimulation of four histamine receptors. H1 receptors are more important and their stimulation causes the production of potent vasodilator substances: increased sensitivity of cell-H1 histamine receptors could explain our results. Some histamine

actions at a cellular level are exerted under the simultaneous influence of mediators such as leukotrienes (16) or the calcitonin-gene-related peptide (17). Any 'histamine-releasing factors' could enhance histamine release from mast cells (18). Moreover, some molecules can 'prime' the skin mast-cell for an enhanced histamine release caused by other molecules (19). All these mechanisms singly or in concert could explain the geographic differences or the temporal trend to an increased histamine wheal dimension.

Histamine is also responsible for the stimulation of sensory nerve endings, which through an antidromic axon reflex induces the liberation of substance P and of other mediators. Substance P produces a dose-related wheal and flare response in human skin (20). The fact that substance P is also capable of inducing the secretion of histamine from human skin mast cells (21) proves that a positive feedback loop exists. Substance P is mainly cleaved by neutral endopeptidase, an enzyme that can be down-regulated by irritative stimuli (18). An increased skin concentration of neuromediators such as substance P due to enhanced production or reduced cleavage could therefore substantiate a neurogenic inflammation capable of explaining our results.

These interpretations leave open the possibility that the differences or time-related increase in the prevalence of positive allergen skin-prick tests may, at least in part, reflect true differences in the skin concentration of specific IgE antibodies. This well documented phenomenon (10, 22) may nevertheless be independent from and variably associated with a broader and less specific change in cell reactivity manifested in the skin by increased reactivity to histamine. Whether the two phenomena are independent or both depend on a common pathogenetic mechanism induced by environmental or genetic factors remains to be established.

On a more practical level, differences in histamine skin sensitivity between populations could at least partly explain differences in allergen skin-test reactivity previously reported (2–7). Our results suggest that the interpretation of skin tests (e.g. cut-off points) may be affected by nonallergen-specific factors that differ among populations with different lifestyles. If so, when assessing the results of allergen skin-tests, we should obviously consider differences in histamine skin-test reactivity. Our study strongly suggests that the current method for taking into account such individual differences, i.e. considering as positive only those wheals half the size of the 10 mg/ml-histamine wheal or larger, is inadequate. Because only one child in the population we studied had a 10 mg/ml-histamine wheal larger than 6 mm, all our children who had an allergen wheal of at least 3 mm fulfilled the criteria of having allergen wheals larger than half the 10 mg/ml-histamine wheal.

The findings in this epidemiological study leave open the question of criteria that could account better for individual levels of histamine sensitivity. We believe that if epidemiological studies investigating the role of the major risk factors on specific and nonspecific components of the human response towards environmental allergens include serial histamine dilutions, they will obtain more meaningful results.

Skin reactivity to histamine has been thoroughly discussed as an age-dependent factor contributing to skin reactivity to allergens (11, 23). Some reports suggest that this variable is largely determined by genetic factors (24). Our observations suggest that skin reactivity to histamine is substantially influenced by environmental factors. This finding may partially explain the geographic differences in the prevalence of positive responses to allergen-specific skin-prick tests. Increased sensitivity to histamine in the skin and in organs other than the skin may also be a key to understanding the differences in the prevalence of atopic diseases (asthma, eczema and allergic rhinitis) reported to exist worldwide.

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