Skin test reactivity and clinical allergen sensitivity in infancy

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We examined the development of skin test reactivity and clinical allergen sensitivity in infancy. Seventy-eight infants of atopic parents were skin prick tested every 4 mo from 4 to 16 mo and an additional 57 of these infants were tested at 20 mo. Wheal diameters were recorded for histamine (1 mg/ml) and specific allergen reactions by use of cow's milk, egg albumen, wheat, and Dermatophagoides pteronyssinus. *The histamine mean wheal diameter was significantly lower at 4 and 8 mo compared to the older infants. Infants at 20 mo also had significantly smaller wheals than adult controls. Histamine reactivity was greater in atopic infants at 4 mo compared to nonatopic infants. Reactions to ingested allergens occurred early in infancy but were usually transient. There was a good correlation between skin sensitivity and clinical immediate-food hypersensitivity to the food concerned. In contrast, reactions to the inhaled allergen,* D. pteronyssinus, *occurred later in infancy, were persistent, and increased in size with age. Although we found no relationship between the acquisition of skin reactivity to* D. pteronyssinus *and development of the respiratory symptoms of atopic disease during the period of the study, it is possible that inhaled allergen reactivity may be related to respiratory symptoms at later ages. Despite the decreased histamine reactivity in early infancy, skin tests proved reliable markers of clinical disease in ingested but not inhalant allergen sensitivity. (J* ALLERGY CLIN IMMUNOL *73:381-386, 1984.)*

Allergen skin prick tests have been demonstrated to correlate well with specific-IgE levels and therefore provide a reliable noninvasive measure of atopy.[1] Statements such as "in children below the age of two, skin tests are unreliable and do not always correlate with specific IgE"[2] or "children, especially less than 3 yr of age, do not predictably exhibit positive skin tests"[3] have questioned the reliability of skin tests during infancy. The factors that may contribute to a positive skin prick test include the presence of specific IgE, mast cell numbers, releasability, and the nonspecific vascular reactivity of the skin.[4] Stevenson et al.[5] found, when skin testing 18 babies with anti-IgE from birth, that all babies had skin test reaction by 39 days. This finding suggested that infants have mast cell–bound IgE by about 1 mo of age. Sulzberger and Baer[6] reported normal whealing response to histamine and codeine sulphate in eight full-term neonates, although the wheal sizes were smaller than in adult controls. Furthermore, positive allergen skin tests have been reported in a newborn infant and confirmed by a Prausnitz-Küstner test reaction.[7] Thus current evidence suggests that infants are potentially capable of producing a positive skin test response to an allergen.

The incidence of positive skin tests in patients with atopic disease is lowest during infancy.[8, 9] Some studies demonstrate a good correlation between allergen–skin test reactivity and specific-IgE levels even in young children,[8, 10] whereas other studies demonstrate variable correlation.[11] The factors that contribute to the lower incidence of positive reactions in infancy may include a reduced whealing capacity of the skin and the delayed acquisition of specific allergen reactivity.

To examine skin tests in infancy, we followed infants of atopic parents from 16 to 20 mo while observing the skin test reactivity to histamine and a number of allergens.

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Abbreviations used
IgE: Immunoglobulin E
w/v: Weight/volume
PNU: Protein nitrogen units
RAST: Radioallergosorbent test

SUBJECTS AND METHODS

Seventy-nine infants born at two major obstetric units participated in the study. The infants were selected to favor atopic individuals. Twenty-six had biparental history of atopic disease, 46 had a uniparental history of atopic disease, and only seven had no parental history of atopic disease. The study was approved by the Ethics Committees of both hospitals, and signed informed consent was obtained from parents with regards to participation in the study. All infants were followed from birth to 16 mo, and 59 were followed to 20 mo. Each infant was observed by one of us (P. V. A.) every 4 mo, and a clinical history, physical examination, and allergen skin tests were obtained. One family declined to have skin tests performed at all ages, and a further family declined at 20 mo.

Skin tests were performed by the skin prick method.[1] The following solutions were used: histamine, 1 mg/ml; control and whole cow's milk, 1:20 w/v in 50% glycerine (Hollister-Stier Laboratories, Spokane, Wash.); egg albumen, 0.5 mg/ml in 0.5% phenol (Commonwealth Serum Laboratories, Melbourne, Australia); wheat, 0.5 mg/ml, each wheat water-soluble proteins; wheat bran proteins and wheat gluten digest (Commonwealth Scientific and Industrial Research Organization, Sydney, Australia); and *D. pteronyssinus*, 1500 PNU/ml in 0.4% phenol and 50% glycerine (Bencard, Middlesex, U.K.). Reactions were read after 10 min, and wheal sizes recorded by use of pen tracings and micropore tape. Wheal sizes for histamine and allergens were measured to the nearest 0.5 mm and expressed as the mean of the maximum and minimum diameters.

Atopic infants were defined as those with a positive skin test reaction (any wheal) to one or more of the allergens. The presence of atopic disease was noted in all infants as follows: atopic dermatitis (on examination), asthma (on history), or allergic rhinitis (on examination). Atopic dermatitis was defined by the presence, on one or more of the four monthly visits, of typical areas of dermatitis that involved particularly flexural folds, face, cheeks, or behind the ears but excluded those with xeroderma alone. Asthma was defined as two or more episodes of wheezing. Allergic rhinitis was defined as the presence, on one or more of the four monthly visits, of swollen pale or bluish nasal turbinates with or without nasal obstruction or nasal discharge. In addition a history of any food reaction for the foods tested was noted in all infants.

Comparisons were performed by use of a Student t test, and 2-tailed t tables, or chi-square test with Yates correction where it was appropriate. A p value of <0.05 was considered significant.

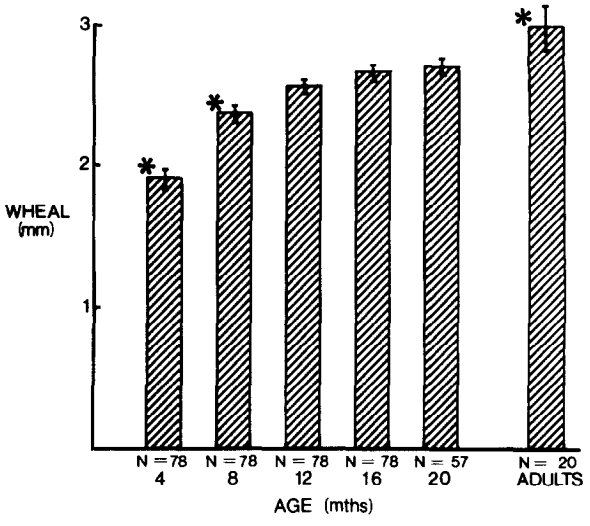


Fig. 1. Histogram of histamine skin reactivity with age. Mean wheal diameter (± SE) in millimeters on vertical axis and age in months on horizontal axis; N is number of patients who were skin tested; $*p < 0.001$ at 4 mo; $p < 0.005$ at 8 mo; $p < 0.05$ for adults.

RESULTS

Histamine reactivity

The mean wheal sizes for histamine at the different ages is illustrated in Fig. 1. The mean histamine wheal size differed significantly at 4 ($p < 0.001$) and 8 mo ($p < 0.005$) from the older age groups but did not significantly alter between 12 and 20 mo. When compared to a group of 20 adult controls, the mean histamine wheal size was significantly less ($p < 0.05$) at 20 mo of age.

The mean wheal sizes for the atopic infants were compared to the nonatopic infants. There was a significantly larger mean wheal size to histamine in the atopic group at 4 mo of age (2.2 mm versus 1.8 mm, $p < 0.005$) but not at any other age. We also compared the wheal sizes of those with atopic dermatitis to wheal sizes of infants without evidence of atopic disease and found a similar result. The mean wheal size was significantly larger (2.1 mm versus 1.8 mm, $p < 0.05$) in those with atopic dermatitis at 4 mo but not in the older age groups.

Allergen reactivity

Nineteen of the 78 infants reacted to at least one of the allergens over the 20 mo period. The reactions were cow's milk (six infants), egg albumen (11 infants), wheat (three infants), and *D. pteronyssinus* (nine infants). Five infants reacted to more than one of these allergens. To assess the usefulness of skin tests in infancy as a marker of atopy, we compared the presence of skin test positivity to one or more al-

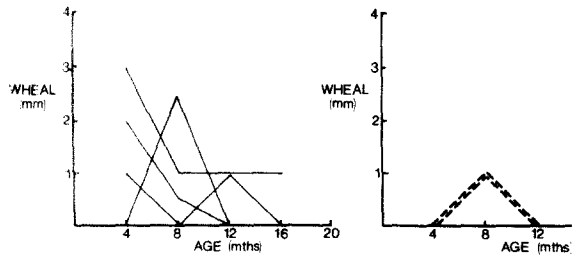


Fig. 2. Wheal size for cow's milk-positive infants with age of symptomatic patients *(unbroken lines)* and of asymptomatic patients *(broken lines)*. Wheal diameter in millimeters on vertical axis and age in months on horizontal axis.

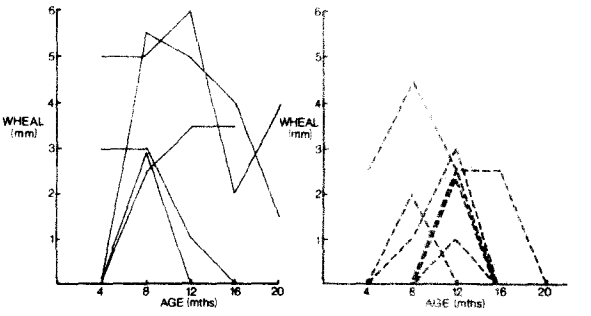


Fig. 3. Wheal size for egg albumen-positive infants with age of symptomatic patients *(unbroken lines)* and of asymptomatic patients *(broken lines)*. Wheal diameter in millimeters on vertical axis and age in months on horizontal axis.

lergens with clinical atopic disease. Skin test positivity was significantly ($p < 0.001$) associated with the presence of atopic dermatitis. Thus 16 (42%) of 38 infants with atopic dermatitis were skin test positive as compared to three (8%) of 40 infants without atopic dermatitis. However, there was no significant association with skin test positivity when comparing infants with or without asthma (36% versus 22%) or allergic rhinitis (32% versus 21%). Overall 17 (90%) of the 19 skin test–positive infants had clinical atopic disease compared to 33 (56%) of the 59 skin test–negative infants, a significant difference ($p < 0.02$).

The wheal sizes of the reactions to whole milk and egg albumen are illustrated in Figs. 2 and 3. Four of the six infants who had a positive skin test to cow's milk gave a positive history of an immediate–type hypersensitivity reaction to cow's milk, whereas two infants were asymptomatic. The mean wheal size in the symptomatic infants was 2.1 mm compared to 1.0 mm in the asymptomatic infants. Four skin test–negative infants gave a history of reaction to cow's milk. In all cases this was a delayed reaction with diarrhea or exacerbation of rash occurring as the symptoms. Five of the 11 infants whose skin reacted to egg albumen gave a history of an immediate reaction to egg, whereas six infants were asymptomatic. The mean wheal size in the symptomatic infants was 4.3 mm compared to 2.7 mm wheal size in the asymptomatic infants. Five skin test–negative infants had a clinical history of reactions with egg. Three infants had delayed reactions (12 to 48 hr), whereas two infants had vomiting immediately after ingesting egg custard but tolerated other forms of egg. None of the patients who reacted to wheat gave a clinical history of wheat sensitivity although two of the three infants were allergic to cow's milk and egg. Four infants still had positive skin tests to foods at the end of the study, and only two of these infants were still intolerant of the food (both egg).

The wheal sizes to *D. pteronyssinus* are illustrated in Fig. 4. Reactions to *D. pteronyssinus* commenced later than reactions to ingested allergens and generally increased in size with increasing age. None became negative. As inhalant allergic reactions are often implicated in atopic disease, we examined the relationship between a positive reaction to *D. pteronyssinus* and atopic disease. A positive skin test to *D. pteronyssinus* was significantly associated ($p < 0.05$) with the presence of atopic dermatitis. Thus eight (21%) of 38 infants with atopic dermatitis were skin test positive to *D. pteronyssinus* as compared to only one (3%) of 40 infants without atopic dermatitis. However, only two of the 25 infants with allergic rhinitis and none of the 22 infants with asthma were skin test positive to *D. pteronyssinus*. There was no association between the acquisition of positive skin test to *D. pteronyssinus* and onset of respiratory symptoms or atopic dermatitis.

DISCUSSION

By use of histamine reactivity as a marker of nonspecific skin sensitivity, we found all infants to wheal in response to histamine, as described previously in neonates,[6] and there is a rise in this reactivity with age. Histamine skin reactivity is significantly less in the 4- and 8-mo-old age groups compared to the skin reactivity in older age groups. In addition there is a significantly smaller wheal size in 20-mo-old infants compared to wheal size in adults. This increase in responsiveness to histamine probably indicates an increase in the vascular reactivity of the skin with age. Since patients with asthma, allergic rhinitis, and atopic dermatitis have evidence of target organ hyperreactivity,[12–14] we wondered whether this may be reflected in the histamine skin response in infants. We therefore compared atopic to nonatopic patients with atopy defined by skin test positivity or the presence of atopic dermatitis. We found an increased his-

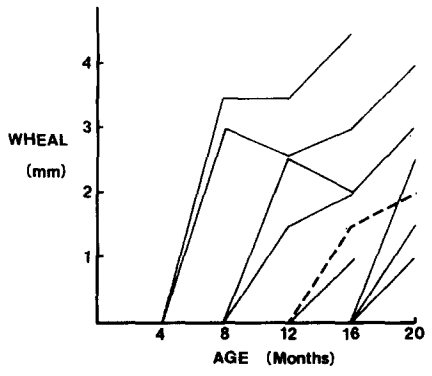


Fig. 4. Wheal size for *D. pteronyssinus*-positive infants with age of infants with evidence of atopic disease *(unbroken lines)* and infants with no atopic disease *(broken lines)*. Wheal diameter in millimeters on vertical axis and age in months on horizontal axis.

tamine reactivity in the atopic groups at 4 mo of age but not at any other age period. This increased nonspecific reactivity in the skin of patients with atopic dermatitis may contribute to its early onset as a symptom of the atopic state.

A question with important ethical and practical implications for this study is whether repeated skin testing may have promoted sensitization. Frick et al.[15] in a prospective study of 13 potentially atopic infants reported that they were precluded from performing serial skin testing by the Human Experimentation Committee, although this theoretical possibility had never been demonstrated. However, another paper from the same institution reported on 94 infants of atopic parents who were skin tested by the scratch technique to 11 allergen preparations every 4 mo.[16] In addition there have been three studies from the United Kingdom[11, 17, 18] in which serial skin tests on infants of atopic parents have been performed. Although specific reference was not made in any of these four studies, presumably the Ethics Committee approval was obtained, which indicated a body of opinion that considers that such studies conform to accepted standards. In addition our study was passed by the Ethics Committee of both hospitals that were concerned. Review of information on prospective studies of potentially atopic infants does not suggest that repeated skin testing can promote sensitization. The presence of IgE positivity by RAST testing of milk in prospective studies in infants of atopic parents was two (15%) of 13 infants,[15] three (8%) of 36 infants,[19] 16 (22%) of 74 infants,[20] and five (10%) of 48 infants.[21] This compares to three (5%) of 58 infants,[11] 15 (36%) of 42 infants,[17] and six (8%) of 78 infants (present study) where serial skin testing has been used. Thus in summarizing the results of these studies, the prevalence of cow's milk sensitization that was observed after serial skin testing (16%) was not different from the 14% prevalence that was observed by RAST testing. Furthermore the prevalence of positive RAST tests to egg allergen in the study of Dannaeus et al.[19] was seven (29%) of the 36 infants, which is in fact higher than 11 (14%) of 78 infants skin test positive to egg in the present study. As skin testing is considered to be somewhat more sensitive than RAST testing,[22] this evidence does not suggest that repeat skin prick testing with foods is promoting increased sensitization. Similar considerations apply to the inhaled allergen, *D. pteronyssinus*. We previously found that five (26%) of 19 infants with asthma less than 2 yr of age were skin test positive to *D. pteronyssinus*,[9] whereas none of the present subjects with asthma reacted to this allergen. Finally, of the nine immediate-food reactions that occurred in seven subjects, five had positive skin tests to the relevant food at the first test, which indicated that repeated skin testing was not responsible for sensitization in these cases.

The pattern of reactivity to the allergens was different for the ingested and inhaled allergens. Skin test reactions to ingested allergens developed in early infancy and were usually transient. The majority of cases reverted to negative by the end of the study. In contrast skin test reactions to *D. pteronyssinus* generally occurred later in infancy and were persistent. The wheal sizes increased with age. Similar patterns have been observed previously. Orgel et al.[8] in a cross-sectional study of 203 selected children with atopic disease found that the 29 children under 12 mo of age showed skin test reactivity more commonly to foods, particularly milk and egg, whereas older children reacted to inhalant antigens. Foucard[10] found IgE antibodies to cow's milk and egg on RAST in nine of 46 infants under 12 mo of age, whereas only one had a positive reaction to *D. pteronyssinus*. Neither study, however, followed skin test response prospectively, although Foucard[10] reported decreasing IgE levels to cow's milk and egg with increasing age. Soothill et al.[11] in a previous prospective study following 58 newborn infants during the first year of life noted, in contrast to our findings, transient reactions to inhalant rather than ingested allergens. However, they did observe an increasing prevalence of reactions to inhaled allergens with age. We chose *D. pteronyssinus* as the inhalant allergen to study as it showed the highest prevalence in asthmatic infants in a previous study[9] and thus is likely to reflect the acquisition of inhalant sensitivity in atopic infants. The difference in the nature of reactions to orally administered versus inhaled allergens suggests that the route or possibly the dose of allergen is important in determining the develop-

ment of sensitivity. A possible explanation may lie with recent animal studies that suggest that the dose of allergen is important in determining enhancement or suppression of the IgE response.[23]

Clearly the best measure of the reliability of an allergen skin test is its correlation with clinical sensitivity to that allergen. This is much easier to measure for ingested allergens since an acute hypersensitivity reaction to a food is obvious and clinically reproducible. We found a good association between clinical immediate-food hypersensitivity reactions and positive allergen skin tests to the food involved. In fact all infants with a convincing immediate–food hypersensitivity reaction to cow's milk or egg were skin test positive to the appropriate allergen. In addition the allergen skin test wheal size reflected the clinical sensitivity. The mean wheal size of the symptomatic patients was larger than the mean wheal size of asymptomatic patients with only one asymptomatic patient having a wheal size over 3 mm. This patient avoided egg until 10 mo of age because of the positive skin test and thus may have had a clinical reaction if exposed to egg earlier in infancy. In the present study it would appear that a wheal size of 3 mm or more differentiates between clinical sensitivity and asymptomatic sensitization for egg, whereas a 2 mm wheal size for cow's milk is a good indicator of clinical sensitivity. The majority of reported reactions to cow's milk and egg in skin test–negative patients were delayed, and it is generally accepted that such reactions are not IgE mediated.[24] The two infants with immediate reactions to egg custard tolerated other forms of egg. Thus it is unlikely that egg was the offending allergen

The relationship between a positive skin test to *D. pteronyssinus* and atopic disease was less clear cut. There was a significant association between atopic dermatitis and a positive skin test to *D. pteronyssinus*. However, a considerable number of infants with atopic dermatitis were skin test negative to *D. pteronyssinus*, and the acquisition of skin test reactivity to *D. pteronyssinus* did not correlate with the onset of atopic dermatitis. Although some investigators have recently suggested a mechanism for *D. pteronyssinus*-induced atopic dermatitis,[25] we believe the positive skin test is simply a marker of the atopic state rather than reflecting a specific pathogenic mechanism. There was no association between asthma or allergic rhinitis and a positive *D. pteronyssinus* skin test reaction. Thus the acquisition of sensitization to inhaled allergens in infancy did not correlate with the acquisition of the respiratory symptoms of the atopic state. Although a correlation between skin tests and bronchial provocation tests to *D. pteronyssinus* has been demonstrated in older subjects,[26] our data suggest that *D. pteronyssinus* is unlikely to be responsible for asthma or allergic rhinitis in infancy. It is possible that this allergen may become more important as a provoking factor with increasing age and that other non–IgE mediated pathogenetic mechanisms predominate during infancy. Continued follow up of these patients may resolve this question.

We found 34% of our infants with clinical atopic disease were skin test positive to the allergens that were used, which was similar to previous studies during infancy.[8–10] This lower rate of occurrence as compared to that in older children reflects the increasing reactivity to inhaled allergens with age. If the acquisition of skin test positivity during infancy merely reflected the increase in histamine skin reactivity in the first 12 mo of life, it would be expected that both ingested and inhaled allergen reactivity would increase in occurrence with age. In contrast our results demonstrated that the incidence of positive skin tests at different stages in infancy reflects the change from a predominance of ingested to inhaled allergen reactivity. Skin test reactivity to ingested allergens was more reliable during infancy in predicting clinical response than reactions to inhaled allergens. In fact the good correlation between skin test reactivity and clinical immediate–food hypersensitivity reactions suggests that skin tests are more reliable as a predictor of clinical response than at a later age, possibly because of subsequent acquisition of subclinical reactivity.

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