
Isolated late cutaneous reactions to allergen skin testing in children

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Background: Occasionally parents report a reaction developing at the site of an allergen skin test several hours after application of the test, despite there having been no immediate reaction. The medical literature contains little information regarding isolated late reactions (ILRs) to allergen skin testing.

Objective: The goal of this project was to determine the incidence of ILRs in children undergoing allergen skin testing.

Methods: Prick and intradermal (ID) skin testing was performed for routine clinical indications in an allergy clinic. Children with a positive histamine control, and at least one negative immediate reaction to allergen skin testing were enrolled in the study. The parents were given detailed instructions to examine the skin test sites 6 hours later, and to record the size of any erythematous indurated sites. Circles of various diameters were included on the report form to assist the parents' size estimates.

Results: Fifty-seven children enrolled in the study and 50 returned the forms. No patients reported ILRs to prick skin tests. Eighteen of the 50 respondents reported 40 ILRs to ID tests, of ≥ 5 mm diameter; 7 of these were ≥ 10 mm. The most common allergen causing ILR was cockroach, accounting for 20% of the ILRs. Each of the other allergens also caused ILRs. The clinical history did not show a definite correlation of symptoms with exposure to the allergens causing ILRs, although all 14 patients with ILRs to indoor allergens had year-round symptoms. There was no correlation between the incidence of ILRs and age, gender, or diagnosis of asthma.

Conclusion: ILRs to allergen skin testing occurred in 36% of pediatric allergy clinic patients. The clinical significance of such reactions is unknown.

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INTRODUCTION

It is not uncommon in a pediatric allergy practice to find negative results to allergen skin tests in children whose symptoms and physical findings are suggestive of respiratory allergy. Occasionally parents report a reaction (induration, erythema) developing at the site of an allergen skin test several hours after application of the test, despite there having been no immediate reaction. The medical literature contains little information regarding isolated late reactions (ILRs) to allergen skin testing. An informal survey of

clinical allergists regarding this phenomenon revealed that most allergists are aware that cutaneous ILRs sometimes occur, but attach no clinical significance to such reactions and do not routinely attempt to identify them. Some allergists do record such reactions, as reported by the patient or parents, on the patient's clinical record and consider them equivalent to an immediate wheal-and-flare reaction, including the causative allergens in environmental control measures and/or immunotherapy extracts.

The goals of this project were to determine the incidence of ILRs in children undergoing allergen skin testing and to assess any relationship between ILRs and the age of the child, diagnosis of asthma, and clinical presentation.

METHODS

Prick and intradermal skin testing were performed for routine clinical indications in an allergy clinic. The allergen extracts were obtained from Bayer Corp, Pharmaceutical Division, Spokane, WA. The extracts used for prick testing included the histamine (positive control), 1 mg/mL, and diluent (negative control). Molds 1 (*Alternaria*, *Hor-mo-dendrum*, *Helminthosporium*, *Peni-cillium*, *Aspergillus fumigatus*, *Aspergillus niger*), molds 2 (*Monilia*, *Mucor*, *Rhi-zopus*), molds 3 (*Fusarium*, *Phoma*, *Pullularia*), cockroach, dog, wheat, peanut, and soybean extracts were used in concentrations of 1:10, wt:vol. Egg, milk, codfish, and pollen extracts were used in concentrations of 1:20, wt:vol. Pollen extracts included trees (ash, beech, birch, box elder, cottonwood, elm, hickory, poplar, red oak, sugar maple, sycamore, walnut, and black willow), grasses (bermuda, johnson, Kentucky blue, orchard, redtop, sweet vernal, and timothy), weeds (cocklebur, kochia, lamb's quarter, marsh elder, pigweed, plantain) and ragweed (giant and short). Dust mite extracts for prick test included *Dermatophagoides farinae* and *D. pteronyssinus*, at 30,000 allergen units/mL. Cat pelt extract was used at 10,000 biological allergen units/mL. Prick testing for the pollens, molds, and dust mites was performed utilizing either the individual allergens or (in most cases) mixes that included all allergens in a given category. When the mixes were used, the concentrations of the individual allergens was decreased proportionally to the number of allergens in the mix, since the final concentration of the mixture was 1:10 wt:vol for the molds, 1:20 wt:vol for the pollens, and 30,000 allergen units/mL for the dust mites.

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Intradermal testing utilized 30 allergen units/mL of dust mite (*Dermatophagoides pteronyssinus* and *D. farinae* mixed, so each was present as 15 allergen units per milliliter), 100 biological allergen units/mL of cat pelt, and 1:1000 concentrations of the three mold mixes, ragweed, grass, tree and weed pollen mixes, dog, and cockroach extracts. Intradermal testing was not performed for food allergens.

Prick testing was performed by lightly pricking the skin with the point of a lancet through a drop of allergen extract. The tests were read 15 minutes after application. A positive histamine control was defined as a wheal of 2 mm diameter or greater, with surrounding erythema. Allergen prick test results were considered positive if a wheal of at least half the size of the histamine control was present, with surrounding erythema. The negative (diluent) control had to show no wheal-and-flare reaction for the prick test results to be considered valid. Allergen extracts that caused no reaction on the prick test were reapplied with intradermal tests in most cases, except for food extracts. (Intradermal tests were omitted in a few cases due to time constraints or the child's intolerance of the procedure.) Intradermal injections were made via a 25-gauge needle, with volume sufficient to raise an approximately 4-mm wheal. Results were read 15 minutes after application. Children under 8 years of age had local anesthesia with prilocaine/lidocaine cream (EMLA, Astra Pharmaceuticals) prior to intradermal testing, which was usually performed on the back. In older children, intradermal testing was usually performed on the forearm without anesthetic cream. Tests were read 15 minutes after application. A positive histamine skin test was defined as a wheal of greater than 4-mm diameter, with surrounding flare. Intradermal allergen skin tests were read as positive if they had a wheal of greater diameter than the diluent control, and at least 4-mm diameter or half the size of the histamine control, whichever was larger. When the topical anesthetic cream was used, in many cases the

intradermal tests would show a wheal but no flare due to the vasoconstrictor effect of the lidocaine and prilocaine. The wheal size alone was used as the criteria for skin test interpretation in these cases.

Both prick and intradermal test sites were re-inspected just before the child left the clinic (usually within 15 minutes of completing the intradermal testing, and about 45 to 60 minutes after the initial reading of the prick tests).

Children with a positive histamine control, and at least one negative immediate reaction to allergen skin testing were enrolled in the study. The study was approved by the institutional review board and informed consent was obtained from all subjects.

After the immediate reactions were read, the initials labeling each skin test site were left on the child's skin. The parents were given a form listing the allergens in the order they were applied. They were asked to examine the prick and intradermal skin test sites 6 hours later, and to record the size of any erythematous indurated lesions noted at sites which had not shown an immediate reaction. Circles of various diameters were included on the form to assist the parents' size estimates. The parents then mailed the form back to the clinic.

An ILR was defined as an area of induration and erythema of at least 5-mm diameter, present 6 hours after skin testing at a skin test site that had not shown an immediate hypersensitivity reaction.

The patients' ages, gender, and diagnosis were noted. The clinical history, obtained prior to allergen skin testing, was assessed for evidence that exposure to the allergens causing ILRs was associated with increased symptoms.

Statistical analysis

The Epistat program was used for correlation analysis (2 x 2 tables for retrospective data) to check for correlation between the occurrence of ILRs and patients' age, gender, and diagnosis of asthma. Specifically, female gender, diagnosis of asthma, age less than 8 years and age less than 6 years were

assessed as potential risk factors for development of ILRs.

RESULTS

Fifty-seven children enrolled in the study. Of these, 50 returned the forms. The children ranged in age from 1.25 to 13.5 years; 35 were boys. In all cases, the positive allergen skin test sites were fading or completely resolved by the time the patient completed their clinic visit; no new onset of skin reactions was observed at this time.

No patient reported ILRs to prick skin tests. Eighteen of the 50 respondents (36%) reported 40 ILRs of ≥ 5 mm diameter to the intradermal tests; 7 of these were ≥ 10 mm.

The most common allergen extract causing ILR was cockroach, accounting for 20% of the ILRs and four of the seven largest ILRs. Each of the other allergen extracts also caused ILRs. One parent recorded a 5-mm ILR to the diluent control. Table 1 shows the relative frequency of ILRs to each type of extract, versus the number of initially negative skin test sites that were checked for reactions.

All 14 patients with ILRs to extracts of indoor allergens had year-round symptoms. Of the four subjects with ILRs only to pollen extracts, none gave a history of symptom exacerbations during the pollen season.

There was no correlation between the incidence of ILRs and age less than 8 years, gender, or diagnosis of asthma (Table 2). Since anesthetic cream was used in children under 8 years of age, there was no indication that it influenced the incidence of ILRs. The results for subjects less than versus older than 6 years were similar to those for less than or greater than 8 years (not shown in the table). We were unable to assess the incidence of ILRs in very young children, as only two of our subjects were 2 years old or younger. Neither of these subjects had ILRs. Only one of the subjects had atopic dermatitis in addition to respiratory allergy. This patient did not have any ILRs.

DISCUSSION

This study demonstrates that isolated late reactions to allergen skin testing are a surprisingly common phenomenon. Although such reactions were recognized in the 1920s and 1930s, and were briefly noted in W T Vaughan's 1939 allergy text,¹ the topic has not received much attention in the medical literature. The pathogenesis and clinical significance of isolated late reactions are unknown, although dual phase reactions (ie, the occurrence of a late-phase reaction following an immediate hypersensitivity reaction) have been well described.

In dual-phase skin reactions, the immediate wheal-and-flare reaction develops within minutes after challenge, peaks at about 15 minutes, and resolves within 30 to 60 minutes. The late phase reaction begins to appear at 3 to 4 hours and peaks at 6 to 8 hours, resolving by 24 to 48 hours.² Frew and Kay demonstrated that the late phase reaction is dose-dependent and can be induced in all skin test-positive subjects if a high enough dose is injected. Continuous observation of skin test sites developing late phase reactions revealed that the immediate reactions persisted at the same size or increased steadily in size by 6 hours after-skin test, rather than fading and reappearing.³ In the current study, parents were not asked to examine the skin test sites until 6 hours post-testing, so we are unable to determine when the ILRs first began to appear.

Histologic studies of the dual phase cutaneous reaction reveal that the immediate reaction is characterized by vasodilation and edema of the papillary dermis, with a perivascular infiltrate of neutrophils and eosinophils.⁴ The late phase reaction is characterized by a mixed cellular infiltrate of lymphocytes, monocytes, eosinophils, neutrophils, and basophils.⁵ Edema and sometimes fibrin deposition are noted.⁶ There is no evidence of immunoglobulin or complement deposition. Biopsies of skin test sites 6 hours following an immediate hypersensitivity reaction, even in the absence of a visible

Table 1. Number of ILRs Versus Number of Initially Negative Skin Test Sites Observed for ILRs for Each Type of Extract

Skin Test Reagent	Prick Tests: ILR/No. of Sites	Intradermal Tests: ILR/No. of Sites (%)
Histamine	0/50	0/50 (0%)
Diluent	0/50	1/50 (2%)
Molds 1	0/43	3/43 (7.0%)
Molds 2	0/45	2/44 (4.5%)
Molds 3	0/42	1/42 (2.4%)
Ragweed	0/44	4/36 (11.1%)
Grass	0/45	4/38 (10.5%)
Trees	0/43	5/41 (12.2%)
Weeds	0/43	5/38 (13.2%)
Dust mite mix	0/39	3/37 (8.1%)
Cockroach	0/43	8/42 (19.0%)
Cat	0/44	4/33 (12.1%)
Dog	0/49	1/34 (2.9%)
Milk	0/9	
Egg	0/6	
Wheat	0/7	
Fish	0/7	
Peanut	0/5	
Soy	0/6	

Table 2. Potential Risk Factors Versus Occurrence of ILR

Risk Factor	ILR +/- Risk Factor + (%)	ILR +/- Risk Factor - (%)	2-Tailed P	Odds Ratio (95% CI)
Age ≤ 8 yr	11/32 (34%)	7/18 (39%)	0.76	1.13 (0.54–2.40)
Female gender	8/15 (53%)	10/35 (28%)	0.12	2.86 (0.69–12.11)
Diagnosis of asthma	10/26 (38%)	6/23 (26%)	0.38	1.77 (0.45–7.34)

late phase reaction, show similar changes.⁷ It has been shown that immediate and late phase reactions can be induced by injection of anti-IgE antibody,⁸ as well as by 48/40, the mast cell secretagogue compound,⁵ implying that mast cell degranulation initiates the complex sequence of immunologic events resulting in the dual phase reaction. The potential roles of various mast cell-derived mediators in the pathogenesis of the LPR have been studied. Injected histamine does not cause LPRs and antihistamines do not block the development of the LPR.⁹ Other mast cell-generated mediators, such as leukotrienes C₄, B₄, and D₄, prostaglandin D₂¹⁰ and/or platelet acti-

vating factor,¹¹ can be detected in the skin during the time frame of LPR development and may be involved in the pathogenesis of the reaction.

Isolated late phase reactions develop within the same time frame and have a similar clinical appearance to the late phase of the dual cutaneous reaction. However, preceding the ILR there are no signs of mast cell degranulation such as pruritus or immediate wheal-and-flare formation. No previous studies have systematically documented cutaneous ILRs and a review of the medical literature reveals no histologic studies of this phenomenon. Green and colleagues¹² studied delayed reactions to allergen skin testing 48 hours fol-

lowing application of the skin test. They found that 5% of all skin tests had isolated delayed reactions at 48 hours, whereas 13% of skin test sites with positive immediate wheal-and-flare reactions showed delayed reactions at 48 hours. Biopsies of the isolated delayed reactions showed an infiltrate of lymphocytes and histiocytes indistinguishable from those of the tuberculin controls; thus, these delayed reactions to allergen injection appeared to be type 4 hypersensitivity reactions. Unfortunately, no data is given regarding the time course of the evolution of these delayed reactions. Typically, delayed hypersensitivity reactions are said to first be visible at about 12 hours after the skin test,⁷ which would argue against the possibility that the reactions noted at 6 hours post-skin test in the current study represent an early stage in the evolution of such reactions.

Another possible immunopathologic mechanism for the development of the ILR is an Arthus or type 3 hypersensitivity reaction. Arthus reactions develop 4 to 10 hours after injection of an antigen into sensitized skin. They are characterized by perivascular deposition of immune complexes, which activate complement via the classical pathway. The activated complement fragments C3a and C5a attract an intense infiltration of neutrophils, which often result in local tissue necrosis.¹³ Arthus reactions are thought to be uncommon in humans, although they have been reported in some diabetics with late phase reactions to insulin.¹⁴ The ILRs noted in this study were not intensely inflamed, and no instances of necrosis have been reported following allergen skin testing. It is possible, however, that the ILRs represent a mild form of Arthus-type inflammation. Biopsy studies will be necessary to shed further light on the immunopathology of cutaneous ILRs.

Isolated late reactions to allergen have been described in the airway. These reactions are characterized by a mixed inflammatory cell infiltrate of eosinophils, lymphocytes, and neutrophils in the bronchoalveolar lavage

fluid (BAL).¹⁵ Lam and colleagues studied serial BAL cell counts, total protein and albumin following bronchial challenge with plicatic acid in subjects with proven red cedar asthma. Twenty-one of the 44 subjects developed isolated late reactions; the remaining subjects had dual asthmatic reactions. No significant difference was found in the cellular or protein changes following challenge in patients manifesting isolated versus dual asthmatic reactions.¹⁶ These findings would imply that in the airway, the ILR is physiologically similar to the better-recognized dual phase reaction. Interestingly, most of the studies reporting isolated late asthmatic reactions have involved occupational asthma antigens, such as plicatic acid or isocyanates, in contrast to pollen or dust mite allergens. This would imply that ILRs are more readily triggered by certain kinds of antigens, possibly through a different mechanism than the classic IgE mast cell-mediated bronchial reaction. Most bronchial challenge studies are designed in such a way that only subjects with positive immediate reactions are included; thus, it is possible that isolated late asthmatic reactions to allergen occur more frequently than recognized. The same situation holds true for most studies of late phase cutaneous reactions; only subjects with positive immediate reactions are enrolled, so little data is available regarding ILRs.

The clinical data available from the present study do not show a convincing correlation between exposure to the allergens causing ILRs and increased clinical symptoms, although most of the implicated allergens were indoor allergens and each of the affected patients had symptoms throughout the year. The four patients with ILRs only to pollen allergens did not report a seasonal increase in symptoms. Nasal or bronchial challenge with allergens causing ILRs would be helpful in elucidating the clinical relevance, if any, of these reactions.

None of the subjects in this study reported ILRs at prick test sites, in contrast to the results of Umemoto and

colleagues¹⁷ who found an equal frequency of dual phase reactions to prick and intradermal tests. This difference in results could reflect a difference in the mechanism between the dual phase reaction and the ILR, or could be related to diminished skin reactivity to prick tests in children.

The accuracy of the results obtained in this survey could be affected by inaccuracies in the parents' observations. Although the parents were carefully instructed in the correct reading of the late reactions and were given a key for estimating the diameter of the reactions, some degree of reporting error is likely. The report of a 5-mm late reaction to the diluent control, for example, probably represents either erroneous identification of the skin test site or an overreading of the reaction. It is also possible that the requirement for the parents to mail the late reaction report back to the clinic selected in favor of subjects with positive late reactions, who would be more interested in reporting their positive findings. Even if the seven subjects who failed to return their reports are counted as negative, however, 32% of the subjects had at least one ILR.

The occurrence of ILRs following allergen skin testing is a frequent event in pediatric patients, which has not been thoroughly studied. Further investigation is needed to evaluate the clinical relevance of cutaneous ILRs.

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