

Apparent cross-reactivity of airborne molds and the dermatophytic fungi

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Forty-one asthmatic children, 10 to 16.5 years of age, were studied for evidence of infection with dermatophytic fungi. No evidence of past or current infection could be obtained, yet 16, or 39 per cent, showed an immediate allergic reaction to a galactomannan peptide (trichophytin) extracted from Trichophyton mentagrophytes. Thirteen of these 16 children (81 per cent) also had immediate allergic reactions to antigens extracted from nonpathogenic, airborne molds. The antibody, presumably IgE, responsible for type I allergic reactions to airborne molds, apparently cross-reacts with antigens from the fungi pathogenic for human skin.

The dermatophytic fungi are pathogenic only for the superficial skin and do not invade the deeper tissues or internal organs. Infections caused by the dermatophytes (members of the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*) are found throughout the world. Infected humans regularly become hypersensitive to antigens extractable from dermatophytes.¹⁻³ The immunologically active material (trichophytin) is a nitrogen-containing polysaccharide,¹ which Cruickshank and his co-workers³⁻⁶ purified and demonstrated to be a galactomannan peptide of approximately 40,000 molecular weight. Basarab, How, and Cruickshank⁷ showed that the glycopeptides from different dermatophytic species are chemically similar and that they cross-react.

Airborne molds are nonpathogenic for human skin, but they are important as extrinsic inhalant allergens in patients with respiratory atopy. The distribution of these organisms varies with temperature, humidity, and air currents, but they are truly ubiquitous.^{8,9} This study focuses on the possible cross-reactivity between purified trichophytin and airborne mold allergens.

METHODS AND MATERIALS

Forty-one asthmatic children, 13 girls and 28 boys, ranging in age from 10 to 16.5 years, were the subjects of this study. All children were living at the Childrens Asthma Research Institute and Hospital (CARIH), Denver, Colorado. As part of the routine evaluation, each child had been skin tested with a battery of 16 airborne mold or fungal antigens (Table I). The basic extracts were obtained from Greer Laboratories, Lenoir, North Carolina, and were dialyzed,

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diluted, and glycerinated for scratch testing. Standard intradermal tests were made with 0.02 c.c. of a 500 protein nitrogen units per cubic centimeter concentration, or using the same concentration via the Sterngun technique. All medications, including steroids, were stopped 24 hours before skin testing. Those children showing at least a 5×5 mm. or greater wheal-and-flare reaction, with or without accompanying itching and pseudopod formation at the 15 minute reading, were classified as positive to that allergen.

The children were evaluated for evidence of past or present dermatophytosis. Specifically, they were questioned for a history suggestive of tinea capitis, tinea corporis, tinea pedis, and tinea cruris. Each child was examined for evidence of current infection. Skin scrapings were taken from all interdigital spaces, the soles and margins of both feet by scraping with a No. 15 Bard-Parker blade, and the scales were inoculated onto plates containing dermatophyte test media (DTM). The mycologic cultures were placed in an incubator at 35° C. for 10 days and identified after the criteria of Ajello and associates¹⁰ and Rebell and Taplin¹¹ for the identification of dermatophytic fungi.

Most of the children were on sympathomimetics or antihistamines or both, and 23 of the 41 were on alternate-day prednisone ranging from 5 mg. to 85 mg. per dose. All systemic medications were stopped 24 hours before skin testing intradermally on the volar forearm with 0.05 c.c. saline containing $1.2 \mu\text{g}$ of Cruickshank's purified trichophytin. The results of the skin tests were read at 20 minutes, 40 hours, and 7 days. The 20 minute reading was classified as positive if the wheal and flare were both greater than 8 mm. in diameter. At 40 hours and 7 days the size of the reaction was measured across the test site.

RESULTS

The history and physical examination produced no evidence of clinical dermatophytosis in any subject. The results of mycologic cultures indicated that no dermatophytic fungi were present. In the vast majority of cases, i.e., > 85 per cent, an *Alternaria* sp. was recovered from culture of the normal foot skin.

Twenty-four of 41 (59 per cent) of the children had at least one positive immediate skin test to the battery of airborne mold allergens. For those 17 children showing no positive airborne mold tests, only 3 (18 per cent) showed a reaction to trichophytin. Conversely, 13 of 24 (54 per cent) positive to the airborne molds were also positive to trichophytin. The interrelationships between immediate skin test reactions to the airborne molds and trichophytin are shown in Table I.

Overall, there was no consistent association between immediate reactions to trichophytin and positive tests to any single or group of mold allergens. There was an association between multiple positive airborne mold tests and reactions to trichophytin (Table I). Only one of 7 children with a single positive mold test was trichophytin-positive, whereas 5 of 9 with 2 to 3 positive mold tests and 7 of 8 with 4 or more positive mold tests were trichophytin-positive. The average size of the reaction was 14 mm. in those with ≥ 4 positive mold tests, 10 mm. in those with 2 or 3 positive mold tests, 4.4 mm. in those with one positive test, and 3.6 mm. in the 17 subjects not reacting to the molds.

Table 1. Relationship between immediate skin test responses to airborne mold allergens

Subject	Tricho- phytin	Total (+) mold allergens	<i>Alternaria</i>	<i>Helmintho- sporum</i>	<i>Neurospora</i>	<i>Rhinocladiella</i>	<i>Cepi spo</i>
1	+	8	+	+	+		
2	+	6		+	+	+	
3	+	6	+	+	+	+	
4	+	6	+	+			
5	-	6	+	+	+		
6	+	6	+	+	+	+	
7	+	5			+	+	
8	+	4	+	+		+	
9	+	3	+	+	+		
10	+	3	+	+			
11	-	3			+	+	
12	-	2				+	
13	+	2	+		+		
14	+	2	+	+			
15	+	2	+			+	
16	-	2	+				
17	-	2	+	+			
18	+	1	+				
19	-	1		+			
20	-	1		+			
21	-	1	+				
22	-	1	+				
23	-	1			+		
24	-	1				+	
25-41 (17)	3	0	0	0	0	0	
Totals							
41	16	69	16	13	10	9	

None of the 41 children manifested a definitely positive delayed hypersensitivity reaction to trichophytin. Four of the 41 children had some minimal erythema at the 40 hour reading, which appeared from questioning to be a residual Arthus type response, but skin test readings were not made by the authors 3 to 12 hours after the injections. From previous experience with trichophytin, we know that delayed-type hypersensitivity reactions do not fade between the second and seventh days, but usually increase in size the first 3 to 5 days and persist 7 to 10 days or longer.¹² In these atopic children there was no erythema at the skin test site on the seventh day, indicating the absence of delayed sensitivity to trichophytin.

DISCUSSION

What is the evidence that these children had ever been infected with dermatophytic fungi? The clinical history, physical examination, and mycologic culture findings indicate that these 41 children were currently free of infection and in all probability had never been infected. The clinical manifestations of tinea capitis and corporis, the types of infection most common in children, are usually so dramatic that it seems unlikely that all of the 16 children who reacted to trichophytin would have forgotten a prior infection.

While contact with the etiologic agents of dermatophytosis is certainly possible, these children had most likely not become infected because they had not been exposed to the proper conditions for an adequate period of time. Several investigators have shown that delayed sensitivity to trichophytin is a good cor-

trichophytin

<i>Fusarium</i>	3- grain smuts	<i>Penicillium</i>	<i>Aureobasidium</i>	<i>Candida</i>	<i>Chaetomium</i>	<i>Mucor</i>	<i>Aspergillus</i>	<i>Rhizopus</i>
+	++		+					
+		+			+			
+			+			+		
				+	+		+	
	++	+				+		
+				+				
		+						
			+					
				+				
0	0	0	0	0	0	0	0	0
4	4	3	3	3	3	2	1	0

relate of past or present infection^{1-3, 12}; thus, in these children the absence of delayed sensitivity is further evidence that they had never been infected with dermatophytic fungi.

In surveying a group of adult male prison inmates for dermatophytosis, two of us (H. E. J. and M. G. R.) found that 14 per cent manifested immediate skin test reactions to trichophytin.¹² The majority of the prisoners with immediate trichophytin reactions were chronically infected with dermatophytic fungi. It is interesting then that 39 per cent of these asthmatic children manifested an immediate skin test reaction to trichophytin. The possibility exists that the reactions to trichophytin represent cross-reactions from sensitivity to airborne molds. It is certain that the children had encountered these extrinsic inhalant allergens because members of the airborne mold group (*Alternaria* sp.) were recovered from the normal skin of more than 85 per cent of these children. Ignoring the fact that the children had in all probability never been infected with dermatophytic fungi, there was a correlation between positive tests to trichophytin and the airborne molds. Fifty-four per cent of those positive to mold skin tests were positive to trichophytin, whereas only 18 per cent having negative mold skin tests reacted to trichophytin. Furthermore, 81 per cent of those who were positive to trichophytin were also positive to mold allergens.

Other investigators have noted a serologic cross-reactivity between certain mold allergens and the dermatophytic fungi.¹³⁻¹⁵ Serologic studies would necessarily have detected antibody of the IgG, M, or A class, and it does not appear that cross-reactivity between these two groups of fungi, as reflected by the

immediate wheal-and-flare skin test responses, has been reported. The immediate skin test cross-reactivity of allergens extractable from several of the airborne molds had been firmly established.⁹ This paper presents evidence that there is a similar cross-reactivity between immediate reacting antibody raised by the non-pathogenic airborne molds and trichophytin extracted from infectious dermatophytic fungi.

Cross-reactivity of allergens must be considered from at least two basic aspects. First, within a group of chemical-related compounds, the more molecular and chemical similarities that any of the members possess the greater is the likelihood that such compounds will cross-react in an individual allergic to any member of the group. Little is known of the chemical nature of the allergens extractable from the airborne molds. The allergens of *Aspergillus niger*, *Botrytis cinerea*, and *Penicillium frequentans* each appear in a fraction of molecular weight > 40,000.¹⁶ We previously mentioned that the trichophytins are galactomannan peptides of approximately 40,000 molecular weight. Until these antigens are further characterized, it is impossible to study their allergic cross-reactivity and taxonomic relationship on an immunochemical basis.

Secondly, it would seem that cross-reactivity of any group of compounds may also depend on the capacity of the individual to become allergic to that family of allergens. Individuals who exhibit cross-reactivity to several members of a family of compounds could conceivably form antibody specific for a particular antigen, which in addition possesses unusually broad group specificity. Alternatively, the antibody could be specific for the sensitizing antigen, and the cross-reacting antibody could have arisen from what would have been to other individuals a suboptimal stimulus from a related allergen. The frequency of immediate allergic reactions to all extrinsic inhalants, including airborne mold extracts, is much greater in atopic than in nonatopic individuals. Also, there are subgroups of atopics that react more frequently to certain groups of antigens (e.g., airborne molds). In this study, the children who had multiple positive mold allergen tests more frequently reacted to trichophytin. This we take as evidence that the cross-reactivity of dermatophytic fungi and airborne molds is dependent not only on the antigenic relationship of the organisms but also upon the capacity of the individual to respond to that class of antigens.

The biologic importance of the hyperresponse of certain atopic individuals to the airborne molds and the resulting cross-reactivity with antigens of infectious dermatophytes is currently being studied.

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