

Atopic Dermatitis, Patch Testing, and House Dust Mites: A Brief Review

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The association between house dust mites (HDMs) and atopic dermatitis (AD) has long been a contested issue in both dermatology and allergy/immunology. As AD continues to burden millions of individuals and even whole societies, the commercial availability of a HDM allergen mix for epicutaneous testing has revived efforts to identify a definitive link between HDMs and AD. Conclusive evidence of a role for HDMs in the pathogenesis of AD would justify patch testing with mite allergens and would further the use of allergen-specific immunotherapy in the management of such patients; an absence of involvement would refocus our investigative efforts on other conspirators. We present a thorough review of the dissenting scientific evidence and expert opinions on this controversial topic. The association of HDMs with AD has remained elusive, and additional investigation is necessary to resolve this vexing riddle.

THE TERM “ATOPY” refers to a state of susceptibility to hay fever, asthma, and atopic dermatitis. “Atopic dermatitis” (AD) specifically represents a dermatosis particularly common among children. Epidemiologic data indicate that the prevalence of AD in developed countries has increased substantially over the last 40 years and in some societies may now be as high as 17%.^{1,2} While predominantly a disease of childhood, the condition may persist in up to 60% of adults, and late-onset disease, while unusual, has been reported.³ Accordingly, the costs to society brought about by this affliction continue to worsen. Recently, Ellis and colleagues estimated the annual cost of AD in the United States at \$0.9 to \$3.8 billion.⁴

Given this socioeconomic burden, much research has been conducted to elucidate the etiology and pathophysiology surrounding this condition. The house dust mite (HDM), a ubiquitous organism, has been implicated in the etiology and exacerbation of the disease. With the recent commercial availability of a HDM-derived allergen mix for epicutaneous testing, controversy has arisen regarding the utility of this testing modality. Moreover, a systematic review of two large double-blind placebo-controlled trials on HDM avoidance in adults with asthma revealed no

clinical improvement, raising the question of whether such is true for AD as well.⁵ Therefore, it is useful to review the evidence implicating HDMs in AD and the potential role of patch tests with HDM allergens in managing these patients.

AD and Immunity

AD is a chronic and recurrent skin disorder characterized histopathologically by an inflammatory T-cell infiltrate,⁶ which suggests a role for these cells in its pathogenesis. Patch testing has demonstrated that a specific sequential activation of T helper (Th) cells occurs in AD.^{7,8} Within 24 hours of allergen exposure, interleukin-4 (a Th2 cytokine) messenger ribonucleic acid and protein levels in AD skin increase and then progressively decline to background levels over the next 24 hours. During this decline in Th2 cytokine production, the initially undetectable expression of interferon- γ (a Th1 cytokine) is markedly up-regulated. On the basis of this model, AD is believed to exhibit a biphasic immune pattern wherein a Th2 profile predominates at onset and during acute exacerbations whereas a Th1 profile prevails during chronic stages of the disease.^{9,10}

Recently, the focus of research has shifted to the role of antigen-presenting cells (APCs), particularly dendritic cells (DCs) and skin-resident Langerhans' cells (LCs), in the pathogenesis of AD. LCs and DCs play a pivotal role in immunity by virtue of an ability to induce primary immune responses through the presentation of antigens to, and activation of, naïve T cells.¹¹ Studies have demon-

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Reprints not available.

DOI 10.2310/6620.2008.07068

strated an up-regulation of the high-affinity immunoglobulin E receptor (FcεRI) upon LCs and DCs present in lesional atopic skin but not among the same cells in nonlesional atopic skin or nonatopic skin.^{12–14} This increased expression correlates with high serum immunoglobulin E (IgE) levels.¹³ More importantly, it has been demonstrated that LCs and DCs present allergens to T cells 100- to 1,000-fold more effectively if the allergen is targeted to FcεRI on these cells by allergen-specific IgE.¹⁵ These phenotypic and functional characteristics equip LCs and DCs in AD with a markedly enhanced ability to initiate T-cell-mediated immune responses against allergens.

HDMs and Their Mechanistic Role in AD

The European HDM, *Dermatophagoides pteronyssinus* (Der p), and the American HDM, *Dermatophagoides farinae* (Der f allergen) (Fig 1), are the most common mites that thrive in household dust,¹⁶ one of the major environmental aeroallergens to which AD patients are hypersensitive. According to the official list of allergens maintained and updated (March 7, 2007) by the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies, 14 Der p and 14 Der f allergen groups have been identified thus far (Table 1).¹⁷ Of these allergens, group 1 (Der p 1 and Der f 1) and group 2 (Der p 2 and Der f 2) are most allergenic^{18,19}; specifically, they bind IgE in 80 to 100% of tested sera

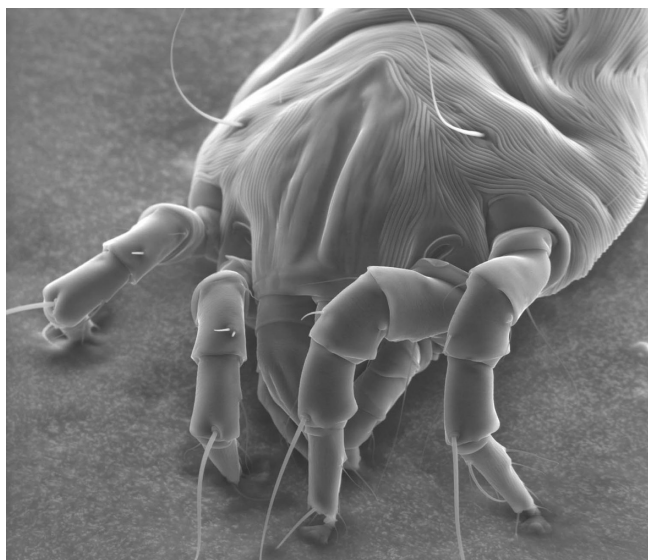


Figure 1. Scanning electron micrograph of a *Dermatophagoides farinae* mite. (×1300 original magnification).

Table 1. *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* Allergens

Group Allergen	Biochemical Name	
	Der p	Der f
1	Cysteine protease	Cysteine protease
2	NPC2 family	NPC2 family
3	Trypsin	Trypsin
4	Alpha amylase	NI
5	Unknown*	NI
6	Chymotrypsin	Chymotrypsin
7	Unknown*	Unknown*
8	Glutathione-S transferase	NI
9	Collagenolytic serine protease	NI
10	Tropomyosin	Tropomyosin
11	Paramyosin	Paramyosin
12	NI	NI
13	NI	Fatty acid-binding protein
14	Apolipoprotein	Apolipoprotein
15	NI	Chitinase
16	NI	Gelsolin/villin
17	NI	Calcium-binding protein
18	NI	Chitinase
19	NI	NI
20	Arginine kinase	NI
21	Unknown*	NI
22	NI	Unknown*

Adapted from Thomas WR, Smith W.¹⁶

Der f = *Dermatophagoides farinae* allergens; Der p = *Dermatophagoides pteronyssinus* allergens; NI = not identified; NPC2 = Niemann-Pick type C2.

* Produced by the mite, but exact nature and function have yet to be determined.

obtained from patients allergic to HDM.²⁰ Moreover, several studies have consistently shown a high rate of positivity for specific IgE antibodies to group 1 and 2 allergens in HDM-sensitized patients.^{21–25}

The group 1 allergens Der p 1²⁶ and Der f 1²⁷ are 25 kD cysteine proteases that have 30% sequence homology with papain.²⁸ They have an amino acid sequence homology of 81%²⁷ and incite both cross-reacting and species-specific antibodies.²⁹ The group 1 cysteine proteases are believed to be secreted into the mite's gut,³⁰ probably as digestive enzymes, and accumulate in mite cultures mostly in association with fecal balls (the remainder is associated with whole mites and cuticles).³¹ Fecal particles from mite cultures have been found to contain as much as 10 mg/mL of Der p 1.³¹ It has been suggested that as little as 2 µg of

group 1 allergens per gram of dust is needed to sensitize an individual to HDMs.³²

Der p 2³³ and Der f 2,³⁴ on the other hand, are 14kD proteins that have a very high sequence homology (88%)³⁴ with each other, accounting for the almost completely cross-reactive antibody responses they elicit.³⁵ In the past, the group 2 allergens were believed to be lysozymes,³⁶ but this hypothesis has been disproven.³⁷ Although the exact nature and function of these proteins has remained elusive, there is mounting evidence that the group 2 allergens may be involved in the recognition and binding of lipids.³⁸ Der p 2 and Der f 2 are currently classified under the Niemann-Pick type C2 (NPC2) family of proteins (see Table 1).¹⁷ NPC2, a distant homologue of the group 2 allergens,³⁹ is a soluble lysosomal glycoprotein that binds cholesterol.⁴⁰ Furthermore, recent studies have shown that the group 2 allergens have structural characteristics that are very similar to those of MD-2,^{39,41} a protein that recognizes bacterial lipopolysaccharides (by forming a complex with Toll-like receptor 4 and CD14 on the cell surface) and initiates an immune response against them.⁴²

Although definite causality has yet to be determined, it has been alleged that HDMs play a role in the immunopathogenesis of AD. Not only do AD patients have elevated levels of serum IgE antibodies specific to HDM allergens, biopsy specimens of AD lesional skin have also been shown to be infiltrated with T lymphocytes that recognize Der p.⁴³ Through cysteine protease activity, group 1 allergens mediate several mechanisms by which HDMs might promote AD. For example, it has been shown that Der f 1 may facilitate its entry into AD skin by enzymatically breaking down the epidermal barrier⁴⁴ in much the same way that Der p 1 is able to increase the permeability of bronchial epithelium to serum albumin *in vitro*.⁴⁵ This protease-mediated epithelial disruption, coupled with a genetically determined barrier defect recently found in AD patients,⁴⁶ provides for the easy entry of various allergens that may promote and perpetuate the disease.

Another proposed mechanism by which HDMs may promote AD is through their potent immunomodulatory effects. Mite allergens are able to activate keratinocytes and induce them to produce and secrete proinflammatory cytokines.⁴⁷ Moreover, Der p 1 is able to polarize immunity toward a Th2 type by (1) cleaving the low-affinity IgE receptor (CD23) on B cells, resulting in up-regulation of IgE synthesis⁴⁸; (2) cleaving the alpha subunit (CD25) of the interleukin (IL)-2 receptor on T cells, causing inhibition of T-cell proliferation and reduced production of interferon (IFN)- γ , a Th1 cytokine⁴⁹; and

(3) cleaving CD40 on DCs, leading to decreased production of IL-12, another Th1 cytokine.⁵⁰ Recently, it was demonstrated that Der p 1 also has the ability to cleave DC-SIGN and DC-SIGNR, C type lectin receptors expressed by DCs that interact with T cells to promote Th1 immunity.⁵¹ Cleavage of these receptors abrogates Th1 immune responses, giving way to the uninhibited propagation of Th2 immunity.⁵¹

Additionally, it is believed that delayed-type hypersensitivity (DTH) reactions characteristic of eczematous AD skin lesions may result from potentiated ability of LCs and DCs to process allergens. IgE-bearing LCs, as opposed to those lacking surface IgE, present HDM allergens to T cells with greater efficiency.⁵² Also, DCs of atopic patients that are sensitized to HDMs express increased levels of mannose receptors, compared to those of nonatopic subjects, and more efficiently capture Der p 1 by a mannose receptor-dependent mechanism.⁵³ The independent role that LCs and DCs play in the pathogenesis of AD is supported by the fact that not all AD patients with elevated serum mite-specific IgE antibodies (an indicator of immediate-type hypersensitivity) show a positive response to patch testing (a model for DTH) with mite allergens.^{54,55}

Clinical Evidence for the Association of HDMs with AD and for the Utility of Patch Testing

Although the role of HDM allergens in asthma and allergic rhinitis has been studied and largely accepted by those in the field, the involvement of these same allergens in the pathophysiology of AD remains a matter of debate (Table 2). Exacerbation of AD by HDMs was first proposed by Tuft in 1949.⁵⁶ Careful environmental analysis in Scotland has demonstrated increased exposure to HDMs among atopic adults,⁵⁷ and several authors have described dramatic clinical improvement when mite elimination and avoidance strategies were employed.^{58–61} In 1982, Mitchell and colleagues showed that purified Der p 1 could induce eczematous lesions when placed on the skin of atopic individuals allergic to HDMs.⁶² More recently, Tupker and colleagues demonstrated the worsening and/or development of new plaques of dermatitis in atopic patients exposed to intrabronchial HDM allergens.⁶³

Nevertheless, there are significant barriers to the acceptance of a major role for HDMs in AD. For instance, the study by Mitchell and colleagues is flawed in that they abraded skin prior to the application of Der p 1, and biopsy specimens of the lesions contained significant amounts of neutrophils, a finding that is not typical of

Table 2. Summary of Evidence **for** and **against** an Association between House Dust Mites and Atopic Dermatitis

<i>Evidence for Association</i>	<i>Evidence against Association</i>
• High rate of positivity for HDM-specific IgE in AD patients • T lymphocytes that specifically recognize HDM allergens found in AD skin biopsy specimens • Clinical improvement with HDM elimination and avoidance strategies • Epicutaneously applied Der p 1 induces eczema • Development of new eczematous plaques after intrabronchial exposure to HDMs • Clinical improvement with HDM-specific immunotherapy	• Lack of improvement of clinical symptoms with mite elimination and/or avoidance • Subgroup of AD patients sensitized to HDM fail to show positive patch-test results • Increased prevalence of AD in dry and northern latitudes • Patch-test reaction rates among atopic patients are not higher than among controls

AD = atopic dermatitis; Der p 1 = *Dermatophagoides pteronyssinus* 1 allergen; HDM = house dust mite; IgE = immunoglobulin E.

the histology of eczema. It is therefore unclear whether the skin lesions in this study represented “true eczema” or were instead a result of inflammation (secondary to intensive local stimulation by abrasion). Likewise, the lesions induced in the study by Tupker and colleagues could not be ascertained to be those of AD because biopsies were not performed. Moreover, many of those lesions developed within a few hours after intrabronchial HDM exposure and then immediately resolved. In addition, like many of the aforementioned studies, that of Tupker and colleagues involved a relatively small number of subjects and did not include an appropriate control population. Association also does not necessarily imply causality. For example, increased transepidermal water loss and copious shedding of squamous cells (on which the mites feed) are common in severe AD and may support an enhanced mite population in the bedroom/mattress environment of these patients, independent of a causal relationship.^{57,64,65} Still other experts have cited the following as evidence against direct involvement: (1) a lack of improvement of AD symptoms despite aggressive measures to eliminate and/or avoid exposure to HDMs (as demonstrated by at least five randomized controlled clinical studies),^{66–70} (2) the failure of a group of AD patients sensitized to HDMs to show a positive patch-test result^{54,55} or to develop lesions when challenged topically with Der p,⁷¹ and (3) the increased prevalence of AD in dry and northern latitudes, where HDM populations are sparse.^{72,73} Moreover, some experts believe that positive patch-test results in AD patients are rarely indicative of a specific allergy to HDMs. Instead, they believe that such a response is an irritant reaction to the proteolytic activity of mite enzymes⁷⁴ in the setting of an intrinsically lowered irritancy threshold in AD patients.⁷⁵

Before the advent of standardized HDM preparations for patch testing, and despite the inconclusive evidence linking HDMs to the causation of AD, several investigators performed epicutaneous tests on atopic patients with a variety of mite antigens, with positive results reported for 21 to 72% of patients.^{76–78} Interestingly, when patients with an aeroallergen-type distribution (face, neck, hands, feet) were examined separately, a significantly higher percentage of patients had positive results.⁷⁹

Recently, Chemotechnique Diagnostics (Malmö, Sweden) began the commercial marketing of a standardized HDM allergen mixture (Der p and Der f in a 50:50 mix) in petrolatum in concentrations of 20% and 30%.⁸⁰ Several investigators have since conducted studies to determine the optimal concentration of this allergen mix and the rate of positivity among atopic patients who are patch-tested with it. Jamora and colleagues⁸¹ found the 20% formulation to have a very high false-positive rate (83%) as it elicited many decrescendo or persistent reaction patterns characteristic of irritant substances. However, when diluted to 0.1%, the allergen mix more appropriately differentiated rates of positivity among healthy controls, respiratory atopic patients, and those with AD. Davis and colleagues⁸² performed a similar study and also found the 20% formulation to have a very high rate of positivity (55%). With the 0.1% concentration, a much lower rate of reaction was seen (6.8%), but it was still high when compared with the reaction rates of other allergens included in their study. Interestingly, the researchers did not find this reaction rate to be higher in atopic subjects than in healthy controls.

This latter finding was echoed by Brasch and colleagues,⁸³ who reported that of 571 patients patch-tested with the HDM allergen mix in Germany, 188 had

positive reactions that were deemed irrelevant; specifically, there was no association between positive patch-test reactions to mite allergens and an atopic diathesis. However, in this particular series, those reacting to HDM allergens were statistically more likely to have additional positive reactions to other allergens, suggesting that unidentified factors that contribute to positive reactions to HDM allergen may also predict enhanced generalized responsiveness to other substances.

Despite these contradictory findings, other investigators believe adamantly that HDM patch testing has an important place in the management of AD patients. In a study population consisting of AD patients in remission, patch testing more specifically correlated with a history of HDM-induced eczema flares reported by these patients than did skin-prick testing or specific serum IgE measurement.^{84,85} Furthermore, it has been shown that patch testing, compared to skin-prick testing, has an apparent ability to identify relevant mite sensitization in AD patients in the absence of specific serum IgE.⁸⁶

The value of HDM patch testing in the management of AD is also supported by accumulating evidence suggesting that specific immunotherapy (SIT) against HDM sensitization is an effective curative therapy against atopic diseases.⁸⁷ SIT involves subcutaneously or sublingually administered incremental doses of allergen extracts to which an individual is sensitized. Studies have shown that SIT results in improved clinical severity of AD^{88–91} and reduction of specific IgE levels against HDM allergens,^{89–91} with concomitant increases in the serum levels of the Th1 cytokine IFN- γ ⁸⁹ and the tolerogenic cytokine IL-10.^{89,91} As promising as they might seem, these studies are flawed in that they were not controlled with a placebo group. Hence, the above data are of limited value and are not powerful enough to justify the routine use of SIT in the management of AD.

Like all patch-testing results, the relevance of positive reactions to HDM allergens must be determined, and this issue is far from being settled. Many studies from different investigators hailing from disparate parts of the world have reported conflicting data. With the availability of the standardized allergen mix and a now standardized patch-test technique developed by the European Task Force on Atopic Dermatitis,⁸⁴ scientists from different parts of the world may employ uniform materials and reproducible methodologies in their studies. There is no question that additional investigation into the relevance of positive patch-test reactions to mite allergens is needed to move beyond anecdotal evidence in regard to the role of HDMs in the pathogenesis of AD and the utility of allergen

avoidance and other novel therapeutic strategies like allergen-specific immunotherapy in improving AD.

Conclusion

The link between house dust mite (HDM) allergy and atopic dermatitis (AD) is supported by several laboratory and clinical translational studies that have cumulatively shown that (1) HDM allergens are able to incite specific and enhanced Langerhans' cell and dendritic cell functioning to yield T-cell-mediated immune responses in AD, (2) HDM allergens have the capacity to polarize immunity toward a Th2 response, and (3) at least some subgroup of AD patients with positive HDM patch-test results improve with mite avoidance and elimination strategies. Admittedly, these studies are at best circumstantial. While strongly suggestive, a definite association between HDM allergy and AD still remains to be firmly established, especially because the overwhelming weight of the clinical evidence shows that avoidance of HDMs does not improve clinical disease activity. Further discussion and investigative pursuits in this arena are necessary.

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