

Atopic Dermatitis and Delayed Hypersensitivity to Dust Mites

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Introduction

In 1923, atopy was defined (1) as a state of hypersensitivity to foreign proteins from the individual or familial environment. The use of prick tests and intradermal reactions has shown that this type of immediate hypersensitivity is very frequent, not only among asthmatics and patients with hay fever, but also in those who suffer from the chronic eczema, known as Besnier's prurigo, or infantile eczema and now known as atopic eczema or atopic dermatitis (AD) (2).

The allergens that may elicit positive tests in 20 min are essentially extracts of dusts and mites, hair, skin scales, feathers, or antigens from animal saliva, pollens, and molds.

A definite link between these tests and the encountered pathology was quickly brought about in the allergies of the respiratory mucosae, therefore, the allergens are also called "aeroallergens." On the other hand, it was only in the 1930s that the aeroallergens were suspected to have an effect on the cutaneous condition of atopics. Thus, Rost (3) in 1932 observed that the patients who had AD and allergy to house dust were improved by staying away from home, in rooms without dust.

There is a significant correlation, verified again recently (4), between immediate skin reactions (prick tests) and positive radio-allergosorbent tests (RAST) to mites and the respiratory history and/or family atopy. The forms of uncomplicated skin atopy are mostly associated with negative immediate tests to mites.

During the 1980s, several authors were interested in the potential responsibility of mites for causing dermatological lesions in AD (5-9); the authors particularly emphasized in some cases the benefit of clean-

ing houses, vacuuming dusts, and removing the patient from the allergenic domestic environment (10). The influence of the environment on the subject was also promoted from measurement of the domestic mite, loading, for example, by relatively simple, semiquantitative techniques (11). The condition of the patient seems to be better correlated with mite than dust content of the ambient air (12).

Over the last 20 yr, the correlation between mite allergy and skin lesions was also consistently demonstrated as the extracts of mites were better identified and purified, notably since Spieksma and Voorhorst proved the antigenic role of mites in domestic dust (13). Furthermore, the method of preparation of the mites has considerable influence on skin tests; for example, extracts of live mites are more often positive than the same extracts prepared from lyophilized mites (14).

AD and Delayed Hypersensitivity

It is currently stated that in AD, delayed hypersensitivity is clinically rare (patch tests, intradermal reactions). This idea is inaccurate and has given rise to publications stating that, on the contrary, it seems to play an important part (15), although not as prominent statistically as in contact eczema. Moreover, use of universal allergens such as dinitro-2,4 chlorobenzene (DNCB) shows that in the minor forms of AD, this delayed hypersensitivity is present, but it disappears clinically in stronger cases, as though the immune system was otherwise occupied (16). Finally, it is possible that delayed hypersensitivity seems to ever hardly occur because the most common allergens are not tested by the best technique. Consequently, it seems to be reasonable to test in the delayed way the current aeroallergens to try to approach logically the pathophysiology of AD.

Allergy to human scales in AD was reported in 1945 (17) and again in 1976 (18), but was not paid much attention before the 1980s. Mitchell and Platts-Mills-group (5,19) published for the first time in 1982 the results of positive delayed patch tests to several types of aeroallergens in AD. Subsequently, many other authors have studied patch tests in AD (20–32), with the most studied allergens being the house dust mite (33), *Dermatophagoides pteronyssinus* (Dpt), and its major antigen P1. Some authors also tested *D. farinae* (Df). The demonstration in vitro of T-lymphocyte stimulation by monocytes and mites (or antigen P1) in AD is a supplementary argument in favor of participation of delayed hypersensitivity to aeroallergens in the genesis of AD (34,35).

The discovery by Bruijnzeel-Koomen et al. in 1985 of the immunoglobulin E (IgE) receptors on the Langerhans cells (LC) in atopic eczema (36) has relaunched studies on the influence of aeroallergens on the cutaneous lesions of AD. This discovery has been confirmed subse-

quently (37,38) and refined. It is now known that the IgE receptor CD23 is of low affinity and that its expression may be induced by interleukin 4 (IL4) and gamma-interferon (IFN γ) (39,40). Finally, the recent work of Bieber et al. (41) suggests that this receptor on the LC is not absolutely specific for AD, but may also be seen in lesional skin in most dermatological inflammatory affections if the IgE titer is high (which is nevertheless relatively rare except in AD). It happens if the IgE titer induces the appearance of receptors on some cells that participate in immunity.

The discovery of the CD23 receptors has given rise to the hypothesis that in AD, the aeroallergens having penetrated the skin (20) are captured by the LC that carry the specific IgE (21,22), and are presented to T-lymphocytes, initiating an overlapping train of events not very different from contact eczema, but involving IgE (42). Nevertheless, there are other differences with contact eczema: The presence of specific IgE and mastocytes or basophils may initiate an early reaction that precedes the lesions of eczema (19), and the eosinophils obviously play an important role in delayed lesions.

The immunohistological study of Tanaka et al. (21,22) has shown the penetration into the skin of mite allergens; it was done on five patients who were biopsied at different reaction times (1, 6, 24, and 48 h), after simple patch test to mites (Dpt, Df). By double immunolabeling, with immunofluorescence and electron microscopy, they traced the mite allergens during their penetration into the tissues. The mite allergens were found after 6 h in the epidermis, especially on the surface of LC OKT6+ (CD1+), which carry surface IgE. Then, after 24 and 48 h the mite allergens were found in the dermis, where they were captured by macrophages and so brought into close contact with T-lymphocytes. Everything seemed to happen in the same way as in the inductive phase of contact eczema, but with one difference, the presence of IgE on the LC. The significant participation of polynuclear eosinophils should also be noted.

For Bruijnzeel-Koomen (43), a positive patch test to an aeroallergen in AD is really a specific allergic reaction to an antigen: The culture of T-lymphocytes extracted from skin biopsies of lesional skin of AD reveals the presence of specific T-lymphocytes for atopens (aeroallergens). After antigenic stimulation these T-lymphocytes produce IL4 and IL5 and only a little or no IFN γ ; thus, they appear to be of TH2 phenotype. The presence of aeroallergens in the skin initiates, in 12 h, an influx of TH2 lymphocytes specific for the allergen. They persist for at least 48 h at the site of the allergen application. The epidermal LC of injured skin cannot present aeroallergens to T-lymphocytes unless they have surface-bound-IgE. Clinically, there is a significant correlation between the presence of LC with surface IgE in the epidermis and the appearance of positive patch tests to aeroallergens.

In the first 24 h after the reaction, the lymphocytes produce IL4, suggesting that they are of the TH2 subgroup, but in fact most of them also produce IFN γ showing that they are instead of the TH0 type. Around 48 h, the IL4 reduces, the response tends toward TH1, and the production of cytokines approaches that found in damaged skin in AD.

Kubota et al. (44) studied 23 AD patients who had a positive patch test to Dpt antigens. Stimulation of the blood monocytes with Dpt antigens in 72 h produced IL2 and IFN γ , but no IL4. These results suggest that the T-lymphocytes produced at the time of the reaction are of TH1 type and able to behave as in usual contact eczema.

Patch Tests to Mites: Methodology

Choice of Patient

This is fundamental: The patients with AD included in various studies of the literature, often conform with the Hanifin and Rajka AD criteria (45), but this is not systematic and some authors do not mention their criteria for patient inclusion in the study (*see* Table 1). Diagnosis of AD may thus be doubtful sometimes. The number of patients tested is of great importance. Total numbers of AD tested are very different between studies and vary from 6–354. It is certain that if only a few patients are tested the study becomes of little significance. Most of the authors compare the test results in AD with those of control subjects, perhaps atopic but without skin symptoms, perhaps healthy subjects, sometimes both. But rarely, it may be not done or not specified. (23,30). Some authors add restrictive inclusion criteria to their study. It considerably modifies the significance by introducing an additional bias, the aim of which is to boost the chance of success of the test. To be included in the study, the patients must have at least prick tests or RAST positive to mites, or a clinical allergy to dust.

Choice of Allergen

The antigens used include at least a mite allergen (Dpt, Df, or antigen P1) in isolation or associated with other aeroallergens (mites, pollens, animal antigens, molds) tested by prick test (19,23,25). These allergens are sometimes prepared by the authors, but are mostly obtained as commercial allergens (Allerbio, Aquagen, Bencard, Dome-Hollister-Stier [DHS], Diephus Laboratorium [DL], Haarlem Allergen Laboratorium [HAL] Spectralgen, Stallergènes) as solutions for prick test or lyophilized allergens. They may be tested as is, in saline solution or glycerol, or in creams that enhance the penetration into the skin, but the composition of which is not always specified (20,23), or finally included in Vaseline to mimic the most common method for

Table 1
Principal Studies about Patch Tests to Mites in AD

References	Year	AD tested	Restrictive criteria	Control subjects	Antigens	Method	Results, % PT+
Brujinzeel-Koomen et al. (25)	1988	15	+	+	Dpt + grass	Stripping-PT ^a	80
Clark and Adinoff (27)	1989	12	+	-	Dpt + others	PT	31
Castelain et al. (26)	1993	272	-	+	Dpt	PT	22
Castelain et al. (32)	1994	450	-	+	Dpt + Df + others	PT	18.4
Gondo et al. (20)	1986	6	+	+	Df	Scratch-PT	83
Langeland et al. (23)	1989	35	+	-	Df + others	PT	17
Mitchell et al. (19)	1982	17	+	+	Df + others	Stripping-PT	100
Norris et al. (28)	1988	30	+	-	Dpt	Repeated-PT	33
Reitamo et al. (29)	1986	17	+	+	Dpt + birch	PT	35
Tanaka et al. (22)	1990	20	+	+	Dpt + Df	PT	70
Voorst Vader et al. (30)	1991	21	+	-	Dpt + Ag P1	Stripping	29
Young et al. (31)	1985	18	-	+	Ag P1	Stripping-PT	83

^aPT = Patch Test.

^bAg P1 = antigen P1.

patch tests. The concentrations are greatly variable from 1–1000 times the concentration of the most widely used prick tests.

Test Methods

These are very variable:

1. Simple patch tests, with immediate (30 mn) and late readings, at 48–96 h.
2. Scratch-patch tests, i.e., patch tests on previous skin reactions. The test material is removed after 24 h and readings made at 24 and 48 h (19,20).
3. Stripping-patch tests, i.e., patch tests on skin previously abraded by repeated application and removal of adhesive tape (10–15 times). Here again, the test material is removed at the 24th h and readings performed at 24 and 48 h (25,30,31).
4. Open test repeated on healthy skin or damaged skin, over 5 d (28).

The criteria for reading are not always those of the International Contact Dermatitis Research Group (ICDRG); it may bring an additional point of divergence (20,23,28,31).

Results

These are very different according to the subject inclusion criteria, the methods and concentrations of the tests and the positive tests may spread from 17–100% (Table 1). None of the different studies has experienced a complete check. The whole problem is to define the relevance and to know, among the many proposed techniques, which is the best or most adaptable.

De Groot and Young (46) already made a summary of some studies in 1989, to underline the fundamental problems that arise from the different techniques used for the tests, as well as the disparity of materials and the sources of allergens, vehicles, and units. Furthermore, the allergens used are prepared from time to time, in small amounts, with variable units and so are not always comparable. The results must be examined with care and if a tendency can be drawn, there is still no standardization of the methods for delayed tests with aeroallergens.

In the experiments organized by GERDA (Groupe d'Etudes et de Recherches en Dermato-Allergie) two successive studies were carried out. The first (26) involved only Dpt and had the objective of defining a vehicle, an appropriate test concentration, and a comparison of different supplies of allergens in varied vehicles and with different scales of concentration on 272 AD-tested and 22% of patients who were positive to Dpt.

In the second study, which is still proceeding with 450 AD patients already tested, preliminary results have been published (32). The range

Table 2
Second GERDA Inquiry: Results of Mite PT in AD

Aeroallergen	Prick +	Percent	PT +	Percent
Dpt	207	45.9	67	14.9
Df	111	24.6	55	12.2
Dpt or Df +			83	18.4
Dpt and Df +			39	8.7

of antigens in this study has been increased with Df and seven other aeroallergens. The concentrations are close to those in the environment (twice that of prick tests), extrapolated from the first study, and the methods are strictly modeled on those of the ICDRG so as to reduce as much as possible the risk of errors, while allowing the allergists to use a technique to which they are accustomed. All the allergens were tested in atopics, like the contact allergens, by simple patch tests, without either scratch or stripping, with material in petrolatum and two readings at 48 and 72 or 96 h.

The results are summarized in Table 2. Of AD patients, 14.9% had a positive patch test to Dpt; this is slightly less than in the first study, where several concentrations of allergen were used that were greater than those used here. Df was positive in 12.2% and the two mites simultaneously in 8.7% of all cases. Overall, 18.4% of patients had a test that was positive to one of the two, or to both mites. The mites were clearly the stars in this study because they occurred in 18.4% of patients, whereas the total figures for all nine pneumoallergens only gave 24.9% positives. Among the 225 control subjects, only 3 gave a positive patch test to mites (1.3%).

As a matter of interest, the results from the ICDRG standard series of patch tests are given in Table 3. The results show that 39.6% of the atopics had at least a classical positive contact test which proves that delayed hypersensitivity is preserved in AD. Dpt had a greater level of positivity than nickel, the first among the contact allergens in this study.

Several points arise from this second study:

1. Patch tests to aeroallergens with delayed readings seem to be a method of effective performance in AD, as the tests are mostly negative in control subjects. The number of subjects who had at least a positive patch test to mites is proportionally 13 times greater in AD than in control subjects.
2. AD with asthma or rhinitis had a weakly higher incidence of positive patch tests to aeroallergens (30.3 and 28.5%, respectively) but this difference cannot be considered as significant.
3. The classical technique of patch test, without artifacts of stripping, or scarifying, seems to be satisfactory.

Table 3
Second GERDA Inquiry:
10 Most Often Positive PT (ICDRG) in AD

Allergens	PT+	Percent
Nickel sulfate	52	13.9
Balsam of Peru	26	7.0
Fragrance mix	23	6.1
Potassium bichromate	19	5.0
Cobalt chloride	15	4.0
PPD	10	2.7
Wool alcohols	10	2.7
Thiuram mix	9	2.4
Colophony	8	2.1
Neomycin sulfide	5	1.3

4. If the readings were made only at 48 h, 27.5% of the positives would be lost, whereas if they were only read at 72 h the loss would be 31.3%. The two readings are indispensable.
5. More than two-thirds of positive patch tests to mites paralleled a corresponding positive prick, but this agreement is weaker for the other aeroallergens.
6. As in the first study, some syndromic reactions and "angry back" were detected (10/450). This induced hyperreactivity is an argument in favor of the appropriateness of the tests, even though it invalidates the reading. Furthermore, undoubtedly the allergens should be retested, separately. Children seem to be much more "hyperreactive" than adults (9 of the 10 angry backs were observed in children).

Some of the tests were biopsied and showed a picture of contact eczema (spongiosis, vesiculation, inflammatory dermis infiltration), always with a high number of polynuclear eosinophils in the dermis.

Relevance of the Tests: Practical Applications

The positive tests to mites showed that a delayed type hypersensitivity exists, caused by some aeroallergens in AD, similar to that of contact eczema. The problem is to know if these tests are relevant. In the older study of Young et al. (31), stripping patch tests with high antigen concentrations (about 20 to 50 times that of prick tests) were used, and these gave 100% of positive tests, but this did not seem to be appropriate, as 100% of the patients tested did not note a Cutaneous worsening by ambient aeroallergens. Moreover, the stripping method artificially increases the sensitivity of the tests by facilitating the skin penetration of the allergens and thus probably altering the physiology. Finally, this method gave a number of false positives (30). The more recent studies,

such as those of GERDA (26,32), performed with a greater number of patients and control subjects has the advantage of using the classical patch test methods, without artificial tricks such as stripping or scratch patch test and without restricted inclusion in the tests. All the AD patients were tested by this method, regardless of their clinical histories concerning dust and mites, or of the RAST results. This method aims at detecting the percentage of patients positive to patch tests with mite allergens, although in the first studies the emphasis was to know if such positivity actually existed.

A percentage of positive patch tests to mites in AD between 15 and 20% seems to reflect more faithfully the reality than the higher percentages of the earlier studies. It should be noted that the percentage is comparable with that of contact allergens which are more often the cause of contact eczema and which are not negligible. In the absence of consensus now on the allergen concentrations used in patch tests to mites, these percentages may still vary.

If these tests are relevant, they must have practical clinical applications, i.e., make sure that delayed allergy to mites is effectively responsible for the creation or worsening of skin lesions (46). The observation of the evolution of the patient when exposed to reduced mite allergens allows verification of the suitability of the patch test. This effect has thus been shown for some, for example by reducing greatly the amount of dust that is present in the environment of the patient (5,10,27,47), but for others the clinical results have not always been confirmed (33,48).

The existence of syndrome reactions triggered by the tests (26,32) is in favor of the suitability of patch tests for mites. The presence of positive tests to aeroallergens only in patients in clinical remission of their AD (19) reproduces the phenomena seen with "universal" types of allergens such as DNCB (16) in contact eczema. In fact, all the studies should have a longer follow-up period for the positive patients to try to find incriminated aeroallergens, perhaps by removal where this is possible, or by specific desensitization, which is discussed later. The future of this type of test will depend mostly on the therapeutic consequences that are found.

Conclusion: Therapeutic Consequences

Delayed hypersensitivity to mites exists in some patients with atopic eczema and this can be shown by patch tests. The involvement of this delayed hypersensitivity in the creation of skin lesions is not yet certain, but seems to be probable, owing to syndromic reactions produced by the tests, the worsening of some patients in the presence of allergens, as well as the improvement of patients who are sensitive to dust or its allergenic fractions by removal of dust from the habitat.

The problem at present is that of definition of a new standard of patch tests to aeroallergens. Recent studies diverge over almost everything—materials, vehicle, test method, concentration, source of allergens, way of reading the tests, and so on. To progress down the road of standardization, it is essential, first, to define standard units for the measurement of allergens amounts, that will be common for all allergists, because they are presently varied: Weight/volume only reflects the method of preparation of the extract, usually in grams (raw allergen/mL solution). Protein nitrogen units (PNU) indicate the amount of total protein in an allergen extract. Among the biological activity units, the following may be mentioned: activity units by RAST (AUR), histamine equivalent by prick (HEP), and index of clinical reactivity (IR) (49).

Finally, in the interest of rationalization and simplification, it would be desirable that the test technique approaches, as quickly as possible, that of the usual patch tests, as has already been done for contact allergy. The allergen should be prepared in a laboratory that is accustomed to the manufacturing and packing of allergens for patch tests so that the materials are reliable and identical from one syringe to another as well as from the start to the end of the same syringe.

These new ideas must include therapeutic consequences. With regard to hypersensitivity to mites, their complete eradication from the environment of the patient is illusory, except at high altitudes, but they can be greatly reduced in amount and this problem is already very well known by pneumoallergists. Isolation in a hospital environment or dust removal by repeated aspiration of the habitat (particularly the bedroom) of atopics is followed in an appreciable proportion of cases by a significant improvement of skin lesions. The action of acaricides is more or less effective, but the efficacy diminishes with time. Action on the ambient humidity (for example, with a dehumidifier) is an attractive means of fighting the mites for which the optimum relative humidity is between 75 and 80%. The new, simple techniques for measurement of the amount of mites in the environment enable a better control of the measures taken.

There are also possibilities for immunological therapy, such as immunotherapy (50), and, more recently, acting in the idiotypic network, the injection of antigen-antibody complexes that should induce the formation of anti-idiotypic antibodies and/or suppressor T-lymphocytes. The first studies, made on 10 patients allergic to Dpt were promising, but must be repeated on a much greater number of subjects and for other allergens (51–53). The other studies are not yet further than projected, or sketched out, based on production of anti-CD23 monoclonal antibodies, or anti-IL4.

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