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Atopy patch testing – a diagnostic tool?

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Atopic dermatitis (AD) is an inflammatory skin disorder with increasing incidence, characterized by severe pruritis, a chronically relapsing course, and clinically distinctive morphologic features and distribution of skin lesions. It is often associated with a personal or family history of atopic diseases (1). Although genetic factors underlie the development of the disease (2), widespread factors seem to be necessary to provoke it. Provocative factors include aeroallergens, food, microbial organisms, sex hormones, stress, sweating, and climate (3).

Atopic dermatitis and aeroallergens

There has been no clear consensus that aeroallergens are important in the pathogenesis of AD; however, several studies have found a role of aeroallergens, especially house-dust mite, in the pathogenesis of AD (4–6). There is evidence that the homes of patients with AD have a higher level of exposure to house-dust mite than the homes of controls (7). In addition, Beck & Korsgaard showed a clear dose-response relationship between the disease activity of AD and exposure to house-dust mite in patients' beds (8), although other authors could not demonstrate such a correlation (9).

In the past, several authors have found improvement of AD after the adoption of measures to avoid house-dust mite in the homes of the patients (10–13), while others did not (14). However, the studies on avoidance measures are difficult to compare, because of differences in effectiveness of the measures used and differences in follow-up parameters. In a double-blind, placebo-controlled study, Tan et al. showed that a combination of measures to reduce house-dust mite, such as Goretex bedcovers, benzyltannate spray, and a high filtration vacuum cleaner in the homes resulted in a reduction in Der p 1 concentrations in parts of

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the domestic environment and a clinical improvement of AD (15).

How aeroallergens aggravate AD is still a matter of debate (3). Early studies have shown that inhalation of house-dust or pollen extract can provoke exacerbations of skin lesions (16, 17). More recently, Tupker et al. demonstrated pruritic, erythematous skin lesions in 9/20 patients with AD after placebo-controlled bronchial challenges with house-dust mite (18). All the responders had a history of asthma, and 8/9 patients had developed an early bronchial reaction after allergen challenge, suggesting that the inhalation route is especially relevant to a subset of patients with AD who also have respiratory symptoms. Brinkman et al. (19) also found a flare-up of skin lesions in AD patients after inhalation of house-dust mite, cat allergen, or tree pollen, and this flare-up was more pronounced in patients with concomitant allergic asthma than patients who suffered only from AD. A possible explanation of this phenomenon is that allergen-induced inflammation in the airways might result in the release of products from activated inflammatory cells, such as mediators and cytokines, for possible distribution to the skin, which is already primed in AD. It is also possible that as a result of allergen exposure in the airways, allergens enter the circulation and are transported to the skin (18, 19).

Apart from the inhalation route, there is also evidence that allergen penetration through the skin may occur. Barnetson et al. suggested that large amounts of house-dust-mite allergens may be scratched into the skin during clinical exacerbations (20). In experimental models, eczematous lesions can be provoked by applying allergens to the skin for 24–48 h, in the so-called atopy patch test (APT) (see below). Repeated applications of allergens to the skin can also induce eczematous lesions (21, 22). The presence of antigens of house-dust mite in the epidermis of AD lesions (23) and APT reactions to house-dust mite (24) have been demonstrated. Recently, Riley et al. demonstrated the presence of house-dust mite on the skin by vacuum cleaning (25).

The atopy patch test (APT)

Introduction

In 1982, Mitchell et al. (26) demonstrated that epicutaneous application of several allergens on the uninvolved, abraded skin of patients with severe AD could induce eczematous lesions only in patients who also showed a positive immediate skin reaction to the same allergen. Thereafter, many groups have used the APT as a model to study the role

of aeroallergens in AD (21, 22, 27–32). The outcome of APT in the different studies shows large variations, due to differences in patient selection and, more importantly, differences in methodology (6, 33).

The patch-test reaction to aeroallergens is specific for sensitized AD patients, and does not occur in healthy volunteers or in patients suffering from asthma or rhinitis (34).

Methodology

In the APT, the allergen solution is applied to the skin epicutaneously (Fig. 1). As was described before, the methodology of this test varies widely in the different studies. These differences include the following.

Differences in type of allergen tested

Although most studies on the APT have used extracts of house-dust mite, as being the most important aeroallergen in AD, some authors have also used other allergens, such as pollen, animal dander, and molds in the APT. De Groot & Young (6) reviewed studies on the APT in 1982–8 with different allergens. The number of positive tests seems not to be related to the type of allergen that is used. Clark & Adinoff (28) studied 12 AD patients with positive prick tests to several aeroallergens; only aeroallergens known to precipitate dermatitis by history or that were identified in patients' home environments elicited positive patch reactions. Seidenari et al. (31) performed patch tests with two types of mite antigen, whole-mite culture and purified mite extracts (*Dermatophagoides pteronyssinus* and *D. farinae*). Fifty percent of patients with AD and specific IgE to mites had positive reactions to whole-mite culture and 52% had positive reactions to the purified extracts (31).

Differences in concentration of allergen and vehicle

There is a large variation in the allergen concentration that is used in the different studies on the APT. Some studies use the commercial solution for prick testing (22, 26), while others use 10–1000-fold the concentration used for prick testing (30, 33, 35, 36), 500-fold the concentration of the prick test standardized to one histamine equivalent prick (37), or $\times 100$ the concentration used for intracutaneous testing (29).

van Voorst Vader et al. (30) compared three concentrations of house-dust-mite allergen (2000, 10 000, and 50 000 AU/ml) used for patch testing and found most positive responses with the highest allergen concentration. Langeveld-Wildschut et al. (33) could not demonstrate an increase in



Figure 1A. Atopy patch tests performed on back of atopic patient, showing positive reactions at 48 h to house-dust mite (upper side), tree pollen, and grass pollen. Lowest reaction is control spot.

Figure 1B. Detail of positive atopy patch test.

positive APT reactions with 100 000 instead of 10 000 AU/ml. Darsow et al. performed the APT with different vehicles and allergen concentrations, and found most positive reactions with an allergen concentration of 10 000 PNU/gm and petrolatum as a vehicle (36).

Differences in skin condition

The APT has been performed on normal skin (28, 37, 38), normal skin after pretreatment of the skin by scarification or stripping with adhesive tape (21, 26, 29, 39), and lesional skin (22).

Mitchell et al. (26) performed patch tests on skin areas which were gently abraded by removing the upper layer of the epidermis without causing capillary bleeding. In this way, allergen can more easily penetrate the skin, a situation which is also apparent after scratching. Gondo et al. (21) succeeded in reproducing an eczematous lesion on the

apparently normal skin of a patient with AD by scratching and continuous application of allergen.

Another way of facilitating allergen penetration is tape-stripping with adhesive tape, resulting in a reduction of the corneal layer. van Voorst Vader et al. (30) found a higher number of positive APTs after rigorous tape-stripping ($\times 15$) compared to $\times 8$ stripping or no stripping. However, the number of nonspecific reactions also increased, especially after a 48-h reading. Seidenari et al. (31) reported the highest number of positive reactions after simple application of the allergen compared to pretreatment of the skin with stripping ($\times 4$), 0.02 ml dimethyl sulfoxide (DMSO), 0.05 ml of 10% sodium lauryl sulfate (SLS), and slight abrasion with scalpel, or on skin having undergone prick test. The different pretreatment techniques partially or greatly reduced the skin reactivity. An increased number of positive APTs was found after stripping $\times 10$ (27/56 patients) compared to no stripping (20/56 patients) by Langeveld-Wildschut et al. (33).

No difference in the incidence and intensity of the APT reaction was found between 10 and 20 tape-strippings. Neither 10 nor 20 tape-strippings induced nonspecific reactions.

Occasionally, allergen is also applied to lesional skin. Repeated daily application of allergen on mildly eczematous skin resulted in a marked or moderate local deterioration after 5 days (22). This was also true, although to a lesser extent, in areas which initially were clinically uninvolved.

Although performing the APT on uninvolved skin after stripping offers the advantage of a way of standardization, repeated application of allergen to the skin without pretreatment, with scratching allowed, most closely resembles the “real life” situation (26).

Differences in localization of the test site

Although most investigators performed the APT on the back of patients (29, 30, 37, 38), some authors also used other locations, such as the antecubital or popliteal fossa (22) or the extensor side of the forearm (21). Langeveld-Wildschut et al. (33) patch-tested on the uninvolved skin of the back and the antecubital fossa in 10 AD patients, failing to find differences in response between the two test sites. Norris et al. (22) described an increased incidence of immediate pruritic reaction on the antecubital fossa as compared with the back after epicutaneous application of allergen to the uninvolved skin, possibly due to local differences in cutaneous absorption (40), itch points (41), or mediators of pruritus (42). Although the back seems the most practical location for testing, it is also suggested that the best reproduction of AD requires various conditions, such as the site of normal distribution of the lesions (21).

Differences in reading time

In the different studies on the APT, there are large differences in the duration of allergen application and reading time. Most studies use a single, prolonged allergen application and reading times of 24, 48, and 72 h. A number of studies also report immediate reactions at 10–20 min (6).

When evaluating the APT at four different time points (20 min, 24 h, 48 h, and 72 h), Langeveld-Wildschut et al. (33) found nine different reaction patterns. Although most patients had positive responses at 24 h, persisting until 48–72 h, 7/34 patients started reacting at 48 h. This latter group had a significantly lower specific IgE level than patients who started reacting after 20 min or 24 h. van Voorst Vader et al. also found more specific reactions after a 48-h reading than a 24-h reading alone (30). It was also suggested that stripping of the stratum corneum results in more positive reactions at

24 h, whereas the reactions to patch testing on normal skin may not be maximal until 72 h (6).

The APT has good reproducibility. When repeating the APT after 6 months, Langeveld-Wildschut et al. found the same clinical score and reaction pattern of the APT in 5/5 patients (33).

The APT as a model for allergen-induced inflammation in AD

Because of the macroscopic and microscopic resemblance between a positive APT and lesional skin, the APT is widely used as a model to study the onset of the allergic response to aeroallergens in the skin (Fig. 2). Early histopathologic examination of positive patch tests induced by human dander showed spongiosis and a moderate, mainly perivascular lymphohistiocytic infiltrate, consistent with eczema (39). Mitchell et al. found that eczematous lesions induced by purified allergen of house-dust mite over 48 h, contained mononuclear cells, basophils, eosinophils, and neutrophils (26). However, their hypothesis that the patch-test reaction could be explained by cutaneous basophil hypersensitivity was not confirmed by others.

An influx into the dermis of activated eosinophils that were in close contact to Langerhans' cells was found by Bruijnzeel-Koomen et al. in positive patch-test reactions after 24–48 h (29). Recruitment and activation of eosinophils in the skin of AD patients might result from Th2-cell derived cytokines, such as GM-CSF, IL-3, IL-4, and IL-5 (34, 43). A dermal infiltrate consisting of CD4+ T cells and activated eosinophils was found in both lesional skin and APT reaction at 24 h (44). After patch-testing for 48 h, the numbers of CD3+, CD4+, CD8+, RFD1+ (dendritic cells), and RFD7+ cells (mature macrophages) were not statistically different from lesional skin (45). House-dust-mite-specific T cells, producing IL-4 and IL-5, were cultured from APT reactions in house-dust-mite-sensitive patients with AD (46, 47), as in the lesional skin of AD patients (48).

Extravasation of leukocytes to inflammatory sites, such as an APT reaction, is driven by chemoattractive agents and increased expression of adhesion molecules. Although the expression of adhesion molecules is already increased in the nonlesional skin of patients with AD, a further increase in ICAM-1, VCAM-1, and E-selectin was observed during the APT reaction (49).

Using immunocytochemical double-staining with IL-4 and IFN- γ antibodies in combination with membrane markers, Thepen et al. demonstrated a shift from a Th2 response (IL-4) in the initiation phase of the APT reaction to a Th0/Th1 response (IFN- γ) in the late-phase APT reaction

Hypothetical scheme of cellular mechanisms after APT

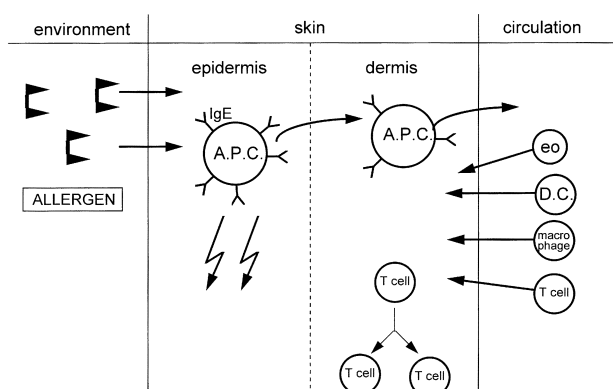


Figure 2. Schematic representation of cellular mechanisms after APT. A.P.C.: antigen-presenting cell; D.C. dendritic cell; eo eosinophil.

and lesional skin (45). The ratio between IL-4 and IFN- γ in lesional skin was comparable with the ratio found in 48- and 72-h patch tests.

T-cell activation can result from antigen presentation by IgE-bearing Langerhans' cells (50). IgE-bearing Langerhans' cells are present in clinically involved and uninvolved skin in patients with AD (51). Also after APT, antigen-bearing Langerhans' cells coexpressing IgE were found in the epidermis after 6 h and predominantly in the dermis after 24 and 48 h (21, 24, 51).

Marked differences were found by macroscopic and microscopic comparison of the APT reaction with the late-phase reaction (LPR) after intradermal allergen challenge (44). With regard to the allergic status of a patient, it is clear that the skin prick test or the intradermal allergen challenge reveals sensitization to a specific allergen irrespective of AD. However, APT reactions mostly occur in AD, and not in allergic rhinitis or allergic asthma (33).

Macroscopically, APT reactions resemble AD lesions by showing erythema, induration, papules, and/or vesicles, whereas the LPR is characterized by diffuse edema, erythema, slight induration, pruritis, and tenderness. Microscopically, APT reaction sites demonstrate acanthosis, spongiosis, and a dermal infiltrate of CD1+ cells, CD4+ lymphocytes, and eosinophils. Neither spongiosis nor acanthosis is observed after the LPR, but edema and mast-cell degranulation are found. Moreover, a clear influx of mononuclear cells, eosinophils, neutrophils, and basophils is observed (52–55).

Although the LPR after intradermal allergen challenge (approximately 8 h after allergen challenge) is considered to reflect closely the inflammatory allergic reaction, this most probably does not hold for AD. If we take into account the AD constitution, and macroscopic and microscopic reac-

tions after allergen testing, the APT appears more relevant to AD than does the LPR after intradermal challenge.

Outcome of the APT related to clinical features

Although the APT is an *in vivo* model for allergic inflammation in AD, the diagnostic value of this test is more controversial. Can the APT be used for further classification of AD patients or for selection of patients who will benefit from allergen-specific treatment, such as allergen avoidance? In other words, what distinguishes sensitized AD patients with a positive APT reaction from sensitized AD patients with a negative APT reaction?

Clinical features

When combining the results of the APT and IgE specific for dust-mite antigens, Imayama et al. (32) classified patients with AD into four groups, each group with its own distribution and morphologic features of skin lesions. Patients with an elevated specific IgE and a positive APT to dust-mite allergens were characterized by extensive erythematous and lichenified skin lesions and a high percentage of facial lesions (89%). These patients might be diagnosed as typical cases of AD. Patients with an elevated level of mite-specific IgE and a negative APT reaction showed generalized skin lesions and a relatively high incidence of involvement of each skin area.

Darsow et al. (56) found that patch-test positivity was related to the distribution pattern of eczema. The group of patients who had eczematous skin lesions predominantly on air-exposed parts of the skin such as hands, forearms, head, and neck showed a significantly higher frequency of positive patch-test reactions (69%) to house-dust mite, grass pollen, and cat dander than a group of patients who did not have this "predictive" distribution of skin lesions (39%). It was concluded that in patients with eczematous lesions predominantly in areas not covered by clothing, the APT may provide an important diagnostic tool (56). In contrast, neither Langeveld-Wildschut et al. (57) nor Wistokat-Wülfing et al. (58) could find a relationship between the outcome of APT reactions and the severity or distribution type of eczema in AD patients.

Peripheral parameters

When comparing patients with AD and a positive APT to patients with AD and a negative APT, Langeveld-Wildschut et al. (33) found a statistically higher total serum IgE level and allergen-specific IgE in the APT-positive AD patients.

Patients who started to react after 48 h showed statistically significantly lower allergen-specific IgE levels than patients who started to react earlier (20 min, 24 h). van Voorst Vader et al. (30) also found more positive APT reactions in patients with higher serum IgE (>1000 ku/l); however, Darsow et al. could not find a significant correlation between total serum IgE and APT positivity (56).

In a study of 96 AD patients, Wistokat-Wülfing et al. demonstrated that allergen-specific proliferation and the increase of the binding of CD45 and CD30 on peripheral blood T cells corresponded to the outcome of the APT with that specific allergen (58). Langeland et al. (27) reported that all the strongly positive patch-test reactions occurred in AD patients with a strongly positive prick test to the same allergen.

Exposure

In moderate to severe AD patients with positive prick tests to numerous aeroallergens, Clark & Adinoff (28) and Adinoff et al. (38) found that only aeroallergens known to precipitate dermatitis by history, or that were identified in the home environments of the patients, elicited positive patch reactions. Negative aeroallergen patch tests were not implicated as precipitating factors by history. In the study of Reitamo et al. (37), 3/4 birch-pollen-allergic patients with seasonal exacerbations of their atopic eczema in spring had a positive APT reaction.

The APT seems to act as a marker of exposure and may be viewed as a direct provocative allergen challenge of the skin in patients with AD (28).

Local factors

When comparing 10 house-dust-mite-allergic AD patients demonstrating positive APT (APT+) with 10 house-dust-mite-allergic AD patients demonstrating negative APT (APT-), Langeveld-Wildschut et al. found differences in the number of IgE-bearing CD1+ cells in clinically uninvolved skin. Epidermal IgE+ CD1+ cells were detected in all APT+ patients but only in 3/10 APT- patients. There were no significant differences between the groups in composition of cellular infiltrate or presence of allergen-specific T cells in clinically uninvolved skin (57). From these data, it seems that the presence of IgE+ CD1+ cells in the epidermis is important for the development of a positive APT reaction. In patients with IgE+ CD1+ cells in the epidermis, exacerbation of eczema might result from skin contact with allergens. In contrast, exacerbation of eczema in APT- patients might not be caused by skin contact; however, the effect of inhalation of aeroallergens on eczema may be present in both groups of patients (57).

Concomitant airway disease

van Voorst Vader et al. found that in all AD patients with a positive APT, there was a history of concomitant airways disease (30). These data suggest that aeroallergen-induced exacerbation of AD occurs in those sensitized AD patients in whom active airway disease is present. When the provocative effect of aeroallergens in AD patients is explained through the inhalation route, active airway disease seems necessary for distribution of mediators and cytokines from activated inflammatory cells in the airways to the skin. Inhibition of inflammatory processes in the airways by anti-inflammatory treatment might result in a reduction of provocative effects by aeroallergens on AD.

The role of the APT in monitoring the effect of local treatment

In a recent study, Langeveld-Wildschut et al. (59) evaluated the macroscopic and microscopic effects of local treatment with a topical steroid (triamcinolonacetonide 0.1%) and tar (pix liquida 10%) on the APT. Treatment with both topical steroids and tar resulted in a reduction in macroscopic outcome of the APT reaction and in a reduction of influx of inflammatory cells. The APT reaction at 24 h showed a considerably lower number of CD3-, EG2-, CD1-, and RFD1- positive cells in the steroid- or tar-pretreated skin than in the vehicle-treated skin. Local treatment also had an inhibitory effect on the allergen-induced increase of cells staining for IFN- γ and IL-4.

It was concluded that the APT may be a useful model to study the effect of topical treatment. In addition, this study demonstrated the importance of the withdrawal of topical anti-inflammatory drugs before patch-testing to avoid false negative results. It is recommended that topical corticosteroids and tar be withdrawn for at least 2 weeks before the APT is performed (59).

Conclusion

The outcome of the APT might be related to the following factors:

- 1) clinical factors, such as the distribution pattern of the eczema and concomitant airway disease
- 2) peripheral parameters, such as total/specific IgE and allergen-specific T-cell proliferation and activation in peripheral blood
- 3) local factors, such as the presence of IgE+ CD1+ cells in the epidermis
- 4) environmental factors, such as high allergen exposure.

In addition, the APT is a suitable model to study allergic inflammation in AD and might serve as a useful tool in the follow-up of topical anti-inflammatory treatment.

Currently, the effectiveness of allergen avoidance in APT+ and APT– patients is under assessment.

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