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Original Article

Evaluation of patch testing in atopic dermatitis using commercially available environmental antigens

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ABSTRACT

Background: In the present study, we measured specific IgE radioallergosorbent test (RAST) levels for house dust, mites, Japanese cedar, kamogaya (*Dactylis glomerata*) and ragweed antigens in a group of atopic dermatitis (AD) patients and healthy volunteer subjects in our outpatient clinic. We also performed closed patch testing with patch test reagents using the aforementioned antigens prepared from commercially available skin reaction antigens.

Methods: The dermatologic symptom severity index, duration of disease, total serum IgE, lactate dehydrogenase (LDH) and eosinophil counts were assessed. The relationship between each of these parameters and the intensity of the response to the patch test was then evaluated.

Results: The patch test positivity rate increased in proportion with the duration of antigen application, with a maximum of 15.6% for mite antigen and a minimum of 4.7% for cedar antigen by 72 h. There were no antigens showing negative results in any patients. The positivity rate was lower for patch tests applied on highly keratinized areas of skin. The 72 h positive patch test responses were significantly correlated with total serum IgE, LDH, symptom severity index and specific IgE RAST levels. Most positive patch test responses were considered to be possible late phase reactions of the type I allergic response. However, a small number of patients had IgE RAST negative antigens but positive patch tests.

Conclusions: These findings suggest the presence of more than one mechanism by which a positive reaction is elicited.

Key words: atopic dermatitis, late phase reaction, patch testing.

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INTRODUCTION

Some atopic dermatitis (AD) patients have positive patch tests for environmental antigens. This serves as a basis for theories emphasizing the role of type IV allergic responses in AD.[1–3] However, many patients with positive patch tests also have antigen-specific IgE antibodies, immediate intradermal reactions and positive scratch tests.[2,4] This lends support to theories that implicate type I allergic responses.

In 1986, Bruynzeel-Koomen *et al.* confirmed the presence of IgE antibodies on intra-epidermal Langerhans' cells[5] and Tanaka *et al.* described transepidermal invasion of mite antigens that bind to these antibodies.[6] Since then, many investigators have postulated that transepidermal invasion of environmental antigens plays some role in inflammation in the skin, which, in turn, acts as an exacerbating factor in AD. Thus, patch testing has become an important diagnostic study. However, the lack of commercially available patch test antigens in Japan has been one obstacle in diagnosing patients with AD.[7] The objective of the present study was to evaluate the relationship between patch test responses and levels of various antigen-specific IgE. The patch test reagents were prepared from commercially available intradermal solutions containing environmental antigens for diagnostic and therapeutic use. Symptom severity index, duration of disease, total serum IgE, lactate dehydrogenase (LDH) and eosinophil counts were also assessed. We then evaluated the relationship between these clinical parameters and patch test

responses in order to investigate the overall significance of patch testing in the diagnosis of AD.

METHODS

Subjects

This study enrolled a total of 64 AD patients who were evaluated in our outpatient department. A diagnosis of AD was based on evaluation criteria established by the Japanese Dermatological Association.[8] The study included 33 men and 31 women, ranging in age from 16 to 48 years (mean age 28 years). In addition, a total of 10 healthy volunteers without subjective symptoms or objective findings of disease were enrolled in the study as control subjects. This group consisted of six men and four women, ranging in age from 29 to 63 years (mean age 38 years). There was no restriction with respect to antigen-specific IgE levels at the time of enrollment.

Preparation of patch test reagents

Allergen extracts for diagnostic, therapeutic and scratch tests were purchased from Torii Pharmaceuticals (Tokyo, Japan). These extracts included house dust (therapeutic use; 1%), *Dermatophagoides farinae* (scratch test extract; 1%), cedar (therapeutic use; 1%), kamogaya (diagnostic use; 5%), and ragweed (therapeutic use; 1%). Each extract solution was added to white Vaseline (Yoshida Pharmaceutical, Tokyo, Japan) containing 0.1% sodium lauryl sulfate to prepare 0.5% antigen concentrations. These were mixed in a mortar and emulsified. The above allergen extracts (with the exception of house dust extract) contained 50% glycerin. The final glycerin concentration in the prepared reagents was 25%. There were three types of control reagents: (i) white Vaseline; (ii) white Vaseline containing 0.1% sodium lauryl sulfate; and (iii) white Vaseline containing 0.1% sodium lauryl sulfate and 25% glycerin.

Patch testing

The patch test reagents were applied using a Finn-chamber® (Epitest, Tuusula, Finland) for 24 and 48 h to the medial aspect of the upper arm (rash-free areas) and results were evaluated after 24, 48 and 72 h from the start of application. Test results were evaluated in accordance with International Contact Dermatitis Research Group (ICDRG) criteria.[9] In some subjects, reagents were applied to the medial aspect of the thigh (rash-free areas) for 48 h to compare differences with respect to patch test site and results were evaluated after 72 h from the start of application.

Clinical parameters

The following clinical parameters were investigated in the present study.

1. Duration of disease. This was estimated by examining patients' medical histories.
2. Severity index. This was estimated using a partially modified method of Yoshiike *et al*.[10] Estimations were based on total numerical values, excluding pruritus scores because of difficulties in subjective assessment (Table 1).
3. Peripheral blood eosinophil count. This was calculated from blood smear preparations.
4. Serum LDH was measured by the UV method.
5. Total IgE was measured by the radioimmunosorbent test (RAST).
6. Antigen-specific IgE levels were also determined by the RAST.

Statistical analysis

Data correlation was analyzed by the Spearman rank correlation coefficient.

RESULTS

Patch testing of each antigen on the medial aspect of the upper arm

After 24 h, the positivity rate for mite antigen was 6.5%. Testing for other antigens was negative. After 48 h, the positivity rates were 4.6% for house dust, 4.6% for mites, 3.1% for ragweed, 1.6% for kamogaya and 3.1% for

Table 1 Atopic dermatitis severity scores

	None	Mild	Moderate	Severe	
Face–Neck	0	1	2	3	A
Trunk	0	1	2	3	B
Extremities	0	1	2	3	C

Severity index = (A+B+C).

Table 2 Positive ratio in patch testing

Allergen	Positive ratio (%; $n = 64$)		
	24 h	48 h	72 h
House dust (1%)	0	4.6	12.5
Dermatophagoides farinae (1%)	6.5	4.6	15.6
Ragweed (1%)	0	3.1	6.3
Kamogaya (5%)	0	1.6	10.9
Cedar (1%)	0	3.1	4.7
White Vaseline	0	0	0
White Vaseline containing 0.1% SLS	0	0	0
White Vaseline containing 0.1% SLS and 25% glycerine	0	0	0

SLS, sodium lauryl sulfate.

Table 3 Patch testing at different sites

	Allergen		
	House dust	Mites	Cedar
Patient 1			
Upper arm	–	+	–
Thigh	ND	+	ND
Patient 2			
Upper arm	+	–	–
Thigh	+	ND	ND
Patient 3			
Upper arm	–	–	+
Thigh	ND	ND	–
Patient 4			
Upper arm	–	ND	ND
Thigh	–	ND	ND
Patient 5			
Upper arm	+	ND	ND
Thigh	+	ND	ND
Patient 6			
Upper arm	–	ND	ND
Thigh	–	ND	ND
Patient 7			
Upper arm	–	ND	ND
Thigh	–	ND	ND
Patient 8			
Upper arm	–	ND	ND
Thigh	–	ND	ND
Patient 9			
Upper arm	–	ND	ND
Thigh	–	ND	ND
Patient 10			
Upper arm	–	ND	ND
Thigh	–	ND	ND
Patient 11			
Upper arm	ND	–	+
Thigh	ND	ND	+?
Patient 12			
Upper arm	–	+	–
Thigh	ND	+	ND

ND, not done; +?, questionably positive.

cedar. After 72 h, the positivity rates were 12.5% for house dust, 15.6% for mites, 6.3% for ragweed, 10.9% for kamogaya and 4.7% for cedar (Table 2). The results in all control subjects were negative at all evaluation time points. The positivity rates for each antigen (except mite antigen) increased with time. The highest final rate was 15.6% for mite antigen and the lowest was 4.7% for cedar antigen.

Patch testing at different sites

Patch testing was performed at two different sites (medial aspects of the upper arm and thigh) in 12 AD patients. These results are shown in Tables 3,4. Four patients (patients 1, 2, 5 and 12) showed a positive response for some antigen on both the upper arm and thigh. Two patients (patients 3 and 11) with a positive response on the upper arm had a negative or a questionably positive (+?) response on the thigh. Six patients (patients 4, 6, 7, 8, 9 and 10) showed negative responses on both the upper arm and thigh. None of the patients with a negative response on the upper arm showed a positive response on the thigh. These results suggest that a positive patch test response is less easily obtained on the thigh compared with the upper arm. In general, keratinization of the thigh is greater than that of the upper arm. The lower patch test positivity rate on the thigh seems to be attributable to inhibition of antigen penetration by the horny layer of the skin.

Clinical parameters

Clinical parameters such as severity index, duration of disease, total serum IgE, LDH and eosinophil counts, were recorded (data not shown).

Correlation of each parameter with the results of patch testing

The present study found no significant correlation between positive patch test results after 24, 48 and 72 h for any single antigen (house dust, mites, ragweed, kamogaya and cedar) and the corresponding antigen-specific IgE level. We then combined the patch test results for all antigens and evaluated the correlation between the intensity of the patch test results for each antigen and the corresponding antigen-specific IgE levels. Analysis of the results at 72 h showed a significant correlation between the intensity of the positive patch test responses and specific IgE levels ($P < 0.03$; $r = 0.35$; Fig. 1).

Among these cases, specific IgE RAST levels were negative in two patients (3.1%) for house dust, in three patients (4.7%) for mites, in two patients (3.1%) for ragweed, in three patients (4.7%) for kamogaya and in one patient (1.6%) for cedar.

Table 4 Summation of patch testing results at different sites

	No. responses (%)	
	Upper arm	Thigh
Reactions		
+	6 (50)	4 (33.3)
+?	0	1
–	6	7
Total	12	12

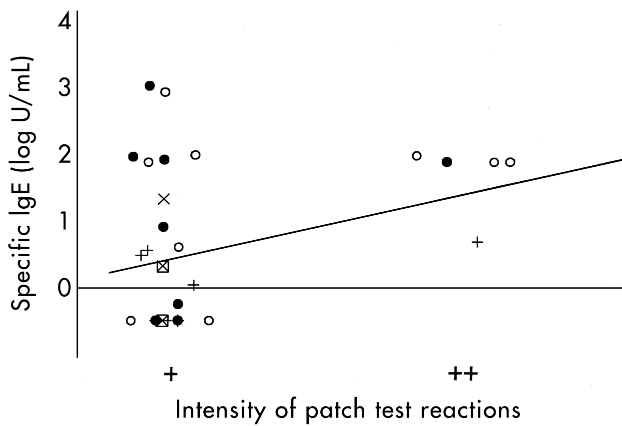

Fig. 1 Correlation between patch test reactions (72 h) and specific IgE levels ($r = 0.35$; $P < 0.03$). All antigen-specific IgE levels (●, house dust; ○, *Dermatophagoides farinae*; ×, *Datylis glomerata*; +, *Ambrosia artemisifolia*; □, *Cryptomeria japonica*) and positive patch test results after 72 h are plotted on the one graph.

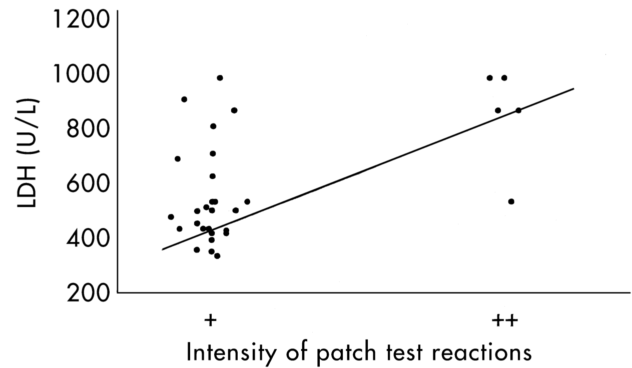

Fig. 3 Correlation between patch test reactions (72 h) and lactate dehydrogenase (LDH; $r = 0.52$; $P < 0.002$).

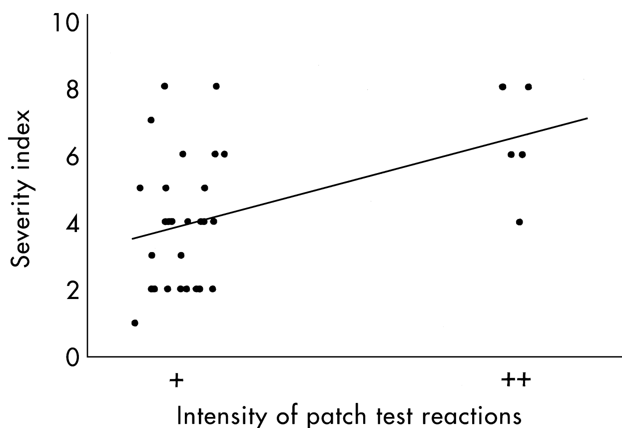

Fig. 2 Correlation between patch test reactions (72 h) and severity index ($r = 0.44$; $P < 0.006$).

Fig. 4 Correlation between patch test reactions (72 h) and total IgE ($r = 0.56$; $P < 0.001$).

Significant correlations were also found between the intensity of positive patch test responses at 72 h and severity index ($P < 0.006$; $r = 0.44$), LDH ($P < 0.002$; $r = 0.52$) and total IgE ($P < 0.001$; $r = 0.56$; Figs 2–4).

DISCUSSION

Much debate still exists regarding the role of allergic reactions in the pathogenesis of AD. Proposed theories concerning allergic pathogenesis can broadly be categorized into those emphasizing the role of type I reactions and those emphasizing the role of type IV reactions. The reasons for emphasizing the involvement of type I allergic reactions in AD include the high incidence of other atopic disorders (e.g. allergic rhinitis and bronchial asthma), the presence of high total serum IgE levels and the detection of specific IgE antibodies to antigens in many AD patients,[2] elevated IgE levels in the skin, the binding of IgE antibodies to Langerhans' cells and mast cells and binding of these cells with specific transepidermal invasive antigens[6] and the gradual recognition that prolonged inflammation is possible even in a type I allergic response.[11–13] The reasons for emphasizing the involvement of type IV allergic reactions in AD include distinct clinical and histopathologic similarities with allergic contact dermatitis[2] and the fact that, despite the reported predominance of T helper (Th) 2 cells in AD, Th1 cells may eventually predominate after a period of time.[14–16]

Patch testing has been used for a long time in the clinical detection of skin allergens. Patch testing has also been used for the evaluation of AD. In addition to mite antigens, patch testing with human grime,[17] cedar pollen[18,19] and *Staphylococcus aureus*[20] has been reported. However, the positivity rates and significance of these results have varied. We have summarized the findings from a number of previous studies regarding patch testing for house dust and mites in Table 5.

As shown in Table 5,[3,6,14,15,21–41] various types of mite antigens have been used in previous studies. No specific antigen for standardized testing has been used. A variety of mite antigens has been used, including mite scratch extract (Torii Pharmaceuticals),[42] *Dermatophagoides farinae* (Df) crude antigen,[4] *Dermatophagoides pteronyssinus* (Dp) extract antigen[21,22] and lyophilized mite antigen.[3] The concentrations of these preparations also varies. Thus, patch test results with specific mite antigens reported by one investigator often cannot be confirmed by other investigators, which implies that they have not been practical from a clinical standpoint. Therefore, the present study was undertaken using patch test reagents prepared from readily available allergen extracts in Japan.

Kligman[43] has described the use of sodium lauryl sulfate (SLS) in conventional patch testing to increase the transepidermal penetration of contact antigens, with an optimal SLS concentration of 10%. However, irritant reactions with 0.5% SLS have been reported.[24] A comparison study conducted by Yamamoto[44] of various preparations found that a concentration of 0.1% SLS was optimal for demonstration of mite contact antigens in AD patients, with a high positivity rate of 48%. We also compared different concentrations of SLS in 10 healthy volunteer control subjects. Although 0.2% SLS caused irritation, 0.1% SLS did not. Therefore, we used a concentration of 0.1% SLS in the present study.

The allergen extracts we used contained a final glycerin concentration of 25% (except for the house dust antigen). However, 25% glycerine was confirmed to have no irritant effect in the 10 healthy control subjects.

Many previous studies have reported pretreatment of patch test sites by stripping or sandpaper abrasion. However, such pretreatment can cause secretion of large amounts of cytokines from keratinocytes.[45] This may lower the irritation threshold. Thus, optimum reagent concentrations must be carefully evaluated in healthy control subjects. Moreover, the skin of AD patients is prone to irritation. Irritative stimuli that produce no inflammation in healthy subjects may produce inflammation in patients with AD.[3] Some previous reports have described a positive response in control subjects and some did not even mention the use of controls. Thus, many studies may not be able to exclude the possibility of irritation due to these other factors.

Considering potential problems, the protocol of the present study does not contain any pretreatment procedures such as stripping. We performed patch testing using reagents easily prepared from commercially available antigen extracts for diagnostic and therapeutic use. These were mixed with white Vaseline containing 0.1% SLS. Each antigen tested gave some positive responses. In addition, the positivity rates increased in proportion with the duration of antigen application. When patch testing was performed on the medial aspects of the upper arm and thigh in the same patients, the positivity rates on the thigh (highly keratinized) seemed to be less than that on the upper arm. Thus, we think the patch test results in AD patients are related to the degree of keratinization at the patch test site. Accordingly, our present data may

Table 5 Reports of mite patch tests

Reference	Allergen	Concentration	Base	Method of fixation	Pretreatment	Patch test site	Positive ratio (%)	Eligible AD patients
22	Antigen P1 extract from Dp	As is	Physiologic saline	Unknown	Stripping	Unknown	100	Severe AD
3	Lyophilized mite antigen solution	0.5%	Distilled water	Patch	None	Upper back	75	No restriction
21	Antigen P1 extracted from Dp	10 μg/mL	Physiologic saline	Steri gauze	None	Flexor aspect of forearm, lateral aspect of upper arm or medial aspect of scapula	100	AD diagnosed by positive intradermal Dp test
31	Dp	500 × prick test concentration	Distilled water, white Vaseline	Finn chamber	None	Back	35.3	No restriction
27	Unknown	0.5%	0.1% SLS + Pet	Unknown	None	Unknown	60	No restriction
32	Dp	100 × i.d. test concentration	Unknown	Unknown	Stripping	Back	80	No restriction
6	Df crude antigen	0.1%	0.1% SLS + Pet	Finn chamber	None	Back	67	No restriction
26	Df whole culture extract; Dp whole culture extract	0.1%	0.1% SLS + Pet	Finn chamber	None	Back	Df 65; Dp 70	No restriction
23	Frozen dust mite antigen	i.d. test concentration	Distilled water, white Vaseline	Finn chamber	None	Back	17.6	No restriction
28	Crushed live mites	As is	None	Finn chamber	None	Back	26	No restriction
28	Crushed dead mites	As is	None	Finn chamber	None	Back	0	No restriction
30	Mite body derived antigen	0.01, 0.1, 1.1 mg/mL	Physiologic saline	Finn chamber	Stripping	Medial aspect of upper arm	10	Dp RAST score ≧ 2
30	Mite feces-derived antigen	0.3, 0.6, 3.2 mg/mL	Pet	Finn chamber	Stripping	Medial aspect of upper arm	62.5	Dp RAST score ≧ 2
34	Dp antigen solution for prick test	0.2, 1, 5%	Unknown	Silver patch	Stripping	Back	29	Positive prick test for house dust mite and RAST score ≧ 2
35	House dust mite	12 500 units/ 25 μL	Unknown liquid	Unknown	Stripping	Back	66.7	Severe AD
37	Solution of proteins extracted from mites	0.008, 0.8, 0.8%	As is	Unknown	Stripping	Back	100	No restriction (however, only conducted in two patients)

Table 5 Cont.

Reference	Allergen	Concentration	Base	Method of fixation	Pretreatment	Patch test site	Positive ratio (%)	Eligible AD patients
42	Mite antigen extract	Stock solution and 10%	Unknown	Unknown	Stripping	Back	34	No restriction
36	Dp frozen extract	Unknown	Unknown	Finn chamber	None	Back	100	No restriction (however, only conducted in five patients)
23	Proteins extracted from Dp	0.8, 0.08 mg/mL	Unknown liquid	Finn chamber	None	Back	100	No restriction (however, only conducted in five patients)
38	Mite antigen	100 × i.d. test concentration	Unknown	Finn chamber	Stripping	Back	90	No restriction
39	Mite antigen	100, 400, 1600 AUR/mL	Glycerin solution, white Vaseline	Unknown	None	Back	20.8	No restriction
40	Live mites	1%	White Vaseline	Finn chamber	Stripping	No restriction for healthy subjects	77.8	Severe AD and high RAST score for mites
24	Lyophilized mite antigen	1000, 10 000 protein nitrogen units	White Vaseline containing 10% isopropyl myristate and methylcellulose hydrogel containing 10% propylene glycol	Finn chamber (including 0.5% SLS in test panel)	None	Back	47	Moderate–severe
41	Dp α fraction	40 000 AUR/mL	Buffered saline/ glycerol 50% petrolatum	Unknown	None	Unknown	25.15	No restriction
	Dp α fraction	20 AUR/mL	Petrolatum and petrolatum oil	Unknown	None	Unknown	54.48	No restriction
	Dp + Df	20%	Petrolatum and petrolatum oil	Unknown	None	Unknown	51.59	No restriction
	Dp	10%	Petrolatum and petrolatum oil	Unknown	None	Unknown	21.38	No restriction
	Df	10%	Petrolatum and petrolatum oil	Unknown	None	Unknown	24.56	No restriction
16	Frozen Dp	100 × i.d. test concentration	Distilled water containing 0.03% human albumin	Leucotest chambers	Stripping	Back	100	No restriction (however, only conducted in five patients)

Table 5 Cont.

Reference	Allergen	Concentration	Base	Method of fixation	Pretreatment	Patch test site	Positive ratio (%)	Eligible AD patients
25	Df antigen	1%	Pet	Finn chamber	Stripping	Back	67	Dp, Df RAST score ≧ 5
29	Crushed live mites	As is	None	Plastic disk	Partial abrasion with sandpaper	Back	35.6	No restriction
29	Mite secretions (citral)	2%	Pet	Finn chamber	Partial abrasion with sandpaper	Back	11.1	No restriction
29	Mite fatty acids (eicosapentaenoic acid)	As is	None	Mini plaster	Partial abrasion with sandpaper	Back	47.7	No restriction
15	House dust mite	1000 AU/mL	Unknown	Unknown	Stripping	Back	65.7	No restriction

P1, an extract from Dp; Dp, Df, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, respectively; SLS, sodium lauryl sulfate; Pet, petrolatum.

have included several false-negative results. The findings suggest that enhanced transepidermal antigen penetration may markedly increase the positivity rates. However, the previously mentioned pretreatment procedures (e.g. stripping or sandpaper abrasion of the horny layer) may cause inflammation due to factors other than an immunologic mechanism. This may taint the original purpose of patch testing. Our patch test method is designed to improve selectivity, albeit at the expense of some sensitivity.

The results of the present study show a correlation between the intensity of patch test responses and antigen-specific IgE levels. Specific IgE antibodies and antigens cause type I allergic reactions with a wheal response, which may be followed by a late phase reaction (LPR). In addition, the release of cytokines from mast cells elicits the involvement of inflammatory cells, such as eosinophils. Prolongation of this response can lead to clinical manifestations of dermatitis. In general, an LPR begins between 2 and several hours after intradermal antigen injection, reaches maximum intensity by 6–12 h and disappears after 24 h.[46] In contrast with antigens used in intradermal tests, the antigens used in patch tests require a much longer time to penetrate the stratum corneum and reach intradermal sites. These antigens gradually penetrate the epidermis over a 48 h period and activate IgE bound mast cells in the dermis. Subsequent degranulation, an LPR and dermatitis can prolong the observed response for up to 72 h. In addition to a correlation with specific IgE levels, the positive patch test responses in the present study also showed a correlation with total IgE, LDH and severity index. A positive patch test response in many cases of AD may represent a LPR following a type I allergic response. However, positive patch tests also develop in a small number of patients with low levels of antigen-specific IgE. Thus, the role of some other mechanism in addition to that of a type I allergic reaction cannot be excluded. Atopic dermatitis is caused or exaggerated by a variety of factors and has a variable clinical presentation. Similarly, patch testing in AD may also cause inflammation by more than one mechanism.

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