

Original article

Atopic dermatitis and allergic reactions to individual fragrance chemicals

Background: Allergic contact dermatitis prevalence is reported as equal in atopic and nonatopic dermatitis. Atopic dermatitis is under-represented in those with allergic contact dermatitis to agents having cutaneous and dietary exposure. We compared rates of atopic dermatitis between patients with allergic contact dermatitis arising out of individual fragrance chemicals with known oral/cutaneous exposure against exclusively cutaneous exposure.

Methods: Between 1982 and 2007, 37 065 dermatitis patients were tested with Fragrance mix I. Those who were positive were tested for individual fragrance allergy. Chemicals were categorized according to whether their exposure pattern was solely cutaneous, oral or mixed. Current and past atopic dermatitis rates were compared between the whole population and groups allergic to individual fragrances. Age and gender were controlled.

Results: Cinnamic alcohol and cinnamal allergy groups had reduced rates of both 'current' [24/266 (9.0%) $P = 0.0008$, 38/364 (10.4%) $P = 0.0005$] and 'past' atopic dermatitis [44/266 (16.5%) $P = 0.009$, 70/346 (19.2%) $P = 0.037$]. Atopic dermatitis rates in groups allergic to Evernia prunastri and hydroxycitronellal (cutaneous exposure only) were not reduced [120/597 (20.1%) and 41/153 (26.8%)]. Groups allergic to cinnamic alcohol ($P < 0.0001$, $P < 0.0001$) and cinnamal ($P < 0.0001$, $P < 0.004$) had reductions in 'current' and 'past' atopic dermatitis, compared with Evernia prunastri.

Conclusions: Patients allergic to individual fragrances with dietary exposure have reduced rates of atopic dermatitis. This suggests that patients with atopic dermatitis have heightened oral tolerance to dietary haptens, in contrast to the known close association of atopic dermatitis with food-protein allergy. Haptens may interfere with food protein tolerance by binding to soluble protein to alter its configuration and immunogenic profile.

**J. M. L. White¹, I. R. White¹,
I. Kimber², D. A. Basketter¹,
D. A. Buckley¹, J. P. McFadden¹**

¹Department of Cutaneous Allergy, St John's Institute of Dermatology, St Thomas' Hospital, London; ²Faculty of Life Sciences, University of Manchester, Manchester, UK

Key words: allergic contact dermatitis; atopic dermatitis; fragrance; hapten.

J. M. L. White
Department of Cutaneous Allergy
St John's Institute of Dermatology
St Thomas' Hospital
London SE1 7EH
UK

Accepted for publication 10 August 2008

Allergic contact dermatitis to contact allergens in the European baseline series has consistently been reported as being equally prevalent in atopic and nonatopic dermatitis patient groups (1, 2). We have also previously shown that there is no significant difference in the frequency of allergic contact dermatitis to Fragrance mix 1 (FM1) (The 'Larsen' mix) between atopic and nonatopic dermatitis patients (3). This mixture was originally devised in the 1970s and contains eight individual fragrance chemicals, which commonly cause allergic contact dermatitis, all at a concentration of 1%, with sorbitan sesquioleate added as an emulsifier. Although all these allergens have broadly similar skin exposure patterns from fragrance and toiletries, with less allergenic chemicals such as geraniol being used more frequently and at higher concentration (4), they do differ in the amount of dietary (gastrointestinal; GI) exposure. Cinnamic alcohol, cinnamal, eugenol and alpha-amyl cinnamic aldehyde are

present in foods in significant amounts while geraniol and isoeugenol are present in trace amounts only; Evernia prunastri and the synthetic fragrance chemical hydroxycitronellal are not known to be present in the diet (D.A. Basketter, personal communication).

Although type I, or immediate, allergy to food proteins is closely associated with atopy, it has been our experience that this does not hold true for contact allergy to haptens, which are also in the diet. Preliminary work by our group has analysed a number of different subgroups to see whether atopy rates are equal in subjects positive to haptens with no gut exposure, compared with haptens where there is both skin and gut. Cutaneous allergy to haptens having GI exposure, as well as cutaneous exposure (skin + GI+), e.g. the garlic hapten diallyl disulfide, is reduced in atopic dermatitis (5). This is in contrast with type I allergy to dietary proteins, which is usually seen in association with atopy (2).

To investigate whether this is a consistent observation, we have analysed the incidence of atopic dermatitis in subjects allergic to the individual fragrance chemicals which comprise FM1.

Methods

Between 1982 and 2007, 37 065 dermatitis patients, both atopic and nonatopic, attended the Department of Cutaneous Allergy at St John's Institute of Dermatology, London. In such of those patients who were FM1 patch-test positive, it has been standard practice to test subsequently for all the individual fragrances in FM1, all at 1% pet (Trolab[®]; Hermal, Reinbek, Germany). Allergens were applied to the skin on 8 mm Finn chambers[®] (Epitest Oy; Tuusula, Finland) under Scanpor[®] tape (Beiersdorf, Hamburg, Germany). Patch-test readings were performed at days 2/3 and 4/5, according to standard ICDRG criteria (6). A positive (+, ++, +++) patch-test reaction signified allergy. Wherever possible, patients who were allergic (patch-test positive) to FM1 were then patch-tested to the individual ingredients of the mix, all at 1% pet.

This was a retrospective, anonymized study of all patients testing positive to one of the individual ingredients of the FM1 series. A comprehensive database recorded demographic data on all patients tested. This includes age, gender, 'current' atopic dermatitis and a history of ('past') atopic dermatitis. Past atopic dermatitis was determined by clinicians taking a history of involvement of the flexures in childhood with pruritus. These features have subsequently been adopted as national consensus criteria for the diagnosis of atopic dermatitis (7), although this decision was taken some 16 years after our database began. These criteria have a sensitivity of 80%, specificity of 97% and positive predictive value of 80% for a 1-year prevalence. Current atopic dermatitis status was determined by clinical examination using the standard criteria of Hanifin and Rajka (7).

Data entry limitations on our database mean that retrospective discrimination between +, ++ or +++ reactions and therefore any analysis of the strength of patch reaction (or indeed differential reactions between days 2/3 and 4/5) was not possible.

Individual fragrances were categorized according to their degree of GI exposure; cinnamal, cinnamic alcohol and eugenol were considered to have significant GI exposure ('skin + GI+'), isoeugenol and geraniol as having 'trace amounts' ('skin + GItrace') and Evernia prunastri and hydroxycitronellal are not known to be present in the diet to any significant degree (skin + GI-).

In our patch-test population, there was a marked variation of 'current' and 'past' atopic dermatitis in relation to age and gender (Figs 1 and 2). To control this, the frequency of observed 'current' atopic dermatitis or 'past' atopic dermatitis was categorized for the entire patch-test population of 37 065 in relation to age (individual year) and gender. Using this data, we were then able to calculate the predicted frequency of current and past atopic dermatitis for each group of subjects positive to an individual fragrance on the basis of their age and gender profile, which could then be compared with the observed frequency of current and past atopic dermatitis for that group. Statistical analysis was performed with the chi-squared test for observed and predicted values, the null hypothesis being that the observed numbers from a population equate with the expected frequencies $[(O - E)^2/E]$.

Positive reactions to alpha-amyl cinnamal were too few for meaningful analysis, and were hence excluded from the results.

For comparison of atopic dermatitis, frequencies between skin + GI+ (cinnamal, cinnamic alcohol, eugenol) and Evernia prunastri (skin + GI-), the frequency of current and past atopic

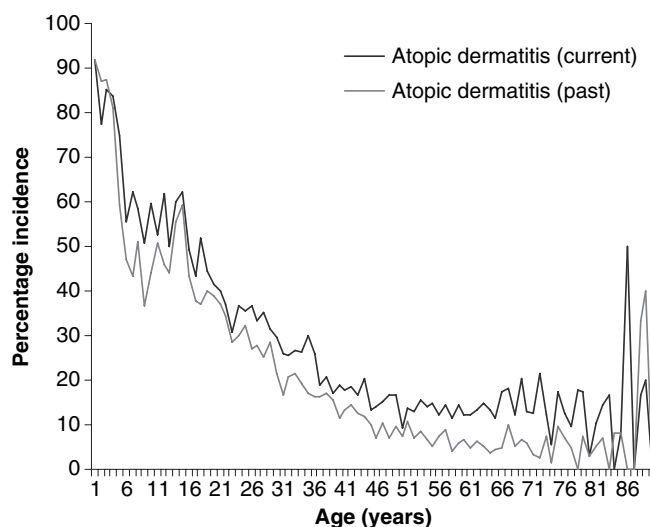


Figure 1. The frequency of both current and past atopic dermatitis decreases with age. There may be some recall bias at higher ages when considering past atopic dermatitis. Variability at higher ages is probably because of lower overall numbers.

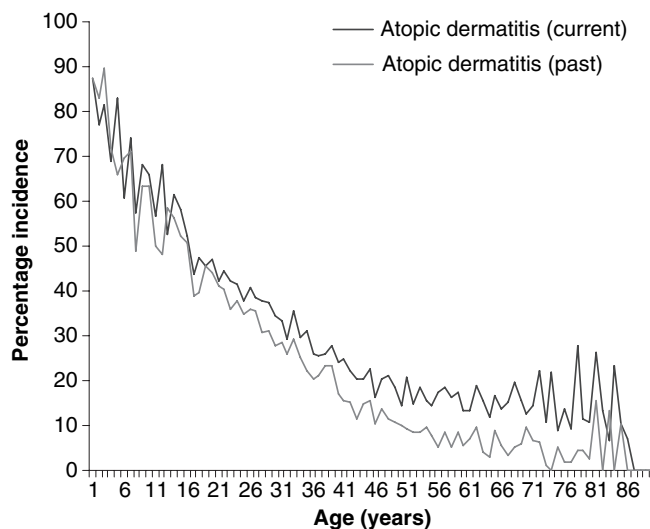


Figure 2. Data from female subjects is similar to that from males; however, higher overall numbers at all ages reduces variability and yields a smoother curve than that seen in males.

dermatitis for the population patch-test positive to Evernia prunastri was categorized with regard to age (by year) and gender in the same way as with the entire patch-test population. Subjects who were allergic to both a 'skin + GI+' and 'skin + GI-' fragrance chemical were excluded from this analysis.

Results

MOAHLFA (male occupation atopic dermatitis, hand, leg, face involvement) (8) for all groups are given in

Table 1 (see Tables 2 and 3). The association between fragrance and hand dermatitis has previously been described (9). Of the 37 065 subjects attending the clinic, 8320 (22.4%) had ‘current’ atopic dermatitis and 10 506 (28.3%) had ‘past’ atopic dermatitis.

A breakdown of results to the individual components of FMI is shown in Fig. 2. In comparison with the total patch-test population (Table 3), subjects patch-test positive to the ‘skin + GI+’ chemicals cinnamic alcohol and cinnamal had reduced frequencies of current atopic dermatitis (24/266 9.0% $P = 0.0008$, 38/364 10.4%

$P = 0.0005$), but the reduced frequency observed in eugenol patch-test positive subjects did not reach statistical significance. There was no significant difference in frequency rates of current atopic dermatitis between the total patch-test population and the ‘skin + GI–’ fragrance chemicals Evernia prunastri and hydroxycitronellal (120/597 20.1% $P = \text{NS}$, 41/153 26.8% $P = \text{NS}$).

With respect to the frequency of ‘past’ atopic dermatitis, there were again significantly reduced levels in subjects allergic to cinnamic alcohol and cinnamal (44/266, or 16.5%, $P = 0.009$, 70/364, or 19.2%, $P = 0.037$)

Table 1. Population patterns of patch-test positive (cutaneous allergy) to single fragrance chemicals

	Total patch-test pop. (%)	Cinnamal PT+ (%)	Cinnamic alcohol PT+ (%)	Eugenol PT+ (%)	Isoeugenol PT+ (%)	Evernia prunastri PT+ (%)	Hydroxycitronellal PT+ (%)	Geraniol PT+ (%)
Exposure pattern		Skin+GI+	Skin+GI+	Skin+GI+	Skin+GI+trace	Skin+GI–	Skin+GI–	Skin+GI+trace
Number	37 065	364	266	225	357	597	153	89
Male	14711 (39.7)	128 (35.2)	88 (33.08)	103 (45.8)	109 (30.53)	199 (33.3)	38 (24.8)	36 (40.45)
Occupational case?	17%*	52 (14.3)	49 (18.42)	36 (16.0)	54 (15.13)	91 (15.2)	20 (13.07)	13 (14.61)
AD current	8320 (22.4)	38 (10.4)	24 (9.02)	30 (13.3)	77 (21.58)	120 (20.1)	41 (26.80)	15 (16.85)
AD past	10 506 (28.3)	67 (19.2)	44 (16.54)	53 (23.4)	106 (29.69)	165 (27.6)	49 (32.03)	22 (24.72)
Hand	10 087 (27.2)	121 (33.2)	88 (33.08)	82 (36.4)	105 (29.41)	136 (22.8)	38 (24.84)	22 (24.72)
Leg	1482 (4.0)	12 (3.3)	8 (3.00)	8 (3.6)	14 (3.92)	22 (3.7)	5 (3.27)	8 (8.99)
Face	7756 (20.9)	88 (24.2)	59 (22.18)	43 (19.1)	73 (20.45)	139 (23.3)	31 (20.26)	16 (17.98)
Age > 40 years	15 692 (42.3)	206 (56.6)	149 (56.02)	127 (56.4)	161 (45.10)	333 (55.8)	58 (37.91)	51 (57.30)

Population characteristics of all eczema patients attending the Department of Cutaneous Allergy, St John’s Institute are given in first column. ‘Hand’ ‘leg’ ‘face’ refer to involvement patterns of eczema patients.

GI+, significant exposure through gastrointestinal tract; ‘GI+trace’, only low-level exposure; ‘GI–’, negative or minimal exposure; AD, atopic dermatitis.

*Estimate of occupational cases only.

Table 2. Frequency of ‘current’ and ‘past’ atopic dermatitis in subjects allergic to individual fragrance chemicals

Fragrance	Patch-test +ve, <i>n</i>	Exposure pattern	Atopic dermatitis current observed (%)	Atopic dermatitis current expected (%)	<i>P</i> -value (95% CI)	Atopic dermatitis past observed (%)	Atopic dermatitis past expected (%)	<i>P</i> -value (95% CI)
Cinnamic alcohol	266	Skin+GI+	24 (9.0)	44.4 (16.7)	0.0008 (0.06–0.13)	44 (16.5)	61.9 (23.3)	0.009 (0.13–0.21)
Cinnamal	364	Skin+GI+	38 (10.4)	64.1 (17.9)	0.0005 (0.07–0.14)	70 (19.2)	87 (23.9)	0.037 (0.16–0.24)
Eugenol	225	Skin+GI+	30 (13.3)	40.3 (17.9)	0.073 NS	53 (23.5)	53 (23.5)	NS
Isoeugenol	357	Skin+GI+trace	77 (21.6)	76.9 (21.5)	NS	106 (29.7)	96.9 (27.2)	NS
Geraniol	89	Skin+GI+trace	15 (16.9)	14.9 (16.7)	NS	22 (24.7)	21.5 (24.2)	NS
Hydroxycitronellal	153	Skin+GI–	41 (26.8)	33.7 (22.0)	NS	49 (32.0)	42.1 (27.5)	NS
Evernia prunastri	597	Skin+GI–	120 (20.1)	104.0 (17.4)	NS	165 (27.6)	143.9 (24.12)	Raised! 0.045 (0.24–0.31)

Table 3. Comparison of frequency of atopic dermatitis in subjects allergic to individual skin+GI– fragrance chemical (Evernia prunastri) vs skin+GI+ (cinnamic alcohol, cinnamal and eugenol) fragrance chemicals

Fragrance	Patch-test +ve, <i>n</i>	Exposure pattern	Atopic dermatitis current observed (%)	Atopic dermatitis current expected (%)	<i>P</i> -value	Atopic dermatitis past observed (%)	Atopic dermatitis past expected (%)	<i>P</i> -value
Evernia prunastri	501	Skin+GI–	107 (21.4)			141 (28.2)		
Cinnamic alcohol	231	Skin+GI+	18 (7.8)	48.4 (21.0)	< 0.0001	34 (14.7)	65.2 (28.2)	<0.0001
Cinnamal	317	Skin+GI+	31 (9.8)	69.9 (22.1)	< 0.0001	60 (18.9)	88.1 (27.8)	<0.004
Eugenol	181	Skin+GI+	23 (12.7)	41.9 (23.2)	0.0008	41 (22.7)	51.3 (28.3)	0.088 NS

Individuals allergic to both skin+GI+ and skin+GI– fragrance chemical excluded from analysis.

AD, atopic dermatitis.

but no significant reduction was seen with eugenol. With hydroxycitronellal, there was no significant difference in the frequency of rates of 'past' atopic dermatitis compared with the whole patch-test population; the rates for *Evernia prunastri* were moderately raised in comparison with expected values (165/597 (27.6%) $P = 0.045$).

A direct comparison of frequency of atopic dermatitis between the group patch-test positive to *Evernia prunastri* ('skin+GI-') and groups allergic to 'skin+GI+' fragrance chemicals, cinnamic alcohol, cinnamal and eugenol showed reduced frequencies of 'current' atopic dermatitis to a highly significant degree (18/231, or 7.8%, $P < 0.0001$, 31/317, or 9.8%, $P < 0.0001$, 23/181, or 12.7%, $P = 0.0008$, respectively). With respect to frequency of 'past' atopic dermatitis, cinnamic alcohol and cinnamal allergic groups again showed reduced frequencies to a highly significant degree (34/231, or 14.7%, $P < 0.0001$, 60/317, or 18.9%, $P < 0.004$, respectively). The reduced frequency of 'past' atopic dermatitis observed in the eugenol allergic group did not reach significance (41/181, or 22.7%, $P = 0.088$).

Discussion

Contact allergens are low molecular weight haptens, which must form stable associations with proteins in order to be able to trigger cutaneous immune responses and therefore sensitization. Contact allergens embrace a wide range of chemistry and include metals, fragrances, preservatives, plant products, medicaments and resins.

Cinnamal and cinnamic alcohol are used as flavoring agents as well as in perfumes. They are present in some beverages, cakes, chewing gum, mouth wash and toothpastes. Eugenol is contained in spices and cloves, and is used in some toothpastes. Geraniol can be found in trace amounts in some fruits such as in lemon peel (10) and candy. Isoeugenol is present in foods at smaller amounts as compared with eugenol, though it is contained in nutmeg oil (11). Neither *Evernia prunastri* nor hydroxycitronellal are usually present in foods.

This study shows a significant reduction in the rate of 'current' atopic dermatitis in the group of patients allergic to skin+GI+ contact allergens/haptens when compared with the group allergic to skin+GI- contact allergens/haptens. As suggested in a FM1/atopy paper recently accepted for publication by *Contact Dermatitis*, to interpret the data better, the 'opposite way round' analysis might be preferable; in other words look at all atopics patch tested between 1982 and 2007 *vs* all nonatopics patch tested during the same period, to evaluate the rate of allergy to, for example, cinnamal in atopics *vs* nonatopics and compare that with, say, *Evernia prunastri* in atopics and nonatopics; in that event, we could categorically say that atopics in our patch-test population are less likely to be allergic to

cinnamal and a little more likely to be allergic to *Evernia prunastri*. There is also a smaller, significant reduction in the rate of 'past' atopic dermatitis. The findings here are also consistent with our recent study showing a negative association between contact allergy to the garlic hapten diallyl disulfide, in caterers and home cooks, and atopic dermatitis (5). This negative association of atopic dermatitis with allergy to haptens with GI exposure is in direct contrast to the strong association between food-protein hypersensitivity and atopic dermatitis (2). In a retrospective study, one must always consider confounding factors, such as differences in the use of skin care products by subjects with chronic skin disease (as in atopic dermatitis). However, in comparing individual fragrances, it is difficult to explain these highly significant differences as being caused by behavioral differences. If atopics were avoiding fragrances, they would probably avoid both skin+GI+ and skin+GI- fragrances. The most likely explanation, therefore, is that atopic dermatitis patients more effectively tolerate haptens through GI exposure than nonatopic dermatitis patients. This is in complete contrast to food glycoproteins, where allergy is strongly associated with atopy. This heightened tolerance to GI haptens could be a secondary manifestation of the atopic state. An alternative explanation is that oral tolerance of haptens is contributing to the atopic state (the hapten-atopy hypothesis).

Of all the body surfaces interacting with the environment, the GI tract has the largest amount of associated lymphoid tissue and also the biggest surface area (12). Interactions with foreign proteins/haptens in the GI tract have a tolerogenic influence on interaction elsewhere such as in the skin (13, 14). Whilst most food protein allergens are water-soluble glycoproteins ranging from 10–17 kDa in size (15), haptens are much smaller, being < 800 Da. Oral tolerance to food proteins and haptens have similarities, such as the use of T-regulatory cells (16, 17), though not necessarily the same subsets, and involvement of cytokines such as TGF- β (18). There are some data to suggest that Peyer's patches are required for oral tolerance to food proteins, but not for haptens (19). It is therefore feasible (but not proven) that oral tolerance to food proteins and haptens may be either antagonistic or competitive. However, haptens, by their very nature, possess the ability to alter configuration and immunogenic profile of proteins and peptides. Therefore, dietary hapten may bind to soluble food protein and alter the protein's immunogenicity. On the one hand, the presence of these haptens renders the food proteins more allergenic by binding to them; on the other hand, the same haptens may induce self-tolerance. There is emerging evidence that fragrance haptens do bind to milk protein, thus changing the chemical properties of the fragrance ingredient (20). It may also be that, conversely, the hapten-protein complexes alter recognition of proteins by the immune system, altering the balance between allergy and tolerance.

Over the last 50 years, there has been a marked increase in atopic disease. This increase has coincided with use of processed food and milk in the Western diet and use of oral antibiotics. Processed food has artificial chemical/hapten content with preservatives, antioxidants, flavorings and colorants. A recent survey found, on average, 10 haptens contained in formula milk (21). Immune recognition of antibiotics is generally thought to be through their haptenic properties (22, 23). Oral antibiotics may therefore increase the chemical/hapten load recognized by the GI immune system. There has also been artificial cutaneous exposure to haptens/contact

allergens in skin care products. It is of interest to note that animal models of atopic dermatitis are based on repeated cutaneous hapten exposure (24, 25).

We speculate that hapten exposure through the GI tract in the form of formula milk, antibiotics and food additives, through the skin in the form of skin care products and through respiratory tract in the form of airborne chemicals, has the potential to interfere with any epithelial tolerance mechanisms to self-proteins and peptides, which may predispose to atopy/protein allergy while at the same time the haptens may induce self-tolerance.

References

1. Cronin E, McFadden JP. Patients with atopic dermatitis do become sensitized to contact allergens. *Contact Derm* 1993;**28**:225–228.
2. Schäfer T, Böhler E, Ruhdorfer S, Weigl L, Wessner D, Heinrich J et al. Epidemiology of food allergy intolerance in adults; associations with other manifestations of atopy. *Allergy* 2001;**56**:1172–1179.
3. Buckley DA, Basketter DA, Denis K-KY, White IR, White JML, McFadden JP. Atopy and contact allergy to fragrance: allergic reactions to the Fragrance Mix 1 (The Larsen mix). *Contact Derm* 2008.
4. Buckley DA. Fragrance ingredient labelling in products on sale in the U.K. *Br J Dermatol* 2007;**157**:295–300.
5. McFadden JP, White JML, Basketter DA, Kimber I. Reduced allergy rates in atopic eczema to contact allergens used in both skin products and foods; atopy and the 'hapten-atopy' hypothesis. *Contact Derm* 2008;**58**:156–158.
6. Wahlberg JE, Lindburg M. Patch testing. In: Frosch PJ, Menné T, Lepoittevin J-P, editors. *Contact Dermatitis*, Chapter 22, 4th edn. Berlin: Springer, 2006:365–390.
7. Williams HC. Diagnostic criteria for atopic dermatitis. *Lancet* 1996;**348**:1391–1392.
8. Schnuch A, Geier J, Uter W, Frosch PJ, Lehmacher W, Aberer W et al. National rates and differences in sensitization to allergens of the standard series. *Contact Derm* 1997;**37**:200–209.
9. Buckley DA, Rycroft RJ, White IR, McFadden JP. Contact allergy to the fragrance mix constituents in relation to primary site of dermatitis. *Contact Derm* 2000;**43**:304–305.
10. Hausen BM, Kulankamp D. Geraniol contact allergy. *Z Hautkr* 1990;**65**:492–494.
11. Janssens J, Laekeman GM, Pieters LA, Totte J, Herman AG, Vlietinck AJ. Nutmeg oil: identification and quantification of its most active constituents as inhibitors of platelet aggregation. *J Ethnopharmacol* 1990;**29**:179–188.
12. Moog F. The finding of the small intestine. *Sci Am* 1981;**245**:116–125.
13. van Hoogstraten IM, Boos C, Boden D, von Blomberg ME, Schepers RJ, Kraal G. Oral induction of tolerance to nickel sensitization. *J Invest Dermatol* 1993;**101**:26–31.
14. Kahn G, Phanuphak P, Claman HN. Propyl gallate – contact sensitization and orally-induced tolerance. *Arch Dermatol* 1974;**109**:506–509.
15. van Wijk F, Knippels L. Initiating mechanisms of food allergy: oral tolerance versus allergic sensitization. *Bio-med Pharmacotherapy* 2007;**61**:8–20.
16. Brandtzaeg P. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci* 2002;**964**:13–45.
17. Dubois B, Chapat L, Goubier A, Papiernik M, Nicolas JF, Kaiserlian D. Innate CD4+ CD25+ regulatory T cells are required for oral tolerance and inhibition of CD8+ T cells mediating skin inflammation. *Blood* 2003;**102**:3295–3301.
18. Faria AM, Weiner HL. Oral tolerance. *Immunol Rev* 2005;**206**:232–259.
19. Fujihashi K, Dohi T, Rennert PD, Yamamoto M, Koga T, Kiyono H et al. Peyer's patches are required for oral tolerance to proteins. *Proc Natl Acad Sci USA* 2001;**98**:3310–3315.
20. Hansen AP, Heinis JJ. Benzaldehyde, citral and *d*-Limonene flavor perception in the presence of casein and whey proteins. *J Dairy Sci* 1992;**75**:1211–1215.
21. White JML, McFadden JP. Contact allergens in food ingredients and additives: atopy and the 'hapten-atopy hypothesis'. *Contact Derm* 2008;**58**:245–246.
22. Bondaruk J, Curcio-Vonlanthen V, Schneider CH. Basic aspects related to penicillin-allergy skin testing: on the variability of the hapten–paratope interaction. *Allergy* 1995;**50**:671–676.
23. Spinks CA, Wyatt GM, Lee HA, Morgan MR. Molecular modelling of hapten structure and relevance to broad specific immunoassay of sulfonamide antibiotics. *Bioconjug Chem* 1999;**10**:583–588.
24. Man MQ, Hatano Y, Lee SH, Man M, Chang S, Feingold KR et al. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008;**128**:79–86.
25. Matsumoto K, Mizukoshi K, Oyobikawa M, Ohshima H, Tagami H. Establishment of an atopic dermatitis-like model in a hairless mouse by repeated elicitation of contact hypersensitivity that enables to contrast functional analysis of the stratum corneum with various non-invasive biological instruments. *Int Arch Allergy Immunol* 2004;**133**:389–397.