

Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization

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Summary

Background Food allergies are an important cause of life-threatening hypersensitivity reactions. Oral tolerance can be considered the default immune response to dietary antigens, with immune deviation resulting in allergic sensitization. However, primary sensitization to food allergens may not solely be through the gastrointestinal mucosa, as strong T-helper type 2 (Th2)-biased immunity can result from exposure to protein allergens on barrier-disrupted skin.

Objective The purpose of this study was to examine whether exposure to allergens through the skin may interfere with the normal development of oral tolerance and promote allergic sensitization to food proteins.

Methods Female BALB/c mice were exposed epicutaneously to peanut protein and induction of systemic oral tolerance through high dose feeds of peanut protein was subsequently assessed. Other mice were rendered tolerant prior to epicutaneous peanut exposure. Sensitivity to peanut was determined by assessing delayed-type hypersensitivity, proliferative, cytokine and antibody responses.

Results Epicutaneous exposure to peanut protein induced potent Th2-type immunity with high levels of IL-4 and serum IgE. Primary skin exposure prevented the subsequent induction of oral tolerance to peanut in an antigen-specific manner. Upon oral challenge, mice became further sensitized and developed strong peanut-specific IL-4 and IgE responses. Furthermore, animals with existing tolerance to peanut were partly sensitized following epicutaneous exposure.

Conclusion Epicutaneous exposure to peanut protein can prevent induction of oral tolerance, and may even modify existing tolerance to peanut. Epidermal exposure to protein allergens selectively drives Th2-type responses, and as such may promote sensitization to food proteins upon gastrointestinal exposure.

Keywords epicutaneous sensitization, food allergy, IgE, oral tolerance, peanut, skin

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Introduction

Food allergy is a group of disorders characterized by adverse immune and hypersensitivity reactions to food proteins. Food-induced allergic reactions is an important problem in western countries and has, in common with other atopic diseases, significantly increased in prevalence during the last two to three decades (reviewed in [1]). The key feature of the disease is a T-helper type 2 (Th2)-predominant allergen-specific immune response, with the production of IgE antibodies specific for the food allergen. The aetiology of food allergy remains unclear; a failure to develop, or a breakdown in the maintenance of, oral tolerance (orally induced systemic hyporesponsiveness) may be responsible.

Patients may also be sensitized via other routes than the immune system of the gastrointestinal tract and the gut-associated lymphoid tissue (GALT).

Clinically, food allergy is closely associated with eczema. Both diseases show systemic allergic responses and are associated with specific IgE antibody that can passively sensitize mast cells, basophils and dendritic cells throughout the body. Eczema usually predates the development of other atopic diseases such as food allergy, asthma and allergic rhinitis (AR) by several years [2, 3]. Impaired function of the epidermal skin barrier is characteristic of eczema, and is seen even in areas of the skin without lesions [4, 5]. Considering the epidermal permeability dysfunction in eczema and other dermatoses, primary sensitization to allergens through the skin is a possibility. A cohort study has recently suggested that the use of peanut-containing creams was more common in children who subsequently became allergic to peanut than in control infants [6], implying that some sensitization to peanut may have occurred through the skin. It has also been

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suggested that food allergy and eczema may just represent distinct organ manifestations of a common systemic allergic response [7]. It may be that a common sensitization pathway, such as resulting from epicutaneous exposure, exists for both diseases.

Peanuts consist of particularly potent allergens and the prevalence of peanut allergy is rising in both children and adults, making it one of the most common food allergies in the UK and the US [8–10]. Most (~95%) peanut-allergic individuals suffer from other atopic diseases, eczema being particularly prevalent. Eighty percent of these subjects react to their first known oral exposure to peanut, implying some prior occult sensitization [11]. This primary sensitization may in some instances be acquired via skin contact. Experimental evidence in mice has demonstrated unequivocally that primary sensitization to proteins can occur through the skin [12–16]. We have recently shown that epicutaneous exposure to peanut protein causes primary sensitization, but that systemic sensitization is primarily caused by exposure on barrier-disrupted skin. Disruption of the outmost skin layer – the stratum corneum – followed by epicutaneous exposure to peanut protein results in potent Th2-biased immunity with large amounts of peanut-specific IgE and IL-4 [12].

Skin manifestations are the most frequent complication associated with food allergy [1, 17], but little is known of whether sensitization through the skin can affect mucosal immunity in the GALT. Epicutaneous sensitization has been found to affect systemic immunity in another mucosal organ, by priming mice to develop airway hypersensitivity. Prolonged epicutaneous exposure to chicken egg ovalbumin (OVA) under occlusion followed by inhalation of OVA elicited a systemic allergic response and hyper-responsiveness to methacholine [14]. Of crucial relevance, it was recently reported that similar prolonged epicutaneous exposure to OVA-induced histological changes in the intestine upon oral challenge [18].

In the present study, the effects of epicutaneous exposure to peanut protein on systemic and mucosal responses were analysed. The influence of epicutaneous peanut exposure on the induction and maintenance of oral tolerance was explored. We report that epicutaneous exposure to peanut protein on barrier-disrupted skin prevents the normal induction of oral tolerance and further enhances Th2-type responses and IgE production upon gastrointestinal exposure. Epicutaneous exposure to peanut protein may additionally modify existing tolerance. The novel experimental system presented here using epicutaneous sensitization and oral challenge may be considered a physiologically relevant model for food-induced sensitization.

Methods

Mice

BALB/c mice were bred and maintained on a special diet free of peanut, OVA, soy and cows milk (the diet made and supplied by Harlan Ltd, Bicester, UK). They were kept under specific pathogen-free conditions and provided water *ad libitum*. Female mice aged 6–8 weeks were used in this study

in accordance with Home Office regulations under the Animals (Scientific Procedures) Act 1986.

Peanut protein

Partly de-fatted peanut flour was obtained from the Golden Peanut Company (Alpharetta, GA, USA). A concentrated peanut protein extract was prepared using a modification of the method described by de Jong et al. [19]. In brief, the flour was de-fatted with hexane five times and then extracted with 0.1 M NH_4HCO_3 . The supernatant was removed and precipitated with 60% $(\text{NH}_4)_2\text{SO}_4$ for 2 h at 4 °C. The precipitate was centrifuged, dissolved in a minimum volume of phosphate-buffered saline (PBS) and dialysed extensively against PBS. This method uniformly yielded 85–90% pure peanut protein. Peanut protein was biotinylated using standard methods [20].

Epicutaneous sensitization and oral challenge

The stratum corneum was removed from both sides of the earlobe by application and removal of cellophane tape (Scotch™ (3M, Cergy-Pontoise Cedex, France)) five to eight times. Twenty-four hours later, 25 µL of peanut protein in PBS (4 mg/mL) were applied to both sides of the earlobe with a cotton bud. The application of peanut protein to the skin was repeated on the next 2 consecutive days. An estimated maximum of 100 µg of protein was deposited on the ear by this technique. Antigen applied to intact skin that had not been tape stripped and PBS without antigen applied to stripped skin and intact skin were included as controls. At 6 or 20 days after the initial epicutaneous immunization the mice were killed and the draining cervical lymph nodes (CLN), spleen and serum collected.

Six or 20 days after the epicutaneous exposure animals were fed a tolerogenic dose of peanut (100 mg peanut protein as described previously [21]) by intragastric gavage. Naïve mice were fed PBS as controls. One week after the gavage, all animals were tail-base immunized with 100 µg peanut protein emulsified 1 : 1 in complete Freund's adjuvant (CFA). After 3 weeks mice were challenged with 100 µg peanut in the footpad and the delayed-type hypersensitivity (DTH) responses measured. Seven days later para-aortic lymph nodes (PLN), spleen and serum were collected.

Alternatively, animals were fed a tolerogenic dose of peanut protein (100 mg) or sham-fed PBS by gavage 1 week prior to epicutaneous sensitization. Twenty days later CLN, spleen and serum were collected and analysed.

Tables indicating the order of experimental procedures in different *in vivo* experiments are provided with the figures. Between six and 10 animals were used per group in all experiments ($n \geq 6$).

Delayed-type hypersensitivity response

To elicit a DTH response, mice were challenged 20 days after the last epicutaneous or subcutaneous immunization by an injection of 100 µg peanut protein in PBS into the left hind footpad. Net footpad swelling was measured using a microcalliper (Mitutoyo, Siwa, Japan) 24 h after challenge. Mice were killed 1 week after the 24 h footpad measurement.

T cell proliferation and cytokine production

Spleen and lymph node cell suspensions were obtained by mechanical disaggregation, and 2×10^5 cells cultured in 96-well flat-bottom plates in a total volume of 200 μ L RPMI 1640 medium supplemented with 10% FCS, 50 μ M 2-ME and 5 μ g/mL gentamycin. Peanut protein was added at concentrations ranging from 5 to 450 μ g/mL. Control responses to a control antigen (OVA) or Concanavalin A (ConA) at 1 μ g/mL were also determined. Cultures were incubated at 37 °C for 90 h and pulsed with 1 μ Ci of [^{3} H]-thymidine (Amersham Pharmacia, Little Chalfont, UK) for the last 18 h. Cells were harvested and thymidine incorporation determined by liquid scintillation counting on a MicroBeta (Wallac, Turku, Finland). Supernatants were assayed by ELISA for IL-4, IL-10, IFN- γ and transforming growth factor- β (TGF- β) using antibodies from PharMingen (San Diego, CA, USA), according to the manufacturer's protocol. Recombinant mouse IL-4, IL-10 and IFN- γ and recombinant human TGF- β_1 from PharMingen were used as standards. The detection limit of the assays was 5 pg/mL for IL-4 and 40 pg/mL for IL-10, IFN- γ and TGF- β .

Antibody responses

At the end of each experiment, mice were bled by cardiac puncture and sera prepared for specific antibody determinations. For IgG and IgG1/2a antibodies, 96-well Maxisorb plates (Nunc, Roskilde, Denmark) were coated with peanut protein at 250 μ g/mL in carbonate bicarbonate buffer (CBB) at 4 °C overnight. Appropriately diluted sera were added (100 μ L in PBS) and the plates incubated at 37 °C for 90 min. After washing, alkaline phosphatase-conjugated polyclonal goat anti-mouse IgG Fc (Sigma, Gillingham, UK), rat monoclonal antibody to mouse IgG1 (Zymed, San Francisco, CA, USA) or rat monoclonal antibody to IgG2a (PharMingen) was added for 1 h at 37 °C. The alkaline phosphatase substrate pNPP (Sigma) was then added and absorbance measured at 405 nm. Antigen-specific IgE was measured by an IgE capture method. Sera to be tested were added to Maxisorb microtitre plate wells coated with 1 μ g/mL of rat monoclonal anti-mouse IgE (PharMingen). Biotinylated peanut was then added at a concentration of 10 μ g/mL and incubated for 2 h at 37 °C. After washing, alkaline phosphatase streptavidin (PharMingen) was added for 1 h followed by pNPP substrate.

Statistical evaluation

The statistical significance of differences between experimental groups with regard to DTH responses and serum antibody levels was determined using two-tailed Student's *t*-test for unpaired data. Statistical significance in T cell proliferation between experimental groups over the entire dose-response curve was determined by a two-tailed *t*-test on the slope of straight-line fits to the data, equivalent to a one-way ANOVA on two groups. If the slopes were not significantly different, the intercept was also compared using a two-tailed *t*-test and was equivalent to an analysis of covariance on two groups [22]. Differences were regarded as significant when $P < 0.05$.

Results

Epicutaneous exposure to peanut protein induces potent systemic T-helper type 2-biased immunity

Administration of peanut proteins on barrier-disrupted skin (24 h following removal of the stratum corneum by tape stripping) induced dose-dependent peanut-specific T cell proliferation *in vitro* both at 6 and 20 days after the epicutaneous exposure (Figs 1a and c). Strong proliferation of T cells from both CLN and spleens was detected in response to peanut protein, but no proliferation to control antigen. At 6 days after the epicutaneous exposure, cytokine responses in the draining CLN were mixed Th1 and Th2 with relatively high levels of IFN- γ (~ 3500 pg/mL) and with IL-4 levels (~ 35 pg/mL) well above the detection limit. After 20 days, Th2 responses were dominating with IFN- γ secretion declining (< 600 pg/mL) while IL-4 levels were rising (~ 185 pg/mL) (Figs 1a and c). A similar pattern of proliferation and cytokine responses was observed with splenic T cells (results not shown). Additionally, the epicutaneous exposure to peanut protein induced high levels of peanut-specific IgG1 and IgE ($P < 0.0001$) in all animals, but little or no IgG2a. This antibody pattern was evident after 20 days and is characteristic of a Th2 response (Figs 1b and d).

Epicutaneous peanut exposure 6 days prior to a tolerogenic feed modifies oral tolerance and specifically prevents suppression of T-helper type 2 immunity

Feeding 100 mg peanut protein to naïve animals induces oral tolerance as demonstrated by suppression of both cellular and humoral responses [21]. Peanut-specific DTH responses, T cell proliferation, secretion of IFN- γ , IL-4, IL-10 and production of both specific IgG and IgE antibodies are abrogated or reduced, while TGF- β production is enhanced, in response to a secondary peanut challenge, compared with saline-fed controls (Figs 2 and 3: SPPP compared with -SPP and detailed in [21]). Feeding a tolerogenic dose of peanut protein 6 days after epicutaneous exposure to peanut still induced specific hypoproliferative T cell responses as demonstrated by suppressed DTH responses and *in vitro* T cell proliferation to peanut protein (Figs 2a and b, PPPP). IFN- γ and IL-10 production from draining PLN cells was also suppressed. In contrast, IL-4 was markedly enhanced compared with tolerant animals epicutaneously exposed to saline prior to the tolerogenic feed. IL-4 was even higher than saline-fed peanut-sensitive controls, while TGF- β was lower than that in tolerant control animals (Fig. 2c). This apparent specific loss of Th2-type tolerance was strikingly evident when peanut-specific antibodies were examined. While levels of peanut IgG and IgG2a antibodies were still significantly suppressed, IgG1 and especially IgE were significantly higher than in tolerant controls (Fig. 2d). Animals exposed to peanut protein on barrier-disrupted skin 6 days prior to a tolerogenic feed not only failed to suppress IgE responses, but produced levels of peanut-specific IgE significantly higher than saline-fed peanut-sensitive controls ($P = 0.0001$) (Fig. 2d).

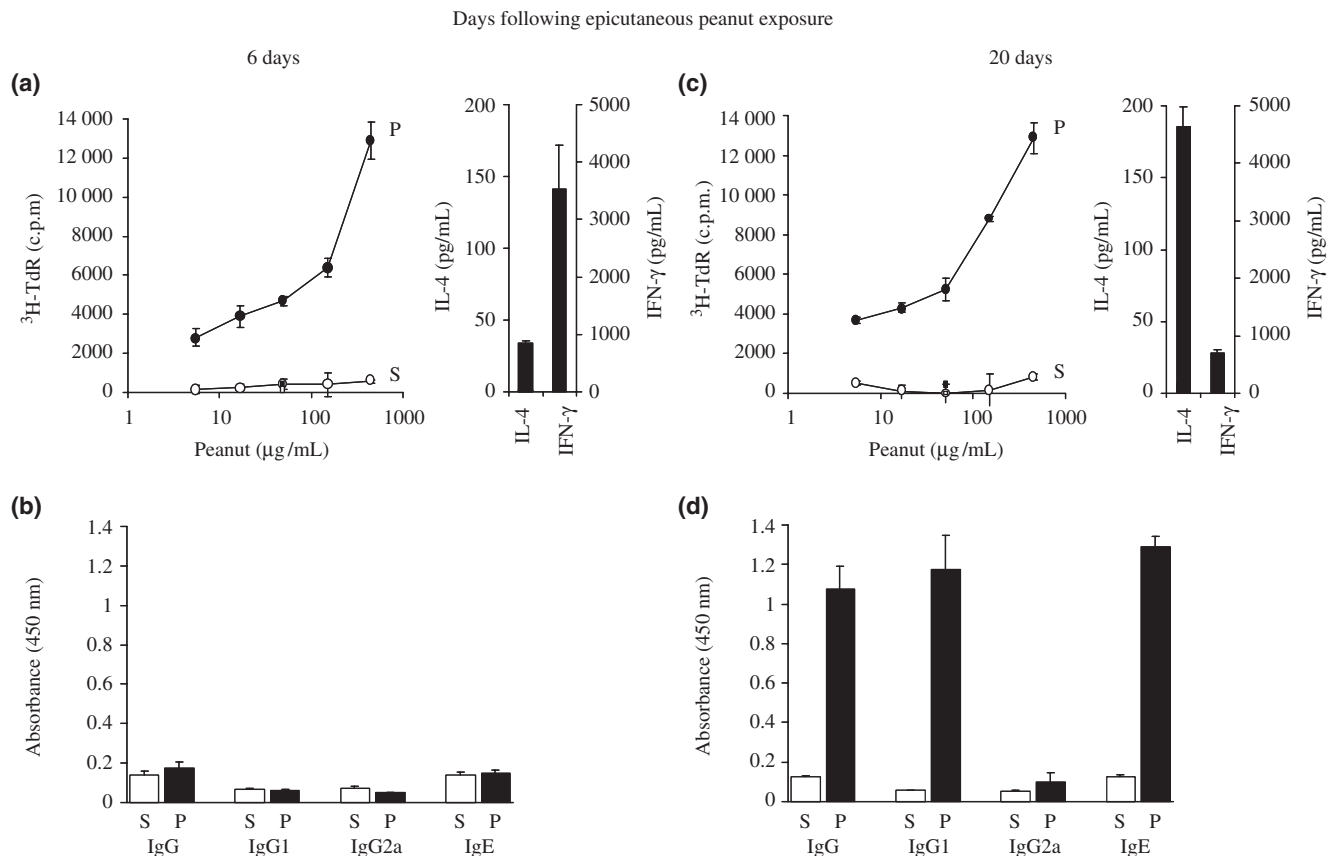


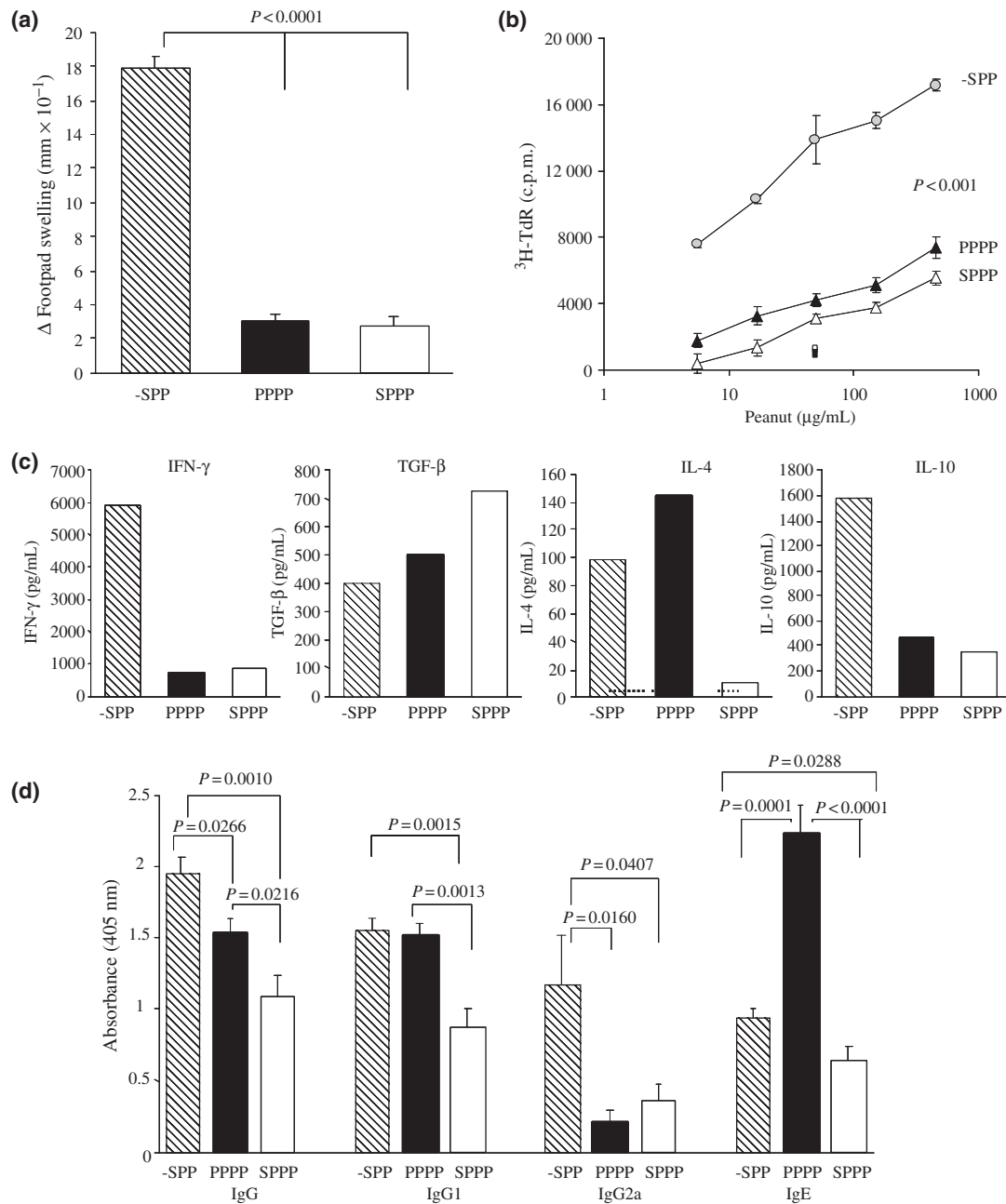
Fig. 1. Peanut-specific cellular and humoral responses following epicutaneous exposure to peanut protein on barrier-disrupted skin. Cervical lymph nodes (CLN) cells were harvested and cultured with peanut protein 6 (a) and 20 (c) days after the initial epicutaneous exposure and T cell proliferation measured by ^3H -thymidine incorporation. Results are expressed as mean cpm from triplicate cultures $\pm$ 1 SEM. Background proliferation from cells cultured with no antigen has been subtracted. Culture with control antigen is shown by (■). IFN- γ and IL-4 production by CLN cells taken 6 (a) and 20 (c) days after epicutaneous peanut exposure was determined by ELISA after 96 h culture with peanut protein. Results are expressed as pg/mL $\pm$ 1 SEM of eight individual experiments. No detectable cytokines were produced in the saline exposed group, nor were any cytokines produced to control antigen or when cells were cultured without antigen. Peanut-specific antibody responses were measured from serum 6 (b) and 20 (d) days after epicutaneous peanut exposure. Specific IgG, IgG1, IgG2a and IgE were diluted 10 times and measured by ELISA. Results are expressed as mean OD $\pm$ 1 SEM ($n \geq 6$). Similar results were obtained from eight individual experiments [S (○); skin exposed to saline; P (●), skin exposed to peanut].

Epicutaneous peanut exposure 20 days prior to a tolerogenic feed prevents oral tolerance and allergic sensitization is further enhanced

Feeding a tolerogenic dose of peanut protein 20 days after epicutaneous exposure to peanut no longer induced oral tolerance and the epicutaneous sensitization was dominant in all parameters assessed. Animals epicutaneously exposed to peanut protein had significantly increased DTH responses as well as *in vitro* T cell proliferation to peanut protein compared with tolerant animals, which were epicutaneously exposed to saline (Figs 3a and b). Levels of secreted IFN- γ , IL-4 and IL-10 were all significantly elevated over tolerant control animals, while TGF- β was reduced (Fig. 3c). Similarly, levels of peanut-specific IgG and IgE antibodies were all significantly higher in animals epicutaneously exposed to peanut prior to feeding than in the tolerant controls (Fig. 3d). Levels of IL-4 and IgE were additionally significantly higher than in the saline-fed peanut-sensitive group of animals ($P = 0.0015$), indicating that the oral peanut instead of tolerizing the epicutaneously exposed animals had sensitized them further.

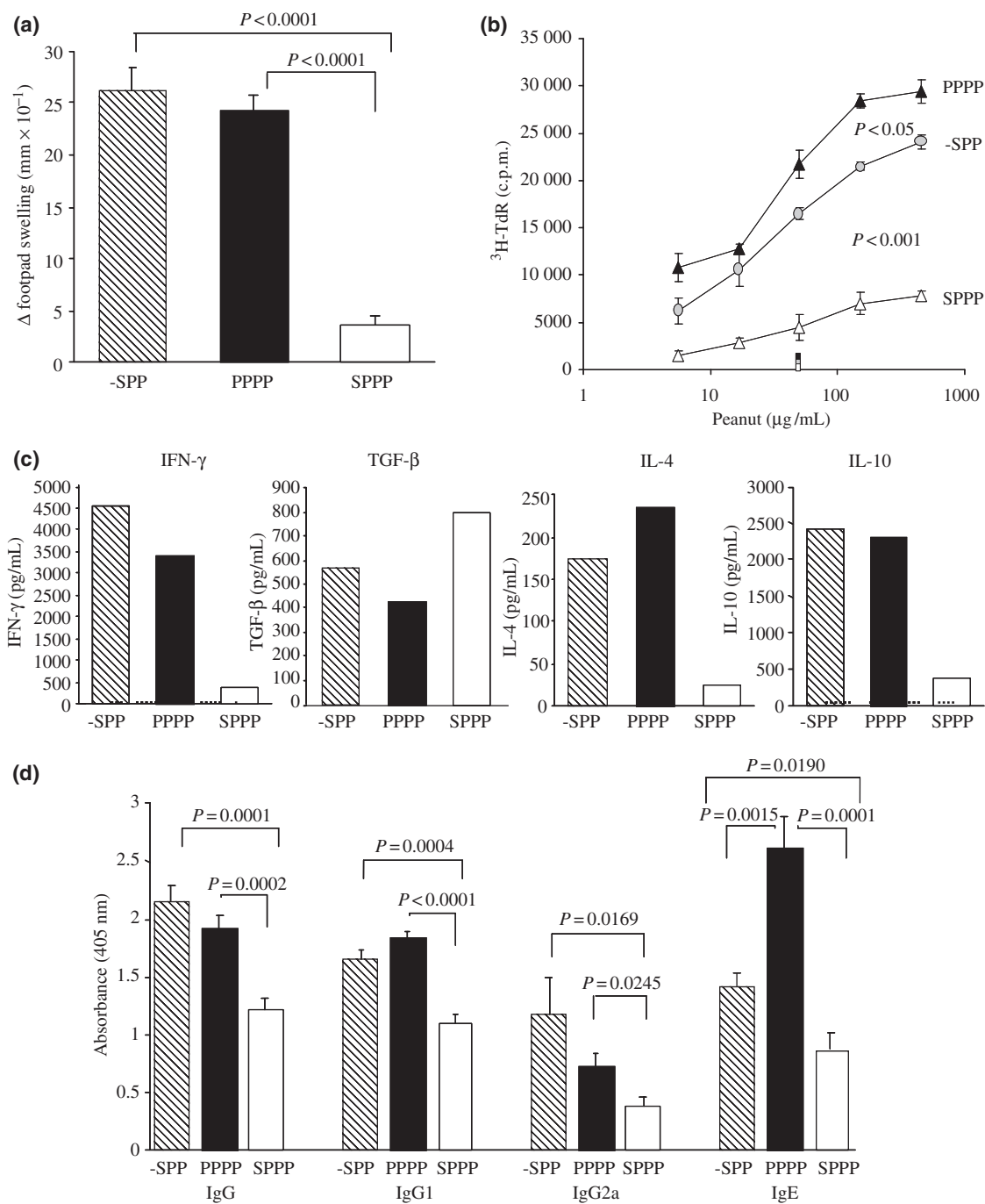
Tolerance to peanut may be disrupted by epicutaneous exposure

To investigate whether an existing state of peanut-specific tolerance, or orally induced systemic hyporesponsiveness, could be modified by epicutaneous exposure to peanut protein, animals were fed a tolerogenic dose of peanut protein 7 days prior to epicutaneous exposure on barrier-disrupted skin. Three weeks later, a peanut footpad challenge demonstrated that T_{DTH} cells were no longer hyporesponsive in animals epicutaneously exposed to peanut, but had become activated (Fig. 4a, PPP). These animals showed DTH responses well above that of animals fed peanut protein but only exposed to saline on the skin (PSP) and even significantly higher than tolerant animals subsequently immunized with peanut in CFA ($P < 0.0001$). However, the tolerogenic feed did confer some protection from sensitization as the DTH responses were lower than in animals fed saline prior to epicutaneous peanut protein exposure ($P = 0.0061$) (Fig. 4a, SPP). The existing state of oral tolerance also reduced the *in vitro* T cell proliferation to peanut following epicutaneous peanut exposure ($P < 0.001$) (Fig. 4b). Peanut-specific T cell



Immune status	Procedure	Epicutaneous exposure	Feed Day 6	Subcutaneous + CFA	Footpad challenge
	Abbreviation				
Sensitive	-SPP	-	Saline	Peanut	Peanut
	PPPP	Peanut	Peanut	Peanut	Peanut
Tolerant	SPPP	Saline	Peanut	Peanut	Peanut

Fig. 2. Tolerance to peanut is modified 6 days after epicutaneous exposure. Delayed-type hypersensitivity responses (a) were measured 24 h after single peanut protein challenge in the footpad and expressed as mean increment of footpad swelling $\pm$ 1 SEM ($n = 8$). T cells taken from para-aortic lymph nodes (PLN) draining the subcutaneous + complete Freund's adjuvant immunization site were cultured for 90 h with peanut protein or control antigen (■) and proliferation determined by ^3H -thymidine incorporation (b). IFN- γ , IL-4, transforming growth factor- β (TGF- β) and IL-10 production by PLN T cells cultured with peanut protein for 96 h was measured by ELISA (c). PLN cells were pooled from eight mice per group and analysed in triplicate. Dashed horizontal lines show the detection limit of the assay. Peanut-specific IgG, IgG1, IgG2a and IgE were measured in serum 6 weeks after the initial epicutaneous exposure (5 weeks after oral exposure) (d). Serum samples were diluted 1 : 1000 for IgG and IgG1, 1 : 200 for IgG2a and 1 : 10 for IgE prior to analysis. Each bar represents mean antibody level $\pm$ 1 SEM ($n = 8$). The entire *in vivo* experiment was repeated twice with similar results. Abbreviations, experimental procedure and peanut immune status of control animals are listed in the inset table.



Immune status	Procedure	Epicutaneous exposure	Feed Day 20	Subcutaneous + CFA	Footpad challenge
	Abbreviation				
Sensitive Tolerant	-SPP	-	Saline	Peanut	Peanut
	PPPP	Peanut	Peanut	Peanut	Peanut
	SPPP	Saline	Peanut	Peanut	Peanut

Fig. 3. Oral tolerance to peanut is prevented 20 days after epicutaneous exposure. Delayed-type hypersensitivity responses (a) were measured as mean footpad swelling $\pm$ 1 SEM 24 h after a single peanut protein challenge in the footpad ($n = 8$). Proliferation of T cells from para-aortic lymph nodes (PLN) draining the subcutaneous + complete Freund's adjuvant immunization site was determined by ^3H -thymidine incorporation after 90 h culture with peanut protein or control antigen (■) (b). Production of IFN- γ , IL-4, transforming growth factor- β (TGF- β) and IL-10 by PLN T cells cultured with peanut protein for 96 h was measured by ELISA (c). PLN cells were pooled from eight mice per group and analysed in triplicate. Dashed lines represent the detection limit of the cytokine ELISAs. Peanut-specific IgG, IgG1, IgG2a and IgE were measured in serum 8 weeks after the initial epicutaneous exposure (5 weeks after oral exposure) (d). Serum samples were diluted 1:1000 for IgG and IgG1, 1:200 for IgG2a and 1:10 for IgE prior to analysis. Each bar represents the mean antibody level $\pm$ 1 SEM ($n = 8$). The experiment was repeated twice with near identical results. Abbreviations, experimental procedure and peanut immune status of control animals are listed in the inset table.

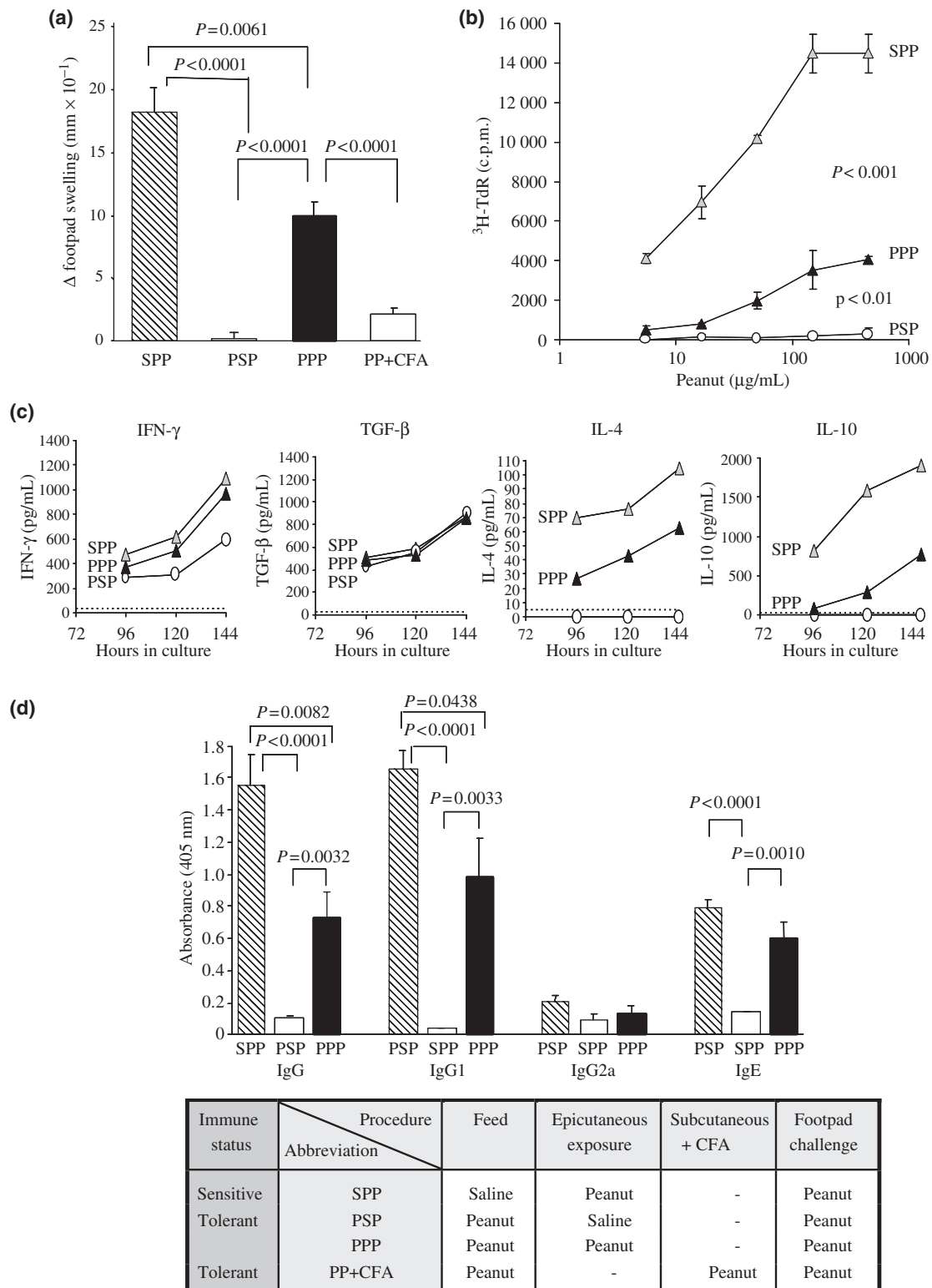


Fig. 4. Existing tolerance to peanut is partially broken by epicutaneous exposure. Delayed-type hypersensitivity responses (a) were measured 24 h after footpad challenge and is represented as the mean increment of footpad swelling $\pm$ 1 SEM ($n=6$). Proliferation of T cells from cervical lymph nodes (CLN) draining the epicutaneous site of peanut exposure was determined by ^3H -thymidine incorporation after 90 h culture with peanut protein or control antigen (■) (b). Cytokine production from CLN cells was measured by ELISA following 4–6 days reactivation with peanut *in vitro* (c). CLN cells were pooled from six mice per group and analysed in triplicate. Peanut-specific IgG were measured in serum 3 weeks after epicutaneous exposure (d). All serum samples were diluted 1 : 10 prior to analysis. Bars represent mean antibody concentration $\pm$ 1 SEM ($n=6$). Abbreviations, experimental procedure and peanut immune status of control animals are listed in the inset table.

proliferation was, however, enhanced also in tolerant animals following epicutaneous exposure to peanut protein compared with epicutaneous exposure to saline ($P < 0.01$) (Fig. 4b). Similar proliferation patterns were observed with splenic T cells (data not shown). T cells from draining CLN of animals fed saline prior to epicutaneous peanut exposure (SPP) produced high amounts of IL-4 and IL-10, which increased over the 6-day culture period. Animals fed a tolerogenic dose of peanut prior to epicutaneous exposure (PPP) also produced high levels of IL-4 and moderate levels of IL-10, both of which were above tolerant controls, although lower than saline fed peanut-sensitive animals (Fig. 4c). There was no difference in secretion of IFN- γ and TGF- β between groups. A similar pattern of cytokine production was observed from splenic T cells (not shown). The partial break of oral tolerance was also evident when examining peanut-specific IgG antibody responses. While IgG and IgG1 responses were significantly higher than those in tolerant controls, they were lower than those in epicutaneously exposed animals only fed saline. IgE responses, however, were no longer suppressed at all, but as high as saline fed, peanut-sensitive animals and significantly higher than tolerant controls ($P = 0.0010$) (Fig. 4d). No IgG2a was produced in any group indicating the Th2-biased nature of the epicutaneous sensitization route.

Discussion

Peanuts contain potent allergens which may cause severe allergic reactions in sensitized children and adults. The clinical features of peanut allergy are usually expressed as an acute IgE-mediated reaction after ingestion or inhalation of peanut protein [23, 24]. However, the primary route of sensitization is not firmly established and may not always be through the gastrointestinal tract. In this study we demonstrated in principle that peanut allergy as assessed by significantly increased peanut-specific IL-4 and IgE upon oral challenge can be induced by exposure to peanut protein through barrier-disrupted skin. Similar results were obtained with OVA (data not shown), which suggests that the strongly sensitizing effects of epicutaneous allergen exposure are a more general phenomenon and not restricted to the highly allergenic proteins in peanut.

Primary epicutaneous exposure to peanut (or OVA) interfered with normal oral tolerance induction. A time-dependent threshold for the induction of oral tolerance was observed. Feeding soon after the epicutaneous exposure (6 days) still induced T and B cell hyporesponsiveness in most parameters assessed. However, TGF- β secretion was reduced, perhaps as a beginning sign of failing tolerance induction. Importantly, IL-4 was increased and specific IgE production was significantly higher in epicutaneously exposed animals following the oral feed (Fig. 2). Twenty days after epicutaneous exposure, the systemic Th2 response was persistent and could not be suppressed by oral tolerance (Fig. 3). This supports the notion that it is more difficult to suppress an ongoing immune response than to induce tolerance *de novo* [25]. However, epicutaneous exposure to peanut protein not only prevented the normal induction of tolerance, but also

affected mucosal responses in a way that led to further enhanced Th2 responses including increased IgE production upon gastrointestinal exposure. Following gastrointestinal challenge, the epicutaneously sensitized mice also developed symptoms of systemic anaphylaxis. The symptoms appeared 10–15 min after challenge and included puffiness around the eyes, decreased activity, pilar recti and cyanosis around the eyes, tail and feet. Some animals were inactive even after prodding, but no animals were lost during oral challenge and they usually recovered within 3 h of challenge. The saline-sensitized control group showed no symptoms after challenge. The symptoms were, however, not formally evaluated using a quantitative scoring system as has been reported in other murine models of hypersensitivity [26]. The results described here demonstrate that skin exposure to allergens can mediate systemic disease manifestations. It also reinforces ours [12] and others [13–16] previous findings that epicutaneous exposure to allergens specifically drives Th2 responses.

An existing state of orally induced systemic hyporesponsiveness was demonstrated to confer some protection from epicutaneous sensitization (Fig. 4). Immune responses following epicutaneous exposure to peanut protein on peanut-tolerant animals were not as high as in naïve animals exposed to peanut protein in an identical way. However, IgE was a notable exception, with high production of peanut-specific IgE also following epicutaneous exposure on tolerant animals (Fig. 4d, PPP). This suggests that existing oral tolerance can be modified by subsequent epicutaneous exposure to peanut protein and may partly explain why some patients develop food allergies, such as peanut allergy, after years of tolerance [27].

In the experiments presented here, the epidermal stratum corneum was disrupted by mild abrasion, prior to peanut administration, to mimic the desquamated skin of eczema and other inflammatory skin conditions. Indeed, epicutaneous sensitization and the possibility of systemic disease manifestations may be particularly relevant for patients already suffering from eczema and other desquamating skin conditions. However, excessive washing and the frequent use of soaps and syndets may cause disruption of the stratum corneum in children. In adults, washing, shaving, waxing and the frequent use of various exfoliants and cosmetics may cause a similar barrier disruption. The mechanisms of this barrier disruption may be via mechanical abrasion of the skin surface (washing with rough materials, shaving, use of exfoliants) and via metabolic events induced by raised skin surface pH and directly deleterious effects on stratum corneum lipid structure (excessive use of water and soap) [28, 29]. Repeated barrier disruption will cause continued activation of resident epidermal Langerhans cells [12], which may lead to a breakdown in tolerance. It is tempting to speculate that such repeated mild disruption of the skin surface may be enough to cause sensitization in genetically susceptible individuals (reviewed in [30]). Recent evidence suggests that peanut allergens can persist on skin, tabletops, etc. if not suitably cleaned after peanut contamination [31]. Such environmental peanut allergen exposure may be a potential source of epicutaneous sensitization as well as topical application of lotions and creams, where peanut oil containing small amounts of protein is a common ingredient [6].

Antigen exposure at mucosal surfaces, such as the gut, nose and lung, generally leads to immunological hyporesponsive-

ness [26, 32–34]. In contrast, antigen exposure through barrier-disrupted skin results in strong Th2 immunity, and may play an important role in giving rise to various allergic diseases. As demonstrated here, epicutaneous exposure to allergens can cause food allergy-like systemic responses with high levels of specific-IgE upon oral challenge. Similar results were recently published in a study showing that prolonged epicutaneous exposure to OVA may result in elevated levels of plasma histamine as well as histological changes in the intestine and lungs following oral challenge with OVA [18]. Epicutaneous exposure to OVA can also result in asthma-like airway hyper-responsiveness following challenge in the lung [14]. These studies did not intentionally disrupt the stratum corneum prior to application of OVA but used a patch method where the skin was shaved and exposed to OVA for prolonged periods under occlusion. However, both shaving and changes in hydration as a consequence of prolonged occlusion cause stratum corneum disruption and thereby probably alter skin immune function. On the basis of these results, we propose that allergen exposure through barrier-disrupted skin could serve as a natural sensitization pathway for food allergy and other allergic hypersensitivity diseases. It may be that diseases such as asthma, AR and food allergy reflect different facets of a single disease, where the disease manifestation is determined by the route of challenge. Epicutaneous sensitization could potentially represent a common sensitization pathway for these allergic diseases.

Most studies examining sensitization and allergy to foods use intraperitoneal sensitization protocols and in addition strong adjuvants such as cholera toxin and aluminium hydroxide [35, 36]. A murine model of peanut allergy, where animals are sensitized through the oral route, exists, but relies on the concomitant and repeated feeding of cholera toxin during peanut sensitization [23]. Indeed, allergic sensitization via the gastrointestinal tract might be facilitated by injury in an as yet undefined way. Several studies, including ours, have demonstrated that the route of antigen delivery profoundly affects the nature of an immune response. Models using intraperitoneal food sensitization and challenge are thus not satisfactory for studying human food allergy, which is provoked by mucosal allergen exposure. The novel model presented here, using epicutaneous sensitization and oral challenge, could be used as a physiologically relevant model for studying peanut or other food-induced sensitization and clinical anaphylaxis. It may additionally be useful for studying interactions between eczema and food allergy as well as the effects of epicutaneous sensitization on other allergic hypersensitivity diseases.

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