

ORIGINAL ARTICLE Basic Mechanisms in Allergic Disease

Staphylococcal-derived superantigen enhances peanut induced Th2 responses in the skin

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Clinical & Experimental Allergy

Summary

Background The allergen-induced activation and expansion of IL-4 producing T helper type 2 (Th2) cells is a key event in the initiation and progression of allergic disease. Intriguingly, concomitant early childhood staphylococcal skin infections are being increasingly implicated in the allergen-induced switch of primary T cell responses towards the Th2 phenotype.

Objective We sought to identify whether or not staphylococcal-derived superantigen can influence the primary T cell response in the skin to food allergens with a view to determining whether such exposures create the immune pathology that predisposes to the development of food allergy.

Methods Using a novel Th2 reporter model, we determined the ability of the staphylococcal superantigen (SEB) to influence priming in the skin of IL-4 expressing Th2 cells by peanut extract (PE). Factors including the effect of SEB on the magnitude of the Th2 response in the skin draining lymph nodes, T cell receptor V region usage and the influence of endotoxin were evaluated.

Results Primary exposure to PE and SEB lead to significantly enhanced PE specific Th2 responses when the mice were subsequently exposed to PE alone. The enhancement of the Th2 response was dependent on the V β -binding properties of the SEB, but was not affected by endotoxin-mediated TLR-4 effects or strain differences in the mice.

Conclusions and Clinical Relevance These results identify that in the skin environment, the presence of SEB can significantly increase the numbers of allergen-induced Th2 cells which develop in response to subsequent allergen exposure. These data highlight the process by which individuals may become pathologically sensitized to food allergens in early life.

Keywords food allergy, peanut, SEB, skin sensitization, superantigen, Th2 response

Submitted 28 November 2010; revised 08 July 2011; accepted 29 July 2011

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Introduction

Recent analysis of the available epidemiological trends suggests that dietary allergen avoidance has not proven to be effective for the prevention of food allergy. This raises the issue whether oral exposure is necessarily a prerequisite for food allergy sensitization and whether other routes of exposure such as the skin could play a role [1]. Skin infections and skin exposure to allergens are increasingly being considered an important cofactor in the development of food-induced allergic sensitization. This is substantiated by the observations that eczema in early childhood closely correlates with the development of food allergy [2–7] and by several mur-

ine studies that demonstrate that allergic sensitization can occur as a consequence of epicutaneous exposure to allergen [8–14]. Most patients with eczema are colonized with *Staphylococcus aureus* [1] and of the *S. aureus* strains that have been isolated from lesional skin, up to 65% have been shown to produce biologically relevant quantities of exotoxins with superantigen properties [2]. These observations lead us to propose that in patients with eczema, superantigens might act as an additional trigger factor in the development of food allergen sensitization in the skin. This is supported by evidence demonstrating a subset of patients with eczema mount an IgE response to staphylococcal superantigens grown from their skin [3], that lymphocytes

isolated from eczema patients secrete T helper type 2 (Th2) cytokines in response to superantigens [4], that *S. aureus* colonization can cause severe exacerbation of eczema in many patients [5] and in some situations antimicrobial treatment leads to amelioration of eczema [1, 6].

Superantigens bind directly to MHC class II molecules and to a subset of T cell receptor (TCR) V β chains [7, 8]. Unlike conventional antigens, superantigens do not require processing by antigen-presenting cells [9] and are able to directly activate the subset of T cells expressing the appropriate V β chain. In this study, we have sought to identify whether appropriate skin exposures to *S. aureus* superantigen, staphylococcal enterotoxin B (SEB) at doses observed in lesional skin, can influence a concomitant skin primary Th2 immune response to peanut allergens. To quantify the skin-draining lymph node CD4⁺ Th2 response to peanut we utilized the G4 mouse strain, which expresses GFP as a surrogate for interleukin (IL)-4 expression, making it possible to track IL-4-producing immune cells *in situ* [10, 11]. Similarly, the effect of SEB on Th2 mediated eosinophilia in response to skin exposure to peanut was followed.

Herein, we report that initial exposure of the skin to SEB and allergen leads to a significant enhancement in the number of CD4⁺ Th2 cells in the draining lymph nodes and the level of Th2 mediated eosinophilia which is induced by subsequent 'allergen only exposures in the skin'. Depleting anti-V β studies reveal that this enhancement occurs through the direct activation of the SEB reactive V β subset of T cells. Our data highlight the potential of skin exposure to microbial products for significantly increasing primary allergen-induced Th2 responses. We propose this skin response to SEB may model events in food allergy sensitization, whereby the enhanced Th2 response that individuals make when infected with *S. aureus* and exposed to food allergens such as peanut through the skin predisposes them to developing an allergic reaction in the gastrointestinal (GI) tract when exposed through the oral route later in life.

Methods

Animals

All mice were maintained in a specific pathogen free barrier facility at the Malaghan Institute of Medical Research, and all animal manipulations were performed with approval of Victoria University animal ethics committee. There was no serological, histological or pathological evidence of viral, bacterial or parasite infection in sentinel mice maintained with the colony. No serological evidence of viral infection or histological evi-

dence of bacterial infection was detected in any mice at necropsy. The generation of GFP/IL-4 reporter (G4) mice has been reported previously [10]. The G4 mice were backcrossed to BALB/c mice for 10 generations before being obtained from the National Institute of Allergy and Infectious Diseases contract facility at Taconic Farms (Germantown, NY, USA). The BALB/c were obtained from NIAID facility and C57BL/6 mice were originally sourced from the Jackson Laboratory (Bar Harbor, ME, USA). C57BL/6^{IL-4⁻/G4} TLR-4^{-/-} mice were obtained by crossing G4 mice with C57BL/6 TLR-4^{-/-}. All mice were housed according to institutional guidelines. Water was available continuously, and a commercial mouse diet was provided *ad libitum*. Experiments were performed on age- and gender-matched mice (6–8 weeks old).

Peanut extract

Peanut extract (PE) was prepared in-house using established protocols. Briefly, commercially sourced roasted peanuts were ground in a coffee grinder (Breville, Botany, NSW Australia) in 0.1 mol/L ammonium bicarbonate (pH 9.0). Insoluble material was removed by means of centrifugation after 4 h incubation at room temperature. The supernatant was dialyzed overnight at 4°C against 0.15 mol/L NaCl fractionated by 25–80% ammonium sulphate precipitation. Material for immunization was prepared by dialysis against 0.15 mol/L NaCl.

Skin sensitization protocol

Mice were injected intradermally (i.d.) with 100 μ g of PE in 30 μ L saline for both the priming immunization and subsequent boosting immunizations. For mice primed to PE in the presence of SEB, 10 μ g SEB (Sigma Aldrich S4881, St. Louis, MO, USA) was either injected with the PE in 30 μ L saline or injected by itself in 30 μ L saline in the outer dermis of the ear. The mice receiving PE immunizations (both PE and PE + SEB) were then subsequently boosted twice; 2 weeks apart with PE alone in 30 μ L saline. Control mice were injected with 30 μ L saline at the same time-points.

Flow cytometry

At the indicated time-points following immunization mice were killed, the ear draining lymph nodes taken and single cell suspensions prepared for flow cytometry analysis. Before staining, all cell suspensions were first incubated with 2.4G2 to block non-specific binding of antibodies to Fc receptors. To analyse the T cell populations the following mAbs were used: PE anti-mouse CD4 (GK1.5), APC anti-mouse CD4 (RM4-5) PE

anti-mouse CD8 α (56-6.7), APC anti-mouse CD62L (MEL-14), PE anti-mouse CD44 (IM7), PE anti-mouse CD69 (H1.2F3), PE anti-mouse V β 8 (F23.1) and biotin anti-mouse V β 10 (B21.5) with Streptavidin-PE conjugate as the detection antibody. Conjugated antibodies were sourced from BD Biosciences PharMingen (Franklin Lakes, NJ, USA). Biotin anti-mouse CD3 ϵ (2C11) was purchased from eBioscience (San Diego, CA, USA) with Streptavidin-PE conjugate used as the detection antibody (BD Biosciences PharMingen). GFP was used to identify IL-4 producing cells and 7-AAD to identify non-viable cells. To analyse blood eosinophils the following mAbs were used: anti-mouse Fc ϵ RI α PE (MAR-1, eBioscience), anti-mouse CD45 Pacific Blue (30-F11, Biolegend) and anti-mouse CCR3 AF647 (83103, BD Biosciences PharMingen). Flow cytometry data were acquired using a FACSCalibur or LSRII (Becton Dickinson, Franklin Lakes, NJ, USA) flow cytometers. Data were analysed using FlowJo (Tree Star) software.

In vivo antibody treatment with depleting anti-V β T cell receptor antibodies

To deplete V β 8⁺ or V β 2⁺ T cells mice were injected i.v. with 100 μ g of antibody (mouse anti-mouse V β 8 TCR, F23.1 BD Pharmingen or rat anti-mouse V β 2 TCR, B20.6 eBioscience) 20 h prior to intradermal sensitization with PE or PE and SEB. The antibodies were prepared for *in vivo* use by dialysis in NaCl to remove azide and the final protein concentration was determined by NanoDrop (ND-1000, Biolab, Wilmington, DE, USA) spectrophotometer.

Statistical analyses

Statistical significance between each of the mouse treatment groups was determined by using the Mann–

Whitney *t*-test. In experiments comparing multiple experimental groups, statistical differences between groups were analysed using the one-way ANOVA and a Bonferroni multiple comparisons post-test. $P < 0.05$ was considered significant. All analyses were performed using Prism 5.0 software. Bar graph data are expressed as mean $\pm$ standard error of the mean (SEM).

Results

Exposure to staphylococcal enterotoxin B during peanut extract (PE) sensitization enhances subsequent generation of PE induced IL-4 producing Th2 cells

Previous studies have demonstrated that epicutaneous sensitization with SEB induces Th2 cytokine expression in the skin leading to elevated serum levels of IgE and IgG1 [12]. We modified the experimental regime [13] to determine whether the generation of PE induced CD4 Th2 cells in skin could be influenced by simultaneous exposure to SEB. Heterozygous G4/IL-4 reporter mice received i.d. PBS, PE, or PE in the presence of 10 μ g SEB (Fig. 1a). The number of CD4⁺ GFP⁺ Th2 cells induced in the draining lymph node 24 h following the second boost on day 28 was determined by flow cytometry. Repeated exposures to PE in the presence of SEB did not increase the absolute number or proportion of CD4 Th2 cells as compared to PE alone (Fig. 1b and c). Of major interest was the observation that PE boosting of mice whose exposure to SEB and PE was restricted to the initial day 14 stage of sensitization lead to significantly greater increases in the CD4⁺ GFP⁺ T cell responses compared to mice only receiving either PE alone or PE + SEB in the sensitization and boosting phases (Fig. 1b and c). These data suggest that a single exposure to SEB in the presence of PE resulted in enhanced CD4 Th2 responses on subsequent exposure to the PE alone.

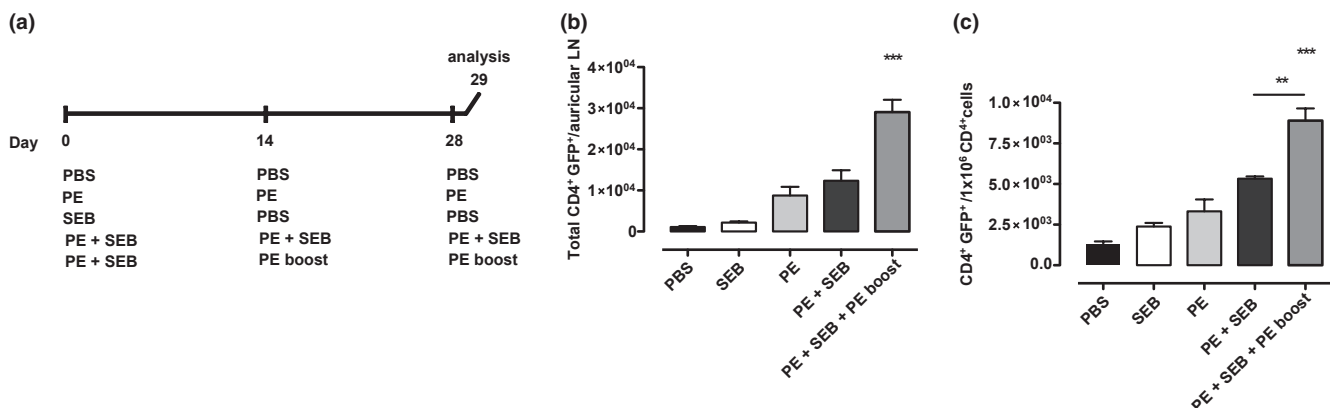


Fig. 1. Exposure to staphylococcal enterotoxin B (SEB) when being primed to peanut extract (PE) leads to an enhanced PE-dependent CD4 Th2 response. (a) Protocol and timeline of SEB exposure and PE prime/boost regimes. (b) Absolute number and (c) proportion of CD4⁺ GFP⁺ T cells present in the draining auricular lymph node 24 h after final intradermal boost. Bar graphs show mean $\pm$ SEM, *** $P < 0.001$, ** $P < 0.01$. Data are representative of multiple separate experiments, containing four mice/group.

We next determined whether or not the enhanced CD4⁺ GFP⁺ Th2 responses we observed following SEB exposure also extended to expression of CD4 Th2 cell effector function. Of particular relevance is the effect of SEB on PE-induced eosinophilia, as eosinophilia has been demonstrated to be a predictor of food allergy in patients with atopic dermatitis [14]. We quantified circulating eosinophil percentages on days -1, 3 and 7 following the final i.d. boost, using CCR3 to identify the eosinophil population in the blood. Prior to CCR3⁺ selection, cells are gated on CD45⁺ SSC^{hi} cells (granulocytes). This gating strategy eliminates any basophils (CD45^{int}, SSC^{lo}) and Th2 cells (CD45⁺ SSC^{lo}) that may also be CCR3⁺. Mast cells, which have also been shown to express CCR3, migrate as progenitor cells and mature in the tissue. In contrast to humans, CCR3 has not been demonstrated on mast cell progenitor populations in mice [15]. Therefore, this gating strategy can accurately identify the CCR3⁺ population as eosinophils in the blood. Primary exposure to PE or SEB alone failed to change circulating eosinophils (Fig. 2). Conversely, skin exposure to PE in the presence of SEB with subsequent re-exposure to PE promoted a significant increase in eosinophils 3 days after the final peanut allergen re-exposure (Fig. 2).

The staphylococcal enterotoxin B induced enhancement of the Th2 response is strain- and TLR-4-independent

Recent investigations have shown that GI exposure to *S. aureus* may contribute as an environmental trigger in food allergy through its ability to break oral tolerance [16]. Interestingly, Ganeshan et al. show that their findings extend to mice on a C57BL/6 background, which is in contrast to the very strain dependent observations found using other recognized experimental food allergy mouse models [16–18]. We analysed both

BALB/c and C57BL/6 strains in our model and observed no strain-related difference in the generation of the Th2 response resulting from prior exposure to PE in the presence of SEB, following PE boosts (Fig. 3a and b). Further, to exclude the possibility that the SEB enhancement of the Th2 response could be due to endotoxin lipopolysaccharide (LPS) contamination of our PE preparations [19] we performed similar studies as above in TLR-4 deficient G4 reporter (IL-4 heterozygous) mice. The inability to signal through TLR-4 did not alter the absolute number or proportion of the Th2 response (Fig. 3c and d). Accordingly, the generation of the Th2 response following skin exposure is neither strain-specific nor a result of LPS contamination.

The staphylococcal enterotoxin B enhanced CD4 Th2 response is allergen-specific

To obtain evidence of whether or not the PE boosted response was allergen specific we tested whether another food allergen, ovalbumin (OVA), could boost the PE + SEB primed response. The number of CD4⁺ GFP⁺ Th2 cells induced in the draining lymph node 24 h following the second boost with either OVA or PE was determined by flow cytometry (Fig. 4). As described previously boosting with PE resulted in a significant enhancement in the total number and proportion of CD4 Th2 T cells as compared to mice primed with PE alone. However, boosting mice with OVA did not stimulate an enhanced CD4⁺GFP⁺ response in mice previously receiving PE + SEB, indicating that the enhancement was allergen specific. Interestingly, the PE + SEB-primed mice boosted with OVA did produce a significant increase in the total number of CD4 Th2 cells compared with PE + SEB-primed only (Fig. 4a). This result would be consistent with previous investigations demonstrating that IL-4 can drive collateral

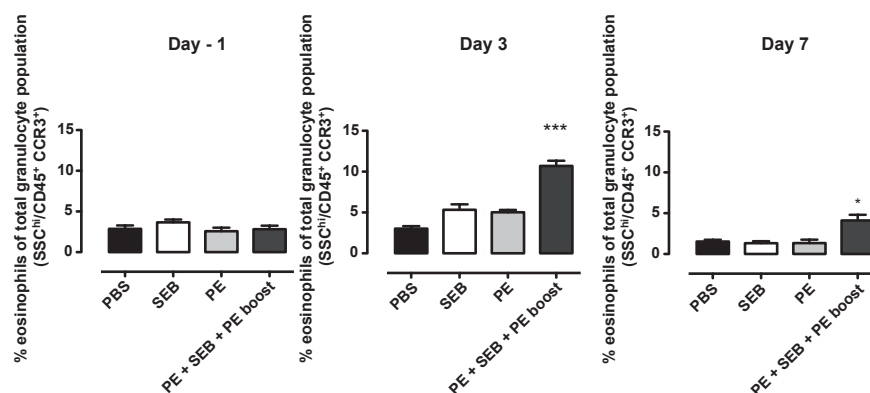


Fig. 2. Skin exposure to staphylococcal enterotoxin B (SEB) enhances peanut extract (PE) induced Th2 effector immune responses. Percentage of circulating eosinophils on day -1, 3 and 7 following the final exposure to PE or PE + SEB. Bar graphs show mean $\pm$ SEM, * P < 0.05 and *** P < 0.001. Data are representative of two independent experiments, containing at least four mice/group.

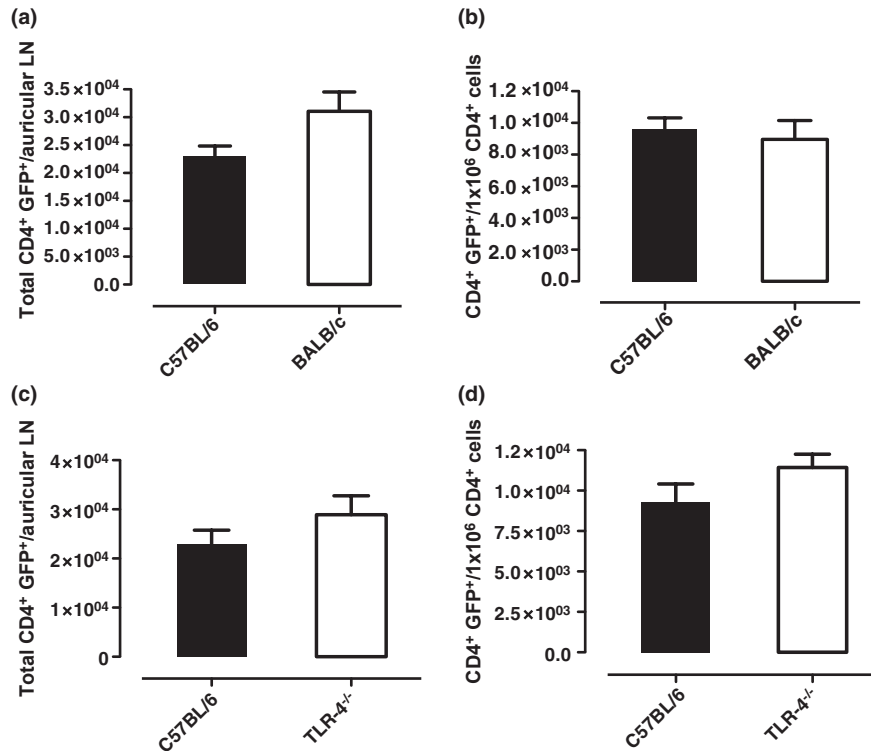


Fig. 3. The generation of the CD4⁺ Th2 response is strain- and TLR-4 independent. (a) Absolute number and (b) proportion of CD4⁺ GFP⁺ T cells from G4 reporter C57BL/6 and G4 reporter BALB/c mice present in the draining auricular lymph node 24 h after final intradermal boost with peanut extract (PE). (c) Absolute number and (d) proportion of CD4⁺ GFP⁺ T cells from G4/IL-4 reporter TLR4^{+/-} and G4/IL-4 reporter TLR-4^{-/-} mice present in the draining auricular lymph node 24 h after final intradermal boost with PE. Bar graphs show mean $\pm$ SEM for five mice/group and data representative of two independent experiments.

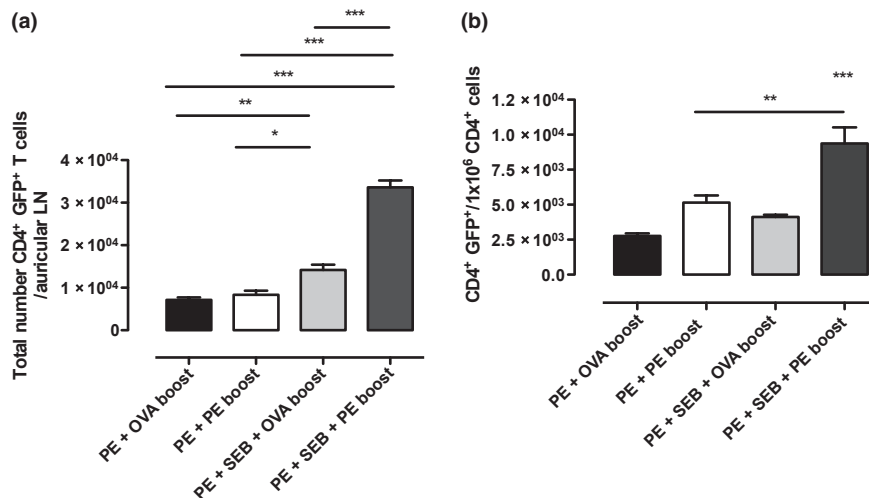


Fig. 4. Maintenance of the CD4 Th2 response is allergen specific. (a) Absolute number and (b) proportion of CD4⁺ GFP⁺ T cells present in the draining auricular lymph node 24 h after final intradermal boost. Bar graphs show mean $\pm$ SEM for at least four mice/group, * P < 0.05, ** P < 0.01 and *** P < 0.001. Data are representative of two separate experiments, containing four mice/group.

priming, a state that results when activated Th2 cells facilitate the subsequent priming of endogenous naïve T cells to new antigens [20]. Taken together, these data suggest that the CD4 Th2 response driven by initial

exposure to PE in the presence of SEB can be maintained in a PE-specific manner and that SEB exposure in the presence of allergen may allow for collateral priming to other allergens.

The fate of V β 8⁺ T cells following staphylococcal enterotoxin B and peanut extract administration

When SEB alone is administered to Balb/c mice it has been shown to drive an initial expansion of superantigen-reactive V β 8⁺ T cells with a subsequent contraction over a 10-day period through a process dependent on SEBs specificity for V β 8⁺ TCR on T cells [21]. In addition, epicutaneous sensitization with SEB has been shown to result in a transient, selective and early infiltration of superantigen-reactive cells in the dermis, followed by their depletion [12]. Therefore, we were interested to compare the fate of the V β 8⁺ T cells (both GFP⁺ and GFP⁻) following immunization with either PE or PE + SEB during initial exposure. Non-SEB responsive V β 2⁺ T cells were also monitored to control for non-V β 8 specific effects of the SEB and PE. Although the percentage of V β 8⁺ T cells present in the CD4⁺ GFP⁺ population, which appeared after the PE boosts, was reduced in SEB-treated mice (Fig. 5a), the absolute number of the V β 8⁺ T cells among the CD4⁺ GFP⁺ pop-

ulation in mice exposed to PE following initial exposure to PE + SEB were significantly greater than the number of V β 8⁺ cells in mice receiving PE alone during priming, or OVA during boosting immunizations (Fig. 5c). This suggests that the V β 8⁺ CD4⁺ GFP⁺ T cell population activated by SEB is not depleted when a concomitant immune response to PE occurs. Interestingly, PE exposure alone (4530 ± 1018) as compared to SEB exposure (906.1 ± 96.45) or PBS control (392.1 ± 112.0) increased the V β 8⁺ T cell population among the CD4⁺ GFP⁺ T cells, implying a preferential skewing of this subset. OVA has also been demonstrated to activate V β 8⁺ T cells that regulate IgE production [22]. However, subsequent exposure to OVA following priming with PE in the presence of SEB did not result in an increased number of V β 8⁺ T cells among the CD4⁺ GFP⁺ T cell population, suggesting this skewing occurs via an allergen-specific manner (Fig. 5c). No changes in the proportion of the V β 2⁺ T cell populations were observed in any of the treatment groups (Fig. 5b). Conversely, the total number of V β 2⁺ T cells

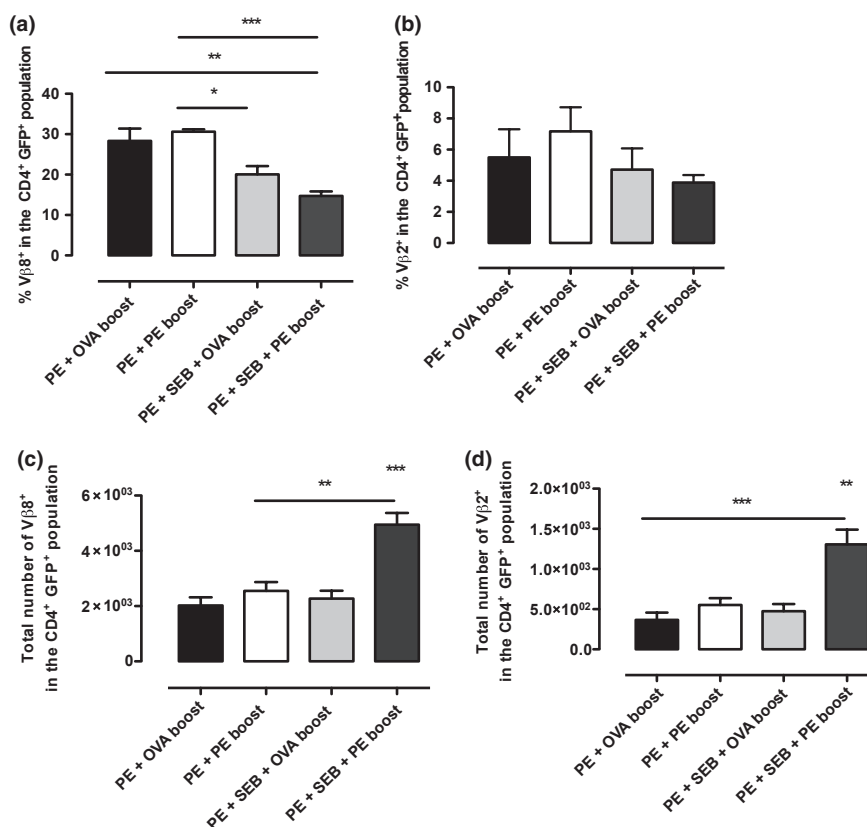


Fig. 5. V β 8⁺ cells contribute to the staphylococcal enterotoxin B (SEB) enhanced generation of peanut extract (PE) activated CD4⁺ Th2 cells. Expression of SEB reactive V β 8 and SEB non-reactive T cells (V β 2) in the CD4⁺ GFP⁺ Th2 population. (a) Percentage of V β 8⁺ T cells among the CD4⁺ GFP⁺ T cell population. (b) Percentage of V β 2⁺ T cells among the CD4⁺ GFP⁺ T cell population. (c) Absolute number of V β 8⁺ T cells among the CD4⁺ GFP⁺ T cell population and (d) absolute number of V β 2⁺ T cells among the CD4⁺ GFP⁺ T cell population. Draining lymph nodes were harvested and analysed 24 h after the last intradermal boost with PE. Bar graphs show mean $\pm$ SEM for five mice/group, * P < 0.05, ** P < 0.01 and *** P < 0.001 and data are representative of two experiments, which gave similar results.

in the $CD4^+ GFP^+$ T cell population was significantly increased as compared to other groups immunized with PE or PE and SEB during initial exposure (Fig. 5d). In addition, the total number of $V\beta 6^+$ and $V\beta 10^+$ T cells among the $CD4^+ GFP^+$ T cell population in mice exposed to PE following PE in the presence of SEB during initial exposure also significantly increased (data not shown). These data validate our conclusion that collateral priming is occurring in this PE + SEB response, and that other $V\beta$ TCR T cells are amplified following the initial exposure to SEB in the presence of PE. PE will contain a number of peanut proteins that may act as allergens, binding to various $V\beta$ TCR repertoires. As such, these T cells are driven to a Th2 phenotype via collateral priming that is facilitated by the $V\beta 8^+ CD4^+ GFP^+$ T cell population activated by SEB, and the immune response induced by PE impedes the SEB-mediated induction of anergy of $V\beta 8^+$ T cells.

Depletion of $V\beta 8^+$ T cells abrogates staphylococcal enterotoxin B induced enhancement of $CD4^+ GFP^+$ Th2 cells

Recently, it has been shown that concurrent exposure to SEB and OVA resulted in OVA-specific Th2 differentiation that was dependent on TIM-4 expression on intestinal dendritic cells [23]. In contrast, other investigations suggest that the SEB enhanced development of Th2 immunity may be due to a loss in immune suppression by impairing the function of Treg responses *in vivo* [16]. We investigated the potential mechanism(s) by

which prior exposure to SEB augmented the PE-induced Th2 response, via depletion of $V\beta 8^+$ T cells with intravenous administration of mouse anti-mouse $V\beta 8^+$ TCR mAb (F23.1) prior to i.d. administration of PE in the presence of SEB. We used anti- $V\beta 2^+$ TCR antibody as a control for depletion of non-SEB reactive $V\beta$ TCR-bearing T cells. Like SEB, administration of F23.1 results in programmed cell death of $V\beta 8^+$ T cells, maximum depletion occurring 12–24 h after injection [21]. However, unlike SEB, F23.1 is not a potent inducer of cytokines *in vivo*, thus its administration allows us to remove SEB reactive T cells to assess the role of SEB during the peanut-induced Th2 response, without concomitant activation of T cells. Using this protocol we found that greater than 95% $V\beta 8^+$ T cells were depleted in both the blood and the auricular draining lymph node 20 h after injection of either anti- $V\beta 2$, or anti- $V\beta 8$ (data not shown). Most significantly, prior treatment with the anti- $V\beta 8^+$ TCR antibody abrogated the SEB-induced enhancement of the $CD4^+ GFP^+$ response as compared to anti- $V\beta 2^+$ TCR antibody control (Fig. 6a and b). These data suggest that SEB enhances the PE-induced $CD4^+ GFP^+$ Th2 response through direct activation of T cells expressing the $V\beta 8$ chain and that once activated these cells can be sustained by repeated immunization with PE alone.

Discussion

Recent clinical and experimental investigations suggest an intriguing correlation between peanut allergy,

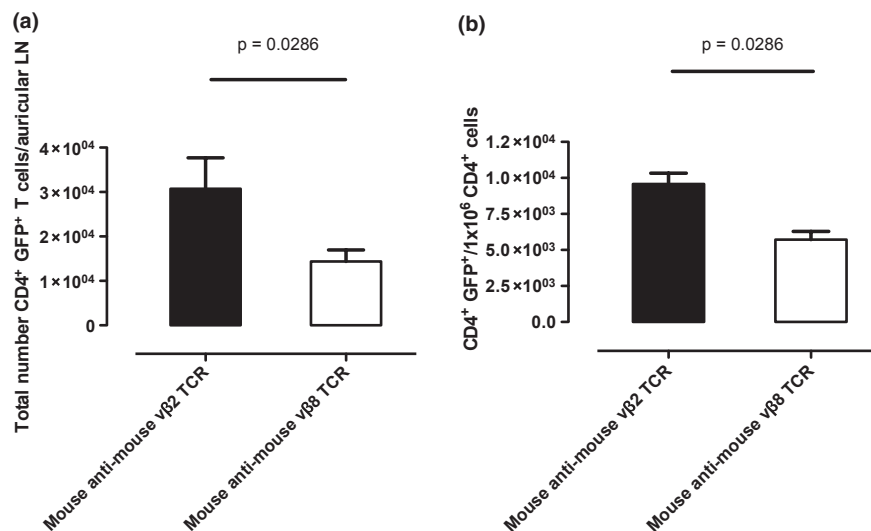


Fig. 6. Staphylococcal enterotoxin B (SEB) enhancement of peanut extract (PE) activated $CD4^+ GFP^+$ T cell responses is $V\beta 8^+$ T cell dependent. (a) Absolute number and (b) proportion of $CD4^+ GFP^+$ T cells found in the draining lymph node of mice administered with murine anti- $V\beta 8$ mAb or murine anti- $V\beta 2$ T cell receptor (TCR) mAb 20 h prior to sensitization with PE in the presence of SEB, and followed by boosting with PE. Draining lymph nodes were harvested and analysed 24 h after the last intradermal boost with PE. Bar graphs show mean $\pm$ SEM for four mice/group, and data are representative of two independent experiments.

peanut exposure through the skin and eczema in children, as well as several models of cutaneous sensitization to food allergens [24–30]. Herein, we report that exposure of mice to physiological doses of SEB augments the PE induced polarization and maintenance of Th2 cells in the skin draining lymph nodes of mice, leading to Th2 mediated effector responses. Previous studies have demonstrated that SEB can augment a Th2 response [12, 16, 23], however, the availability of the novel IL-4/GFP reporter mouse strain enabled the effects of SEB on the PE induced CD4 Th2 response to be accurately quantified *in vivo*. SEB enhanced the induction of the CD4⁺ Th2 response in both BALB/c and C57BL/6 strains, demonstrating that there were no strain-specific features of this model. We excluded the possibility that the SEB enhancement of the PE-induced CD4⁺ Th2 response was a result of LPS contamination, as mice unable to signal through TLR-4 still developed enhanced Th2 responses to PE in the presence of SEB. We found that repeated exposure to PE + SEB did not increase the number or proportion of CD4 Th2 cells induced in the draining lymph node, which would fit with SEBs well-documented ability to drive anergy in SEB reactive T cells, and thereby limit amplification of the Th2 response with repeated exposures to SEB [31–33]. Clinical data suggest that peanut-induced Th2 responses in the skin results from multiple skin exposures to allergen, and it is unlikely that this would result in concurrent exposure to SEB each time. Therefore, we have developed a relevant model of skin exposure to PE in the presence of SEB, followed by PE boosting that drives the maintenance of PE-specific CD4 Th2 responses. The SEB-enhanced CD4⁺ Th2 response resulted from direct activation of the SEB reactive V β 8⁺ T cells, as prior depletion of V β 8⁺ T cells abrogated the observed enhancement of the Th2 response. Our studies therefore add to previous studies of SEB in the development of allergic inflammation in the skin [12, 34].

These findings are of particular relevance to the events that predispose individuals to food-induced allergic reactions. Studies have convincingly demonstrated that childhood allergic diseases often start in the skin with eczema and later progress to the GI and respiratory tracts [35–40]. This suggests that eczema may be the cutaneous manifestation of a progressive allergic disorder with the key mediators of allergic inflammation, CD4⁺ Th2 cells trafficking from one site to the other, from sensitization to elicitation of allergic disease at the skin, lung or GI tract. Lymph nodes provide important hubs of immune connection where CD4⁺ T cells are able to enter from the blood and sample allergens presented by antigen-presenting cells draining via the lymphatics from the surrounding tissues. Antigen-experienced CD4⁺ T cells exit from the lymph node to

peripheral tissues and as such are able to provide a much faster and effective memory immune response at the site of allergen exposure [41]. Recirculation also occurs from peripheral sites such as the skin, and their drainage to the lymph node occurs via the afferent lymph [41]. Thus, our studies, which quantified the Th2 response at the draining lymph node allowed us to observe the profound expansion of Th2 cells capable of recirculating and initiating allergic reactions at distant sites such as the GI tract.

These investigations are timely, with respect to the recently proposed eczema therapies, which seek to employ engineered-binding affinity agents with superantigen-neutralizing capacity [42]. Soluble forms of engineered V β proteins have been shown to inhibit SEB-mediated T cell activation [43, 44], and these data suggest that the proposed therapeutics may also be efficacious for food allergy resulting from skin sensitization to food allergen in the presence of staphylococcal superantigens. Moreover, human studies also suggest SEB may play a role in triggering food allergies [45]. Previous investigations demonstrate that SEB provides an adjuvant effect through enhancing epithelial presentation of antigen as well as amplification of the Th2 response [46]. We are further investigating the role of epithelia and their derived factors that could possibly contribute to the CD4⁺ Th2 response following sensitization through the skin. In addition, use of antimicrobials to control *S. aureus* infections has also proved efficacious for the treatment of eczema, and may well help control food-induced allergic sensitization through the skin [1, 47].

In conclusion, these data demonstrate that skin sensitization to PE in the presence of SEB followed by subsequent PE exposure generates a greatly enhanced CD4⁺ Th2 response, via a SEB-dependent, T cell-mediated mechanism. This model highlights the potential importance of the skin site in the generation of the Th2 response to food allergens and thus in identifying possible strategies for the treatment and prevention of food allergy in humans.

Acknowledgements

This work was supported by the Health Research Council (09/347), Foundation of Research, Science and Technology (MALA0801), Lotteries Health Research (265304) and Wellington Medical Research Foundation (2008/159) grants. We thank Prof. Shizuo Akira (Osaka University, Japan) for the provision of TLR-4 deficient mice, Prof. Franca Ronchese (Malaghan Institute of Medical Research, New Zealand) for helpful discussion and Dr Marina Harvie (Malaghan Institute of Medical Research, New Zealand) for technical assistance.

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