

ORIGINAL PAPER

Peanut-specific B and T cell responses are correlated in peanut-allergic but not in non-allergic individuals

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Clinical and
Experimental
Allergy

Summary

Objective We aim to find what is the relationship between B cell antibody responses and specific T cell help in the specific cases of allergy and tolerance to peanuts.**Background** B cell antibody responses to foreign proteins usually depend upon antigen-specific T cell help. However, specific antibody levels can sometimes be maintained lifelong after infections or vaccination.**Methods** We measured peanut-specific proliferation and antibody levels in peanut-allergic and non-allergic children using tritiated thymidine incorporation and UniCAP, respectively. We also investigated the corresponding tetanus toxoid specific responses in both groups.**Results** We found that tetanus-specific IgG did not correlate with lymphocyte proliferation (Spearman rank correlation coefficient $r' = 0.08$, $P = 0.74$) nor with tetanus-specific cytokine production (IFN- γ : $r' = 0.198$, $P = 0.285$; TNF- α : $r' = 0.274$, $P = 0.146$; IL-4: $r' = -0.007$, $P = 0.96$; $P = 0.221$; IL-13: $r' = 0.363$, $P = 0.056$). Conversely, in peanut-allergic donors, peanut-specific IgE (average 21 kU/L, median 2.27 kU/L, range 0.34–100 kU/L) but not peanut-specific IgG was positively correlated with proliferation ($r' = 0.751$, $P = 0.003$). In these donors, specific IgE was positively correlated with peanut-specific Th2 cytokines production: $r' = 0.635$, $P = 0.02$ for IL-4 and $r' = 0.641$, $P = 0.025$ for IL-13 and negatively correlated with Th1 cytokines ($r' = -0.71$, $P = 0.007$ for IFN- γ and $r' = -0.746$, $P = 0.005$ for TNF- α , respectively). However, peanut-specific IgE was not correlated with T cell proliferation or cytokine production in non-allergic individuals. In conclusion, in allergic individuals, B and T cell responses to peanut antigens are correlated whereas normal immune responses B and T cell responses are uncoupled.**Conclusion** Our results support the view that B cell responses to allergens but not those to non-allergenic proteins are correlated with specific T cell responses and therefore specific immunotherapy targeting of such T cells would inhibit allergen-specific B cells.**Keywords** allergy, antibodies, B lymphocytes, cellular proliferation, T lymphocytes

Submitted 29 June 2007; revised 5 February 2008; accepted 5 March 2008

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Introduction

With few exceptions, B cell antibody production is induced by B cell receptor cross-linking associated with cognate T cell help. Further B cell proliferation, maturation of antibody responses and antibody class switching are also dependent upon T helper co-stimulation and cytokines. Maintenance of specific antibody levels in serum (which for long-term responses depends upon antibody production by plasma cells that are predominantly localized in the bone marrow) also depends upon persistent T cell help. In HIV-infected children and reci-

ipients of chemotherapy, impaired T helper function is associated with fewer antigen-specific memory B cell numbers and decreased specific antibody levels [1–4]. Indeed, in the absence of a sustained interaction between T helper cells and B cells, antigen-specific memory B cell numbers progressively decrease leading to a decrease in specific antibody levels as suggested by the decrease in antibody levels observed in children who become immunodeficient due to HIV infection [1–3] or those who undergo chemotherapy [4]. One might therefore predict that specific T cell response to a recall antigen correlates closely with specific B cell response to that antigen. On the

other hand, the relative long lifespan of the plasma cells and the recently published data on the effects of polyclonal activation of memory B cells [5] would suggest that there is little/no correlation between antigen specific T cell and B cell function. Surprisingly, there is a paucity of literature in humans examining the relationships of T cell responses with B cell responses to recall antigens such as tetanus or purified protein derivative.

As a possible explanation, it has been suggested that there are distinct short-lived and long-lived plasma cell populations [6] while other studies suggest that most plasma cells have a very long lifespan [7] and are independent of antigen presence [8]. The relative independence of antigen-specific serum antibody levels on ongoing immune responses was further supported by the finding that tetanus-specific IgG levels were not correlated with the frequency of circulating tetanus-specific B cells [9].

A recent report [10] attempts an explanation of these seemingly contradictory findings: maintenance of serological memory might be ensured independently of antigen/T cell help due to the polyclonal activation of memory B cells by non-specific stimuli such as lipopolysaccharide, CpG DNA, other ligands that bind to Toll-like receptors and bystander T cell activation.

These findings are of considerable interest to clinical allergists who aim toward the optimization of allergen-specific immunotherapy (SIT). Indeed, allergy is caused by long-term, persistent IgE production by B cells whose presence on mast cells and basophils triggers allergic reactions upon contact with allergens. Whereas in seasonal allergies (such as grass pollen allergy), IgE levels may show fluctuations that are related with the degree of pollen exposure, in most perennial allergies as well as in peanut allergy, IgE levels are stable and often progressively increase over the duration of the allergy. Paradoxically, this IgE increase may occur even despite the deliberate avoidance of any allergen exposure and strongly suggests that allergen-specific immune responses are continually active in allergic individuals. Because SIT is thought to act largely upon T helper and antigen presenting cells, it is important to characterize the relationship between T and B cell responses in the allergic state as well as in the non-allergic state. In order to do this, we compared T cell responses to peanut (proliferation and cytokine production) and B cell responses (IgG, IgE) in both peanut-allergic (PA) and non-allergic (NA) children. We also examined the relationship of the T and B cell responses to the control recall antigen tetanus in the same children.

Materials and methods

Donors

Thirty-one children '19 PA and 12 NA' participated in this study, which was approved by the Local Research Ethics

Committee. The diagnosis of peanut allergy was based on a characteristic history of immediate hypersensitivity reactions occurring soon after peanut ingestion and at least one of the following diagnostic criteria: peanut skin prick test weal ≥ 6 mm [11] or peanut-specific IgE ≥ 15 kU/L [12, 13] or a positive peanut challenge. The NA children had never reacted to peanut ingestion and were currently consuming peanuts as part of their diets, tolerating the equivalent of at least one peanut butter sandwich. All children had been vaccinated to tetanus according to the UK schedule. None of the children had been vaccinated or had received a tetanus boost less than 3 months before blood collection.

Reagents

Peanut defatted extract was kindly provided by Dr Henning Løwenstein (ALK Abelló, Hørsholm, Denmark). The lyophilized protein was dissolved in phosphate-buffered saline (PBS), sterilized by filtration and added to the cell culture at a final concentration of 100 μ g/mL. This antigen concentration ensured consistent cellular proliferation *in vitro*, as determined by titration in a preliminary experiment. Tetanus toxoid (TT, Staatens Serum Institute, Copenhagen, Denmark) was used at 10 μ g/mL [14]. Fluorochrome-labelled antibodies specific for human cytokines (IFN- γ , IL-4, IL-13 and TNF- α) were from BD Pharmingen (Cowley, UK); the corresponding clones used were B27 (IFN- γ), MP4-25D2 (IL-4), JES10-5A2 (IL-13) and MAb11 (TNF- α). Carboxyfluorescein succinimidyl ester (CFSE) was from Molecular Probes (Eugene, OR, USA).

Antibody levels

Peanut-specific IgG was measured using a direct ELISA system adapted according to the method described by Hamilton and Adkinson [15]. Briefly, NUNC MaxiSorp ELISA plates (NUNC A/S, Roskilde, Denmark) were coated overnight with 50 μ L/well of 0.1 mg/mL defatted whole peanut extract dissolved in bicarbonate buffer (pH 9.6). The plates were then washed twice with PBS-Tween (0.1%) and blocked with 1% bovine serum albumin for 1 h at 37 °C. Multiple dilutions of plasma were then added to the wells, incubated for 2 h at 37 °C, washed and then bound IgG was measured using a biotinylated anti-human IgG antibody and streptavidin-horseradish peroxidase with tetramethylbenzidine as substrate for detection. Peanut-specific IgG is expressed as relative units/mL using a standard curve obtained using human purified IgG as reference. Antibody levels were determined for all the patients from whom we could collect sufficient plasma. Peanut-specific and TT-specific IgE antibody levels in plasma were measured using the Phadia UniCAP and TT-specific IgG antibody

levels were assessed by a commercial ELISA (Binding Site, Birmingham, UK).

Peripheral blood mononuclear cells (PBMC) isolation, CFSE labelling and cell culture

Venous blood was collected using citrate dextrose (Sigma, Poole, UK) as anticoagulant and centrifuged (600 µg, 10 min, room temperature) to separate plasma. Tetanus-specific IgG and peanut-specific IgE were determined in plasma (or alternately in serum collected separately) using ELISA according to the manufacturers' instructions. PBMC were further isolated by density gradient separation using Histopaque-1077 (Sigma-Aldrich, Poole, UK) and washed in Hank's buffered salt solution (HBSS) containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (HBSS+HEPES). The cells were then resuspended at 5×10^7 cells/mL in HBSS+HEPES and labelled with 5 µM CFSE for 10 min at 37 °C with gentle shaking, then the excess dye was washed [16–18]. CFSE-labelled PBMC were then cultured either with or without antigens in RPMI 1640 (Sigma-Aldrich, Poole, UK) containing antibiotics and supplemented with 5% autologous plasma. Cells were distributed in 24-well flat bottom plates, at a concentration of $3 \times 10^6/2$ mL medium/well in order to ensure minimal proliferation background [19, 20] and were cultured in the presence of peanut and TT for 7 days.

Antigen-specific proliferation assessment

In a similar way as previously published in literature [21–23], after 5 days of culture in the presence of peanut antigens or TT, a 100 µL aliquot was collected from each culture (in triplicate) and transferred to a 96-well, U-bottom plate. 0.5 µCi [3H]-methyl-thymidine were added to each well, the plate was incubated for 6 h and then cells were collected on glass fibre filters using a cell harvester (Packard, Meriden, CT, USA) and thymidine incorporation into DNA was measured using a beta-counter. Cell proliferation was determined for all the patients in whom we could collect sufficient cells. The percentage of CFSE^{low} lymphocytes was determined by flow cytometry on day 7 of the cell culture.

Cell activation and staining for detection of cytokine production by flow cytometry

After 7 days of culture in the presence of antigens, PBMC were restimulated with phorbol-12-myristate-13-acetate (PMA, 50 ng/mL) and ionomycin (1 µg/mL) in the presence of brefeldin A (10 µg/mL) for 6 h as previously described [24]. The cells were then fixed (20 min, 2% paraformaldehyde/PBS, pH 7.4), permeabilized (0.2% saponin) and stained with cytokine-specific antibodies. PBMC cultured in the presence of the same antigens, but not restimulated

with PMA and ionomycin, were used as negative controls. At least 10 000 events were collected for each experimental condition. Cytokine production was determined for all the patients in whom we could collect sufficient cells. Dead cells and debris were excluded by gating on the lymphocytes using their forward scatter/side scatter characteristics. Subsequently, CFSE^{low} cells were gated according to their low levels of CFSE fluorescence in comparison with CFSE^{high} cells and the percentage of cytokine-positive cells amongst the CFSE^{low} cells was determined. CFSE^{low} cells were analysed for their production of cytokines using the WinMDI 2.8 software (Scripps Research Institute: <http://facs.scripps.edu/software.html>).

Results

TT-specific T and B cell responses (lymphocyte proliferation and, respectively, IgG and IgE levels) are normal and equivalent in PA and NA donors.

We compared the T cell and the B cell responses to TT in PA and NA children (Fig. 1). T cell responses were determined by assessing TT-specific lymphocyte proliferation, expressed as stimulation index (SI) measured using tritiated thymidine incorporation after 5 days of PBMC culture in the presence of 10 µg/mL TT. B cell responses to tetanus were determined as tetanus-specific IgG levels, measured by ELISA.

Tetanus-specific lymphocyte proliferation as well as tetanus-specific plasma IgG levels were equivalent in both PA and NA donors (Fig. 1). Tetanus-specific IgE was not detected in either the PA or NA donors, with the exception of a few donors who had relatively low IgE levels. Thus, the allergic status to peanut does not affect the normal immune response to tetanus antigens in children who have received a routine immunization schedule.

Peanut-specific T and B cell responses (lymphocyte proliferation and respective IgE levels) are higher in PA than in NA donors

T cell responses to lyophilized peanut extract and peanut-specific IgE levels as measured by Pharmacia CAP were different in PA and NA children. We found that T cell responses to peanut were significantly greater in children with PA as compared with their NA controls (Fig. 2a). Cell proliferation was again expressed as SIs representing the ratio between the averages of triplicate measurements of antigen-stimulated wells and control wells where no antigen was added in order to take into account background proliferation. Regarding peanut-specific counts-per-minute (cpm) absolute values, proliferation reached median values of 1523 (range 228–4203) in NA and 16 359 (range 411–39 817) in PA donors. For comparison, in the case of TT-specific proliferation, the respective cpm values were 13 711 (range 150–36 676) in NA and 12 788

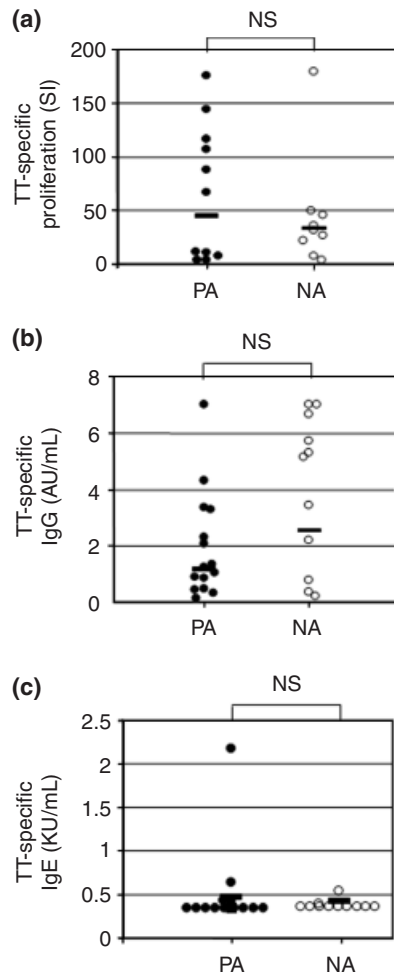


Fig. 1. (a) Tetanus toxoid (TT)-specific proliferation of peripheral blood mononuclear cells from peanut-allergic (PA) ($n = 11$) and non-allergic (NA) ($n = 9$) donors. (b) TT-specific IgG in the plasma from PA ($n = 12$) and NA ($n = 11$) donors. (c) TT-specific IgE in the plasma from PA ($n = 13$) and NA ($n = 11$) donors. Points represent values from individual donors while bars represent median values. Open circles represent NA while closed circles represent PA individuals. P -values were calculated using the Mann-Whitney test (NS, non-significant).

(range 1337–59 136) in PA donors. Not surprisingly, specific IgE levels to peanut were significantly elevated in the PA group and absent in the NA group (Fig. 2b). IgG response to peanut was equivalent in both groups.

T cell response to TT (lymphocyte proliferation) does not correlate with B cell response (IgG and IgE levels) whereas the T cell response to peanut is correlated with the allergic humoral response (IgE levels) but not with the peanut-specific IgG response

We further determined the correlation between T cell (proliferation) and B cell (antibody level) responses to TT and, respectively, peanut antigens (Fig. 3).

While no correlation could be observed in the case of TT-specific responses ($r = 0.08$, $P = 0.74$, Fig. 3a) we did

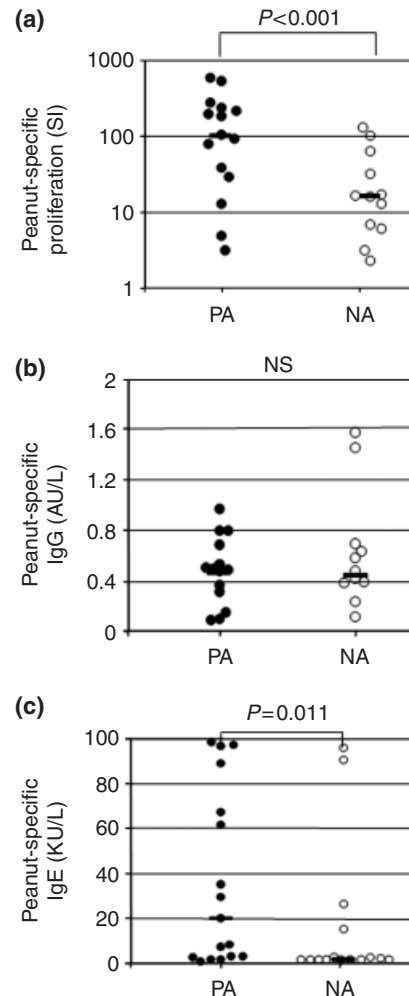


Fig. 2. (a) Peanut-specific proliferation of peripheral blood mononuclear cells from peanut-allergic (PA) ($n = 18$) and non-allergic (NA) ($n = 11$) donors. (b) Peanut-specific IgG in the plasma from PA ($n = 14$) and NA ($n = 11$) donors. (c) Peanut-specific IgE in the plasma from PA ($n = 17$) and NA ($n = 11$) donors. Points represent values from individual donors while bars represent median values. Open circles represent NA while closed circles represent PA individuals. P -values were calculated using the Mann-Whitney test.

observe a strong correlation of T and allergic B cell responses to peanut antigens ($r = 0.741$, $P < 0.001$, Fig. 3d). Conversely, we did not find a correlation between T cell proliferation and IgG responses to peanut proteins (Figs 3b and c). Thus, proliferative responses to TT do not correlate with the humoral immune response. In contrast, T cell responses to peanut allergens correlate strongly with the humoral (peanut-specific IgE) allergy response in the allergic state.

There is no correlation between humoral responses to TT and TT-specific cytokine responses

We identified TT-specific Th lymphocytes using CFSE-labelling as previously described [17]. Indeed, in a CFSE-

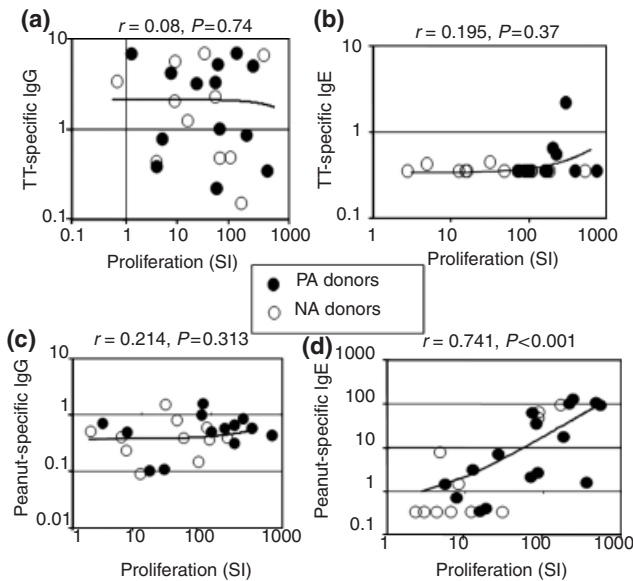


Fig. 3. (a) Correlation between tetanus toxoid (TT)-specific IgG and tetanus-specific proliferation from peanut-allergic (PA) ($n = 12$) and non-allergic (NA) ($n = 11$) donors. (b) Correlation between TT-specific IgG and tetanus-specific proliferation from PA ($n = 12$) and NA ($n = 11$) donors. (c) Correlation between peanut-specific IgG and peanut-specific proliferation from PA ($n = 13$) and NA ($n = 11$) donors. (d) Correlation between peanut-specific IgE and peanut-specific proliferation from PA ($n = 16$) and NA ($n = 11$) donors. Points represent values from individual donors. Open circles represent NA while closed circles represent PA individuals. The r -values represent Spearman rank correlations.

labelled, (CFSE^{high}) PBMC population cultured in the presence of antigens we observed the emergence of a less fluorescent, CFSE^{low} subset which represents the dividing antigen-specific cells. In a previous paper, we have shown by cloning that this emerging CFSE^{low} population is indeed antigen-specific [16]. Identification of the (rare) antigen-specific Th cells among the PBMC in culture also allows us to determine their cytokine-production phenotype while using a non-specific boost with PMA and ionomycin in the presence of brefeldin A as secretion inhibitor. We thus characterize the antigen-cytokine response as the percentage of cytokine-producing cells among the antigen-specific T cell population. We examined the relationships between tetanus antigen responses and IL-4, IL-13, IFN- γ and TNF- α in the tetanus-specific CFSE^{low} population by intracellular cytokine staining and flow cytometry. We did not observe any correlation between the humoral response to tetanus (TT-specific IgG level) and TT-specific cytokine responses (Fig. 4).

Humoral allergic responses to peanut antigens (B-cell responses) are correlated with the cytokine profile of peanut-specific T cell responses

There was a strongly positive statistically significant correlation between the Th2 response (IL-4, IL-13) mea-

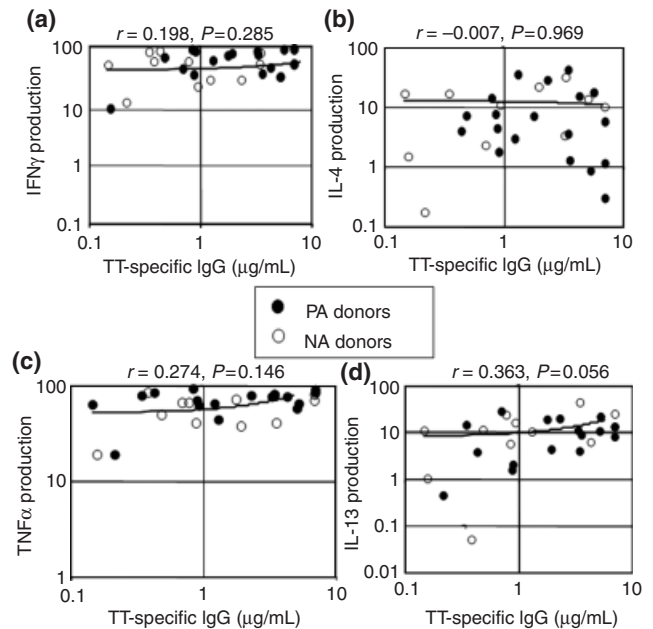


Fig. 4. (a) Correlation between B cell responses to tetanus toxoid (TT) (specific IgG) and TT-specific T cell IFN- γ responses in peanut-allergic (PA) ($n = 17$) and non-allergic (NA) ($n = 11$) donors. (b) Correlation between B cell responses to TT (specific IgG) and TT-specific T cell IL-4 responses in PA ($n = 19$) and NA ($n = 11$) donors. (c) Correlation between B cell responses to TT (specific IgG) and TT-specific T cell TNF- α responses in PA ($n = 17$) and NA ($n = 11$) donors. (d) Correlation between B cell responses to TT (specific IgG) and TT-specific T cell IL-13 responses in PA ($n = 16$) and NA ($n = 11$) donors. Points represent values from individual donors. Open circles represent NA while closed circles represent PA individuals. The r -values represent Spearman rank correlations between the respective values. The y-axis represents the percentage of cytokine-producing cells amongst the carboxyfluorescein succinimidyl ester low population.

sured in the peanut-specific CFSE^{low} population with the specific IgE responses to peanut measured in the plasma. Conversely, there was a strongly negative statistically significant correlation between the Th1 cytokine response (IFN- γ , TNF- α) and the levels of peanut-specific IgE (Fig. 5).

Peanut-specific B and T cell responses are correlated in PA but not in NA individuals

In Table 1, we compare the level of peanut-specific B cell responses (expressed as specific IgE level) with several peanut-specific T cell response parameters (proliferation and specific cytokine production, respectively). We found that indeed there is a strong correlation between B and T cell responses in PA individuals suggesting a permanent ongoing B stimulation by T cells while this correlation has been lost in the case of NA individuals. Thus, in the case of PA individuals, there is a highly significant correlation between peanut-specific T cell proliferation and IgE (Spearman $r' = 0.751$, $P = 0.003$). The correlations between

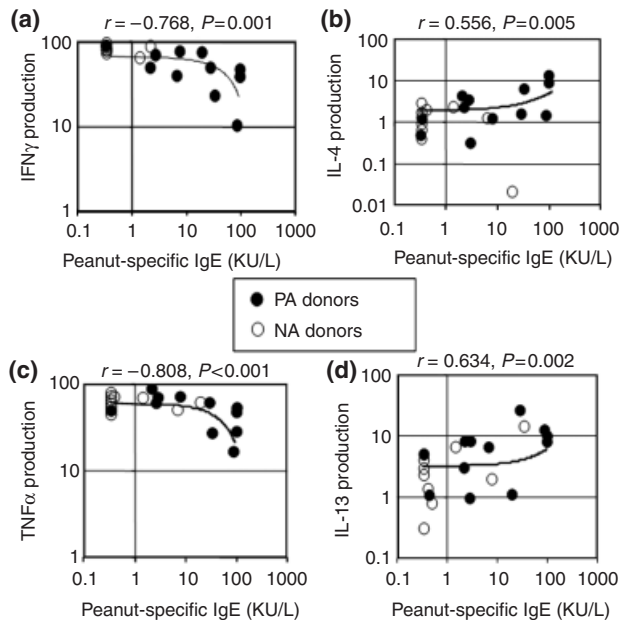


Fig. 5. (a) Correlation between B cell responses to peanut (specific IgE) and peanut-specific T cell IFN- γ responses in peanut-allergic (PA) ($n = 11$) and non-allergic (NA) ($n = 10$) donors. (b) Correlation between B cell responses to peanut (specific IgE) and peanut-specific T cell IL-4 responses in PA ($n = 12$) and NA ($n = 9$) donors. (c) Correlation between B cell responses to peanut (specific IgE) and peanut-specific T cell TNF- α responses in PA ($n = 11$) and NA ($n = 9$) donors. (d) Correlation between B cell responses to peanut (specific IgE) and peanut-specific T cell IL-13 responses in PA ($n = 12$) and NA ($n = 9$) donors. Points represent values from individual donors. Open circles represent NA while closed circles represent PA individuals. The r -values represent Spearman rank correlations between the respective values. The y -axis represents the percentage of cytokine-producing cells amongst the carboxyfluorescein succinimide ester low population.

peanut-specific T cell cytokine production and IgE levels are negative in the case of Th1 cytokines ($r' = -0.71$, $P = 0.007$ for IFN- γ and $r' = -0.746$, $P = 0.005$ for TNF- α , respectively). Conversely, peanut-specific IgE levels are positively correlated with Th2 cytokines: $r' = 0.635$, $P = 0.02$ for IL-4 and $r' = 0.641$, $P = 0.025$ for IL-13. On the other hand, there is no correlation between peanut-specific B cell responses (reflected by the IgE values) and T cell responses in NA individuals.

Discussion

While it is generally accepted that the initiation and maturation of B cell antibody-responses depend upon T cell help, there is little literature on the relationship between antibody levels to a recall antigen and the specific T cell responses in humans. We studied the relationship between T and B cell responses to tetanus and then further examined the relationship between T and B cell responses to peanut in children with persistent peanut allergy as a starting point.

Table 1. Correlation between peanut-specific IgE and peanut-specific T cell responses in non-allergic (NA) and in peanut-allergic (PA) donors

	Spearman correlation	P-value
PA sp IgE values vs.		
T cell proliferation (expressed as SI)	0.751	0.003
IFN- γ (% expression in CFSE ^{low})	-0.71	0.007
TNF- α (% expression in CFSE ^{low})	-0.746	0.005
IL-4 (% expression in CFSE ^{low})	0.635	0.02
IL-13 (% expression in CFSE ^{low})	0.641	0.025
NA sp IgE values vs.		
T cell proliferation (expressed as SI)	0.445	0.317
IFN- γ (% expression in CFSE ^{low})	0.259	0.574
TNF- α (% expression in CFSE ^{low})	0.319	0.538
IL-4 (% expression in CFSE ^{low})	0.556	0.195
IL-13 (% expression in CFSE ^{low})	0.406	0.425

Thus, in the present work, we determined the correlation between peanut-specific IgE levels and peanut-specific T cell responses and, for comparison as a control response, the correlation between tetanus-specific IgG levels and tetanus-specific T cell responses (a typical long-lived memory immune response) in the same donors. IgE levels were measured by established commercial CAP assays (Phadia AB, Uppsala, Sweden). It was found that in several donors, the plasma peanut and tetanus-specific IgE values were below the assay level of detection, but clinically relevant IgE (in peanut or tetanus allergy) would be detected using this assay. Clearly, it is not possible to resolve the question whether the level of detection is inadequate, or there is absolutely no IgE being made to peanut and, respectively, tetanus.

We found to our surprise that tetanus-specific antibody levels did not correlate with tetanus-specific lymphocyte proliferation nor with tetanus-specific cytokine production, a finding that reflects however the recent observations regarding the maintenance of antibody production by polyclonal activation of memory B cells [6]. Indeed, immune responses involving long-term antibody production persisting in the absence of sustained antigen-dependent T cell stimulation have been shown to occur in humans as most vaccines ensure persistent protective levels of antibodies lasting for several years [25, 26]. Several non-exclusive mechanisms have been proposed for this long-term maintenance of antibody responses: (i) long-lived plasma cells, (ii) persistence of cross-reactive antigen or (iii) polyclonal activation of B cells [6].

Conversely, we found that peanut-specific IgE levels were strongly and positively correlated with specific T cell proliferation and Th2 cytokine levels and negatively correlated with Th1 cytokine production. This correlation, that we characterized in detail for the first time in the case of peanut allergy, confirms previous findings of Dorion et al. [27] who showed that the production of IFN- γ to a

peanut allergen was negatively correlated with peanut-specific T cell proliferation. Further studies showed that T cells that respond to dietary antigens in NA individuals display a Th1 phenotype [28]. However, no peanut-specific IL-4 (nor other Th1 or Th2 cytokines) was studied at the time by the respective authors because of technical difficulties in measuring cytokine production. We overcame these impediments using a novel technique that we established for the identification of rare allergen-specific T lymphocytes amongst PBMC using CFSE labelling [16]. In that published report, we also describe the cytokine differences between PA (having Th2-skewed peanut-specific responses) and NA individuals (whose responses are Th1 polarized). Also, in the case of another food allergy (to hen eggs), it was shown that specific IgE values are correlated with T cell proliferation and their combination is predictive for the clinical course of this allergy [13].

Because the peanut-specific B and T cell responses clearly reflect the allergic state (PA or NA) of the donor, we suggest that in active allergic states, B and T cell responses remain in fact correlated whereas in NA, long-term responses (such as that against tetanus antigens) B and T cell responses are uncoupled. A possible explanation for this paradox would be a relatively longer life of IgG-secreting plasma cells (mainly localized in the bone marrow) compared with a shorter lifespan of IgE-secreting plasma cells, many of whom are localized in the tissues and ensure high local IgE production. Localization of IgE production might thus be achieved at the price of a shorter life of IgE producers due to lower levels of microenvironmental support in peripheral tissues in comparison with the bone marrow. Our data is thus consistent with the notion that ongoing normal humoral responses to antigens eventually become independent of T cell help. However, in the case of peanut allergy, the strong positive correlations between antigen specific T and B cell responses support the hypothesis that ongoing IgE production requires constant T cell support with appropriate cytokine production.

Our data suggests that this dependency upon antigen-specific T cell help may be exploited to try to block allergic IgE responses by modulating T cell function. Indeed, alteration of the immune response using SIT is dependent upon the immunomodulation of allergen-specific T cells [29]. Furthermore, rush immunotherapy actually shows that the degree of T cell change is reflected in the clinical response to SIT [30]. Allergen-SIT is currently the only therapeutic approach for type I hypersensitivity that alters the natural history of the disease. Essentially, SIT involves the repeated, long-term administration of relatively high doses of allergen aiming to modulate the allergen-specific T cells. This process is supposed to subsequently terminate T cell support for allergen-specific B cells production of IgE. While the precise immune mechanism leading to the success of SIT is still unclear, it

has been found to correlate with a decrease of Th2 cytokine production by allergen-specific Th cells and an increased production of IL-10 and/or TGF- β [21, 29]. In this respect, it seems probable that active regulatory/suppressor T cells are required in order to decrease allergy-type responses. Comparatively, in a recent publication [16], we have shown that in individuals who have never had allergic T cell responses to tolerated food antigens responses are characterized by low Th2 and high Th1 cytokine production (increased IFN- γ and decreased IL-4). Our results, which are in accordance with previous work describing a Th1 cytokine profile for food-antigen specific Peyer patch T lymphocytes that persist even in states of oral tolerance [28], suggest that physiological states of tolerance to food antigens might be achieved by different immune mechanisms than SIT-induced allergen tolerance which takes place on a pre-existing allergy state.

In the case of peanut allergy, SIT for peanut allergy was indeed used reasonably successfully but with a high incidence of adverse reactions as described by Oppenheimer et al. [31] and by Nelson et al. [32]. It is indeed the high risks associated that led to the development of modified peanut proteins [33] and peptides [34] for immunotherapy and more recently the proposal of using anti-IgE blocking antibodies as a protection for preventing anaphylactic reactions during SIT for peanut allergy [35].

In conclusion, we find that in PA individuals, the allergen-specific B cell response is correlated with its allergen-specific T cell counterpart. Conversely, in the same individuals, the T and B cell responses specific for a non-allergenic control antigen such as TT are not closely correlated. These studies provide a theoretical basis for the targeting of T cell function in different immunomodulatory treatments such as SIT to treat peanut allergy and other allergic diseases.

Acknowledgements

The authors acknowledge Mr Ian Lamb's assistance with the statistical analysis. This work was supported by the Food Standards Agency, UK, and by the National Peanut Board, USA.

Conflict of interest: the authors have no conflict of interest.

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