

## EDITORIAL

# Immunological responses in peanut allergy

Even amongst those with the most sceptical view on the importance and the role of food allergies, few fail to recognize the seriousness of peanut allergy. Peanuts are the most common cause of fatal and near-fatal food-related anaphylaxis [1,2]. Peanut allergy usually occurs early in life [3,4] and indeed we have shown that 80% of British peanut allergic individuals have had a reaction on their first exposure to peanuts suggesting an early sensitization [5]. Unfortunately for the individual, peanut allergy seems to be a life-long problem starting in childhood and persisting into adulthood [6]. Although the first report on peanut allergy was in 1920 [7], there has been a gap of over 60 years before it was investigated systematically. Allergic reaction to peanuts killed at least six people in the UK and there is good evidence to suggest peanut allergy is a condition which is on the increase [8]. A recent prevalence study in UK indicates that peanut allergy affects around 1% of 4-year-olds. The reason for its increase at present remains debatable. Whether its increased introduction to our diet has meant that expectant mothers consume more peanuts during pregnancy and expose their babies to peanut allergen via breast milk, or even intrauterine sensitization, needs to be addressed in a methodical way. Our group has provided some data supporting an association between self-reported increased consumption of peanuts by expectant mothers who subsequently breast-fed their babies and a decrease in age of onset of peanut allergy over the last 10 years [9]. However, our study was a cross-sectional study and there is an element of recall bias which may have influenced our data. Clearly, there is a need for long-term prospective birth cohort studies which will be able to provide answers for these questions.

Peanut allergy is a big challenge to immunologists interested in food allergy. Peanut has a complex protein chemistry containing up to 32 different proteins [10,11]. Some of the major proteins include arachin and its subunits with a molecular weight ranging between 10 and 71 kDa and conarachin and its subunits (mol.wt.: 18–62 kDa). Identification and characterization of allergens within peanut is instrumental to our understanding of mechanisms which underlie immune responses to peanut. Two major peanut allergens (Ara h 1 and Ara h 2) with a mol.wt. of 63.5 and 17 kDa, respectively, were identified in early 1990s [12,13] and recent efforts with these allergen have concentrated on epitope mapping and identifying the relevant IgE-binding epitopes [14,15]. Another allergen, peanut agglutinin (mol.wt.: 31 kDa) was also identified and characterized by the same group [15]. Recently, using sera from over 90

peanut allergic individuals, we have identified 18 proteins bands in a 'crude peanut extract' which were capable of binding specific IgE with many reacting to proteins other than those identified allergens [16] including a protein with a low molecular weight of 12 kDa. Further investigations might demonstrate that it is these lower molecular weight allergens, having the ability to be rapidly absorbed through the mucosal barrier, could be responsible for the quick onset of reaction observed in allergic individuals. Recently, Ara h 3 (mol.wt.: 14 kDa) was identified by using peanut sensitive patient sera absorbed with soy proteins [17]. Subsequent work in characterizing this protein will assess the clinical relevance of this protein in allergic individuals. These investigations suggests that our quest in identifying the full spectrum of allergens within peanuts is far from complete and there are clearly many more allergens which need to be identified and it may be feasible to predict that peanut allergic individuals with different clinical severity may have a specific immune response to one allergen or indeed a particular combination of few allergens.

With regards to immunological responses to peanut and its allergens, in allergic individuals, there are relatively limited studies. Few investigations have addressed the relationship between clinical severity and IgE levels [18] or peripheral blood mononuclear cell (PBMC) responses [19]. We have measured peanut-specific IgE in 346 peanut allergic subjects and found that IgE concentration peak at the 11–15 years of age ( $n = 57$  mean IgE; 36 IU/mL) but did not detect a relationship between clinical severity and IgE levels in any age group investigated [5]. Clearly, in order to understand what makes peanut such a potent allergen, number of factors need to be investigated and high on this agenda will be the host's immune response and the role of lymphocytes and relevant cytokines in particular IL4 and IFN $\gamma$ .

T-lymphocytes are pivotal in controlling cellular immune responses and regulation of IgE expression. Although several studies have shown association between T-cell proliferation, cytokine production and IgE levels in inhalant allergy, very few have addressed this issue in food allergy and specifically in peanut allergy.

The first study investigating lymphocyte reactivity in peanut allergic subjects [20] reported significantly higher levels of PBMC proliferation in response to stimulation with Ara h 2 in nine allergic patients compared with their respective response to other food allergens such as ovalbumin, casein and soy, possibly indicating an increased number of Ara h 2 reactive circulatory lymphocytes. No

difference in proliferative responses between peanut allergic subjects and controls were reported. In the same study, it was shown that after stimulation with Ara h 2, culture supernatants from PBMCs of peanut allergic subjects contained significantly lower levels of IFN $\gamma$  compared with that of the two control groups (six atopics and six non-atopics) and hence exhibiting a 'TH<sub>2</sub>-like' profile. There was also a significant inverse correlation between serum levels of anti-Ara h 2 and IFN $\gamma$  production. This group did not detect IL4 protein in culture supernatant of PBMCs stimulated with Ara h 2 which could possibly be explained by very long incubation (6 days) with the allergen. However, PCR experiments demonstrated IL4 expression in Ara h 2 stimulated PBMCs from both peanut allergic subjects and control subjects.

Using a commercial peanut extract, containing a spectrum of peanut allergens, Higgins *et al.* [21] demonstrated a strong proliferative response towards peanut extract in both groups of nut-allergics ( $n=5$ ) and non-atopic controls ( $n=4$ ) but specific IgE was elevated only in the former group. In a series of elegant experiments, this group raised a panel of peanut-reactive T-cell clones from a peanut and brazil nut allergic individual. They reported that peanut-specific clones produced high levels of IL-4 and consistent with the previous study [20], low levels of IFN $\gamma$  were detected following stimulation with peanut extract also verifying a 'TH<sub>2</sub>-like' profile. Indeed, this finding has now been confirmed by other groups [22,23].

From a slightly different angle and using a paediatric group, in a publication in this issue of '*Clinical and Experimental Allergy*' Laan *et al.* [24] report their findings on the specificity of the T cells in proliferative responses, in children with atopic dermatitis with or without peanut allergy ( $n=13$  and 8, respectively) and their age-matched controls ( $n=10$ ). They report that the proliferative response of PBMCs stimulated with peanut extract amongst children with atopic dermatitis and peanut allergy were significantly higher when compared with controls and children with atopic dermatitis but not peanut allergy. Similar finding have been reported with adult peanut allergic subjects in previous studies [21,22] where similar commercially available peanut extracts were used. Using FACScan analysis Laan *et al.* demonstrated that it was CD4<sup>+</sup> cells that proliferated preferentially after stimulation with peanut extract. The authors state that they were not able to detect IL4 production in culture supernatant in any of the samples. This is in agreement with one previous report [20] where Ara h 2 was used to stimulate PBMCs, although other groups have reported presence IL-4 in culture supernatant following stimulation with peanut extracts [21,22]. In contrast with previous studies, Laan *et al.* did not detect a reduced IFN $\gamma$  production in culture supernatants of the peanut allergic group when their PBMC were stimulated with peanut extract. This is in contrast with previous

investigations and unfortunately had not been addressed in the article. Indeed, IFN $\gamma$  was only detected in culture supernatant of two of the atopic dermatitis group without peanut allergy. They also investigated the IL-4 and IFN $\gamma$  mRNA scan value generated after PCR following peanut extract stimulation and compared this with the scan value of unstimulated PBMC of the same individual. Their results indicated a significant positive correlation between increased IL-4 mRNA expression and peanut specific PBMC proliferative response in the peanut allergic group. No correlation between IFN $\gamma$  mRNA and proliferative responses were observed.

There is now a wealth of evidence suggesting that individuals with peanut allergy, show variable immunological responses that are different to both atopic and non-atopic controls. What is still unclear is what makes an individual susceptible to peanut allergy. Is there something different about the T-cell receptor or the human leucocyte antigen (HLA) class II antigens amongst these individuals rendering them to be more susceptible to develop peanut allergy?

Dorian and Leung have recently addressed the first issue [25] and investigated whether peanut allergics ( $n=8$ ) use a different T-cell receptor variable beta regions (V $\beta$ ) when encountering peanut allergens, compared with controls ( $n=8$ ). They reported that PBMCs from six of the peanut allergic (and none from the controls) selectively expanded their V $\beta$ <sub>2</sub> T cells in response to incubation with crude peanut extract and hence there appears to be a restricted T-cell receptor V $\beta$  usage to peanut.

In a study described earlier [21], Higgins *et al.* showed that T-cell clones raised from a peanut allergic individual recognized peanut allergens in association with HLA-DR and HLA-DP but not HLA-DQ class II molecules and conclude that there is a restriction of T-cell response by the former two class II molecules.

More recently, in an attempt to find out whether HLA class II genetic polymorphisms contribute to the development of peanut allergy, we have carried HLA typing of all individuals from 37 families with at least one peanut allergic subject and nine unrelated peanut allergic individual ( $n=161$  in total) and compared the genotype frequencies with those found in 293 unrelated controls [26]. We demonstrated that HLA class II polymorphism contributes to determining susceptibility to peanut allergy where three class II genotypes (DRB1\*08, DRB1\*08/12 tyr16 and DQB1\*04) were significantly increased in peanut allergic individuals compared with controls.

In summary, to date, although most of our efforts in understanding mechanisms which underlie food allergy and specifically peanut allergy have focused on IgE responses, it is clear from studies published and research in progress that other arms of the immune response are extremely important

in enhancing our understanding of immunological responses to peanut.

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