

EDITORIAL

To bee or not to be? T-cell responses to bee venom PLA₂ in relation to anaphylaxis and immunotherapy

Allergic sensitization results from exposure to an antigen and development of an immune response in the context of a T helper 2 (TH₂) type cytokine environment, which potentiates IgE production and mast cell/basophil reactivity. It is probable that allergenic antigens (allergens) can induce an IgE-independent production of TH₂-type cytokines from certain effector cells such as mast cells on primary exposure, leading to the induction of antigen-specific TH₂-type T cells, isotype switching in B cells and allergen-specific IgE production. Secondary or subsequent exposure can lead to the very rapid IgE-dependent release of inflammatory mediators from mast cells and/or basophils and cause systemic or anaphylactic reactions. The variation in susceptibility to and acquisition of allergic sensitivity is still not fully understood, but has genetic [1], acquired and environmental [2] components.

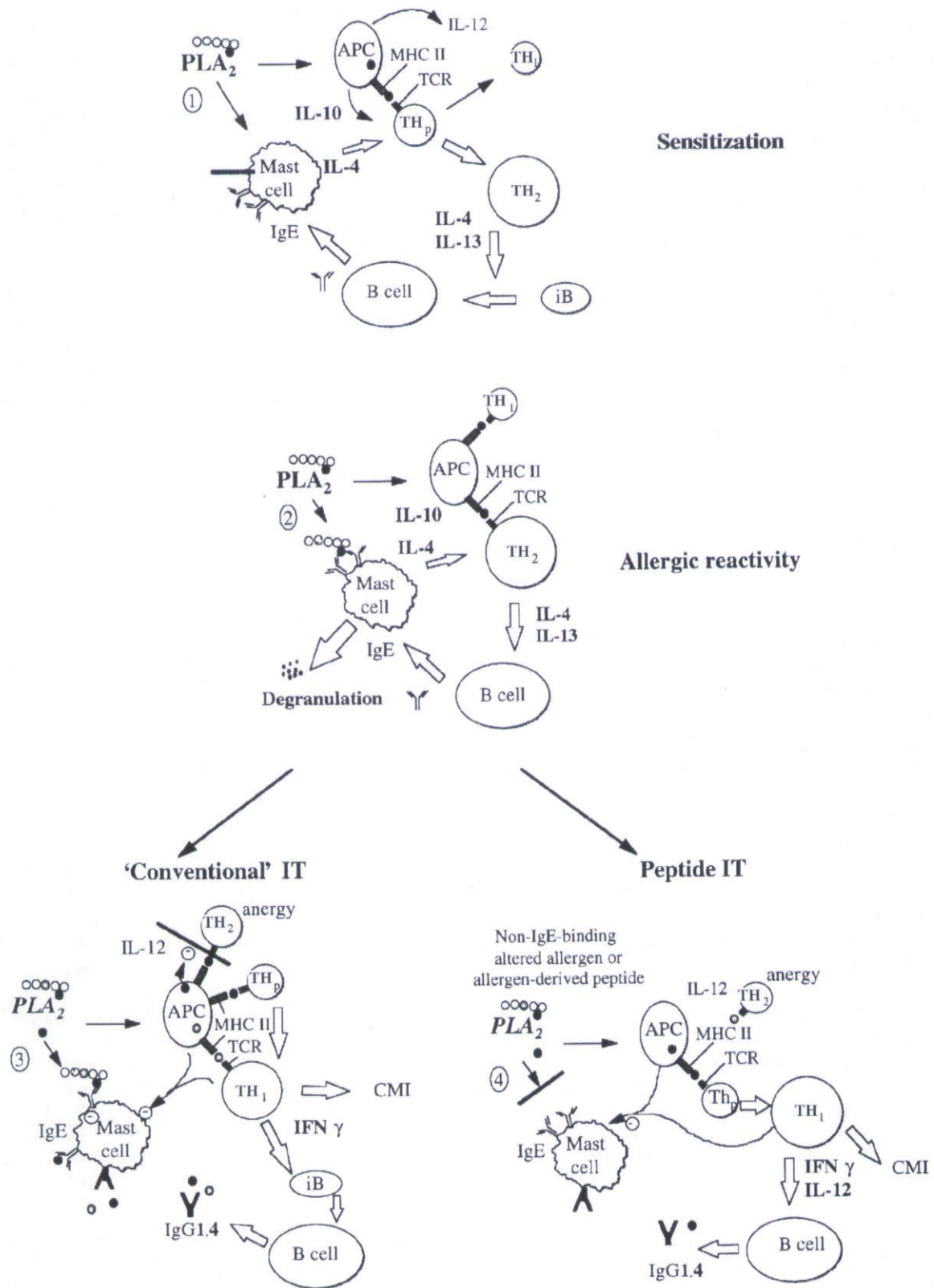
The aetiology of allergic sensitivity is poorly understood and ranges from early or late onset, through the possibility of spontaneous resolution or desensitization, to persistence and the chance of systemic reactivity and anaphylaxis. Severe systemic reactions to bee stings, including life-threatening anaphylaxis, are the principal indicators for immunotherapy to bee venom [3]. This treatment has the advantage of being potentially curative and has a high success rate [4]. Immunotherapy entails administering very low doses of specific allergen (or allergenic peptides) then gradually increasing the dose over variable time periods until clinical desensitization is achieved [5]. The mechanisms involved in desensitization are incompletely understood but recent evidence suggests that modulation of signaling pathways following engagement of immunoglobulin receptors on mast cells/basophils by incremental doses of allergen, and alterations in T-cell cytokine production from TH₂ to TH₁ [6–8] by tolerogenic mechanisms, may effect immediate and longer term hyporesponsiveness, respectively [9].

Bee venom contains a number of allergens including phospholipase A₂ (PLA₂), hyaluronidase, acid phosphatase, melittin, apamin, kinins and various other minor allergens [10,11], any or all of which can be responsible for specific IgE-mediated allergic reactivity. PLA₂ is highly toxic to cells in high concentrations, but at lower concentrations is a powerful immunogen and is the major allergen of bee venom, with greater than 90% of subjects with bee venom anaphylaxis producing IgE antibody to it [12]. It is a relatively small, complete allergen whose crystal structure has been resolved [13]. It has a molecular mass of about

19 kDa (range 14–20 kDa) consisting of 134 amino acids, with a single asparagine-linked glycosylation site. In common with all PLA₂s, bee venom PLA₂ has a calcium binding site and, as its name suggests it has enzymatic activity, specifically hydrolysing the 2-ester bond of L-glycerophospholipids. Studies with specific inhibitors have demonstrated that this catalytic activity is centered around a hydrophobic binding cavity formed between residues at the C-terminal β -sheet and an anti-parallel β -sheet, juxtaposed to a cationic site (on linking residues between two anti-parallel α -helices and between the second α -helix and the first β -sheet) which reacts with polar moieties [14]. This indicates that tertiary structure is important in conferring enzymatic activity, the relevance of which will be discussed below.

The interactions between PLA₂ and surface bound immunoglobulin on mast cells, basophils, eosinophils, B cells and other antigen presenting cells (APCs) relies on specific contact with components of the native molecule. However, taking a step backwards, in initial allergic sensitization there is now strong evidence that the enzymatic activity of PLA₂ causes direct release of preformed IL-4 from mast cells by local cellular disruption, as a result of concentration dependent cytotoxicity either by hydrolysing certain membrane phospholipids [15] or via specific membrane receptor events [16]. Dudler *et al.* [17] have demonstrated that a mutant recombinant PLA₂ with a single amino acid substitution in the active site, although still highly immunogenic, is unable to cause disruption or induce mediator release from a rat mast cell line at equivalent concentrations of recombinant enzymatically active PLA₂. T cells activated in the presence of TH₂ cytokines tend to develop into the TH₂ phenotype [18], proliferating in response to allergen and producing interleukin-4 (IL-4) and IL-13, which cause isotype switching to IgE and subsequent allergic reactivity. For an IgE response to be initiated and maintained T cells have to recognize (via the T-cell receptor) processed allergenic peptides presented in the antigen binding groove of the class II MHC molecules of APCs, and at the same time received obligatory co-stimulatory signals. There is, therefore, considerable interest in the T-cell epitopes derived from PLA₂ which are able to perpetuate or even possibly inhibit this process.

This editorial is based around an article by Kämmerer *et al.* in the current issue of *Clinical and Experimental Allergy* [19] which describes the delineation of T-cell epitopes of PLA₂, comparing short with long overlapping peptides. Their approach was to investigate the ability



of 13 overlapping 15-mer peptides, covering the whole 134 amino acids of PLA₂, to induce CD4 T-cell proliferation from PBMC or T cell lines of bee venom sensitive subjects, and to compare it with the proliferative response induced by three longer (40–60-mer) overlapping peptides. They show that the short linear sequences were not efficient at inducing a T-cell response compared with longer peptides, which enhanced T-cell responses in all patients.

This is not the first report on studies of T-cell epitopes of PLA₂, and, in a wider context, many other allergens have been epitope-mapped [20]. What is generally observed is that major environmental allergens contain multiple T-cell epitopes and that MHC restriction is extremely diverse, although some HLA restriction is evident with some allergens and/or peptide responses [20,21]. By the nature of antigen presentation via MHC II, where short (13–17-mer) peptides are presented to the TCR [22], it would appear likely that linear sequences constitute the majority of

important T-cell epitopes, and indeed there is evidence to support this. Dhillon *et al.* [23] and Carballido *et al.* [24] have independently identified five (two and three respectively) putative major T-cell epitopes of PLA₂. Whilst there was some overlap, the amino acid sequences and peptide lengths were different in these studies, and related sequences in the study of Kämmerer *et al.* [19] did not appear to evoke a T-cell response. This suggests that either direct uptake by MHC II presents the peptides to a number of different MHC restricted clones or that differential processing may occur, depending on the exact composition and length of the synthetic peptides used. Also it is recognized that peptides presented in the absence of adequate co-stimulatory signals from APC will induce T-cell anergy [25]. Clearly it would not be possible to synthesize and test all possible peptide permutations so the approach adopted by Kämmerer *et al.* seems a logical advance, whereby antigen processing of large portions of the PLA₂ molecule would be likely to throw

Fig. 1. Simplified schematic diagram representing an hypothetical progression from 1) allergen sensitization (in this case PLA₂), 2) ongoing allergic reactivity, 3) 'conventional' immunotherapy, 4) peptide or altered allergen immunotherapy.

1) Sensitization

Enzymatic activity of PLA₂ causes IL-4 release from mast cells. Antigen presenting cells (APC) take up PLA₂, process it and present peptides to T helper precursor (TH_p) cells via MHC class II (MHC II) and the T-cell receptor (TCR). In the presence of IL-4, TH_p cells develop and expand into TH₂ cells which release IL-4 and IL-13. These cytokines cause IgE isotype selection during maturation of immature B cells (iB) activated by binding B cell epitopes of PLA₂ via surface IgM, and the subsequent production of IgE. The allergen-specific IgE binds to mast cells, resulting in allergic sensitization. In this model, low doses of PLA₂ cause IL-10 release from APC, whereas high doses cause IL-12 production. In the latter case TH₂ cytokines are inhibited and IFN γ production induced, resulting in a TH₁ response and no allergic sensitization. High and low doses are arbitrary and would depend on genetic predisposition.

2) Allergic reactivity

Secondary and subsequent exposure to PLA₂ results in recognition of native antigen by surface-bound IgE on mast cells, IgE cross-linking occurs and mast cells degranulate causing signs or symptoms of allergic reactivity. Antigen processing and the direct enzymatic action of PLA₂ on mast cells maintains a TH₂-dominated positive feedback loop, which could result in hypersensitivity and lead to anaphylaxis. Natural desensitization could occur by the mechanism outlined for 'conventional' immunotherapy in 3) below.

3) Conventional immunotherapy

Initial very low concentrations of PLA₂ bind to IgE on mast cells but the cross-linking is insufficient to initiate degranulation. However, this does not prevent TH₂ type antigen presentation from APCs and IgE production is maintained. As the PLA₂ dose is gradually increased, progressively more IgE is prevented from triggering the mast cell by mechanisms of receptor aggregation and immobilization, until the mast cell is unable to degranulate to a given high dose (say equivalent to two natural stings). During this process the nature of antigen presentation also changes. Higher and higher doses of PLA₂ induce the production of IL-12 from APC. This has a dual effect of antagonizing TH₂ cytokine production from reactive TH₂ cells and also causing negative feedback on enzymatically released endogenous IL-4 from mast cells. High levels of processed peptides presented via MHC II anergize reactive TH₂ cells. Subsequent antigen presentation to TH_p cells takes place in the presence of IFN γ , induced from bystander cells (e.g. monocytes) by IL-12. This results in the development of new, or expansion of existing, TH₁ cells and augmentation of cell-mediated immune (CMI) mechanisms. Maturing B cells do not undergo IgE isotype selection and produce IgG1 and IgG4, often in high amounts. Allergen specific IgG subclasses bind to mast cells via appropriate Fc γ receptors, further down regulating IgE reactivity, either by competition for allergen or by sterically preventing IgE cross-linking.

4) Peptide immunotherapy

This occurs in much the same way as 'conventional' immunotherapy except that the altered allergens no longer bind IgE. Therefore the desensitization of mast cells would be secondary to the induction of 'protective' IgG subclass antibodies, particularly IgG4. In this model the T-cell epitopes represented by the peptides would have to match the dominant native allergen T-cell epitopes in order to induce TH₂ cell anergy. TH₂ anergy could be induced by high concentrations of peptide without antigen processing. Providing a sufficient repertoire of relevant T-cell epitopes would be essential for this approach if it were to be appropriate for population immunotherapy.

up a high proportion of naturally occurring reactive T-cell epitopes. Furthermore, the large peptide fragments did not bind IgE in most (but not all) subjects, indicating that the major IgE-binding sites were not present in the peptides. The peptides would therefore be unable to cause mast cell/basophil degranulation by cross-linking IgE. This suggests that conformational epitopes of PLA₂ may be important in evoking T-cell mediated immune responses, or that the conformation of the antigen presented to APCs may be important in subsequent processing and presentation. The IgE binding data is very encouraging and suggests that these or related peptides may be useful in allergen immunotherapy, but indicates that caution (i.e. individual *in vitro* testing before IT) may be necessary to avoid potential IgE-mediated reactions in some individuals. The heterogeneity of MHC restriction previously demonstrated by others [20], and possibly to be expected in T-cell responses, makes the concept of large, non-IgE-binding peptide immunotherapy, covering the whole allergen, more attractive than the use of a cocktail of short peptides representing arbitrary T-cell epitopes. The latter would have to be individually tailored to individual HLA restriction patterns, at potentially enormous cost and possibly low effectiveness. There also exists the potential for $\gamma\delta$ T cells to recognize non-peptide antigens [26], increasing the number of T-cell epitopes but, in the context of PLA₂, allergenicity does not appear to be affected by deglycosylation [27]. Another potential limitation on T-cell epitope recognition is the restricted use of TCR variable regions. However, in several studies no bias in variable gene usage has been demonstrated although stable $v\beta$ -gene usage has been demonstrated over several years [20].

Although not the main point at issue here, it is worth mentioning B cell and immunoglobulin recognition of PLA₂. Both B cells and immunoglobulin are believed to recognize conformational epitopes, and this has been demonstrated for PLA₂ [28]. It is noteworthy that the ratios and amounts of IgE and IgG subclasses produced on exposure to PLA₂ are critically effected by the dose of antigen to which APCs are exposed [29]. High doses of antigen induce IL-12 secretion and augmentation of IFN γ production (TH₁ potentiating) whereas low doses induce IL-10 and subsequent IL-4 production from T cells (TH₂). Alteration of PLA₂ to remove its enzymatic or cytotoxic activity by site-directed mutagenesis or reduction and carboxymethylation, respectively, can result in incomplete signaling and induce anergy in reactive T cells [25], and prevent recognition by IgE [30]. In such situations, and in repeated exposure to native allergens, IgG emerges as the predominant isotype with high levels of particularly IgG4 and often IgG1 being produced [31]. With respect to immunotherapy with either native allergens, altered allergens or peptides, the significance of these high levels of IgG subclasses produced by B cells, with help from T cells recognizing non-TH₂-inducing

epitopes, although often suggested as protective, is still unresolved.

In summary (Fig. 1), the enzymatic activity of many allergens may act directly on mast cells to induce TH₂-type cytokine release from these cells, creating a TH₂ environment for subsequent T cell activation and expansion. Destroying or inhibiting the enzymatic activity could serve to remove endogenous TH₂-type cytokines from the local inflammatory environment. The epitopes recognized on re-exposure to allergen by T cells will vary between individuals and between individual reactive T cell clones. Despite evidence that short continuous peptides derived from PLA₂ (with minimal tertiary structure) can be effective T cell epitopes, it seems probable that longer peptide fragments may be more efficient at activating T cells as they will contain more continuous, as well as structurally related epitopes which might be produced during antigen processing. The lack of IgE binding ability of the T cell reactive epitopes may be an important factor in altering T cell responses from predominantly TH₂ to TH₁ with time. This would occur if the recognized epitopes were still immunogenic, as T cell help would be directed away from supporting IgE production. Such epitopes or allergen fragments would also be incapable of binding IgE at the surface of the mast cells and thus would not induce mast cell degranulation. However, it remains to be seen if the desensitizing capacity of such IgE-unreactive epitopes or altered allergens would be efficient down regulators of mast cell sensitivity in the short term, a phenomenon thought to occur in immunotherapy where incremental doses of native allergens act directly via IgE on the IgE receptor and downstream signaling events.

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