

EDITORIAL

Clinical & Experimental Allergy

The specifics of allergen recognition by CD4⁺ T lymphocytes at the epitope level

This editorial discusses the findings of the paper by L. D. Archila et al. [10] pp. 986–998.

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The identification within allergens of immunodominant epitopes restricted to common MHC class II haplotypes has allowed to develop MHC class II peptide tetramers in order to investigate allergen-specific CD4⁺ T cell responses. In the tetramer approach, four MHC class II peptide complexes are coupled to biotin and subsequently multimerized using fluorescently labelled streptavidin (Fig. 1), to yield high avidity reagents to stain and detect epitope-specific T cells by flow cytometry [1, 2]. In the last few years, several groups have successfully used such MHC class II peptide tetramers to assess CD4⁺ T cell responses directed against allergens such as Ara h 1, Art v 1, Bet v 1, Bos d 2, Der p 1, Der p 2, Fel d 1, Lol p 1, Phl p 1 and Phl p 5 [3–9].

The interesting study by Archila et al. published in this issue of CEA relied upon double staining with such tetramers to investigate the cross-reactivity of CD4⁺ T lymphocytes directed to T cell epitopes found in grass pollen major allergens [10]. To this aim, four epitopes from Timothy grass (*Phleum pratense*) group 1 and 5 allergens, that is Phl p 1₉₇₋₁₁₆, Phl p 1₂₂₁₋₂₄₀, Phl p 5a₁₆₇₋₁₈₆ and Phl p 5a₁₉₉₋₂₁₈ were considered as representative immunodominant epitopes presented by the common DR04:01 MHC class II haplotype. In a very elegant approach, dual tetramer staining of circulating allergen-specific T cells was performed as follows: peripheral blood mononuclear cells from grass pollen allergic patients were first stimulated with 13 to 20-mer peptides corresponding to homologues of the four above-mentioned epitopes present in various

Pooideae grass species (e.g. Orchard grass, Kentucky blue grass, Rye grass, Velvet grass...). Such T cell epitopes are similar but not identical to the ones found in Timothy allergens, with typically 1–4 amino acid differences between the two.

After 2 weeks in culture, CD4⁺ T cells were stained using both Allophycocyanin-labelled Timothy tetramers, and Phycoerythrin labelled Pooideae grass homologue tetramers (i.e. tetramers made with DR04:01 molecules complexed to a T cell epitope from group 1 or group 5 from either Timothy grass or a distinct Pooideae, respectively). In this setting, for each single epitope considered, three patterns of co-staining of CD4⁺ T cells with the tetramers were observed in patients, allowing to distinguish i) cross-reactive responses with $\geq 85\%$ of CD4⁺ T cells reactive with the two tetramers, ii) non (or minimally) cross-reactive responses, with $\leq 25\%$ T cells reactive with the two tetramers, iii) partially cross-reactive responses, with intermediate percentages of co-staining with the tetramers.

As a first conclusion of this study, all patients were found having allergen-specific CD4⁺ T cells directed against both cross-reactive and non cross-reactive epitopes in their blood. That patients have cross-reactive CD4⁺ T cells recognizing to a comparable level epitopes from group 1 or group 5 allergens originating from multiple grass species is not surprising, given the well known level of amino acid sequence homology (i.e. 90% and 55–85%, respectively) documented for these allergens, within Pooideae [11]. Interestingly, however, minimally cross-reactive epitopes (e.g. Poa p 1₉₇₋₁₁₆, Lol p 1₂₂₁₋₂₄₀, Poa p 5a₁₉₉₋₂₁₈ and Lol p 5a₁₉₉₋₂₁₈) inducing grass species-specific T cell responses were also identified, confirming that a high homology does not imply a full cross-reactivity. This observation was confirmed in reverse experiments in which CD4⁺ T cells were first expanded by Timothy allergen-derived peptides prior to staining by two tetramers and was also extended to other MHC class II haplotypes (DR07:01,

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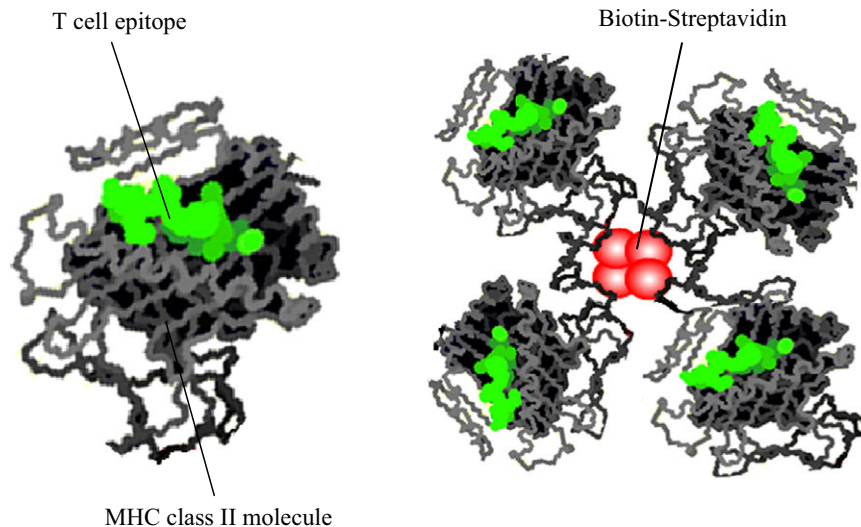


Fig. 1. Development of MHC class II peptide tetramers. T lymphocytes recognize a complex between a single MHC heterodimer (in grey) and a peptide (corresponding to T cell epitope, in green) in a highly specific manner, following engagement of their T cell receptor with amino acid residues located both within the MHC molecule and the peptide. The right panel shows tetramers made from four MHC class II heterodimers assembled in presence of a peptide corresponding to a high affinity T cell epitope. These complexes are coupled to biotin (red spheres), and subsequently multimerized using fluorescently labelled streptavidin to create a high avidity bioprobe to detect epitope-specific CD4⁺ T lymphocytes.

DRB5* 01.01). These results are in agreement with previous reports documenting the presence of both cross-reactive and non cross-reactive T cell epitopes within group 1 and group 5 grass pollen allergens [12, 13]. They also fit with evidence for significant amino acid sequence variations between grass species within 13 out of the 14 MHC class II restricted T cell epitopes identified by computer algorithms within group 1 allergens [14].

On a structural basis, it is now well understood why distinct epitopes with as little as a single amino acid difference can be recognized dramatically differently by T cells. A simple alteration within the primary sequence of a T cell epitope can induce either a change in affinity with the MHC molecule, or a lack of recognition by the T cell receptor, depending upon the orientation of the amino acid (i.e. inward or outward respectively within the MHC molecule groove). Interestingly, a major impact on the recognition by the T cell receptor rather than changes in affinity for MHC molecules is proposed by Archila et al. to explain the existence of non cross-reactive epitopes within grass pollen allergens.

The observations made by Archila et al. are interesting in at least two regards. First, the study sheds light on the physiopathology of Th2 responses to grass pollen allergens. Specifically, this study demonstrates that patients diagnosed on the basis of skin prick test reactivity to a Timothy pollen extract have in fact circulating Th2 cells reactive with allergens from other Pooideae grasses. In this regard, although only 6 patients were included in the study, it should be emphasized that all of them exhibit CD4⁺ T cell

responses against minimally cross-reactive epitopes. Thus, a general inference of this work is that grass pollen allergic patients are coexposed and cosensitized to allergens from multiple Pooideae species.

Furthermore, these non cross-reactive CD4⁺ T cells were confirmed to be memory IL4 producing Th2 cells, easily detected *ex vivo* in peripheral blood, that is without any need for *in vitro* expansion following allergen re-stimulation. In addition, as shown by the induction of the CD38 marker, non cross-reactive CD4⁺ T cells are activated and thus, likely expanded during the pollen season. Collectively, these results suggest that non cross-reactive T cells very likely contribute to allergic inflammation in a vast majority (if not all) of grass pollen allergic patients.

The observation that grass pollen allergic patients have circulating CD4⁺ T cells directed against both cross-reactive epitopes as well as species-specific T cell epitopes is also consistent with parallel evidence that such patients also have IgE antibody responses against cross-reactive and non cross-reactive B cell epitopes within group 1 and group 5 allergens [14]. Collectively, this can be explained by the extraordinary molecular diversity of group 1 and group 5 grass pollen allergens, well documented by mass spectrometry analysis of purified allergens from various Pooideae grass species, and shown to encompass as many as 5–10 and 50–100 species-specific isoforms, respectively, differing in their amino acid sequences [14].

The work from Archila et al. is also important to design appropriate immunotherapeutic approaches. As

shown by the same team in a parallel study, in order to deplete existing allergen-specific Th2 cells or reorient them towards a Th1/TReg profile, immunotherapy should modify allergen-specific CD4⁺ T cell responses in an epitope-dependent manner [15]. The strength of epitope recognition, related to the affinity of T cell receptors for MHC-peptide complexes, is known to impact the type of T cell responses induced, with a high affinity interaction needed to elicit immunoregulatory responses [16]. Consequently, with the aim to reorient existing allergen-specific CD4 Th2 cells directed against a large spectrum of cross-reactive and non cross-reactive

epitopes, Archila et al. recommends to use a mixture of pollens from various Pooideae species to recapitulate the broad repertoire of T cell epitopes to which patients are sensitized. Lastly and importantly, although not discussed by the authors, if this work is confirmed and extended to other allergens, it raises as well the potential limitation of recombinant allergen or peptide based approaches for specific immunotherapy, in that the latter cover only a single (or a few) allergen isoform(s).

Conflict of interests: The author is an employee at Stallergenes SA.

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