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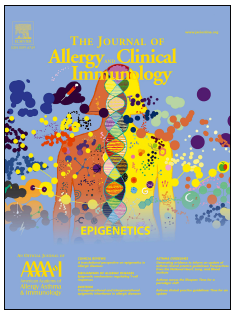

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What makes an allergen an allergen? Formyl-peptidyl receptor 3 and lipocalins: at the crossroads of Th2 induction

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Following the first description of Th1/Th2 polarization by Mossman et al. in the 1980s (1), our knowledge of the complexity and polarization of T cell subsets has greatly expanded. Their pivotal role in the development of immune system deviations and restoration "back to normality" is now better understood, albeit incompletely (2). The uptake and presentation of antigens by antigen presenting cells (APC), including dentritic cells (DC), provide signals that influence the polarization of the naive T cells they interact with. Many of these signals have been elucidated over the past decades, notably the “key axe” IL-12 for Th1-differentiation (3). In contrast, we know surprisingly little about the molecular mechanisms DCs use to drive Th2-immunity, the hallmark of allergic inflammation. In particular, the biochemical and biophysical properties of the antigens influencing their uptake, processing, activation and presentation by APC in this setting remains insufficiently understood. While there is ample evidence that DCs are required for optimal Th2 polarization, DCs paradoxically are not a potent source of the main Th2-promoting cytokine IL-4 (4). Among the DC-related factors suggested to pave the way for Th2 polarization are uptake and intracellular trafficking of the antigens (5) and the lack or low expression of IL-12 (4). The role of the extrinsic key players in the field, the antigens, has been intensively investigated and discussed, including intrinsic or introduced adjuvant properties of allergens (by e.g. coupling to adjuvants), modification of the APC targeting and uptake by coupling to non-oxidized mannan (6), as well as mere biochemical properties like enzymatic activity, hydrophobicity or oligomerisation. In the present study in this issue (7), Klaver et al. addressed the question of how proteins from one family (lipocalins) are taken up and processed by monocyte-derived DC as APC. They suggest a mechanism by which lipocalins known as allergens, in contrast to their non allergenic counterparts, skew DCs towards the Th2-direction and finally induce allergic responses.. Proteins of the lipocalin family, transporters of small hydrophobic molecules, are known as one of the most important animal allergen families (8). Major allergens from dog (*Can f1*) and cat (*Fel d4*) belong to this family and have human non-allergenic homologs, namely Lipocalin-1 (Lcn-1) and major urinary protein (MUP). The existence of two allergenic and non-allergenic lipocalin counterparts with high homology offers an interesting opportunity to investigate the insufficiently characterized pathways that allergens engage to interfere with Th1 priming and thus to facilitate Th2 priming. In a previous study, the authors have demonstrated different gene expression signatures in DC upon exposure to the allergenic Can f1 and non-allergenic human homologue Lcn-1 lipocalins (5). It needs to be mentioned, that one of the selected non-allergenic homologs (MUP), is encoded by a pseudogene (human MUPP), and it is not probable that natural production and thus exposure to MUP takes place (9). However, the natural occurrence of MUP in other species, the recombinant production and the focus on binding of derived peptides and the influence on DCs render recombinant human MUP as a suitable study candidate. Regarding the other lipocalin pair

(Can f1 and Lcn-1), it does not have a direct functional relation, i.e. Can f 1 is an oral protein involved in taste perception, whereas Lcn-1 is a tear protein. These caveats have to be kept in mind for further in-depth pathomechanistic studies.

This work compares gene expression changes in human monocyte-derived DC following exposure to allergenic vs. non-allergenic lipocalins and identified Formyl peptide receptor 3 (FPR3) to be upregulated in the DCs treated with lipocalins known to be allergens only. They hypothesized that binding of allergenic lipocalins to FPR3 could induce a Th2-promoting phenotype in mDC. In a first step, the authors showed lipocalins to co-localize with FPR3 in endosomes. Interestingly, only allergenic lipocalin-derived peptides were co-localizing with FPR3. Co-localization of allergenic lipocalins derived peptides to FGR3 prevented up-regulation of IL-12 by human mDC upon activation by the antigen and in turn a Th2-skewing of naïve T cells. The FGR-3 antagonist WRW4 abolished this effect and instead led to the upregulation of the regulatory cytokine IL-10.

The study presented in this issue investigates the role of peptides derived from allergenic lipocalins inducing IL-12 suppression via FPR3 binding in mDC and subsequent Th2 responses. This constitutes an interesting new mechanism of how allergens skew immune responses towards a Th2 direction. These findings raise the question whether the FPR3-mediated IL-12 suppression / Th2 induction mechanism is specific to lipocalins only or is generalizable to diverse, if not all, major allergen families. The authors' finding that the non-lipocalin major cat allergen Fel d1 fails to induce FPR3-mediated Th2-skewing provides a hint against this as an allergen independent mechanism of pathogenesis. Further investigations in this regard will be particularly relevant when it comes to the potential clinical translation of the study's findings, i.e. whether blocking FPR3 could be of benefit in treating lipocalin-associated allergies or whether this concept could be more widely applicable for other deviations of the immune system, such as autoimmunity.

Beyond the allergen-receptor interaction and related cascade in DC, additional factors play into the equation of inducing (allergic) Th2 responses. Cell-derived danger signals / stimuli such as thymic stromal lymphopoietin or amphiregulin can act as potent enhancers of a Th2 milieu. It will be interesting to further explore how these factors fit into allergic lipocalin-induced immune responses in different experimental settings such as *in vitro* epithelial explants. It is conceivable that allergic lipocalins alone are such potent Th2-inducers, that they act even in the near absence of additional strong co-signals/-factors. Related to this is the question regarding the effect of allergic lipocalins on innate players such as innate lymphoid cells or other types of APC and whether and how they contribute to generating a type 2 response in this setting. From the molecular point of view, the characterization of the

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allergenic lipocalin derived peptides, the binding properties of these peptides to the FPR3 receptor, and finally the potential role of a cargo molecule of small molecular weight of the lipocalins, represent starting points for further studies.

The differential FPR3-mediated mechanism of Th2 induction by allergic and non-allergic lipocalins presented in this study suggests another and novel piece of knowledge in the answer to one of the key questions in allergy research - what makes an allergen an allergen.

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Figure Legend

Figure 1:

A) Lipocalins are proteins serving as transporters of small hydrophobic molecules such as lipids, retinoids, pheromones or steroids. The sequence homology is limited to defined regions but a common overall tertiary structure. Important animal allergens belong to the lipocalin family.

B) Lipocalins are taken up and proteolytically processed by monocyte derived dendritic cells. Co-localization of lipocalin peptides and Formyl-peptide receptor 3 in the same vesicle occurs. Peptides derived from lipocalins known as allergens activate FPR3 signaling and silence Il-12 production.

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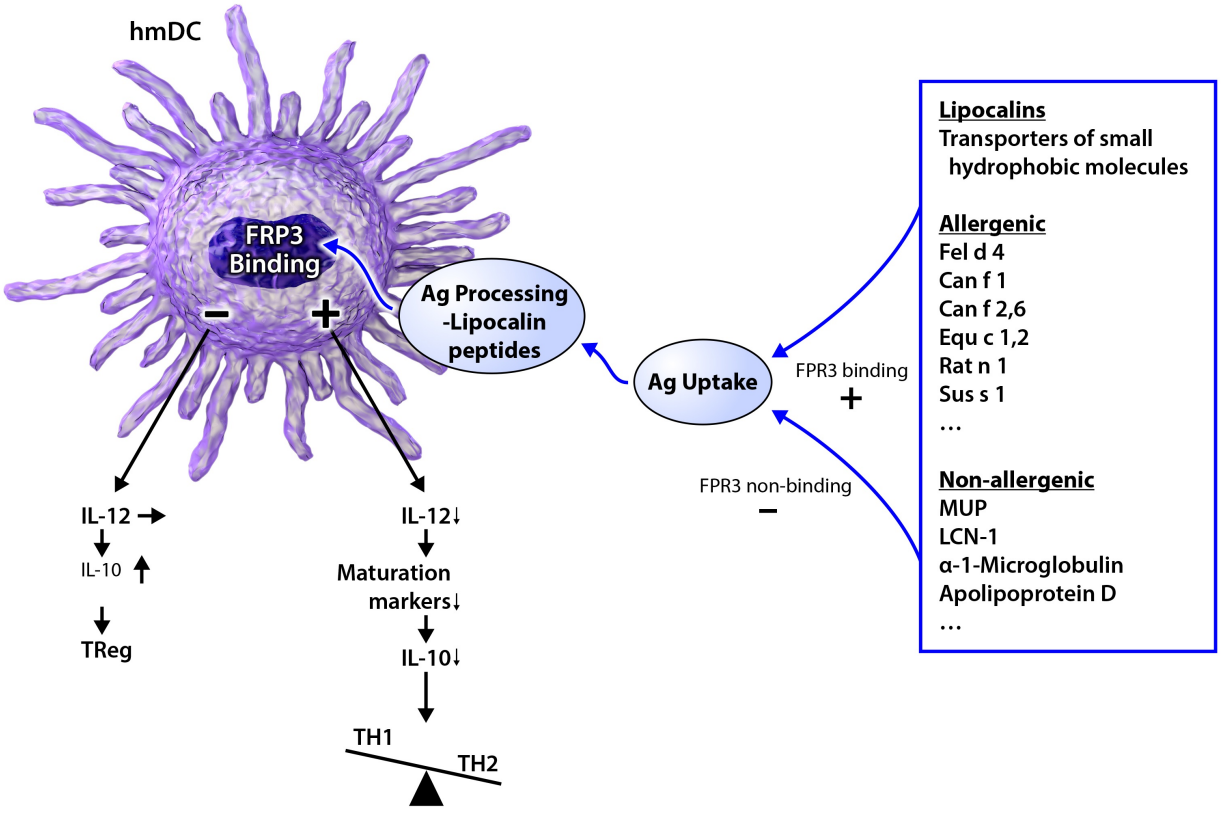
hmDC
FRP3 Binding
Ag Processing -Lipocalin peptides
Ag Uptake
FPR3 binding
+
FPR3 non-binding
−
Lipocalins
Transporters of small hydrophobic molecules
Allergenic
Fel d 4
Can f 1
Can f 2,6
Equ c 1,2
Rat n 1
Sus s 1
…
Non-allergenic
MUP
LCN-1
α-1-Microglobulin
Apolipoprotein D
…
IL-12
IL-10
TReg
IL-12↓
Maturation markers↓
IL-10↓
TH1
TH2

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