

Lipocalin allergens

Most animal-derived major allergens causing respiratory sensitization belong to the family of proteins called lipocalins. Their sequential identity varies but the three-dimensional structure is conserved. They are present in body fluids and secretions. Several lipocalins are able to bind and transport small hydrophobic ligands like retinol. The immunological characteristics of lipocalin allergens are poorly known. Cow dust-derived allergen, Bos d 2, which is a potent inducer of IgE production, was observed to induce the weak proliferative responses of peripheral blood mononuclear cells of asthmatic patients upon stimulation *in vitro*. The responses were Th2-deviated and directed to a few epitopes in Bos d 2. One of the epitopes, situated adjacent to a structurally conserved region of lipocalins, was recognized by the T cells of all patients. Computer predictions suggested that human endogenous lipocalins may contain epitopes in the corresponding region. We have proposed that the allergenicity of lipocalins may be associated with the adaptation of the immune system to the presence of endogenous lipocalins.

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Lipocalin family of proteins

The structural and biological characteristics of lipocalins have been summarized in a recent review (1). A protein to be included in the lipocalin family should fulfil the requirements for sequence homology, biological function and structural similarity (<http://www.expasy.ch/cgi-bin/nicedoc.pl?PDOC00187>, October 15, 2000). The kernel lipocalins contain three conserved sequence motifs. The most conserved is the first motif, GXW, which is also present in the outlier lipocalins. The overall sequential identity between lipocalins is about 20%. In some cases, the similarity can be considerably higher. The human PP14 (2) and the food allergen β -lactoglobulin (Bos d 5) exhibit 43% amino acid identity. A salivary lipocalin from boar exhibits amino acid identity at the 60% level with horse Equ c 1 and rodent urinary lipocalins (3). The Swiss-Prot database contains the complete or partial sequences of about 100 lipocalins (<http://www.expasy.ch/cgi-bin/nicesite.pl?PS00213>, October 15, 2000).

In contrast to relatively low sequential identity, the crystal structure of lipocalins is highly conserved (1, 4). The central β -barrel is composed of eight antiparallel β -strands and it encloses an internal ligand-binding site. An α -helix is situated at the carboxy-terminal end of the sequence (1, 4, 5). Some lipocalins, like Bos d 5 and Equ c 1 (5), are present in a dimeric state, while other lipocalins, like Bos d 2 (4), exist as monomers. Parts of lipocalin allergens are nonglycosylated (Bos d 2, Bos d 5, mouse Mus m 1), while others have putative N-glycosylation sites on their surfaces (dog Can f 1 and Can f 2, Equ c 1, cockroach Bla g 4).

Most lipocalins are produced in the liver or secretory

glands. They act as carriers of retinol, odorants, steroids, lipids and pheromones (1). Some lipocalins exhibit a broad selectivity of binding; and retinol, for example, binds to several lipocalins (6). Some lipocalins are also known to be involved in stress and inflammatory reactions and in the regulation of cell growth and proliferation (1).

Lipocalin allergens

Most animal-derived major allergens causing respiratory sensitization have been shown to belong to lipocalins (<http://www.expasy.ch/cgi-bin/nicesite.pl?PS00213>, October 15, 2000). The physicochemical characteristics of lipocalin allergens are shown in Table 1.

The predominant allergen of mouse is Mus m 1, which is mainly found in mouse urine (7). The Swiss-Prot database contains the sequences of seven highly identical mouse major urinary proteins (MUP). MUP6 equals with Mus m 1. MUP mRNA has been discovered in several secretory tissues (8).

Rat n 1, present in urine, is highly homologous to mouse MUP. The isoallergenic forms, formerly called prealbumin and α_2 -globulin, are now designated as Rat n 1.01 and Rat n 1.02, respectively (9–11).

Cow dander contains three major allergens. Our studies showed that Bos d 2 is the most allergenic of the three proteins (12, 13), and it proved to be a lipocalin (14). Bos d 2 is produced by apocrine sweat glands and assumed to be transported to the skin surface as a carrier of a pheromone ligand (15). Structurally, Bos d 2 is a typical member of a lipocalin protein family (4).

The dog allergens Can f 1 and Can f 2 were cloned

from the parotid gland (16). Can f1 is a major allergen in that 75% of dog-allergic subjects had IgE against it. Twenty-five percent of the subjects had the antibody against Can f2 (16). Can f1 is highly homologous and has a similar tissue distribution to von Ebner's gland proteins, which are closely related between several species and present in saliva.

Two horse allergens, Equ c1 and Equ c2, are lipocalins. Equ c1 is a major allergen (5). It was cloned from sublingual salivary glands (17) and is also expressed in the liver and submaxillary salivary glands.

One of the several cockroach allergens, Bla g4, is a lipocalin (18). Of the cockroach allergic patients with asthma 40–60% had IgE antibodies against the recombinant Bla g4.

Immunological characteristics of lipocalin allergens

Apart from their capacity to bind IgE, the immunological characteristics of lipocalin allergens are poorly known. Bos d5 was found to contain six regions with IgE-binding capacity (19). All patients recognized the amino acid segment 97–108. In another study, the corresponding region was identified as an epitope (20). This region is localized within the conserved motif 2 of lipocalins and it contains the sequence DTDY. The sequence is also found, for example, in human retinol-binding protein (RBP) and human PP14. A monoclonal antibody has been reported to recognize peptides from Bos d5 and RBP comprising this sequence (21).

We observed that IgE reactivity is mainly directed to the carboxy-terminal part of Bos d2 (22). Further studies aimed at reducing the IgE-binding capacity of Bos d2 showed that the elimination of the internal disulfide bond of Bos d2 resulted in a significant decrease in IgE binding when analyzed by ELISA inhibition (23). A more detailed analysis of IgE epitopes of Bos d2 is lacking.

A recent study with Equ c1 suggested that the dominant IgE epitopes are localized in a restricted region on the molecule (5). One of four monoclonal antibodies was able to prevent IgE binding to the recombinant allergen. The authors supposed that the L2 loop and part of the adjacent β -sheet on the surface of Equ c1 form the critical epitope.

Cellular responses against lipocalin allergens are also poorly characterized. We observed that the peripheral blood mononuclear cells of Bos d2-sensitized asthmatic patients exhibited the weak proliferative responses against Bos d2 (24). The maximal number of epitopes that an individual's T cells could recognize was five, which suggests that the allergen contains a limited number of epitopes. One of the epitopes, named epitope G, situated in the α -helix at the carboxy-terminal end of the molecule, was recognized by the T cells of all patients. Two patients' T cells did not recognize any

Table 1. Physicochemical characteristics of lipocalin allergens¹

Allergen	MM ²	pI	Residues	Accession number ³
Bla g4	19.8	6.8	170	P54962
Bos d2	17.9	4.6	156	Q28133
Bos d5	18.3	4.8	162	P02754
Can f1	17.3	5.7	156	O18873
Can f2	18.4	4.9	162	O18874
Equ c1	20.1	4.5	172	Q95182
Equ c2 ⁴	—	—	—	P81216, P81217
Mus m1	18.7	4.8	162	P02762
Rat n1	18.7	5.5	162	P02761

¹ Estimated by the ProtParam Tool without a signal sequence in Swiss-Prot Protein Sequence Database (<http://www.expasy.ch/cgi-bin/lists?allergen.txt>)

² Molecular mass, kDa

³ Swiss-Prot Protein Sequence Database

⁴ Full sequence not available

other epitope. The T cell clones recognizing the epitope G exhibited predominantly the Th2/0-like phenotype. Computer predictions suggested that human endogenous lipocalins could contain an epitope in the corresponding region (24).

Piastra and coworkers have studied the cord blood mononuclear cell responses against Bos d5 (25). Most of the reactivity was directed to the very end of the molecule, not corresponding to the immunodominant epitope in Bos d2. However, the use of digested fragments of Bos d5 did not allow an exact analysis. Murine T cell responses against Bos d5 have also been examined (26–28). BALB/c mice exhibited responses against a few regions in Bos d5. One of the regions which was situated in the carboxy-terminal α -helix (amino acids 130–140) corresponds to the epitope G of Bos d2 (27, 28).

Allergenicity of lipocalin allergens

The basis for the allergenicity of proteins, i.e. capacity to induce Th2-type responses, is not known and therefore it has been the subject of discussion (29). Because only a part of the proteins from an allergen source appear to bind IgE it is logical to infer that they may have an intrinsic capacity to induce IgE production.

To explain the observations made with Bos d2, we have recently presented an hypothesis for the allergenicity of lipocalins (30). We proposed that the allergenicity of lipocalins may be associated with the adaptation of the immune system to the presence of endogenous lipocalins. As described above, human immune response against Bos d2 manifests itself as a weak Th2-dominated cellular response with high IgE production. A similar type of immune response is characteristic for parasitic infections (31, 32). In contrast, Th1-type responsiveness, which is important in antimicrobial defense (31), is also associated with autoimmune pathology (31). Interestingly, strong proliferative or Th1-deviated immune responses against helminths are

associated with more severe forms of the diseases (33–35). In a study of *Schistosoma mansoni* infection with IL-4/13-deficient mice, it was observed that while the removal of IL-13 could be beneficial, correlating with reduced hepatic fibrosis, the absence of IL-4 resulted in high mortality of mice, suggesting a protective role for IL-4 (36).

It can be expected that T cells reactive to (endogenous) lipocalins have been eliminated during thymic maturation. Therefore, weak cellular responsiveness against Bos d2 could be due to the low precursor frequency of Bos d 2-specific T cells. Another possibility is that the survived T cells with their low-affinity T cell

receptors for self, recognize allergen epitopes inefficiently. Although it cannot be excluded that diverse regulative mechanisms are operative in the periphery, it is intriguing to hypothesize that the way T cells recognize epitopes is a factor influencing the quality of immune response against allergens. Studies on epitope recognition suggest that the low affinity of a peptide for an MHC class II molecule and/or a T cell receptor is able to favor Th2-type responsiveness (37). It is tempting to speculate that the Th2-type response in allergy and parasitic diseases may be an attempt to avoid harmful responses, possibly against antigens resembling self.

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