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ALLERGENS (RK BUSH, SECTION EDITOR)

Animal Lipocalin Allergens

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Abstract Lipocalins represent the most important group of inhalant animal allergens. For some of them, three-dimensional protein structures have been resolved, but their functions are still elusive. Lipocalins generally display a low sequence identity between family members. The characterization of new lipocalin allergens has revealed however that some of them display a high sequence identity to lipocalins from another species. They constitute a new group of potentially cross-reactive molecules which, in addition to serum albumins, may contribute to allergic cross-reactions between animal dander of different species. However, the clinical relevance of cross-reactivity needs to be assessed. Further studies are needed to understand which of these animal lipocalins are the primary allergens and which are cross-reacting molecules. The use of single, well characterized allergens for diagnosis will allow the identification of the sensitizing animal, which is a prerequisite for specific immunotherapy.

Keywords Lipocalin · Allergy · Allergen · Mammalian · Cat · Dog · Horse · Cattle · Guinea pig · Rabbit · Hamster · Mouse · Rat · Arthropod · Cross-reactivity

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Introduction

Among the many molecules present in our environment, some have the property to induce allergic sensitization and IgE-mediated reactions. The development of molecular biology tools allowed the isolation of allergenic proteins and the cloning of cDNAs coding for these allergens. During the last 2 decades, a great number of allergens have been isolated from different plant and animal sources. Analysis of known allergens revealed that they are mostly proteins or glycoproteins and that they belong to relatively few protein families [1]. The AllFam database currently lists 1091 allergens (release 12-09-2011; http://www.meduniwien.ac.at/allergens/allfam/). The lipocalin protein family constitutes the most important group of inhalant animal allergens.

The Lipocalin Family

Lipocalins are a group of proteins ubiquitously present in vertebrate and invertebrate animals, plants, and bacteria [2]. They are characterized by a common tertiary structure composing a central β-barrel formed by 8 anti-parallel β-strands. The internal binding pocket carries small hydrophobic molecules such as retinol, steroids, odorants, and pheromones. Despite the highly conserved structural similarity, lipocalins display a very weak amino acid identity, which can be lower than 20 % [3••]. This diversity in sequence matches a variety of functions and mechanisms of action. Lipocalins are small extracellular proteins composed of 150–250 residues. They are classified by the presence of 1–3 structurally conserved regions (SCRs) into kernel (all 3 SCRs) or outlier (1 or 2 SCRs) lipocalins [4] (Fig. 1). The SCR 1 motif, including the conserved residues GxW, is located at the N-terminal end and it is present in about 90 % of all known lipocalins. Based on nucleotide/amino acid similarity and phylogenetic analyses,

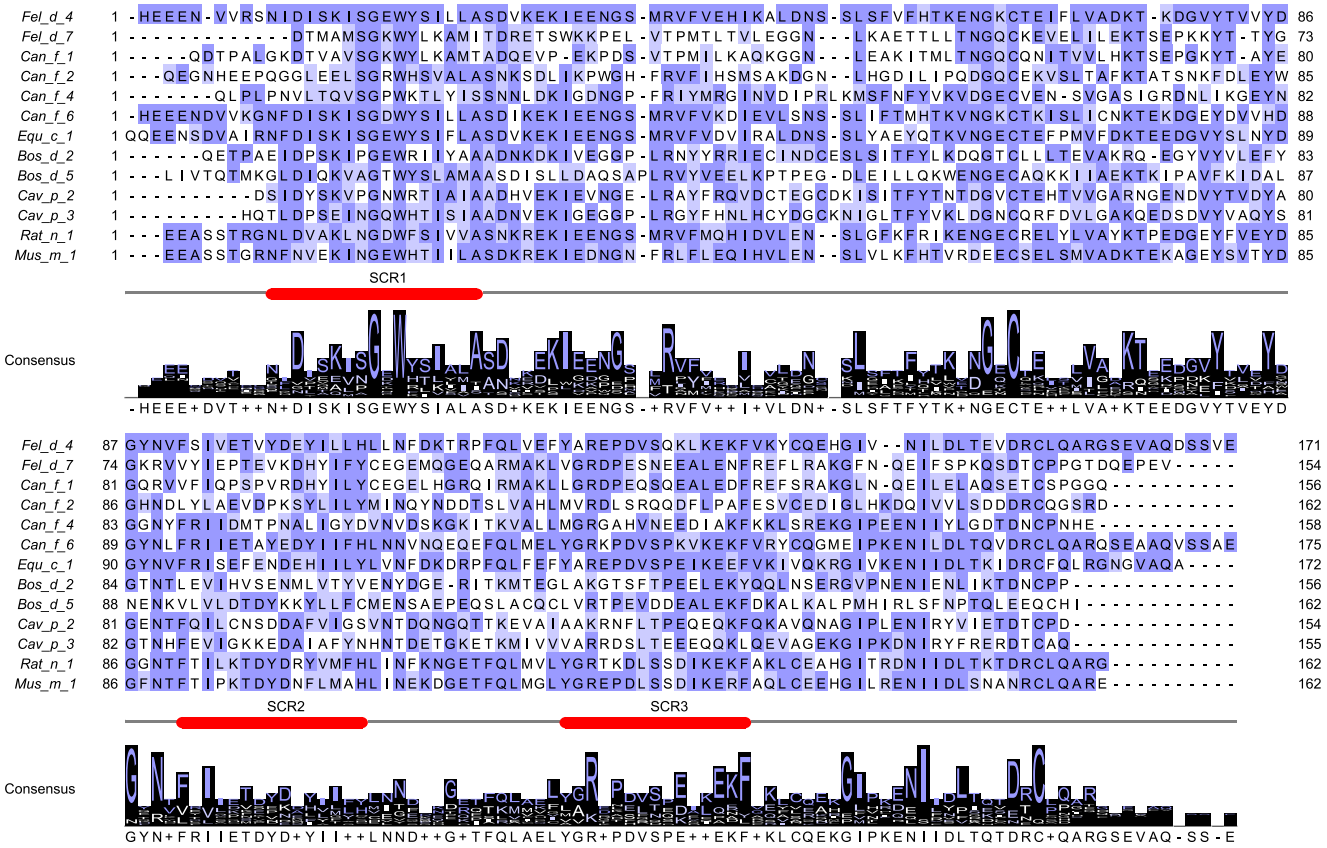

Fig.1 Mammalian lipocalin allergens were aligned without their respective peptide signals using Clustal W. The graphic layout was done with Jalview 2.7 [76]. Amino acids identical to the consensus are colored dark blue, similar amino acids are shaded in pale blue. *SCR*, structurally conserved region. Predominant conserved features are GxW in SCR1, TDYxxY in SCR2, and R/K in SCR3

the lipocalins have been grouped into 13 clades [5]. Interestingly, the gene arrangement of introns and exons is rather conserved and points to a common ancestor which underwent several gene duplications in the animal kingdom [6].

Lipocalin Allergens from Mammals

Several new mammalian respiratory allergens belonging to the lipocalin family have been identified and characterized during the last 2 years: cat Fel d 7, dog Can f 4 and Can f 6, guinea-pig Cav p 2 and Cav p 3. This review summarizes briefly all allergenic lipocalins and gives more extended information about the new members of the lipocalin family (Table 1). A special paragraph is dedicated to the topic of IgE cross-reactivity between lipocalins. A comprehensive compilation of mammalian allergens including lipocalins, but also all other allergens has been summarized by T Virtanen and T Kinnunen [7••].

Cat

Cat dander contains several allergens. The major cat allergen, Fel d 1, is an uteroglobin and was already isolated in 1991 [8]. The first cat lipocalin, Fel d 4 was only described in 2004 by Smith et al [9]. Fel d 4 is produced in the submandibular salivary gland; it is not expressed in the parotid gland, tongue, skin, or liver. The frequency of IgE-reactivity to Fel d 4 was 63 % in 27 cat-allergic patients tested, although titers were low. Fel d 4 has a high amino acid identity (67 %) with horse Equ c 1 and dog Can f 6. IgE-inhibition experiments showed that IgE-binding to Fel d 4 could be significantly reduced by an allergen extract from cow and to a lesser extend by extracts from horse and dog.

A second cat lipocalin, Fel d 7, was identified by Smith and colleagues in 2011 [10]. Fel d 7 was cloned from a tongue cDNA library. It was not detected in skin or other salivary glands such as the parotid or submandibulary gland. Fel d 7 constitutes about 0.2 %–0.4 % of the saliva proteins and it is also detectable in cat hair extract. The prevalence of IgE to Fel d 7 was 38 % in cat-allergic patients. Fel d 7 has a high degree of amino acid identity with Can f 1 (62 %). IgE-reactivity to Fel d 7 could be inhibited with a dog extract in 1 of 2 patient sera. However, it is not clear if this inhibition might be attributed to Can f 1. Inhibition experiments will have to be done with purified molecules.

Table 1 Animal lipocalin allergens

Animal	Allergen	UniProtKB accession No	Apparent MW, (predicted MW) in kDa	Glyco-sylation	Allergen production	Principal source	Sensitization in % [c]	In vitro diagnosis available
Cat	Fel d 4	Q5VFH6	nd (19.7)	yes [b]	Submandibular salivary gland	Saliva	63	yes
	Fel d 7	E5D2Z5	18,20 (17.6)	no [b]	Tongue	Saliva, hair	38	no
Dog	Can f 1	O18873	22–24 (16.5)	yes	Tongue	Saliva	50-75	yes
	Can f 2	O18874	24–27 (18.2)	yes	Tongue, parotid gland	Saliva	22-30	yes
	Can f 4	D7PBH4	16 (17.6)	no [b]	Tongue	Dander	35	no
	Can f 6	E2QYS2	27,29 (20.2)	yes [b]	Submaxillary gland	Dander	61	no
Horse	Equ c 1	Q95182	22 (20.0)	yes	Sublingual gland	Saliva, dander, urine	100	yes
	Equ c 2	P81216 P81217	18.5 (16.0)	no	nd	Dander, sweat	33-50	no
Cow	Bos d 2	Q28133	20 (17.9)	no	Sweat glands	Skin	83-97	no
	Bos d 5	P02754	(18.3)	no	Mammary glands	Milk	88	yes
Guinea-pig	Cav p 1	P83507	20	no	nd	Hair, urine		no
	Cav p 2	F0UZ11	17 (17.1)	no	Harderian gland	Hair	65	no
	Cav p 3	F0UZ12	19 (17.5)	no	Submaxillary gland	Hair	54	no
Rabbit	Ory c 1	nd	17–18	yes	nd	Saliva, hair	nd	no
	Ory c 2[a]	nd	21	nd	nd	Saliva, hair, urine	nd	no
Rat	Rat n1	P02761	17,21 (18.7)	yes	Liver	Urine, saliva, fur fur, saliva	73-87	no
Mouse	Mus m 1	P02762, P11589	17–19 (18.7)	no	Liver	Urine	66	yes
Hamster	Pho s 21 kDa [a]	nd	18, 21, 23	nd	Salivary gland	Hair , urine,	nd	no
Kissing bug	Tria p 1	Q9U6R6	20 (17)	no [b]	Salivary gland	Bite	nd	no
Cockroach	Bla g 4	P54962	(19.8)	yes [b]	Adult male reproductive system	nd	40-60	no
	Per a 4 [a]	B7TYB3, Q1MOY5	(18.9, 20.5)	yes [b]				
Pigeon tick	Arg r 1	Q5GQ85	19 (15.4)	no	Salivary gland	Bite	100	no

Nd no data

[a] Name not deposited in IUIS

[b] Glycosylation site predicted by protein sequence analysis

[c] Sensitization % has been determined by different analytical methods

Additional cat allergens are Fel d 2, cat serum albumin [11], Fel d 3, cystatin [12], Fel d 5, immunoglobulin A [13], Fel d 6, immunoglobulin M [13], and Fel d 8, a latherin-like protein [10].

Dog

Can f 1 and Can f 2 were initially cloned from the parotid gland [14]. Can f 1 is strongly expressed in tongue epithelial tissue and displays high homology to human von Ebner's gland protein (57 %). Can f 2 was found predominantly in the parotid gland. Both are not detected in skin or liver. Although the prevalence of IgE-reactivity to Can f 1 is variable in different studies, Can f 1 seems to be a major dog allergen. IgE-reactivity against Can f 2 without reactivity to Can f 1 was not observed. Can f 1 levels have been analyzed in different dog breeds [15]. In general, males produce more Can f 1 than females, but no difference was found according to hair length or hormonal status. Labrador dogs have lower levels of Can f 1 than other breeds; however, there is a wide variability between individuals and a hypoallergenic breed does not exist.

Can f 1 and Can f 2 were the first isolated dog allergens. However, as the diagnostic sensitivity using both allergens was rather low, it was evident that more dog allergens contribute to sensitization. The next allergens to be identified were Can f 3, dog albumin [16], and Can f 5, a prostatic kallikrein [17].

Can f 4, the third dog lipocalin, was recently isolated by Mattsson et al [18] from dog dander. Thirty-five percent of

dog-allergic sera recognized Can f 4. It was found to cross-react with a 23 kDa odorant-binding protein purified from cow dander. Identity to this protein is 39 %. Besides serum albumins, which have high sequence identities between species and which easily display cross-reactivity, this is a novel association between dog and cow dander. Can f 4 has a low identity to Can f 1 (19 %) and Can f 2 (25 %). Can f 4 is possibly a relevant allergen for diagnosis as one of the reactive sera tested did not show reactivity to any of the other dog allergens.

Can f 6 is the latest lipocalin added to the list of dog allergens [19]. It was identified as a predicted dog lipocalin by searching the databases for proteins with high similarity to Fel d 4 and Equ c 1. The cDNA was cloned from submaxillary gland and the protein was detected in dog dander, confirming the existence of the putative lipocalin. Can f 6 has a high amino acid identity to Fel d 4 (67 %) and to Equ c 1 (57 %). In a group of patients selected for their sensitization to both cats and dogs, 61 % had IgE antibodies to Can f 6. Cross-reactivity between Fel d 4 and Can f 6 was confirmed by inhibition and cross-inhibition experiments (see below).

Horse

Equ c 1 was cloned from the sublingual salivary gland [20]. The glycoprotein is found at high concentrations in saliva and dander as well as in urine from adult animals [21]. The three-dimensional structure of Equ c 1 has been determined. It crystallizes as a dimeric molecule [22]. Equ c 1 has a surfactant property; it significantly lowers surface tension [23].

Equ c 2 was purified from horse dander. Two isoforms were identified and named Equ c 2.0101 and Equ c 2.0102 [24]. Both allergens have not been cloned so far and only N-terminal amino acid sequences are available. Besides Equ c 3, horse serum albumin [25], two other allergens have been characterized, Equ c 4 and Equ c 5 [23]. They are latherins and have surfactant properties [23].

Cow

Bos d 2 is the major respiratory cow allergen. It was cloned from a cow skin cDNA library [26]. It is produced in the sweat glands and transported to the skin [27]. In a study of 49 dairy farmers with clinically diagnosed asthma, all patients reactive in immunoblot with cow dander also had specific IgE to Bos d 2 [28]. The three-dimensional structure of Bos d 2 has been resolved [29]. It is related to lipocalin proteins with transport functions.

Bos d 5, β-lactoglobulin, is the major milk whey protein and constitutes about 10 % of the total protein of cow milk [30]. β-Lactoglobulin is absent from rodent, lagomorph, and human milk. Eighty-eight percent of milk allergic patients had a positive skin prick test to Bos d 5, 73 % had a positive IgE test in ImmunoCAP. In contrast to other lipocalins, β-lactoglobulin has a high sequence identity to the homologous proteins in milk of goat (96 %) or sheep (95 %). Equine lactoglobulin has only 58 % identity to Bos d 5. Sheep's milk and goat's milk are considered more similar to cow's milk as all milk allergens have higher similarity between these species. In cow's milk allergic patients, better clinical results were obtained upon consumption of milk from more distantly related mammals such as horse, donkey or camel [30].

Further bovine allergens are Bos d 3, a small calcium binding protein, Bos d 4, alpha-lactalbumin, Bos d 6, serum albumin, Bos d 7, immunoglobulin, and Bos d 8, casein [7••].

Guinea-pig

Guinea-pig allergens have been detected in dust, dander, fur, urine, and saliva [31]. Two allergens of 20 and 17 kDa, Cav p 1 and Cav p 2 are recognized in IgE-immunoblot by a majority of guinea-pig-allergic patients [32, 33]. The proteins have been isolated from hair and their N-terminal amino acid sequences were determined. Both allergens were assigned to the lipocalin family.

The coding sequence for Cav p 2 was only recently determined [34]. The protein was identified in the harderian gland. This is a lachrymal gland present in most terrestrian vertebrates. In rodents, the gland is under hormonal control and there are marked sex differences which might indicate a function as a producer of social odors [35].

A third lipocalin, Cav p 3, was purified from the submaxillary gland [34]. Cav p 2 and Cav p 3 were aligned to similar mammalian allergens. Significant identities were found between Cav p 2 and Bos d 2 (39 %), Equ c 1 (33 %), respectively. Cav p 3 also aligned best to Bos d 2 (39 %). Rat and mouse urinary proteins had less identity (24 %–30 %). In a group of 26 guinea-pig-allergic patients, 65 % had IgE antibodies to Cav p 2 and 54 % had IgE antibodies to Cav p 3. Cav p 2 and Cav p 3 share 43 % amino acid identity. Cross-inhibition experiments with 6 patient sera showed a minor decrease in IgE-binding for 4 of them using high doses of Cav p 3. Although Cav p 2 and Cav p 3 are independent allergens, limited cross-reactivity might be observed for individual patients depending on their epitope profile. IgE-recognition of Cav p 2 or Cav p 3 seems to be a specific marker of allergy to guinea-pig. Indeed, a group of 16 cat- and dog-allergic patients were assayed for IgE to Cav p 2 and Cav p 3. Only 1 patient had a low IgE titer to Cav p 2, but 14 patients were tested positive using a commercial assay against guinea-pig epithelium. Inhibition experiments confirmed that the false positive results were

probably due to cross-reactivity between cat serum albumin and guinea-pig serum albumin. These results emphasize the importance of single allergens for a correct diagnosis of the sensitizing animal.

Rabbit

Rabbit allergens have not been extensively characterized. Saliva was found to be the most potent allergenic source as it inhibited IgE-binding to fur and urine extracts [36, 37]. A 17 kDa glycoprotein, Ag R1, later Ory c 1, was found to be the dominant allergen of saliva. It was also abundant in fur, but occurred in minimal amounts in urine and dander. A second allergen, Ory c 2, was found in saliva, fur, and urine [38]. In total, 12 allergens were recognized in saliva, 7 in urine, and 7 in fur [36]. The N-terminal sequences of Ory c 1 and Ory c 2 were determined and assigned to the lipocalin protein family. The 20 amino acids of Ory c 2 correspond to an odorant binding protein isolated from rabbit nasal mucosa [39].

Mouse

The major mouse allergen, Mus m 1, belongs to the family of mouse urinary proteins (MUP) [40•]. They are produced by the liver and excreted into the urine of adult mice. The total amount of MUP in male urine is about 5–10 mg protein per day. Female urine contains 4 times less MUPs. Up to 15 forms of MUP have been distinguished in male urine. Analysis of urinary MUPs from different inbred mouse strains revealed a marked heterogeneity [41]. Not all MUPs are expressed in each strain. At least 35 distinct MUP genes have been characterized, about half of them are expressed, and the others are pseudogenes. Besides the liver, some MUPs are expressed in other tissues such as salivary, mammary, lachrymal, and modified sebaceous glands. MUPs bind small natural odorant molecules and they seem to play a complex role in chemosensory signaling among rodents. Mus m 1 consists of 2 isoforms, Mus m 1.0101 (MUP 6) and Mus m 1.0102 (MUP 2). Both isoforms differ by only 2 amino acids. Sequence identity between mouse MUP and rat MUPs is about 64 %. Sensitization to mouse allergens has primarily been related to the occupational setting. About one-third of animal workers develop work-related allergic symptoms [42]. The significance of mouse allergen exposure in residential environment has also been evaluated. Mouse allergen Mus m 1 was found to be an important indoor allergen in inner-city children with asthma [43, 44]. Ferrari and colleagues modified the allergenicity of Mus m 1 in a perspective of immunotherapy. Two single-point mutants of Mus m 1 were constructed and one of those had an impact on the spatial rearrangement of the protein cavity. IgE-binding and basophil release were reduced while T-cell reactivity was maintained [45]

Rat

Rat n 1 is very similar to the mouse MUPs. The protein is glycosylated and has a molecular weight of 17–21 kDa. Formerly, 2 allergen fractions had been isolated called prealbumin and α-$_{2U}$-globulin. More recently, analysis of the 2 proteins revealed that they have a high homology and that they are isoforms of one parent protein, α-$_{2U}$-globulin called Rat n 1.01 (21 kDa) and Rat n 1.02 (17 kDa) [46]. Both allergens are found in high amounts in urine, but also in fur and saliva. Strong IgE responses were found to allergens of 17-21 kDa in saliva and fur. A total of 23 allergens were detected in fur and 17 in saliva [47]. 73 %–87 % of rat-allergic patients reacted to Rat n 1 in dust [48].

Hamster

There are several case reports of anaphylactic reactions to hamster bites [49]. An 21 kDa IgE reactive protein was found in dwarf hamster (*Phodopus sungorus*) saliva using sera from 2 children with hamster bite-induced anaphylaxis [50]. In a case of anaphylaxis following a bite from another dwarf hamster, 3 IgE-binding proteins of 18, 21, and 23 kDa were found in hair, urine and salivary glands [51]. The 3 protein bands seemed to correspond to a single protein and its isoforms. Three peptide sequences were determined. The allergen was identified as odorant binding protein as it displayed a significant homology to a corresponding protein from the blank vole and to aphrodisin from hamster.

Lipocalin Allergens from Arthropods

Four arthropodan allergens belong to the lipocalins: Bla g 4 [52] and Per a 4 [53], cockroach allergens, Tria p 1 [54], a ‘kissing bug’ allergen and Arg r 1 [55], a pigeon tick allergen.

The cDNA coding for Tria p 1 was isolated from the salivary glands of *Triatoma protracta*, a hematophagous bug. These insects inject salivary proteins during the acquisition of a blood meal. In the US, allergic sensitization may develop in 7 % of the general population [56].

The first cockroach lipocalin, Bla g 4, was isolated from *Blatella germanica*, the German cockroach. The prevalence of specific IgE to Bla g 4 was 40 %–60 % in cockroach-allergic patients [52]. Recently, the structures of Bla g 4 and Per g 4, a *Periplaneta americana* (American cockroach) allergen, have been resolved [53]. They adopt a typical lipocalin fold, but in comparison to mammalian lipocalin allergens, they have distinct structural features. IgE prevalence was evaluated in cockroach-allergic Singaporean patients. Despite a low sequence identity between both proteins (21 %), a very high correlation in IgE-binding to

Bla g 4 and Per a 4 was found. Competitive ELISA was done with a patient pool and Per a 4 could completely inhibit IgE-binding to Bla g 4. In contrast, Bla g 4 could only inhibit up to 60 % of IgE-binding to Per a 4 [53]. In Singapore, the American cockroach (*P. americana*) is the dominant species, which might partly explain these results. However, cross-inhibition experiments with individual, well characterized sera will be necessary to elucidate this cross-reactivity between lipocalins of low sequence identity.

The European pigeon tick, *Argas reflexus*, is responsible for anaphylactic reactions after a bite, occurring often at night in the hot season. Arg r 1 [55] is a salivary gland protein with a sequence identity of 25 %–35 % to histamine-binding proteins from the brown ear tick, *Rhipicephalus appendiculatus* [57]. In 13 patients with an anaphylactic reaction after pigeon tick bites, Arg r 1 was found to be the major allergen [55]. In an epidemiologic study including 148 German patients with a history of a pigeon tick bite, 99 % had local reactions and 8 % had systemic reactions [58].

Function

In general, mammalian lipocalin allergens are classified as odorant and pheromone binding lipocalins [59•]. However their functions are largely unknown. The MUPs are more extensively characterized [40•]. They bind small odorant molecules in their hydrophobic pocket and are excreted in the environment. The odorants are released slowly, extending longevity of olfactory marks. MUPS have a different impact on male and female behavior: females are attracted while males are repelled. Recently, behavior of mice to fear-evoking odors from cat, rat, and snake was analyzed [60]. Detection and processing of these signals require the function of the sensory neurons in the vomeronasal organ, a specialized chemosensory organ of terrestrial vertebrates. Surprisingly, the substances promoting defensive behaviour belong to the MUP family. A rat MUP encoded by the gene *MUP13* as well as cat Fel d 4 were able to induce defensive behaviour through the vomeronasal organ. Mouse MUPs excreted in the urine were found to act as pheromones themselves to promote aggression [61]. MUP 1 present in the blood was found to regulate glucose and fat metabolism in mice. Expression of hepatic MUP 1 was reduced in genetic and dietary fat-induced type 2 diabetes. Mice expressing recombinant MUP 1 in the liver showed a markedly reduced hyperglycemia and glucose intolerance [62].

β-Lactoglobulin, as well as human tear lipocalin, were reported to have endonuclease activity. However, its significance is unclear as its activity is more than 1000x lower than that of DNase I [63].

Lipocalin Allergenicity

A physical prerequisite for a protein to be an allergen is its stability and prevalence in the environment. Allergens stick to particles and become airborne [64••]. Mammalian lipocalins are present in urine and on animal dander and thus are easily spread.

Virtanen and colleagues [65••] have summarized the immunological properties of mammalian lipocalin allergens and formulated a hypothesis which could explain why lipocalins are allergenic. Lipocalins are characterized by a weak cellular immune response. Proliferation of peripheral blood mononuclear cells (PBMCs) has consistently been very weak upon stimulation with different mammalian lipocalins such as Bos d 2, Can f 1, Equ c 1, and Rat n 1. The immunodominant epitope of Bos d 2 and an epitope of Can f 1 were further characterized. They were found to be suboptimal. The presence of endogenous human lipocalins may have conducted to the absence of high avidity lipocalin-reactive T-cells due to thymic deletion. A weak cellular reactivity is assumed to favor Th2 reactions.

The mannose receptor (MR) was shown to be involved in the uptake of glycosylated allergens, including Can f 1 [66]. There was further evidence that MR plays a crucial role in allergen-induced Th2 cell polarization after Der p 1 exposure through regulation of indoleamine 2,3-dioxygenase (IDO) activity. Lipocalin-interacting membrane receptor (LIMR) binds human tear lipocalin [67]. It was also shown to mediate uptake of Bos d 5 [68]. The receptor is expressed in a number of tissues including thymus and lymph nodes, but its function is still not clear [67]. Recently, dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) receptor was shown to bind Der p 1 and Can f 1 [69]. Surprisingly, down-regulation of DC-SIGN promoted a Th2 phenotype in DC/T cell cocultures. Uptake of some allergens (Der p 1, Can f 1) seems to be mediated at least by 2 receptors, which may result in different signaling pathways. It can be assumed that the balance of those signals will contribute, among other factors, to the determination of the fate of the T-cell response.

Ige Cross-Reactivity Between Lipocalins

Lipocalins are characterized by a common tertiary structure. Primary amino acid sequences may be very divergent and usually, sequence identities between family members can be as low as 15 %. Structurally, they are grouped into kernel and outlier lipocalins (see above). They can also be classified into functional groups: enzymes, defense and immunity related, odorant and pheromone binding lipocalins. Mammalian lipocalin allergens isolated so far belong mostly to the odorant and pheromone binding lipocalins. They are

produced in secretory glands or in the liver and they are found in saliva, urine, and dander. Despite these functional similarities, identities between most mammalian lipocalin allergens are low (usually between 20 % and 30 %) and IgE cross-reactivity is difficult to conceive. Earlier reports on inhibition studies using animal allergen extracts suggested some cross-reactivity between lipocalins [9, 10, 32]; however these reactions were poorly characterized. Saarelainen and collaborators were the first to analyze IgE cross-reactivity between 5 animal-derived lipocalins and 1 human endogenous lipocalin, tear lipocalin [70]. Serum pools of 3 to 5 sera with a high level of IgE to a specific lipocalin were used in ELISA IgE-inhibition experiments. Recombinant allergens were added in increasing doses up to 100 μg/mL. Can f 1 was able to inhibit IgE recognition of human tear lipocalin at low doses. Sequence identity between Can f 1 and tear lipocalin is 57 %. Can f 1 weakly inhibited IgE-reactivity to Can f 2. Equ c 1 also weakly inhibited IgE directed to Mus m 1. Reciprocal experiments did not show any effect. No cross-reactivity was observed between Bos d 2 and Can f 1, Can f 2, Equ c 1, Mus m 1, and tear lipocalin.

The crystal structure of Can f 2 has been resolved recently [71]. Although the calyx was nearly structurally identical with MUP 1, Equ c 1, and α_{2u} globulin from rat, there was no IgE cross-reactivity detected. However, a patient-dependent cross-reactivity could be demonstrated between Can f 2 and Fel d 4 (up to 58 % inhibition) using high doses of Fel d 4 as inhibitor. Can f 2 and Fel d 4 share only 25 % sequence identity. This fact might explain why only high doses were able to inhibit IgE-reactivity to some extent. In lipocalins with low sequence identity, single conformational epitopes exposed at the surface might be responsible for this limited cross-reactivity. Part of the IgE antibodies will be inhibited from binding, but as the IgE response is polyclonal, the bulk of IgE will still bind and this will result in a weak inhibition.

Another recently isolated lipocalin from dog, Can f 4, was shown to crossreact with a homologous 23 kDa protein from bovine dander [18]. Using 3 patient sera, Can f 4 was able to completely inhibit IgE-binding to the bovine lipocalin when added in excess. Sequence identity between Can f 4 and the bovine homologue is 39 %. This strong cross-reactivity appears surprising. However, it is conceivable that there is at least 1 common epitope between the 2 proteins. Notably at the C-terminal end, there is a stretch of 10 identical amino acids and a sequence identity of 75 % over 20 amino acids. Clinical history of the patients whose sera were used in the experiment was not detailed, except that they were dog-allergic. Nothing is known about their potential contact with cattle, but Can f 4 was supposed to be the primary sensitizer as specific IgE to Can f 4 were much higher than IgE to the bovine protein.

Dog lipocalin Can f 6 has a very high identity to Fel d 4 (67 %) and Equ c 1 (57 %). Inhibition and cross-inhibition experiments were performed using sera from 6 patients allergic to cat or dog [19]. All of them had specific IgE to cat and dog dander. Three patient profiles could be determined: patients allergic either to cat or dog, recognized Fel d 4 and Can f 6, but the respective IgE-binding to the cross-reacting molecule could be strongly inhibited by the sensitizing molecule. Patients sensitized to both cats and dogs showed intermediate inhibition profiles indicating the presence of IgE recognizing epitopes specific for Fel d 4 and Can f 6 and epitopes cross-reactive for both lipocalins. Patients allergic to cat who had IgE antibodies to Can f 6 did not have IgE to Can f 1 or Can f 2.

The cross-reactivity between Can f 6 and Fel d 4 has been confirmed in a recent report [72]. IgE-inhibition experiments performed with 2 patient sera showed variable degrees of cross-reactivity. Equ c 1, which was included in the experiments because of its high sequence identity with Can f 6 and Fel d 4, could inhibit IgE-binding to Can f 6 and Fel d 4. Clinical history of the patients was not detailed and it is not known if they were in contact with horses.

In the frame of our studies on guinea pig allergens, we have identified another lipocalin allergen with high sequence identities to Fel d 4 (54 %), Equ c 1 (47 %), and Can f 6 (53 %) (unpublished data). We assume that a subgroup of highly homologous lipocalins does exist, which is responsible for

Table 2 Amino acid identities (%) between mammalian lipocalins

	Fel d 4	Fel d 7	Can f 1	Can f 2	Can f 4	Can f 6	Equ c 1	Bos d 2	Bos d 5	Cav p 2	Cav p 3	Rat n 1	Mus m 1
Fel d 4													
Fel d 7	19												
Can f 1	24	62											
Can f 2	25	22	23										
Can f 4	25	21	19	25									
Can f 6	67	24	25	24	26								
Equ c 1	67	25	25	24	28	57							
Bos d 2	30	16	23	20	31	27	31						
Bos d 5	25	27	26	20	16	23	20	20					
Cav p 2	31	22	22	14	33	27	33	39	19				
Cav p 3	28	20	19	23	32	27	28	39	18	43			
Rat n 1	55	24	21	28	28	52	47	26	23	30	25		
Mus m 1	49	21	19	26	28	47	46	26	18	27	24	64	

>60% identities
50-60 % identities
40-50 % identities

Lipocalins devoid of their signal peptide were aligned using Clustal W. Pairwise comparisons were made using the generated alignment. Minor variations in the % compared to published data may arise through the positioning of gaps or inclusion of the signal peptide

cross-reactive IgE reactions between mammalians. Upon searching the databases for homologous lipocalins, predicted proteins are indeed retrieved for several animals with identities >50 %. Among those, additional putative lipocalins from horse or cow are displayed. Some of those might be expressed and prove to be allergens.

Reports on clinical sensitization show the difficulty in performing conclusive cross-reactive studies. The clinical history of the patient, such as animal contact and sensitization, is crucial for the outcome of the experiment. Cat and dog allergens are ubiquitous and exposure is not limited to direct contact. They stick to clothes and are transported into public places such as schools or public transport [64••, 73]. Concentrations are low, but may be high enough to cause sensitization [64••]. Consequently, it will be very difficult to conduct a study with patients who are exposed only to cat or only to dog allergens. A certain level of co-sensitization can never be ruled out. However, as more and more allergens are characterized, it will be of crucial importance to assess which molecules will be able to provide a discriminative diagnosis for 1 animal species and which molecules are indicators of cross-reactivity. So far, data are incomplete and this is also due to the commercial unavailability of the molecules. In vitro diagnosis of pollen and fruit allergens has profited enormously from component-resolved diagnosis and cross-reactivities between different species become more and more predictable [74•]. The severity of clinical symptoms has been related to sensitization to different protein families such as lipid transfer proteins or profilins [75]. To date, it is completely unclear if there are specific clinical symptoms related to sensitization to lipocalins, serum albumins or other animal allergens.

Table 2 lists the sequence identities of all known lipocalin sequences so far. These figures can only give indications to putative cross-reactivities as they depict an overall sequence identity. They do not take into consideration short sequence identities or potential identical conformational epitopes.

Conclusions

The number of allergenic animal lipocalins is impressive, and there are probably more to be discovered. The number of endogenous lipocalins is not known for most species. One gene may imply different proteins through splice variants or post-translational modifications. Lipocalins are expressed in high quantities in different tissues, but their primary function for the host is still largely unknown. We will need to analyze the functions of lipocalins in order to understand how they possibly interact and deviate the immune system to an allergic response. The characterization of allergen uptake and processing pathways will be another important issue. A surprising new feature is the existence of a group of highly homologous lipocalins with strong IgE cross-reactivity between different animal species. For the clinician, it will be important to conduct epidemiologic studies to detail the role of lipocalins in clinical symptoms. Purified molecules are needed for diagnosis and for the evaluation of their specificity and possible cross-reactivity.

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- •• Of major importance

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