

Mammalian-derived respiratory allergens – Implications for diagnosis and therapy of individuals allergic to furry animals



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ABSTRACT

Furry animals cause respiratory allergies in a significant proportion of the population. A majority of all mammalian allergens are spread as airborne particles, and several have been detected in environments where furry animals are not normally kept. The repertoire of allergens from each source belongs to a restricted number of allergen families. Classification of allergen families is particularly important for the characterization of allergenicity and cross-reactivity of allergens. In fact, major mammalian allergens are taken from only three protein families, i.e. the secretoglobins, lipocalins and kallikrein families. In particular, the lipocalin superfamily harbours major allergens in all important mammalian allergen sources, and cross-reactivity between lipocalin allergens may explain cross-species sensitization between mammals. The identification of single allergen components is of importance to improve diagnosis and therapy of allergic patients using component-resolved diagnostics and allergen-specific immunotherapy (ASIT) respectively. Major disadvantages with crude allergen extracts for these applications emphasize the benefits of careful characterization of individual allergens. Furthermore, detailed knowledge of the characteristics of an allergen is crucial to formulate attenuated allergy vaccines, e.g. hypoallergens. The diverse repertoires of individual allergens from different mammalian species influence the diagnostic potential and clinical efficacy of ASIT to furry animals. As such, detailed knowledge of individual allergens is essential for adequate clinical evaluation. This review compiles current knowledge of the allergen families of mammalian species, and discusses how this information may be used for improved diagnosis and therapy of individuals allergic to mammals.

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1. Introduction

Allergic diseases affect a significant fraction of the entire population in industrialized countries [1,2]. Domestic animals such as dogs, cats and horses are among the most common allergen sources [3,4], and both sensitization and ownership frequencies for cats and dogs have increased over the last decades [5–7]. In addition, exposure to animals such as mice, rats, guinea pigs, rabbits and cows have been shown to induce allergy. Particularly, sensitization to rodents represents a major health hazard to workers in animal facilities, where 20–30% of all animal workers become sensitized to mammalian allergens [8,9].

Mammalian allergens as inducers of airway symptoms have been frequently studied. Prominently, sensitization to the major cat allergen Fel d 1 has been implicated as a major risk factor for the development of asthma [10,11]. IgE-levels to mammalian allergens have been shown to correlate with an increased risk of

developing new allergies in later childhood [12,13]. A recent study demonstrated that sensitization to several mammalian allergens was associated with bronchial inflammation in children with severe asthma [14]. This relationship was not observed in children with controlled asthma, indicating the major role of respiratory allergens in the development of severe IgE-mediated asthma.

Most allergens from furry animals are found in dust and on airborne particles in homes, but also in public facilities such as schools or day care centers [15–17]. It remains unclear whether early life exposure to cat or dog allergens increase the risk of developing pet allergy later in life [18,19]. Even though this matter has been addressed by several studies, the results are contradictory, and the outcome of early cat and dog ownership has been implicated as either protective [20], unaffected [21], or increasing the risk of developing asthma [22,23]. These studies are likely affected by a number of confounding factors, and further investigation is required to elucidate the variables responsible for the observed differences.

The common wish to have a cat or a dog among pet sensitized individuals has spurred several dog breeding companies to market

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“hypoallergenic cats” and “hypoallergenic dogs” that are supposed to have reduced allergenicity. Even though some cat and dog breeds may have a less grooming/shedding that could imply a reduced allergenicity, it is questionable whether certain breeds would express lower allergen levels as claimed. In fact, a recent study demonstrated higher expression of Can f 1 in hypoallergenic dog breeds than in “normal” breeds [24]. The diverse allergen panels of cats and dogs comprise to date eight and six allergens respectively and it is unlikely to envision animals with a defined allergen pattern, that would suite allergic patient’ sensitization profile in general.

Since cloning of the first allergen from cDNAs in 1988–1989 [25,26], major focus has been towards completing the different panels of various allergen sources. Characterization of recombinant allergens from multiple furry animal species has contributed to improve diagnostics of allergic patients. In addition, single recombinant allergens may be used to formulate allergy vaccines to improve allergen-specific immunotherapy (ASIT) of allergic patients. The identification of single allergen components allows for classification of allergens into distinctive protein families. This might be particularly important when studying the mechanisms of allergenicity, since less than 2% of all protein families constitute known allergens, and proteins from the same family may share similar structural and functional characteristics [27]. Allergens within the same family may also display cross-reactivity, as demonstrated for the mammalian allergen families albumins [28,29], and lipocalins [30,31], discussed below. Cross-reactive allergens harbour similar three-dimensional structures and epitopes that may imply IgE-mediated cross-reactivity, and share similar T cell epitopes, which may translate into T cell mediated cross-reactivity. Clinically, cross-reactivity has implications for the induction of allergic symptoms, since allergic patients may suffer from hypersensitivity to allergen sources they have not encountered previously [32]. Additionally, features of cross-reactivity can be used to simplify diagnostics of related allergies, due to an abundance of shared epitopes between very similar allergens [33,34]. A strong association has been established between allergies to cat, dog and horse in children [3,35], the latter study showing that sensitization to horse alone (mono-sensitization) was uncommon, likely reflecting cross-reactivity between these allergen sources. The role of cross-reactive allergens in cross-sensitization to related mammalian allergen sources is still not fully elucidated. However, recognition of related cross-reactive allergens by antigen presenting cells and T cells is likely to increase the risk of neo-sensitization to that allergen.

Here, we discuss important aspects of allergen families and allergens from mammalian species. Furthermore, we review how recombinant allergens are used to improve diagnostics and treatment of individuals allergic to furry animals. A summary of respiratory allergens characterized to date of various furry animal species is presented in Table 1.

2. Allergen families of furry animals

2.1. Secretoglobins

Fel d 1, is the most important allergen in cat dander and has been extensively studied since its discovery in 1973 [36]. The protein is found in saliva and is transferred to the fur by grooming, and is spread to the environment predominantly via dander [37,38]. The role of Fel d 1 in cat allergy is very dominant, as it has been shown to account for 60–90% of the IgE reactivity to cat dander [39–41], and up to 95% of all cat allergic patients react to Fel d 1 [40,42,43]. It is a tetrameric protein, joined together by two heterodimers consisting of two disulphide linked peptide chains. The molecular weight of natural (n) Fel d 1 is approximately 38 kDa,

while the recombinant (r) version has a molecular weight of 30 kDa [44,45]. The observed difference in molecular weight is probably due to 10–20% N-linked glycosylation on nFel d 1. By recombinant techniques, the two chains of Fel d 1 has been covalently fused and expressed as a folded polypeptide [44]. Importantly, the recombinant protein mimics the structure of the natural protein, which is the basis for maintained allergenicity. The T cell epitope repertoire of Fel d 1 has been studied [46,47], and surface epitopes have been identified with monoclonal antibodies [48–50].

Fel d 1 is special compared to other mammalian allergens by its very dominant role in cat allergy. A human homologue to Fel d 1, Clara cell protein CC16 has been described [51], but the biological role of Fel d 1 is unknown. In 2007, a paper was published describing a potential dog homologue of approximately 20 kDa detected in dander [52]. In several dog sensitized subjects, competitive inhibition with rFel d 1 adsorbed more than 50% of all dog-reactive IgE antibodies, indicating that a majority of all IgE antibodies to dog detected in those individuals could be directed towards a homologue. However, no sequence of the allergen was identified, and replication of this finding should be performed to ascertain the relevance of the described allergen. Recently, a novel secretoglobulin protein was identified as a major allergens in rabbit, the lipophilin Ory c 3 [53]. The allergen was identified in rabbit hair by IgE-immunoblotting followed by mass-spectrometry and N-terminal sequencing. Similarly as Fel d 1, Ory c 3 consists of two chains forming a heterodimeric allergen, and predictions indicated a similar structure as Fel d 1. The allergen bound IgE in 77% of rabbit allergic patients, and is reported as a major allergen. Despite the shared structural similarity, no cross-reactivity was detected between the allergens, possibly due to low sequence identity (~24%). As such, there is still little evidence to support a role for Fel d 1 in cross-reactivity with other mammalian sources.

2.2. Lipocalin

More than 50% of all allergens from all furry animals belong to the lipocalin superfamily. At least one major lipocalin allergen has been identified in every species, Table 1. Virtually all persons who are in contact with household pets, work in animal facilities or practise horse riding will be exposed to lipocalins. Allergen levels have been detected in facilities where pets are not normally kept [15]. As such, lipocalins hold a dominant position amongst mammalian allergens, and in most cases, the major allergens are lipocalins. As an example, the major allergen found in cow dander is the lipocalin Bos d 2 and is recognized by 80–90% of sensitized patients [54]. By its dominant role in cow dander allergy, it has been thoroughly characterized. The three-dimensional structure of Bos d 2 has been determined [55], and like other lipocalins, it contains a hydrophobic calyx for binding of small hydrophobic molecules. In the following sections, we discuss important lipocalin allergens of different furry animals and their implications for cross-reactivity and allergenicity.

2.2.1. Dog lipocalin allergens

Among dog sensitized patients we have identified a broad population that are sensitized to lipocalin allergens. Four out of six currently identified dog allergens, the major allergen Can f 1 (45–70% dog allergic patients sensitized), Can f 2 (~25%), Can f 4 (15–30%) and Can f 6 (~40%) are lipocalins, and the latter two were identified during recent years [31,56,57]. Can f 1 and Can f 2 is found mainly in saliva, but also in dander [58,59]. Both allergens were originally detected in dog dander extracts [56], and have been extensively investigated among dog allergens.

Table 1

Summary of the respiratory allergen panels of important mammalian species 2013-07-01.

Animal	Allergen	Protein family	Source	Amino acids	MM (kDa) [*]	Sensitized (%) [‡]	UniProtKB accession #	Reference(s)
Cat	Fel d 1	Secretoglobulin	Saliva	162	30–38	80–95	P30438, P30440	[40–42]
	Fel d 2	Albumin	Dander, Sera, Urine	584	68	20–35	P49064	[29,40]
	Fel d 3	Cystatin	Dander	98	11	10	Q8WNR9	[98]
	Fel d 4	Lipocalin	Saliva	171	20	63	Q5VFH6	[61]
	Fel d 5	Immunoglobulin, IgA	Saliva, Serum		24, 28, 64	38		[92]
	Fel d 6	Immunoglobulin, IgM	Saliva, Serum		28, 94			[92]
	Fel d 7	Lipocalin	Saliva	154	18	38	E5D2Z5	[62]
	Fel d 8	Latherin	Saliva	208	24	19	F6K0R4	[62]
Cow	Bos d 2	Lipocalin	Dander	156	20	80–90	Q28133	[54]
	Bos d 3	Calcium-binding protein	Dander	101	22		E1BKA1	[99]
	Bos d 6	Albumin	Dander, Meat, Milk, Sera	583	66		B0JYQ0	[83]
Dog	Can f 1	Lipocalin	Saliva, Dander	156	22–25	45–55	O18873	[31,56,57,89]
	Can f 2	Lipocalin	Saliva, Dander	162	22–27	20–30	O18874	[31,56,57,89]
	Can f 3	Albumin	Dander, Saliva, Sera	584	69	15–35	P49822	[31,57,82,89]
	Can f 4	Lipocalin	Saliva, Dander	158	18	15–30	D7PBH4	[57]
	Can f 5	Kallikrein	Urine	236	28	70	P09582	[89]
	Can f 6	Lipocalin	Dander	175	27, 29	38	E2QYS2	[31]
Guinea-pig	Cav p 1 [§]	Lipocalin	Dander, Urine		20	70–87	P83507	[73]
	Cav p 2	Lipocalin	Dander, Tears	154	17–19	55–65	F0UZ11	[73,74]
	Cav p 3	Lipocalin	Saliva	155	18–19	54	F0UZ12	[74]
Horse	Equ c 1	Lipocalin	Dander, Saliva, Urine	172	22	76	Q95182	[30,64]
	Equ c 2 [§]	Lipocalin	Dander		16	50	P81216, P81217	[65]
	Equ c 3	Albumin	Dander, Meat, Sera	583	65	18	P35747	[84]
	Equ c 4	Latherin	Dander, Saliva	208	17–19		P82615	[96,97]
	Equ c 5	Latherin	Dander	208	17		P82615	[96,97]
Mouse	Mus m 1	Lipocalin	Dander, Urine	162	18–21	66	P02762	[30,70]
	Mus m 2		Dander, Hair		21			[101]
Rabbit	Ory c 1 [§]	Lipocalin	Dander, Saliva		17–18			[72]
	Ory c 2 [§]	Lipocalin	Dander, Saliva		21			[72]
	Ory c 3	Secretoglobulin	Dander, Saliva	183	18–19	77	Q9GK63, Q9GK67	[53]
Rat	Rat n 1	Lipocalin	Urine	162	17–21	66	P02761	[30,70]

^{*} Molecular mass and, [‡], IgE-reactivity data were obtained using different methods <http://www.uniprot.org/>.[§] Only N-terminus known.

The full-length sequence of Can f 4 was identified based on cross-reactivity with a putative lipocalin allergen in cow dander. Can f 4 was reported to bind IgE in approximately 30% of dog sensitized subjects [57]. However, when we assessed the sensitization profile for Can f 4 among 100 dog sensitized individuals from Sweden, only 14 subjects were determined positive (Nilsson O.B., et al., unpublished data). As such, Can f 4 might be subject to regional differences or prevalence estimated using a selected patient population.

In 2010, we identified a novel dog allergen based on its high homology with Fel d 4 and Equ c 1. The protein was detected in dog dander, and the allergenicity was confirmed by basophil activation test and ELISA, where it was shown to bind IgE in 38% of dog sensitized patients [31]. We submitted the sequence of the allergen to the WHO/IUIS Allergen nomenclature sub-committee (www.allergen.org) in 2010, and it was designated Can f 6. We also showed an IgE-mediated cross-reactivity of Can f 6 with the cat lipocalin allergen Fel d 4 and the horse lipocalin allergen Equ c 1. Thus, the allergen might be implicated in cross-sensitization to related mammalian species [31]. Can f 6 has been suggested as a major dog allergen (61%) [60]. However, the study was performed with patients sensitized to both cat and dog allergens. Consequently, dog sensitized patients lacking IgE to cat dander extract, and more importantly to Fel d 4, were excluded. Due to the cross-reactive relationship between Can f 6 and Fel d 4 (>80% double-positive patients recognizing either allergen), this patient selection may exclude a proportion of Can f 6-negative patients and thereby increase the number of positive patients. Even though Can f 2, Can f 4 and Can f 6 are minor allergens, they still possess diagnostic and therapeutic values, since some dog allergic patients only recognize minor allergenic components. Preliminary data from our group also show that sensitization to only one lipocalin allergen is uncommon, and most patients are sensitized to two or more lipocalin allergens, with virtually any combination. Also,

there seem to be very little cross-reactivity between the four dog lipocalin allergens. This demonstrates the importance of single allergen components for diagnosis and ASIT of dog sensitized subjects.

2.2.2. Cat lipocalin allergens

Two lipocalin allergens, Fel d 4 and Fel d 7, have been detected in cat and introduced in the official allergen nomenclature [61,62]. Fel d 4 was identified by IgE screening of an expression cDNA library and Fel d 7 was amplified by PCR-techniques based on its homology with Can f 1. Since the discovery in 2004, Fel d 4 has received little attention despite its role as a major allergen. This is most likely due to the dominant role of Fel d 1 in cat allergy. Nevertheless, the importance of Fel d 4 has recently been demonstrated by its cross-reactivity with Can f 2 [63], Can f 6 and Equ c 1 [31]. This allergen may therefore play an important role in cross-reactivity and cross-sensitization with dog, horse, mouse and rat. Fel d 7 was reported to bind IgE in approximately 38% of all cat allergic subjects in a recent study [62]. However, the IgE-binding frequency to Fel d 1 was also relatively low (60%), and whether Fel d 7 might bind more IgE in another population remains to be analysed. Due to its high homology with Can f 1, cross-reactivity between Fel d 7 and Can f 1 is expected. Thus, Fel d 7 may be implicated in cross-sensitization with dog and potentially also with horse (see below).

2.2.3. Horse lipocalin allergens

Two lipocalin allergens, Equ c 1 and Equ c 2, have been identified in horse, and both appear to be major allergens [30,64–66]. The dimeric Equ c 1 has been thoroughly characterized since the

Can f 1 (dog)													
Can f 2 (dog)	23												
Can f 4 (dog)	19	25											
Can f 6 (dog)	25	24	26										
Fel d 4 (cat)	24	25	25	67									
Fel d 7 (cat)	62	22	20	24	20								
Equ c 1 (horse)	27	26	28	57	67	25							
Mus m 1 (mouse)	19	26	27	47	49	19	45						
Rat n 1 (rat)	21	28	28	52	55	24	47	64					
Bos d 2 (cow)	22	19	31	27	30	17	31	28	26				
Cav p 2 (guinea pig)	22	14	33	27	31	22	30	27	30	39			
Cav p 3 (guinea pig)	19	20	32	27	28	20	27	24	25	39	43		
	Can f 1	Can f 2	Can f 4	Can f 6	Fel d 4	Fel d 7	Equ c 1	Mus m 1	Rat n 1	Bos d 2	Cav p 2	Cav p 3	

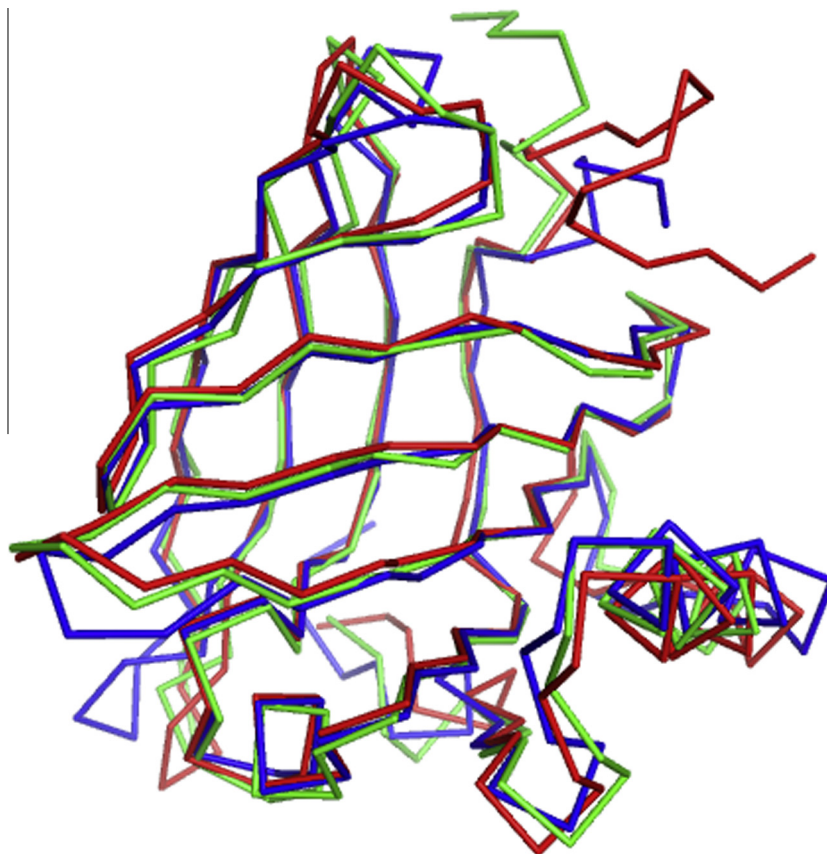


Fig. 1. Structural alignment of the backbone of the lipocalin allergens Can f 2 (dog, highlighted in green), Mus m 1 (mouse, highlighted in red) and Bos d 2 (cow, highlighted in blue). Even though these proteins share similar three-dimensional structures, the primary structure homology is low (<28%), and no cross-reactivity has been demonstrated between the allergens. Figure by Tatyana Sandalova and Adnane Achour, Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden.

recognition of an antigen with partially endogenous T cell epitopes, could induce Th2 responses.

2.3. Albumin

The albumin protein family has been documented to induce allergy from virtually every mammalian allergen source [29,40,72,73,82–86]. However, only the albumin allergens Fel d 2, Can f 3, Equ c 3 and Bos d 6 from cat, dog, horse and cow, respectively, have been introduced to the official list of allergens (www.allergen.org). Albumins are minor allergens, and around 15–35% of cat and dog allergic patients are sensitized to Fel d 2 and Can f 3, and around 15–20% of horse allergic patients appear to be sensitized to Equ c 3 [29,31,40,82,84]. For other mammalian albumins the sensitization profiles have not been thoroughly characterized.

Until recent discoveries with cross-reactive lipocalin allergens, albumins were attributed with being the primary allergens responsible for cross-sensitization to cat, dog and horse, but also to other furry animal species [29,84]. At the same time, the cross-reactivity between different albumins has been described as variable, which indicates that a significant proportion of albumin-specific IgE is directed towards species-specific epitopes. As shown in Table 3, albumin allergens share a high sequence identity compared with lipocalin allergens, and albumins from cat, dog, horse, guinea-pig, cow, pig, rabbit, mouse and rat are all 48.6% identical. Therefore, the observed variability in cross-reactivity is surprising. One study reported that only about one third of patients sensitized to cat, dog and horse had IgE-reactivity to albumins and more than 50% of specific cross-reactive IgE was directed to other allergens [28]. This

gap may be explained by cross-reactivity to lipocalin allergens and other allergen components.

The overall high sequence identity between albumins from different mammalian species may relate to the status of albumins as a minor allergen family, and this is supported by bioinformatics studies [87]. For example, Fel d 2 shares 81.7% homology with human serum albumin, and this could imply tolerance to the allergen in a majority of subjects.

2.4. Kallikrein

The occurrence of a dog kallikrein allergen was indicated in a patient with allergy to human seminal plasma (HSP) after the demonstration of IgE-mediated cross-reactivity with dog dander [88]. A causative allergen of HSP allergy had previously been identified as the prostate-specific antigen (PSA), a member of the kallikrein protein family, and a cross-reactive protein was detected in dog urine. The allergen was later confirmed to be the dog prostatic kallikrein (DPK), designated Can f 5 [89]. Expression of this allergen is mainly restricted to male animals and the protein is secreted in the urine. Compared with dander and saliva, exposure to urinary allergens might be more controversial, and exposure to urinary allergens will be related to how the pets are kept. Nevertheless, Can f 5 has been reported as a major allergen, mainly in a Spanish population where 70% of the subjects were sensitized. Further studies of sensitization frequency to this allergen are warranted. Until now, no other kallikrein allergen than PSA and Can f 5 has been identified in any other source. However, a BLASTp search with the corresponding template gene for Can f 5 identified potential homologues in both cat and horse. Thus, the kallikrein family

Table 3

Amino acid sequence identities (%) between mammalian albumins with known sequences. Signal peptides were deducted from the sequences. Comparisons were made with Geneious 6.1.6 (www.geneious.com), using the Blosom62 matrix. Note that the lowest sequence identity between any of the albumins is 69%.

Can f 3 (dog)								
Fel d 2 (cat)	87							
Equ c 3 (horse)	76	78						
Bos d 6 (cow)	76	78	74					
Mus m albumin (mouse)	72	74	71	70				
Rat n albumin (rat)	73	75	73	71	90			
Cav p albumin (guinea pig)	72	74	71	69	73	73		
Ory c albumin (rabbit)	74	75	71	72	72	73	70	
	Can f 3	Fel d 2	Equ c 3	Bos d 6	Mus m albumin	Rat n albumin	Cav p albumin	Ory c albumin

may play a role alongside lipocalins and albumins as a cross-reactive allergen family, and the role of kallikrein allergens from these sources remain to be elucidated.

2.5. Minor allergen families

A few allergen families that constitute minor allergens in various sources have been identified. During recent years, the carbohydrate epitope galactose- α -1,3-galactose (α -gal), present for instance on cat IgA and IgM and in red meat [90,91], has received much attention for its role in various allergies. Cat IgA and IgM designated Fel d 5 and Fel d 6 respectively [92], have been detected in cat serum and saliva. IgE-binding to the two allergens is mainly limited to the carbohydrate groups on the heavy chains of the antibodies. We have demonstrated that 38% of cat sensitized subjects were positive to Fel d 5. Since α -gal is also found on parasites, IgE antibodies to this epitope has shown to cause impaired allergy diagnostics in parasite-infested areas [93]. Compared to Fel d 5, Fel d 6 has been less thoroughly characterized, although IgE-binding to the two allergens seems to correlate [92]. Until now, the only other immunoglobulin allergen identified is the food allergen Bos d 7, found in serum and milk from cows [94]. However, immunoglobulins from other mammalian sources may exhibit allergenicity similarly to Fel d 5 and Fel d 6, given that they contain the α -gal epitope [95].

Another minor allergen family is the surfactant protein family, latherins. The minor horse allergens Equ c 4 and Equ c 5 are both latherin allergens detected in horse sweat, and are derived from the same peptide fragment [96,97]. The IgE-binding profile of Equ c 4/Equ c 5 is unclear, although one study has reported that a major fraction of horse sensitized subjects react to Equ c 5 [96]. A cat homologue to the horse latherin proteins was recently identified in cat, named Fel d 8. The IgE-binding frequency of Fel d 8 is about 20%, and it is unclear whether latherin allergens from different sources exhibit IgE-mediated cross-reactivity.

A few other minor allergens have also been identified. The minor cat allergen Fel d 3 is the cysteine protease inhibitor, cystatin [98]. The allergen is apparently recognized by about 10% of cat sensitized individuals. The cow allergen Bos d 3 belongs to the family of calcium binding proteins [99], and homologous allergens have been identified in numerous non-mammalian allergen sources [100]. An additional allergen has been detected in mouse dander, termed Mus m 2 [101]. However, this protein has not been characterized.

3. Diagnostics with mammalian allergens

Allergic disease may decrease quality of life in affected patients [14,102], and physician's diagnosis serve as basis for management of allergic symptoms as well as long term curative therapies. Traditional diagnostic methods using crude allergen extracts are well established. An obvious advantage with such methods is that they

ideally mimic the source of natural exposure, obtaining a positive result. Allergen extracts are also associated with major drawbacks. Due to the heterogeneous nature of raw materials, they are inherently difficult to standardize. Major shortcomings have been reported for several commercial dog dander extracts, where important allergen components may have been lacking, or they have only contained the minor dog albumin allergen Can f 3 [103]. Adequate quality control of extracts is also required to avoid contamination from other allergen sources, which may produce false-positive results [104]. As an example, a recent study with dog extracts described the contamination with human serum albumin in one of the extracts [103].

Recombinant allergens may serve as an alternative to natural allergen extracts for diagnosis of allergic patients. For cat allergy, the possibility to replace natural extracts with the major allergen Fel d 1 has been investigated. As previously mentioned, rFel d 1 seems to bind IgE in up to 95% of cat allergic patients [42,43]. Furthermore, IgE-levels to rFel d 1 show a very good correlation with IgE-levels to cat dander extract, and as such, Fel d 1 may replace the natural extract for diagnosis of nearly all cat allergic patients [42]. Allergen-specific IgE to rFel d 1 is also a risk factor for childhood asthma, and may be used as a diagnostic tool alongside the cat dander extract [42]. For other mammalian allergies, no other single allergen holds the same dominant position as Fel d 1. Also, the characterization of other mammalian allergens as diagnostic tools has been less thorough. As exemplified by dog allergy, the current allergen panel comprise six allergens, where Can f 1 and Can f 5 have been classified as major allergens. However, they only diagnose about 55% and 70% respectively of all dog allergic patients, and may not individually replace dog dander extract. Furthermore, whether mixtures of mammalian allergens may replace the natural extracts of the various sources needs to be elucidated. Aside from cat allergy, there is no evidence that a single allergen from mammalian species may account for a major fraction of the total source-specific IgE and thus, possibly, replace the allergenic extract for diagnostics of allergic patients.

As an alternative to single recombinant allergens, modern DNA technology also allows for assembly of several IgE-epitopes into one hybrid molecule, which may replace natural extracts [105]. In addition, artificial extracts may be prepared through combination of several recombinant allergens. However, the main advantage of recombinant allergens for diagnostic purposes is component-resolved diagnostics, where IgE-reactivities towards an allergen source is divided into individual molecules [106]. Recent advances in routine diagnostics involve the use of microarray technologies [107,108], where a broad panel of different allergens from multiple sources are assembled to determine highly specific sensitization profiles of allergic patients.

4. Therapy with mammalian allergens

A first line of interventions for allergy is avoidance of the allergen source, which is a cost effective way of alleviating allergic

symptoms [109–111]. However, mammalian allergens are stable and have been detected in areas where pets usually are not kept [15–17], and as such, allergen avoidance may be particularly difficult. ASIT is disease-modifying, and is the only therapy with the ability of inducing a long-lasting protection to allergic disease [112]. However, the beneficial outcome of immunotherapy with crude mammalian extracts may vary for various sources.

4.1. Immunotherapy with crude allergen extracts

ASIT with cat dander has been shown to be clinically effective [113–115]. Subjects receiving subcutaneous (SC) immunotherapy with cat dander have been documented to obtain increased levels of IgG4, decreased skin reactivity, decreased bronchial reactivity and improved symptom scores compared with placebo groups in double-blind, placebo-controlled studies. The clinical efficacy of immunotherapy with cat dander extract is also dose-dependent, and high allergen doses are required for a potent effect [114,115]. However, immunotherapy for dog allergy does not appear as efficacious as immunotherapy in cat allergy. One study with asthmatic patients allergic to dog or cat evaluated the effects of ASIT. Only patients treated with cat immunotherapy had a decreased sensitivity to bronchial challenge, which is indicative of a lower efficacy of dog immunotherapy [116]. Another study also reported an enhanced clinical efficacy of cat immunotherapy compared with dog immunotherapy, assessed by decreases in bronchial sensitivity and histamine levels, and increases in IgG4-levels in the cat immunotherapy group [117]. Differences between cat and dog immunotherapy may depend on the standardization of cat extracts to Fel d 1, whereas dog extracts may vary greatly in terms of allergen content, as reported previously [103]. Immunotherapy with horse extracts has also been performed, and reported to be clinically efficacious [118]. However, very little data has been published with horse immunotherapy, and more clinical research is desirable.

Immunotherapy with crude allergen extracts is associated with several disadvantages. As previously discussed with regard to dog allergy immunotherapy, commercial crude allergen extracts may contain low levels or even absence of important allergens. Other shortcomings associated with the use of extracts for ASIT include a natural presence of not identified non-allergenic molecules. In addition, inherent instability, presence of proteolytic activity, unknown contaminants and an uncontrolled immunogenicity of individual allergens suggest additional challenges [119,120]. Immunotherapy with extracts may also induce new sensitization, and reactivity to other allergen sources due to cross-reactive allergens or contamination with other allergen sources [121–123]. These major hurdles are difficult to overcome and viable alternatives to crude allergen extracts in ASIT are needed.

4.2. Immunotherapy with recombinant allergens

Recombinant allergens are attractive tools to combat the obstacles that are associated with allergen extracts. A detailed sensitization profile of each patient can be determined with component-resolved diagnostics, and tailored treatment can be engineered using the allergens the patient is sensitized to (also designated component-resolved immunotherapy) [106]. Using this strategy, natural extracts can be replaced and some obstacles avoided [120]. However, the complexity of necessary allergens that need to be included varies between sources. As previously discussed, immunotherapy with cat extracts appears more efficacious than immunotherapy with dog extracts, probably due to complex sensitization profiles in dog allergy and an incomplete dog allergen

panel [31]. For the same reason, it may be difficult to prepare potent allergy vaccines using recombinant allergens for dog allergy.

4.3. Hypoallergens

Successful ASIT implies that the higher the allergen dose, the better efficacy of the treatment [124,125]. However, treatment with high allergen doses is limited by acute side effects and late-phase responses (LPR) [126,127]. To combat adverse events of ASIT, different strategies have been applied to increase safety of the treatment, while maintaining efficacy needed for a beneficial clinical outcome. Formulation of recombinant allergen derivatives with attenuated characteristics, also called hypoallergens, is a widely explored strategy for efficacious and safe ASIT. A general characterization of hypoallergens states that they have a reduced IgE-binding and a retained T cell reactivity, although this may vary for different strategies [120,128–130]. Hypoallergenic derivatives based on mammalian allergens have exclusively been restricted to Fel d 1, due to its dominant role in cat allergy. Fel d 1 has also been extensively characterized in terms of IgE- and T-cell epitopes [46,131,132], which enables for the design of hypoallergenic derivatives. Rational design of hypoallergenic derivatives of rFel d 1 was described [133], where certain vaccine candidates had 400–900 times lower IgE-binding capacity compared to wild type rFel d 1, which could make them safe alternatives for ASIT. This approach showed beneficial characteristics in a mouse model for cat allergy [134], as demonstrated by increased IgG production and reduced airway hyperresponsiveness (AHR) in treated mice. We recently described the generation of hypoallergenic mutants to rFel d 1 with a lower IgE-binding and T cell activation capacity for the reduction of acute and late phase responses [135]. This strategy involved randomly mutated allergen sequences and selection of derivatives with a maintained tertiary structure by phage display, using sera from cat allergic patients. A major advantage with this strategy is that it does not require knowledge of relevant B- and T-cell epitopes for the generation of hypoallergens, and could basically be applied to any allergen.

4.4. Peptide-based vaccines

As an alternative to hypoallergens, T cell peptides containing major allergen epitopes for allergy vaccination have been generated from the primary structures of Fel d 1 [136,137], Can f 1 [138], Bos d 2 [139] and Equ c 1 [140]. Such peptides show no or markedly reduced ability to cross-link IgE epitopes, are not able to cross-link IgE on basophils and mast cells, and thus do not induce acute allergic adverse events. Their mechanism of action is through the induction of T cell tolerance, which may improve immune responses towards the allergen mainly through induction of regulatory T cells and induction of linked epitope suppression [141]. The complexity of peptide vaccines is a major drawback and consequently, only a few have reached clinical trials. Such trials have demonstrated the induction of T cell tolerance, but since the protective effect seems limited to T cells, acute responses are only partially or even unaffected by T cell epitope vaccines [136,137]. In addition, peptide vaccines may induce LPR in vaccinated patients [142], and cannot be deemed completely safe for ASIT.

4.5. Modification of allergens with immunomodulatory derivatives

To improve the efficacy of ASIT, coupling of whole allergens or peptides to compounds with immunomodulatory functions has been performed. B cell peptides corresponding to whole

conformational epitopes (haptens) may be fused to carrier molecules, such as viral proteins [120,129,130]. This approach strengthens the hapten approach by applying T cell help from the carrier protein, thus inducing stronger immune responses. Carrier molecules are attached chemically, or directly fused to the construct using recombinant DNA technologies [143,144]. Recombinant fusion of the viral protein PreS from hepatitis B with rFel d 1-derived peptides has been demonstrated [145]. The produced vaccine had a safer IgE-binding profile, while inducing IgE-blocking IgG antibodies. We also demonstrated the coupling of rFel d 1 to vitamin D3 for improved efficacy and safety of ASIT [146]. The approach showed promising results in a mouse model for cat allergy, with a reduced airway inflammation, AHR and enhanced allergen-specific IgG responses. rFel d 1 was also covalently coupled to carbohydrate-based particles (CBP) for targeting of dendritic cells and enhanced adjuvancy in ASIT [147]. This approach was evaluated as a prophylactic and therapeutic vaccine in a mouse model for cat allergy [148,149]. Prophylactically, it was shown to prevent the induction of airway inflammation, possibly through the induction of allergen-specific IgG and IgM [149], and by a prolonged tissue exposure to rFel d 1. The construct also showed promising results in a therapeutic setting, through reduced airway hyper-reactivity and beneficial humoral responses [148]. rFel d 1 has also been directly fused to peptides with immunomodulatory functions. A fusion protein was created by joining rFel d 1 with a protein targeting human FcγRIIb [150], aiming to cross-link FcεRI with FcγRIIb, neutralizing allergen-specific mast cells and basophils. This fusion protein did not induce mediator release on its own. Instead, it inhibited mediator release in basophils from cat sensitized individuals and mast cells from cord blood. Vaccine potential of the construct was demonstrated in a mouse model for cat allergy [151], where it induced IgE-blocking IgG antibodies and no mediator release *in vivo*. Another successful fusion-based strategy for an enhanced vaccine potential of recombinant allergens through recombinant fusion was recently described. By attaching a truncated invariant chain peptide, and a transactivator of transcription peptide to allergens, they were converted into cytoplasmic proteins, while also targeting the MHC class II pathway. The technology is called modular antigen translocation (MAT), and has been attempted with several major allergens [152]. MAT fusion of recombinant allergens allowed for 10–100 fold lower allergen doses for induction of PBMC proliferation, while also shifting T cell responses to Th1 and IL-10 production. MAT-Fel d 1 showed advantageous responses in immunized mice [153], and a recent paper described a clinical trial using this construct by intralymphatic injection immunotherapy [154]. The construct was safe to use in this setting, and tolerance to rFel d 1 was induced after only three injections. It was concluded that a combination of the MAT technology with intralymphatic injections is a major improvement over classical subcutaneous or sublingual immunotherapy, due to improved safety profile, low allergen doses, few injections, and a short treatment period.

5. Concluding remarks

The expansion of the various mammalian allergen panels is crucial for improved diagnosis and therapy of patients allergic to furry animals. As exemplified by cat and dog allergies, Fel d 1 may replace the natural allergen extract for diagnosis and therapy, while no major dog allergen has been suggested with a similar capacity. Consequently, there is need for improvements of both diagnostics and therapeutics for dog allergy. The dog allergen panel is incomplete, as we may not detect all dog allergic individuals using recombinant allergens. In addition, recombinant allergy vaccines based on mammalian allergens have been restricted to Fel d 1. Particularly in dog allergy where several commercial extracts have

shown to be of poor quality, there is need for a recombinant alternative for efficacious ASIT. Finally, few studies using ASIT for treatment of allergy to other mammals have been conducted, and further studies are needed.

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