World Journal of

Methodology

World Journal of *Methodology*

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Molecular diagnosis in cat allergy

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Abstract

Domestic cats represent one of the most common sources of indoor allergens. All over the world, many households own cats, whose allergens are persistent and widespread. Cat allergy itself is frequent, and its symptoms vary from rhinoconjunctivitis to life-threatening asthma. *In vitro* diagnosis using precision medicine allergy immunoassays is important because natural cat dander extracts may differ in quality and quantity of some of the individual allergen components and other molecules. In the component-resolved diagnosis of cat allergy, singleplex and multiplex specific immunoglobulin (Ig) E assays include use of the cat-specific major allergen, secretoglobin Fel d 1 (as a species-specific molecule), other allergen components (such as lipocalins Fel d 4, cross-reacting with other animal similar molecules, and Fel d 7, present in small quantities in natural extracts), and serum albumin Fel d 2 (related to the cat-pork syndrome). IgA Fel d 5 and IgM Fel d 6 are not available as allergen components in the current commercial IgE immunoassays, but they may impair the *in vitro* diagnostic evaluation of cat allergy because galactose-α1,3-galactose is an IgE-binding epitope of these native feline allergens. The benefits of molecular-based cat allergy diagnosis are continually evaluated, as the role of recombinant allergen components already known is detailed and new other molecules of interest may be discovered in the future.

Key Words: Feline; Allergens; Component-resolved diagnosis; Immunoglobulin E; Immunoassays

Core Tip: Cats are a common source of allergens for humans, and allergy to these pets are frequent and variable in their clinical manifestations. The benefits of molecular diagnosis in cat allergy include use of the species-specific major allergen Fel d 1, cross-reacting allergen components, including those present in small quantities in natural extracts, while considering molecules that may impair the *in vitro* allergy diagnosis. The identification and characterization of molecular cat allergens with clinical significance has allowed their use in singleplex and multiplex immunoglobulin E immunoassays for a precision diagnostic approach.

INTRODUCTION

The domestic cat (*Felis domesticus*, synonym: *Felis catus*) is one of the most common sources of indoor allergens, and allergy to cats in humans is the most common mammalian-origin immunoglobulin (Ig) E-mediated hypersensitivity. Cats have been associated with humans for more than 9500 years and are considered nowadays the most popular pets in the world. In past decades, a high incidence of allergy to these furry animals, especially among children and young adults, has been recorded. Cat allergy is currently estimated to affect approximately 1 in 5 adults worldwide. Many households own cats, indicating that there is a high exposure to their allergens. Moreover, the major and most studied cat allergen, Fel d 1, is persistent and ubiquitously present in indoor habitation spaces, dust samples from homes with or without cats, in public buildings and transportation, making allergen avoidance difficult[1-3]. The symptoms of allergy to cats vary from relatively mild rhinoconjunctivitis to potentially life-threatening asthma exacerbations[2].

Precision medicine allergy immunoassays support the molecular-based diagnosis for cat allergy. Also known as component-resolved diagnostics (CRD), this patient IgE sensitization *in vitro* molecular-level diagnostic approach uses allergenic components.

To date, eight *Felis domesticus* molecular allergens have been recognized as Fel d 1 to Fel d 8 by the World Health Organization/International Union of Immunological Societies (WHO/IUIS)[4]: uteroglobin-like protein Fel d 1, serum albumin Fel d 2, cystatin Fel d 3, lipocalins Fel d 4 and Fel d 7, Igs Fel d 5 and Fel d 6, and latherin-like protein Fel d 8. Cat allergens are involved in the molecular mechanisms underlying IgE-mediated allergic sensitization and different cross-reactivities. Representative isoforms are described for these allergens: Fel d 1.0101, Fel d 2.0101, Fel d 3.0101, Fel d 4.0101, Fel d 5.0101, Fel d 6.0101, Fel d 7.0101, Fel d 8.0101, but none is mentioned as such in the commercial IgE immunoassays. Data on the IgE binding epitopes are scarce, with sequence positions mentioned only for Fel d 1. IgE epitope mapping of this dominant cat allergen revealed five sequential/linear epitopes on chain 1/Fel d 1-A and two on chain 2/Fel d 1-B, in addition to a discontinuous/conformational epitope on chain 1[5], the last one being located on the four helices of the Fel d 1 chain 1 spatially juxtaposed upon protein folding.

Currently, the best characterized and available cat allergenic molecules for commercial IgE assays are Fel d 1, Fel d 2, Fel d 4 and Fel d 7. The two types of such allergen components used in singleplex and multiplex immunoassays are recombinant (r) allergens (produced by recombinant DNA technology) and highly purified natural (n) allergens (purified from natural sources)[6]. All are included in the list of cat allergens presented in the European Academy of Allergy and Clinical Immunology Molecular Allergology User's Guide[7] and in a recent Consensus document on dog and cat allergy[8]. The characteristics of these cat allergens[7-11] are presented in Table 1 together with all other allergenic molecules recognized by the WHO/IUIS database[4].

One major advantage of the CRD is the evaluation of primary sensitization animal source, which is not feasible by using native extracts, and better management of pet

Table 1 Characteristics of cat molecular allergens[7-11] mentioned in the World Health Organization/International Union of Immunological Societies database[4]

Allergen	Biochemical designation	Source of exposure	MW in kDa
Fel d 1[1,2]	Secretoglobin[4]	Saliva, dander	38
Fel d 2[1,3]	Serum albumin	Dander, serum, urine	69
Fel d 3	Cystatin[5]	Dander	11
Fel d 4[1,2]	Lipocalin[5]	Saliva	22
Fel d 5	Immunoglobulin A[4]	Saliva, serum	400
Fel d 6	Immunoglobulin M[4]	Saliva, serum	800-1000
Fel d 7[2]	Lipocalin, von Ebner gland protein	Saliva	17.5
Fel d 8	Latherin-like protein	Saliva	24

The World Health Organization/International Union of Immunological Societies is commonly known by its acronym, WHO/IUIS. Fel d 1, Fel d 2, Fel d 4 and Fel d 7 allergens are available in commercial immunoglobulin E immunoassays:

[1]Available in singleplex immunoassays as recombinant allergen.

[2]Available in multiplex immunoassays as recombinant allergen.

[3]Available in multiplex immunoassays as native purified component.

[4]Presence of glycosylation.

[5]Glycosylation deduced from sequence analysis. MW: Molecular weight.

allergic patients[1]. The importance of molecular-based diagnosis is continually evaluated, as the role of allergen components already identified in cat allergy is detailed, and new molecules of interest may be discovered.

CAT ALLERGEN COMPONENTS FOR MOLECULAR DIAGNOSIS

A deep understanding of the most important cat allergens is crucial for assessing allergen products for *in vitro* molecular diagnosis to evaluate in detail the IgE sensitization profile of patients allergic to furry pets. Other allergen proteins, recently identified and defined, must also be discussed for their potential use in CRD in the future.

Fel d 1

The cat major allergen Fel d 1 is a small tetrameric protein composed of two heterodimers, each containing two distinct chains (chain 1, a polypeptide, and chain 2, a glycopeptide with N-linked oligosaccharide composed of triantennary glycans) linked by disulfide bonds in its native form. This allergen is a secreted globular protein belonging to the secretoglobin family. It is homologous with the human Clara cell 10-kDa phospholipid-binding protein and the progesterone-binding rabbit uteroglobin (uteroglobinlike protein). Fel d 1's biological function for the cat is not clearly established, initially being discussed that it may have a protective role in cat skin[12-16]. Fel d 1 is probably involved in immunoregulation and intra-species chemical communication, binding with good affinity to some fatty acids and steroids, the best ligands being lauric acid (cat pheromone with effects on social interactions) and androsterone (volatile steroid pheromone). Fel d 1 is a thermostable protein produced in various anatomical areas of cats, mainly by the sebaceous glands and anal sacs, but also by salivary and lacrimal glands. Fel d 1 is primarily found in cat skin and hair follicles. As cats groom, Fel d 1 is distributed on the fur, then shed with hair and dander. It is easily airborne and found in various indoor environments, such as homes with and without cats, hotels, schools, buses and trains, occupational and/or leisure environments, including cinemas, animal facilities, pet shops, farms. Pet owner's clothing is a significant source of allergen dispersal. Up to 60% of airborne Fel d 1 molecules are carried by small particles, of which 75% are more than 5 μm in diameter and 25% less than 2.5 μm. This allergen is very pervasive indoors, many airborne Fel d 1 settles out within a couple of days of disturbance, but smaller particles can remain airborne for up to two weeks or even longer. Measurement of this secretoglobin allergen levels in settled dust should not be used as a surrogate for airborne exposure. Moreover, the concept of a specific allergen threshold amount of exposure expected to

provoke respiratory symptoms (such as 8 µg/g of dust) is also probably misleading, mentioning besides that IgE sensitization can occur at much lower Fel d 1 levels [1,3,12,16].

All cats produce Fel d 1 regardless of age, sex, breed, body weight, hair length or housing (indoors *vs* outdoors). Fel d 1 is produced under testosterone control (male cats produce more Fel d 1 than females if uncastrated and 3-5 times less after neutering, while its production could be restored to pre-neutering levels with exogenous testosterone administration)[1]. In the fur of domestic cats, Fel d 1 levels are significantly higher than those of Fel d 4, and cat-to-cat variability was revealed. The quantity of Fel d 1 on cat hair can range from 1 µg/g to more than 1770 µg/g, with high concentrations on hair from the neck region. The hair length does not seem to affect Fel d 1 production. Fel d 1 is also present in cat saliva, but in lower concentrations than Fel d 4. Urine is not a significant source of Fel d 1, but hormonal status affects its urinary levels in male cats, making it possible that litter boxes of intact male cats to be a source of this allergen at home[3,17]. Washing cats is of little benefit, because even if it reduces the amount of Fel d 1 on the skin and fur, the effect does not last long as the amount of Fel d 1 returns to its original level in just 2 d[12]. Feeding cats a diet with an egg product ingredient containing anti-Fel d 1 IgY reduces active Fel d 1 in cat saliva and dander, decreasing the environmental allergen levels[18,19].

The recombinant cat allergen rFel d 1 is produced in an *Escherichia coli* expression system by direct fusion of chain 2 and chain 1. This major allergen accounts for 60%-90% of the total allergenic activity of cat dander extracts, while specific IgE antibodies to rFel d 1 are reported in 90%-98% of European subjects with cat allergy. This is aligned with African data which revealed that nearly 75% of the patients with cat allergy from Zimbabwe have IgE antibodies against rFel d 1[12-15]. Rabbit (*Oryctolagus cuniculus*) Ory c 3 secretoglobin from saliva and dander, belonging to the same secretoglobin family, has very low sequence identity with Fel d 1, with no known IgE cross-reactivity[20]. Sequence similarity of Fel d 1 was reported with the skin brachial gland protein of an arboreal prosimian from Southeast Asia, named low loris (*Nycticebus* spp). Used for communication and defense when mixed with saliva, this gland protein has induced several cases of anaphylaxis in humans, some lethal, reported after the prosimian bites[21,22].

Fel d 1-related epithelial allergens from the majority of "big cats" (Table 2) are cross-reactive with domestic cat Fel d 1[11,23-26]. Sera from cat-allergic patients were analyzed by the first-generation solid-phase isotopic allergosorbent immunoassay using big cat fur extracts, obtained from hair collected by brushing animals (from the Natura Altis Magistra Zoo, Amsterdam, The Netherlands) at the time they were losing their winter fur. All subjects with positive skin test to cat extracts had IgE antibodies reacting with hair extracts from seven Felidae species (lion, Siberian tiger, snow leopard, jaguar, puma, ocelot, serval) but not caracal[23]. Cat-allergic individuals may be uncommonly exposed to such cross-reactive Fel d 1-related allergens in special settings, like zoos, wild parks and circus visits, but only very few cases developed severe allergic reactions upon exposure to lions and tigers in circuses[23-27]. The weight of big cats used in the past in circus entertainment is much greater than that of common domestic cats, and therefore it is likely that they produce large quantities of aeroallergens. Moreover, Siberian tiger hair extract contains 15-times more Fel d 1-like allergens *per* gram than that of lion[23].

rFel d 1 is available in singleplex and multiplex immunoassays, being considered a marker of genuine cat sensitization. It is presented together with other allergens used in singleplex and multiplex IgE assays[7,8,10,28,29] in patients with cat allergy in Table 3.

Fel d 2

The serum albumin Fel d 2 is a minor cat allergen, despite being an important protein in dander. All cats have this allergen. It is an allergen component available as a native purified and recombinant molecule in singleplex and multiplex immunoassays (Table 3). Serum albumin is a large, globular non-glycosylated protein, with α-helical structures stabilized by disulfide bridges. It is synthesized in the liver and represents a main protein constituent of plasma, with important transporter and colloid-osmotic pressure regulating roles. The amino acid identity between cat serum albumin and those of other mammals, such as dog Can f 3, pig Sus s 1, cattle Bos d 6 and horse Equ c 3, is high (75%-85% on average). Fel d 2 is considered a useful biomarker for high risk of cross-reactivity with other serum albumins[29,30-32]. Many patients allergic to cat albumin react to dog and horse albumins. About 15%-25% of cat-allergic patients are sensitized to feline serum albumin. In European allergic patients, monosensitization to Fel d 2 was found in 3.2%-7%[30-33]. There are patients with respiratory

Table 2 Cat Fel d 1 and other cross-reactive Fel d 1-related allergens from big cats (Felidae family)[11,22-25]

Subfamily	Species	Common name	Allergen
Felinae	*Felis domesticus (Felis catus)*	Domestic cat	Fel d 1
	Leopardus pardalis	Ocelot	Leo p 1
	Leptailurus serval	Serval	Lep s 1
	Puma concolor	Puma/cougar	Pum c 1
Pantherinae	*Panthera leo*	Lion	Pan l 1
	Panthera onca	Jaguar	Pan o 1
	Panthera pardus	Leopard	Pan p 1
	Panthera tigris longipilis	Siberian tiger	Pan t 1
	Panthera uncia (Uncia uncia)	Snow leopard	Unc u 1

Table 3 Allergens used in singleplex and multiplex immunoglobulin E immunoassays in patients with cat allergy[7,8,10,28,29]

Protein family	Allergen	IgE sensitization biomarker
Secretoglobins	rFel d 1	Major cat allergen, species-specific biomarker of primary sensitization to cat, as efficient as or even superior compared to natural cat extract in diagnosis
Lipocalins	rFel d 4	Major cat allergen, biomarker of cross-sensitization to other animal lipocalins, cross-reactive with lipocalins dog rCan f 6, horse rEqu q 1, and mouse nMus m 1
	rFel d 7	Minor cat allergen, biomarker of cross-sensitization to dog lipocalin, cross-reactive with lipocalin dog rCan f 1
Serum albumins	n/rFel d 2	Minor cat allergen, biomarker of sensitization to non-human serum albumin, cross-reactive with pork rSus d1/nSus s1 (cat-pork syndrome) and other serum albumins bovine nBod d 6, dog nCan f 3, and horse nEqu c 3
Immunoglobulins	nFel d 5	Minor cat allergens IgA Fel d 5 and IgM Fel d 6 carry α-Gal epitopes involved in the α-Gal syndrome and in impairing cat allergy *in vitro* diagnostics in parasite-infected patients; α-Gal biomarker: nBos d TG

Major cat allergen: Allergen recognized by immunoglobulin E antibodies of > 50% of cat allergic patients; Minor allergen: Allergen recognized by < 50% of the allergic population; IgA: Immunoglobulin A; IgM: Immunoglobulin M; α-Gal: Galactose-α-1,3-galactose; TG: Thyroglobulin, bovine.

allergy who present exclusive IgE sensitization to many serum albumins of furry animals. Regarding the clinical relevance, Fel d 2 sensitization is associated with moderate/severe rhinitis and diagnosis of asthma; it is also associated with severity of respiratory symptoms and with FeNO, as a type 2 biomarker, in young asthmatics. Moreover, high levels of IgE against Fel d 2 are associated with atopic dermatitis in children with cat allergy[34-38]. Fel d 2 is also important in relation to food allergy[1].

Cat-pork syndrome, described below[39-41], is the main food allergy phenotype in cat-allergic patients and it is secondary to the cross-reactivity of Fel d 2 with other albumins from mammals. This entity consists primarily of IgE-mediated respiratory symptoms following exposure to cats, and secondarily of food allergy symptoms after the ingestion of pork meat; therefore, the term "cat-pork syndrome" seems to be appropriate, although it is also frequently referred to as "pork-cat syndrome". The clinical picture varies from oral itching and urticaria to anaphylaxis. Fatal anaphylaxis after eating wild boar meat has also been reported. Symptoms usually occur within 30-45 min after eating pork meat, and it is not related to tick bites. Although most of the patients report reactions only to pork, some (10%-20%) report reactions to beef as well, including broiled beef intestines, but no one to cow's milk. Because albumin is a heat-labile protein, fresh meat, undercooked or dried and smoked pork are more consistent elicitors. Pork grilled meat, ribs, ham, sausages and hamburger have been mentioned as triggers. Only 1%-3% of patients who are allergic to cats seem to be at risk for allergic reactions to pork consumption, keeping in mind that only one-third of subjects who are IgE-sensitized to porcine serum albumin are likely to present food allergy to pork meat. Identification of the component-specific sensitivity pattern related to cat-pork syndrome allowed use of the cat albumin Fel d 2 and swine serum albumin nSus s 1 as markers for CRD in this clinical entity. Domestic pig (*Sus scrofa domesticus*) components nSus s 1 and rSus d 1 are available for IgE singleplex and multiplex immunoassays. These serum albumin molecules also cross-react with dog serum albumin nCan f 3 and bovine serum albumin (BSA) nBos d 6[29,42-45].

A new subphenotype of cat-pork syndrome was recently reported as anaphylaxis to BSA-containing surgical tissue adhesive (45% BSA) used as an adjunct for achieving hemostasis during cardiovascular surgery in a patient with asymptomatic long-term home exposure to cat and IgE sensitization to rFel d 1 and nFel d 2, but not to galactose-α1,3-galactose (α-Gal) containing bovine thyroglobulin. As Fel d 2 sensitization may predict cross-reactivity to nonhuman mammalian serum albumins, preoperative assessment of IgE sensitization to rFel d 2 in cat-allergic patients could be meaningful to avoid bovine and porcine surgical products[46]. BSA contained in culture media used in artificial insemination is an important anaphylaxis risk factor in patients allergic to cats, with sensitization to BSA being another possible cause of allergic reactions to some vaccines[47-49]. Moreover, equine serum albumin (also presenting high sequence identity with Fel d 2) is a causative factor of anaphylaxis to horse serum-based snake antivenom[50].

Fel d 3

Fel d 3 cystatin is a minor allergen, unavailable in commercial immunoassays. The prevalence of IgE reactivity to rFel d 3 is about 10%. It belongs to the cystatin superfamily of cysteine protease inhibitors (CPIs), being part of the stefin family. It is a small acidic protein, without cysteine residues or disulfide bonds, and having 80% sequence identity to bovine cystatin. Another animal cystatin with similar low molecular mass is Can f 8[51]. Besides Fel d 3 from cat dander, IgE-reactive cystatins have been identified in the kiwi fruit *Actinidia deliciosa* (Act d 4), *Ambrosia artemisifolia* weed pollen (Amb a CPI), and the parasitic nematode *Anisakis simplex* (Ani s 4). The sequence similarity between phytocystatin Act d 4 and other cystatins is only 13% to Fel d 3, 27% to Ani s 4, and 40% to Amb a CPI[52].

Fel d 4

The lipocalin Fel d 4 is a major allergen synthesized in cat salivary glands and found primarily in saliva in higher concentrations compared with Fel d 1. This cat allergen is involved in feline chemical communication, serving as a kairomone by eliciting defensive behavior in mice. Cat saliva is the main source of this allergen, which is deposited through grooming on the fur. Fel d 4 levels have no relation to hair length and its salivary levels appeared to be greater in neutered than intact female cats due to hormonal influences[6,8,17].

Fel d 4 is available as a recombinant molecule[10,28] in singleplex and multiplex immunoassays (Table 3). Lipocalins constitute the largest mammalian allergen family and, despite their highly conserved structure, they have variable sequence identities and cross-reactivities. The Fel d 4 cat allergen molecule has sequence identity of 67% with dog lipocalin Can f 6 and similar to horse lipocalin Equ c 1, which explains the moderate-high risk of cross-reactivity with these clinically significant allergen molecules. This is an argument for using such cross-reactive animal allergen molecules in CRD. Although Equ c 1 was regarded as a horse allergen marker, it should be considered as a highly cross-reactive molecule with the cat and dog lipocalins Fel d 4 and Can f 6. Specific IgE antibodies to Can f 6 are present in nearly 40% of patients sensitized to dogs; however, they are present in 60% of patients sensitized to both cats and dogs, which could be related to sequence identity with Fel d 4. There are patients with selective IgE reactivity to Fel d 4 but not to Equ c 1, and patients with IgE reactivity to Fel d 4 but not to Can f 6. Other major lipocalins, rabbit Ory c 4, domestic guinea pig Cav p 6, rat Rat n 1, and mouse Mus m 1, show identities between 47% and 52%. Fel d 4 shows weak cross-reactivity with the other dog lipocalin Can f 2, having less than 22% of their sequences being identical[29,53-55].

It is generally accepted that Fel d 4 lipocalin is the second most frequent sensitizing feline allergen. IgE reactivity to Fel d 4 is found in up to 63% of cat-sensitized subjects. The majority of children sensitized to Fel d 4 are also sensitized to Fel d 1 but not *vice versa*. In Central European cat-allergic patients, the sensitization rate to Fel d 4 is inferior to Fel d 1 but higher compared to Fel d 2, while monosensitization to Fel d 4 is scarce. Sensitization to this allergen molecule has relevance to the clinical presentation, as Fel d 4 is associated with the presence of asthma symptoms. Moreover, high levels of IgE to Fel d 4 are also associated with atopic dermatitis in children with cat allergy[29,33-35,56].

Fel d 5 and Fel d 6

IgA Fel d 5 and IgM Fel d 6 are present in high concentrations as Igs in cat saliva[56] and serum, and also in natural cat dander extracts, but are not used as molecular allergen components in the commercial IgE immunoassays. The IgE reactivity was found to be directed at carbohydrates of these Igs (lack of activity to deglycosylated cat

IgA) and to IgM from other animal species (rabbit, mouse, dog, pig, cow and horse) but not to human Igs. α-Gal is an IgE-binding epitope of both cat allergen Igs Fel d 5 and Fel d 6, which are cross-reactive with each other[57-59]. Serum specific IgE antibodies to the α-Gal carbohydrate epitope cause impaired *in vitro* diagnostic evaluation of cat allergy. These specific Igs may be present in patients with cat sensitization but they are not associated with rhinitis or asthma[15,29].

The glycosylated allergen component nFel d 5 present in cat dander extracts is recognized by nearly 40% of cat-sensitized European patients. Less than 20% of African patients with cat allergy have IgE against Fel d 5 compared with 66% among parasite-infected subjects without reported symptoms of cat allergy; of note, the majority (85%) of nonallergic Zimbabwean subjects with schistosomiasis and/or geohelminth infections showed anti-α-Gal IgE antibodies. The greater IgE binding to α-Gal *vs* Fel d 5 is explained by the lower number of α-Gal epitopes in nFel d 5. There is a strong correlation reported for the IgE antibody levels and cat dander extract, Fel d 5 and α-Gal specifically but not rFel d 1. The α-Gal epitope on IgA Fel d 5 is responsible for IgE anti-α-Gal reactivity to cat epithelia in parasite-infected patients[15]. Moreover, serum IgE antibodies to cat dander extract were detected among African children from rural Kenya without positive skin tests to cat epithelia extract[60], and no significant relationship was found between IgE and positive skin prick test responses to cat among South African children[61]. The α-Gal epitope is present not only on Fel d 5 and Fel d 6 but also on parasites. In addition, IgE antibodies against α-Gal are induced by tick bites. Therefore, nFel d 5 and nFel d 6 are not good markers for cat allergy diagnosis[15].

In order to decipher the problem of α-Gal cross-sensitivity in the cat IgE sensitization *in vitro* assessment, it is recommended to use at least the reliable rFel d 1 and the α-Gal biomarkers from a molecular perspective[15,29,62]. α-Gal-bearing glycoproteins are used in solid-phase immunoassays as biomarkers. Besides α-Gal coupled to human serum albumin and beef (Bos domesticus) carbonic anhydrase nBos d CA, the most widely used α-Gal markers are the recombinant human/murine chimeric monoclonal antibody cetuximab (2.04 μg α-Gal *per* mg) and the beef thyroglobulin (5.6 μg of α-Gal *per* gram). The performance characteristics in immunoassays of the last two biomarkers are relatively similar[63-67]. The bovine thyroglobulin (Bos d) α-Gal carrying molecule is commonly used in the singleplex fluorescence enzyme immunoassay with capsulated cellulose polymer as solid-phase[6,28,68]. Regarding the induction of IgE antibodies against α-Gal in humans, bites of hard ticks from the Ixodidae family are the most important primary sensitization source. The prevalence of α-Gal IgE sensitization depends on the degree of exposure to ticks[69,70]. Individuals from rural areas or with forest-related jobs have higher risk of such but only less than 10% of them present features of α-Gal syndrome[63,71-73].

The α-Gal syndrome consists of IgE-mediated allergy to α-Gal presenting as late-onset anaphylaxis after ingestion of pig, beef or lamb meat/viscera, or immediate-onset anaphylaxis to parenteral exposure to drugs containing α-Gal, such as cetuximab, snake antivenom, gelatin in plasma volume substitutes, and some vaccines[67,70]. In the α-Gal syndrome, most patients experience a decline in α-Gal-specific IgE titers by avoiding tick bites; as such, these levels should be reassessed at regular intervals[74]. The mechanisms by which parasites also induce α-Gal-specific IgE antibodies in subjects with no history of cat allergy are not elucidated but mucosal blood feeding may be involved, such as for urinary blood fluke (*Schistosoma haematobium*) or intestinal blood-feeding hookworms (*Ancylostoma duodenale*, *Necator americanus*)[15]. Keeping in mind that the human blood group B antigen represents a fucosylated α-Gal structure, some studies have revealed that individuals with blood groups AB and B may present a reduced susceptibility to IgE sensitization to α-Gal[63,73].

An association of α-Gal syndrome with anaphylaxis to pork kidney and allergic rhinoconjunctivitis with cat sensitization, presenting serum IgE to cat extract but no specific IgE to Fel d 1, has been reported[75]. Although patients allergic to red meat with specific IgE response against α-Gal are considered not to have IgE antibody responses to plant-derived cross-reactive carbohydrate determinants[67], this association is also possible[67,76]. Interestingly, α-Gal and cross-reactive carbohydrate determinants among the N-glycans of salivary glands of ticks were also reported recently[29,77].

Fel d 7

Fel d 7 is available as recombinant cat lipocalin in the singleplex fluorescence enzyme immunoassay with capsulated cellulose polymer solid-phase and the new generation

macroarray nanotechnology-based multiplex immunoassay[10,28] (Table 3). It was reported to bind IgE in approximately 40% of subjects with rhinoconjunctivitis and/or asthma exposed to cats. Almost 20% of patients with Fel d 7-specific IgE do not have detectable IgE against Fel d 1. Fel d 7 is present in small quantities in natural extracts. The concentration of this lipocalin in cat hair extracts is approximately 0.24 μg/mL. Fel d 7 is a von Ebner gland protein isolated from the posterior region of the cat tongue, known to contain lingual salivary glands. It shares a high sequence identity (62%) with the major dog allergen Can f 1, giving it high potential for cross-reactivity with Can f 1. Thus, Fel d 7 may contribute to respiratory allergy symptoms not only in cat but also in dog-allergic patients. Because the concentration of Fel d 7 in cat saliva is about 4 mg/mL, it is plausible that cat licking may be a route for the sensitization to Fel d 7 along with the inhalation of aerosolized allergen[29,78-81].

Fel d 8

Fel d 8 is a distinct latherin-like protein. The frequency of IgE binding of sera from patients with respiratory cat allergy to rFel d 8 is nearly 20%. The IgE binding to Fel d 8 is highly correlated with binding to Fel d 1. Fel d 8 is not usually detected in natural cat dander extracts, being found in the saliva of cats and isolated from their submandibular salivary gland[78]. It has a high degree of homology to horse Equ c 4 and Equ c 5. Equ c 5 is an allergen that binds IgE in 77% of horse-allergic patients, and rEqu c 4 is available in the new macroarray multiplex immunoassay[10,80,81]. Fel d 8 belongs to the lipopolysaccharide-binding protein/bactericidal permeability-increasing family[81] and it is not yet available in the commercial IgE immunoassays.

Other cat allergens

Fel d S100, a calcium-binding protein detected in cat saliva, and Fel d Hp, a haptoglobin detected in blood, are two additional allergens mentioned in the Allergome database[11,81], also not currently available in commercial immunoassays. S100A12 and haptoglobin are undenominated IgE binding proteins. The IgE antibody response to S100A12 is of low prevalence, but the specific IgE titer could be high in some individuals. This is of interest as it suggests inhalation of this calgranulin inflammatory mediator, known to have interspecies activity. IgE binding to plasma haptoglobin is infrequent, but significantly more IgE binding was found in subjects with cat-allergy than in those without allergy. The likely source of exposure to this acute phase protein is saliva from cats with poor gingival hygiene[81].

Because a frequent association between cat and dog sensitization is known for several decades, and a common question is whether this is due to co-sensitization to different allergen components or cross-reactivity between cat and dog allergenic molecules, a short presentation of additional allergens related to this aspect is needed.

Cross-reactivity between cat and dog allergens is usually explained by high-sequence homologies or structural similarities between lipocalins Fel d 4 and Can f 6, albumins Fel d 2 and Can f 3, as mentioned above, but recently a cat Niemann-Pick type C2 (Cat-NPC2) allergenic protein, a homologue of Can f 7, was also detected in cat dander extracts. Can f 7 shares 78% sequence identity with Cat-NPC2, and this clearly indicates the possible cross-reactivity between them. rCat-NPC2 can bind specific IgE in at least 14.5% of cat-allergic subjects[82]. This newly identified and characterized animal allergen has the potential of becoming a useful tool for CRD, but it is not yet available in commercial IgE immunoassays. Interestingly, cross-reactivity was observed also between Cat-NPC2 and Der f 2 (also belonging to the NPC2 family of proteins) indicating a possible association between IgE sensitizations to cat, dog and house dust mites[82].

Moreover, a previous report demonstrated the presence of a Fel d 1-like allergen with a molecular weight of 20 kDa in dog dander extracts, which may be responsible for *in vitro* double positivity to cat and dog. The clinical significance of this cross-reactivity is not clear since no patients with IgE cross-reactivity to this Can f CRA (Fel d 1 cross-reactive allergen) revealed clinical symptoms to dogs[83].

Regarding kallikrein allergens, no patterns of cross-reactivity of cat allergens with male dog prostatic kallikrein Can f 5 have been identified to date. Therefore, even if there are few case reports of human seminal plasma allergy in women sensitized to Can f 5 from dog urine and dander[29,84-86], no such cross-reactivity reactions have been published in cat allergic patients.

MOLECULAR APPROACH TO CAT ALLERGY

The molecular approach to cat allergy involves allergen components used in singleplex and multiplex immunoassays for *in vitro* diagnosis, presented in Table 3. The designation of allergen names is derived from the source, the first three letters of the genus, the first letter of the species, and a number indicating the chronology of the discovery, for example, Fel d 1 is the first allergen from the domestic cat *Felis domesticus*[87]. The common exposure to these allergen molecules includes different indoor settings, such as homes with cats as pets, but also in schools, daycare centers, public buildings, workplaces, and public transport vehicles, particularly if pet ownership is more prevalent in the area[88] because of their transportability on clothing[89]. A popular misconception persists regarding cat allergy related to the belief that certain cat breeds produce less allergens and are 'hypoallergenic' due to their fur type[90]. The major allergen Fel d 1 is produced by the cat's sebaceous glands, and, together with Fel d 4, is detected in the saliva and distributed on the fur by grooming. In common neutered domestic cats, fur length and color or body size did not relate Fel d 1 levels in reservoir dust from homes. Fel d 4 levels are also not related to hair length, however, neutered female cats have higher levels compared to unneutered ones[17,86,91]. There have been attempts to obtain so-called 'allergy-free' transgenic cats characterized by the absence of Fel d 1, by disrupting the coding sequence of the target gene with a specialized construct[92] or by CRISPR-Cas9-mediated genomic editing of Fel d 1[93]. To date, there are no hypoallergenic or allergen-free cats[1].

The diagnosis of cat allergy may seem uncomplicated at first glance, since most patients react to the main allergen molecule Fel d 1, but it is important to keep in mind that the natural cat dander extracts used for diagnosis, while containing this allergen mainly, differ in the quality and quantity of cat individual allergens and other molecules. Moreover, contamination of commercially available animal dander extracts with house dust mite allergens is possible and may induce *in vivo* false-positive responses. CRD using individual allergenic proteins can improve the diagnosis of mammalian pet allergy[56,94-96].

Recombinant and well-defined allergen components have great advantages for CRD immunoassays used to assess IgE sensitization patterns to cat allergen components; these include primary sensitization and presence of allergy, polysensitization and presence of severe allergy, secondary sensitization, cross-reactivity to other furry animals, and irrelevant sensitization[1,86]. In patients suspected of cat allergy, Fel d 1, Fel d 2 and Fel d 4 seem to be the most important allergen components to assess. IgE sensitization to more than three cat allergen molecules in children is superior in predicting future cat symptoms than sensitization to cat extract, and sensitization to the major species-specific allergen is a predictor of cat allergy at adult age[9,29]. Sensitization to Fel d 1 is associated with asthma, and polysensitization (Fel d 1, Fel d 2 and Fel d 4) is associated with both clinical reactivity to cat and also bronchial responsiveness and increased FeNO as a type 2 inflammation biomarker. Asthmatic children with cat allergy have higher Fel d 1-specific IgE levels than children with rhinitis only. Asthma symptoms to cat exposure are associated with specific IgE antibodies to cat allergens Fel d 1 and Fel d 4 in cat-allergic children. Moreover, IgE sensitization to Fel d 2 and Fel d 4 is associated with atopic dermatitis in children with cat allergy[86].

CONCLUSION

The benefits of molecular diagnosis in cat allergy involve the use of the cat-specific major allergen as a species-specific molecule, cross-reacting allergen components, including those present in small quantities in natural extracts, while considering those impairing the *in vitro* allergy diagnosis. Identification and characterization of molecular cat allergens allowed their use in singleplex and multiplex immunoassays for a precision diagnostic approach, with assessing their clinical significance and the association with cat allergy phenotypes and severity[29].

The manifestations of cat allergy vary widely, from rhinitis and conjunctivitis to severe asthma. Other than respiratory and ocular allergy, cat licks can cause contact urticaria upon exposure to the saliva, while cat bites can cause anaphylaxis in patients sensitized to cats[2,97,98]. IgE sensitization to cat epithelia increases the risk of patients to develop asthma or rhinitis. In addition, persistent atopic dermatitis lesions occur more often in patients sensitized to cat dander. There is also clear evidence for the clinical importance of assessing cat allergen components in relation to both α-Gal and

cat-pork syndrome[29].

Allergenic molecules induce specific IgE sensitization of mast cells and trigger type 2 allergic inflammation upon re-exposure. The availability of natural purified or recombinant allergens improved the understanding of the molecular mechanisms leading to these immune responses, which vary depending on several structural and biological characteristics of these allergens. In addition, other pro-inflammatory properties of some allergens must be mentioned, including late-phase allergic inflammation induced by non-IgE reactive peptides of Fel d 1 *via* major histocompatibility complex-restricted T cell activation[99-101].

The molecular approach for cat allergy allows a better understanding of the exposure and immune response to feline allergens, the relationship of these specific IgE responses to symptoms, and their clinical relevance[29].

Identification of cat allergen-specific IgE antibodies, either bound to mast cells by skin prick tests or in serum by immunoassays, detects IgE sensitization, a condition necessary but not sufficient to make the definitive diagnosis of cat allergy[100]. CRD, with or without *in vivo* tests, must be used within the framework of a detailed clinical history, because IgE sensitization does not necessarily imply clinically relevant allergy[86,99,100]. A deeper *in vitro* analysis with the help of IgE immunoassays using molecular allergens creates the bigger picture of the patient IgE sensitization profile in order to assess genuine sensitization, primary sensitization source, co-sensitization, cross-reactivity and allergy risks, including prediction of allergy severity[1,86].

Precision allergy molecular diagnostic applications (PAMD@) in cat allergy involve several molecular allergens used in commercial singleplex and multiplex IgE immunoassays, Fel d 1, Fel d 2, Fel d 4 and Fel d 7, these being the allergenic components currently available on the market[100]. For other native or recombinant allergenic components to be included in such immunoassays used in clinical practice, they must not only be well characterized and experimentally validated, but must also be clinically validated and available from their production point of view. Moreover, the characteristics of the solid-phase of the immunoassay and the manner by which allergenic molecules are coupled are important to reflect their biochemical properties and specific requirements for stability, preserving epitope complexity. Regarding native IgA Fel d 5 and IgM Fel d 6 allergen components with α-Gal IgE-binding epitopes, their use may be associated with analytical errors and impaired *in vitro* diagnostics in some patients, in such cases bovine thyroglobulin being a good molecular biomarker for α-Gal IgE sensitization[5,15,28,29,86]. Although α-Gal is present on cat Igs, cross-sensitization between cat allergens and the oligosaccharide antigen is not considered clinically relevant[100].

Concerning cat allergen immunotherapy, although some patients may likely benefit more from it, particularly those with moderate-to-severe disease, monosensitized to Fel d 1[102], and a good immune and clinical response to subcutaneous immunotherapy is associated with high doses of major allergens in the cat allergen extracts[103], more data are required from large trials to obtain more definitive conclusions. Summing-up, cat allergy CRD, recently proposed to be termed as PAMD@ by the updated World Allergy Organization consensus document[100], allows for an accurate and detailed assessment of patients' IgE sensitization profiles and may facilitate individualized management options[88,100].

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