

12 Mammalian Allergens

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INTRODUCTION

People come into contact with animals in many different occupations and activities. Household animals are significant sources of allergens in the indoor environment. Cat and dog allergens are especially recognized as being associated with allergic disorders, including asthma. Exposure to these allergens is perennial and not limited to immediate contact with the animals. It is not surprising that sensitization is common, since people in industrialized countries spend some 90% of their time indoors.

Almost all important mammalian respiratory allergens belong to the lipocalin family of proteins, with the exception of cat allergen, Fel d 1 (1). This naturally raises the question whether lipocalin allergens have intrinsic properties that explain this phenomenon (2). However, the allergenicity of lipocalin allergens cannot be explained by simple physicochemical characteristics.

TAXONOMY OF MAMMALS

Figure 1 shows the condensed taxonomy of eight mammals emitting allergens identified at the sequence level. Sensitization also occurs to other members of the order Cetartiodactyla (e.g., reindeer, *Rangifer tarandus*, family Cervidae, and pig, *Sus scrofa domestica*, family Suidae). Several members of the family Felidae, in addition to the house cat, are also possible sources of allergens. Their hair extracts contain allergens similar to and immunoglobulin E (IgE) cross-reactive with the house cat allergen, Fel d 1. Although mouse, rat and guinea pig are the only rodents included in the current list of allergens (<http://www.allergen.org>, July 25, 2007), e.g., hamsters (family Muridae) are known to be significant causes of allergy both in home and in occupational environments (Table 1).

HUMAN CONTACT WITH OTHER MAMMALS

People come into direct contact with mammalian animals in many ways. Household pets, especially cats and dogs, are found in many (30–50%) homes in industrialized countries. Consequently, high levels of Can f 1 or Fel d 1 allergens occur in the homes of dog and cat owners, although variations of several orders of magnitude between houses are observed.

The effects of the exposure depend on a complex array of environmental and genetic factors. In this context, it is of interest to note that high levels of exposure to animal allergens may lead to tolerance. The highest levels of sensitization in children exposed to Fel d 1 are observed with “intermediate” levels of the allergen (1.7–23.0 µg/g of dust) (3). This suggests that the dose-response relationship between exposure to cat and sensitization could have a bell-shaped curve. The protective effect is hypothesized to be due to a modified T-helper type 2 (Th2) response involving the synthesis of specific IgG4. In an experimental human study of nasal neoantigen exposure, specific IgE demonstrated a bell-shaped dose response, while the specific IgG response went up with the increasing exposure (4). There are also studies that provide no evidence for the protective effect of high exposure to mammalian allergens. Several factors, such as the age of the subjects or the nature of the exposure, may account for these conflicting results.

High exposure to mites does not protect against sensitization. It can be speculated that allergens of different origin have distinct optimal concentrations that favor tolerance; perhaps, this level for mite allergens is very high. On the other hand, it has been found that the level of

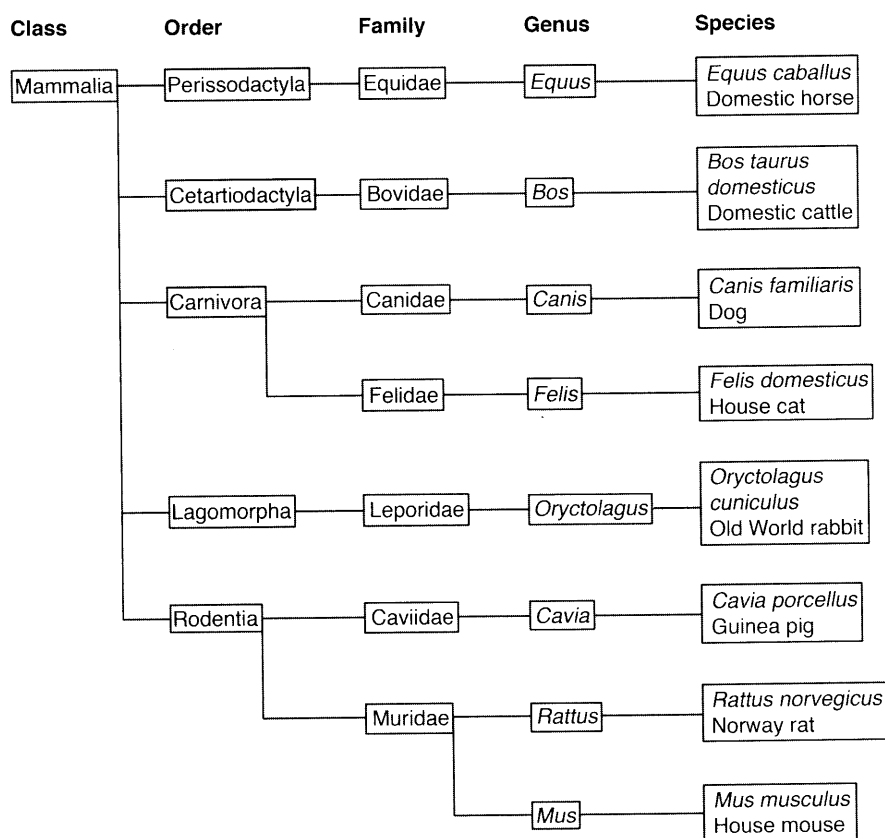


Figure 1 Condensed presentation of the taxonomical location of eight mammals emitting allergens.

Table 1 Internet Resources Referred to in the Text

Description	Web site address
Lipocalin family, documentation in the PROSITE protein database	http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00187
Lipocalins, general information and the list of lipocalins in the PROSITE protein database	http://www.expasy.org/cgi-bin/nicesite.pl?PS00213
List of allergens by Allergen Nomenclature Subcommittee of International Union of Immunological Societies	http://www.allergen.org
PDB, a databank for three-dimensional biological macromolecular structure data	http://www.rcsb.org/pdb/
Serum albumin family, documentation in the PROSITE protein database	http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00186
SWISS-PROT protein database	http://www.expasy.org/sprot/

Abbreviation: PDB, Protein Data Bank.

exposure to mite allergens is essentially lower than that to cat allergens (5,6). Therefore, both high (or "intermediate")-dose (cat) and low-dose (mite) allergen exposures seem to induce sensitization. One possibility is that both types of exposures result in a suboptimal stimulation of specific T-helper cells (see section "Lipocalins"). With cat allergens, suboptimal stimulation would be due to the homology with human proteins (evolutionary relatedness), whereas with mite allergens, it would be due to the low concentration of the allergen (low epitope density on antigen-presenting cells). Other factors, such as the enzyme activity of mite Der p 1 or factors not directly related to allergenic molecules themselves, such as associated substances with adjuvant properties, are probably implicated in the sensitization to cat and mite allergens. For example, it appears that pathogen-associated molecular patterns (PAMPs), such as endotoxin,

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are responsible for the apparent beneficial effect of the farming environment against sensitization (7).

Exposure to pet allergens is not limited only to direct contact. Dog and cat allergens stick to clothing, and they are consistently found in homes without pets as well as in public buildings, including schools and day care centers and public transport vehicles. The concentrations are low, but may be high enough to cause sensitization and symptoms in sensitized persons.

Mice, hamsters, guinea pigs, and gerbils are also popular household pets, and handling the pets and cleaning their cages expose their owners to allergens. The presence of rodent allergens in the home depends not only on the presence of pets but also on the living conditions: mouse Mus m 1 and rat Rat n 1 are detectable in apartments infested with these animals. In U.S. studies, approximately 20% of inner-city children with asthma appear to be sensitized to mouse and rat allergens. Keeping guinea pigs as pets is associated with a more than a threefold increased risk of atopic eczema, an effect not seen with other pets such as cats, dogs, or hamsters. Horse allergy is not a very common health problem, but horseback riding as a hobby causes sensitization and clinical illness.

Sensitization associated with the handling of laboratory animals is a worldwide occupational problem. The exposure occurs through the respiratory tract and conjunctiva and by skin contact. One review of seven studies found that 15.6% of workers in laboratory animal facilities had work-related symptoms and 22.5% were skin prick test-positive for animal allergens (8). The common laboratory animals (mouse, rat, guinea pig, hamster, rabbit, and dog) appear to be equal sensitizers. The level of exposure varies according to the task concerned. The highest concentrations of airborne allergens are encountered during the emptying and cleaning of the cages. However, several factors in exposure to laboratory animal allergens are incompletely understood, for example, whether mean or peak exposures are the most relevant for sensitization (9). The highest prevalence of sensitization to laboratory animals is generally found in subjects with moderate exposure.

An example of work-related allergy caused by domestic animals is occupational asthma in Finnish dairy farmers. An interesting feature is the prolonged exposure time (median, 22 years) before cattle asthma becomes clinically evident. In contrast, symptoms of laboratory animal allergy appear within two to three years of exposure in 70% to 80% of cases.

MOLECULAR CHARACTERISTICS OF MAMMALIAN ALLERGENS

Protein Families of Mammalian Allergens

Lipocalins

Lipocalins are a large protein group (<http://www.expasy.org/cgi-bin/nicesite.pl?PS00213>) comprising proteins from vertebrate and invertebrate animals, plants, and bacteria. In addition to mammalian respiratory allergens, a milk allergen, Bos d 5 (β -lactoglobulin), a cockroach allergen, Bla g 4, a "kissing bug" (*Triatoma protracta*) allergen, Tria p 1, and a pigeon tick (*Argas reflexus*) allergen, Arg r 1, belong to the lipocalin group. Together, with fatty acid-binding proteins, avidins, a group of metalloproteinase inhibitors, and triabin, lipocalins form the calycin superfamily (10). A protein should fulfill the requirements for sequence homology, biological function, and structural similarity to be included in the family (<http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00187>).

Although the overall amino acid identity between lipocalins is usually below 20%, they contain one to three characteristic conserved sequence motifs [structurally conserved regions (SCR)] (10). The first motif, containing the triplet glycine-x-tryptophane, is present in all lipocalins (Fig. 2). While kernel lipocalins contain all three motifs, outlier lipocalins contain only one or two. In some cases, the sequential identity over animal species can be well above 20%. For example, dog Can f 1 exhibits a 57% identity with human tear lipocalin (von Ebner's gland protein), and human epididymal-specific lipocalin-9 exhibits a 40% to 50% identity with rodent lipocalins [the SIB BLAST network service (SBNS) at the Swiss Institute of Bioinformatics, Jul. 25, 2007 (11)]. Lipocalins exist as both monomers and dimers, and they can be either glycosylated or nonglycosylated (Table 2).

Despite the low sequential identity, lipocalins share a common three-dimensional structure (Fig. 3) (10). The central β -barrel of lipocalins is composed of eight antiparallel

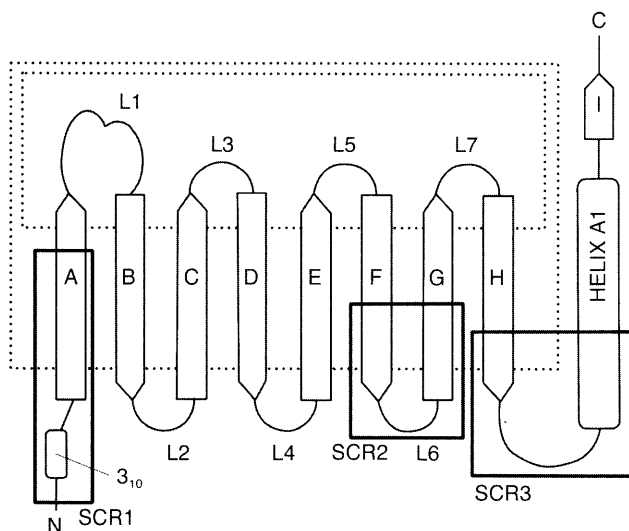


Figure 2 Schematic structure of the lipocalin fold. The nine β -strands of the antiparallel β -sheet are shown as arrows and labeled A-I. The C-terminal α -helix A1 and N-terminal 3_{10} -like helix are also marked. Connecting loops are shown as solid lines and labeled L1-L7. A pair of dotted lines indicates the hydrogen-bonded connection of two strands. One end of the lipocalin β -barrel has four loops (L1, L3, L5, and L7); the opening of the internal ligand-binding site is here, and so it is called the open end of the molecule. The other end has three β -hairpin loops (L2, L4, and L6); the N-terminal polypeptide chain crosses this end of the barrel to enter strand A via a conserved 3_{10} helix, closing this end of the barrel, the closed end of the molecule. Those parts that form the three main structurally, and sequence, conserved regions (SCRs) of the fold (SCR1, SCR2, and SCR3) are marked as heavy boxes. SCR3 corresponds closely with the sequence conserved region rather than the structurally conserved region. *Abbreviation:* SCR, structurally conserved region. *Source:* From Ref. 10.

β -strands, and it encloses an internal ligand-binding site (Figs. 2 and 3). At the N-terminus, there is a 3_{10} helix, whereas at the C-terminus, there is an α -helix. The three-dimensional structures of several lipocalin allergens are known (Table 2).

Lipocalins are typically small, extracellular proteins with the capacity to bind small, principally hydrophobic molecules, to attach to specific cell-surface receptors, and to form covalent and noncovalent complexes with soluble macromolecules (10). Most of the mammalian lipocalins are produced in the liver or secretory glands. Although they were originally characterized as transport proteins for diverse molecules, such as odorants, steroids, and pheromones, they are involved in a wide range of other biological functions.

Some lipocalins show immunomodulatory activity. One such protein, glycodefin (placental protein 14), exerts its anti-inflammatory activity by elevating the T-cell activation threshold and possibly in this way favors the Th2 deviation of immune response (12). Some lipocalins can also be enzymes, such as glutathione-independent prostaglandin D_2 synthase. Two other lipocalins, β -lactoglobulin (Bos d 5) and tear lipocalin, have been reported to have nonspecific endonuclease activity. The glutamic acid at position 128 in tear lipocalin, important for the enzyme activity, is present in several lipocalin allergens, such as Bos d 2, Mus m 1, Rat n 1, Equ c 1, and Can f 1. The amino acid is situated at or adjacent to the immunodominant T-cell epitope in Bos d 2 (13). Can f 1 has also been proposed to act as a cysteine proteinase inhibitor because of its sequential homology with tear lipocalin. Whether this is the case is not known, but the motifs crucial for the function are only partially conserved in Can f 1. Lipocalins also participate in the regulation of cell growth and proliferation.

Why Th2 responses arise against inert inhaled antigens is unknown. One property associated with the allergenicity of a protein is that it is effectively dispersed in the environment. Lipocalin allergens appear to fulfill this requirement, since they are found in animal dander and excretions. However, the crucial element in the sensitization to a protein is its recognition by the immune system. In this respect, lipocalin allergens seem to differ from infectious agents, since they are not known to contain PAMPs, the recognition of which favors the Th1 (or Th2) deviation of immune response. It is not known whether some intrinsic biologic property of lipocalin allergens, e.g., enzyme activity (Bos d 5), would favor the Th2 immunity.

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Table 2 Physicochemical Characteristics of Mammalian Lipocalin Allergens Causing Respiratory Sensitization

Allergen	Animal	MM ^a , kDa	Amino acids	Isoelectric point	Glycosylation	Oligomeric state	SWISS-PROT accession # ^b	Structure, PDB ID code ^c	Key reference
Bos d 2	Cow	20	156	4.2	No	M	Q28133	1BJ7	54
Can f 1	Dog	22-25	156	5.2	Putative	D	O18873		42
Can f 2	Dog	22-27	162	4.9	Putative	D	O18874		42
Cav p 1 ^d	Guinea pig	20		4.3	No	M/D	P83507		66
Cav p 2 ^d	Guinea pig	17		4.3-4.5	No		P83508		58
Equ c 1	Horse	22	172	3.9	Yes	D	Q95182	1EW3	49
Equ c 2 ^d	Horse	16		3.4-3.5	No		P81216		52
							P81217		
Fel d 4	Cat	20	171	4.6	Putative		Q5VFH6		39
Mus m 1	Mouse	18-21	162	4.6-5.3	No	M	P02762	1MUP	62
Ory c 1 ^d	Rabbit	17-18			Yes				68
Ory c 2 ^{d,e}	Rabbit	21							68
Rat n 1	Rat	17-21	162	4.2-5.5	Yes	M	P02761	2A2U	62

^aMolecular mass.

^bSWISS-PROT data base, <http://www.expasy.org/sprot/>.

^cProtein Data Bank, <http://www.rcsb.org/pdb/>.

^dOnly N-terminus known.

^eTentatively named.

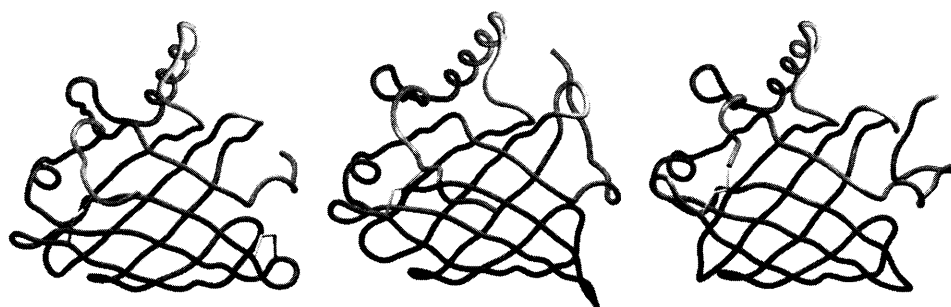


Figure 3 Molecular structure of three lipocalin allergens, bovine Bos d 2, horse Equ c 1, and mouse Mus m 1. Source: Courtesy of Juha Rouvinen, Department of Chemistry, University of Joensuu, Joensuu, Finland.

It is possible that evolutionary relatedness between human proteins and animal allergens is implicated in the allergenicity of the latter (2,6). In line with this view, we have observed an unexpected characteristic in lipocalin allergens: when the peripheral blood mononuclear cells (PBMCs) from allergic patients sensitized to cow Bos d 2 (13), dog Can f 1 (14,15) or horse Equ c 1 (16) are stimulated with the allergens, the cells proliferate very weakly. The stimulation indices, in general, are below two. The PBMC response to rat Rat n 1 is also weak (17). In parallel with these findings, Bos d 2 is a weak immunogen for several inbred mouse strains (18). A weak stimulatory capacity is also a characteristic of another animal-derived (nonlipocalin) allergen, cat Fel d 1 (19,20). Consistent with the hypothesis is the report indicating that the sequences of allergenic proteins have few or no bacterial homologues, in contrast to those of randomly selected control proteins (nonallergens from the same species as the allergen) (21). This observation suggests that allergens may not be clearly recognized as foreign substances by the immune system.

The human T-cell response is directed to a few epitope regions in Bos d 2, Can f 1, and Equ c 1 (13,15,16). For example, the total number of epitopes detected in Bos d 2 is seven, and the maximal number an individual's T cells can recognize is five. Both Bos d 2 and Equ c 1 contain one immunodominant epitope region at the carboxy-terminal end of the molecule. It is of interest that several of the epitope regions in these three allergens colocalize (Fig. 4), including those of Rat n 1 (17), since this points to the possibility that the T-cell epitopes of lipocalin proteins, in general, colocalize. Of all the lipocalin allergens examined, the first epitope region is found to be colocalizing. This region contains the signature motif G-x-W present in all lipocalin proteins, including human endogenous lipocalins.

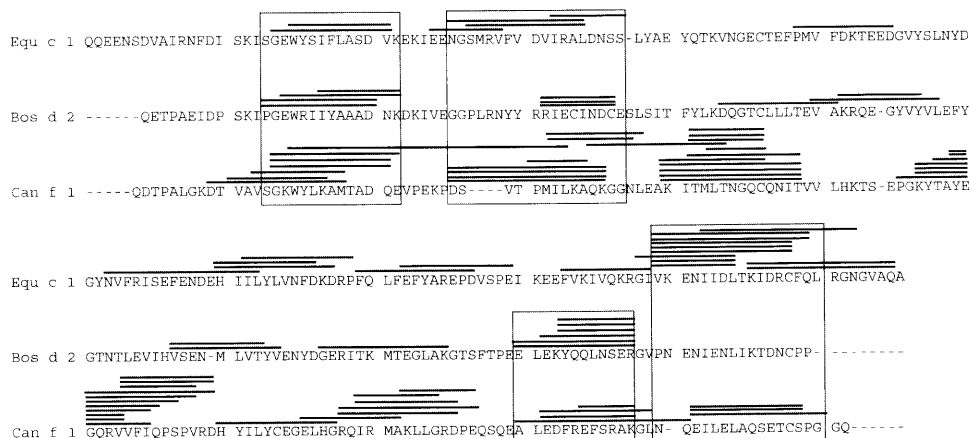


Figure 4 Alignment of the amino acid sequences of the major allergens of horse, Equ c 1, cow, Bos d 2, and dog, Can f 1. Lines above the sequences represent the cores of the epitopes recognized by individual T cell lines and clones. The core sequence is defined as those amino acids that are shared by two to five consecutive peptides able to stimulate the T cells. The most colocalizing epitope regions between the allergens are indicated by boxes. Source: From Ref. 16.

It has been observed in studies with peptide analogues (altered peptide ligands) that the outcome of T-cell response is influenced by the extent of T-cell receptor (TCR) ligation. Weak stimulation favors Th2-type responses, whereas stronger stimulation favors Th1-type responses (22). As lipocalins can exhibit considerable amino acid identity between species, it is possible that high-avidity lipocalin allergen-reactive T cells are deleted during thymic maturation (1,2), as is the case with high-avidity self-reactive T cells. The remaining T-cell population, with low-avidity TCRs, might recognize exogenous lipocalin allergens in a suboptimal way. Therefore, it is noteworthy that the immunodominant epitope of Bos d 2 (23) and at least one T-cell epitope of Can f 1 (R. Juntunen, personal communication) are found to be suboptimal. Their optimal peptide analogues can stimulate human T-cell clones at 10- to 100-fold lower concentrations than the natural ligands. Importantly, the optimal peptide analogue of the immunodominant epitope of Bos d 2 is able to give rise to novel T-cell populations with the Th0/Th1 phenotype *in vitro* (24). However, further studies are needed to assess whether T-cell recognition plays a role in the allergenicity of lipocalins.

Others

Albumins constitute another protein family (<http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00186>) containing respiratory allergens from several mammals. Albumin is produced in the liver, and it is a major constituent of plasma. It is involved in transporting various molecules and in maintaining the colloidal osmotic pressure of blood. The molecular mass of albumins is around 67 kDa (25). Albumins show about 80% amino acid identity among mammals (25). For cross-reactivity among albumins, see section "Allergenic cross-reactivity among mammals."

Allergenic Proteins from Mammals

Cat

Fel d 1. Cat dander contains several IgE-binding components, the most important being Fel d 1 [P30438 and P30440 at SWISS-PROT (<http://www.expasy.org/sprot/>)]. Fel d 1, formerly cat-1, is a potent allergen sensitizing over 90% of cat-allergic individuals (26). It is also responsible for 80% to 90% of the IgE-binding capacity of cat allergen extracts (26). The removal of Fel d 1 from a dander extract decreases the histamine-releasing capacity of the preparation by 200- to 300-fold.

Fel d 1 is a glycoprotein with a molecular mass of 38 kDa (27). It is a tetramer composed of two noncovalently linked heterodimers, each with a molecular mass of about 19 kDa. These dimers comprise 8-kDa chain 1 (α -chain) and 10-kDa chain 2 (β -chain), which are linked together covalently by three disulfide bonds. Chain 1 contains 70 and chain 2 contains 90 to 92 amino acids (28,29). Chain 1 exhibits about 25% amino acid identity with uteroglobins, anti-inflammatory proteins, but considerably higher identities (up to 50%) can be observed with other proteins, mostly rodent androgen-binding proteins (SBNS). Chain 1 is classified as a member of the secretoglobin (uteroglobin) family. Chain 2 shows various degrees of amino acid identity with several proteins, up to 39% with a human protein in a segment of 41 amino acids (SBNS). It belongs to the secretoglobin protein family. Fel d 1 exists in several isoforms (27). It can be produced in a recombinant form. The three-dimensional structure of Fel d 1 is strikingly similar to that of uteroglobin (30).

Genes encoding Fel d 1 chains are expressed in the salivary glands and in the skin (29). Fel d 1 is found in hair roots and sebaceous glands, in dander and saliva, and in high concentrations in anal glands. Male cats appear to produce more Fel d 1 than female cats. The biological function of Fel d 1 is unknown, but it may be related to the protection of epithelia (28). It has been speculated that Fel d 1 could have an intrinsic capacity to promote allergy by sequestering calcium ions from phospholipase A₂ (30). Fel d 1 does not have enzymatic activity (30).

Most of the IgE-binding epitopes on Fel d 1 are conformational, and glycosylation present in chain 2 does not play a major role in IgE binding (31). Analyses with overlapping synthetic peptides suggest that IgE-binding epitopes are localized at residues 25 to 38 and 46 to 59 in chain 1 and at residues 15 to 28 in chain 2 (32). Among the sera tested, the highest percentage of positive reactions (46%) is against peptide 25 to 38.

The proliferative response of PBMC induced by Fel d 1 is, in general, not strong (19,20). In contrast, Fel d 1-specific T-cell clones and lines proliferate vigorously upon stimulation with Fel d 1 (33,34). In two studies, T-cell responses against Fel d 1 exhibited no correlation with human leukocyte antigen (HLA) phenotypes (19,33). A third study found a possible HLA-DR1

excess (odds ratio = 2, $p = 0.002$) among subjects with Fel d 1-specific IgE (35). In another study, no association was found between specific IgE and the alleles of the loci examined (including HLA-DRB1) (36). Human T-cell epitopes are present in several regions of Fel d 1, but T-cell reactivity is more pronounced against chain 1 than against chain 2 of the molecule (19,34). In chain 1, most of the reactivity is concentrated in the N-terminal half of the molecule, in amino acids 18 to 42, while in chain 2, the most reactive region is the C-terminus, amino acids 74 to 92. Two peptides, Fel-1 (IPC-1) and Fel-2 (IPC-2), amino acids 7 to 33 and 29 to 55 of chain 1, respectively, stimulate T cells but bind IgE only at low levels, which suggests that they could be suitable for peptide-based allergen immunotherapy (19). It appears that T-cell epitope recognition does not distinguish Fel d 1-allergic from nonallergic subjects. However, distinct Fel d 1 epitopes may be able to induce qualitatively different T-cell responses (37).

Fel d 2. Fel d 2, cat serum albumin (P49064 at SWISS-PROT), is a minor allergen with IgE reactivity in about 20% of cat-allergic individuals (26), although higher figures are also reported. Its role in cat allergy is unclear, in that dominant IgE response against it is found only in 2% of cat-allergic individuals (26). Moreover, the significance of cat albumin as a primary sensitizer is difficult to assess (26), since albumins exhibit cross-reactivity across animal species (see section "Allergenic cross-reactivity among mammals"). In accordance with IgE determinations, polyclonal T-cell lines, raised with cat dander extract, proliferated only weakly upon stimulation with cat albumin, whereas the response was strong against Fel d 1 (33).

Fel d 3. Fel d 3, cystatin (Q8WNR9 at SWISS-PROT), was cloned from cat skin (38). The prevalence of IgE reactivity among cat-allergic subjects is about 10% when measured using *Escherichia coli*-produced recombinant protein in a solid-phase enzyme-linked immunosorbent assay (ELISA) (38).

Fel d 3 is an 11-kDa protein containing 98 amino acids. There is one potential N-linked glycosylation site in the sequence. Fel d 3 exhibits nearly 80% amino acid identity with bovine and human cystatin A. As endogenous protease inhibitors, cystatins control the function of cysteine proteases. Fel d 3 contains the signature motif conserved in cysteine protease inhibitors. Dog allergens, Can f 1 and Can f 2, which are lipocalins, exhibit some degree of conservation with the sequence motif.

Fel d 4. The sole cat allergen known to belong to the lipocalin family of proteins is Fel d 4 (Q5VFH6 at SWISS-PROT). It is the second major allergen of cat, since 63% of cat-allergic subjects have IgE against it (39), on the basis of measurements using recombinant Fel d 4. In general, the level of the antibody is low compared to that induced by Fel d 1, but about half of the Fel d 4-sensitized subjects have higher Fel d 4 than Fel d 1 IgE levels. The physicochemical characteristics of Fel d 4 are shown in Table 2.

Fel d 4 was cloned from submandibular salivary glands (39). It seems that the expression of Fel d 4 is limited to this tissue, since mRNA for it was not found in several other tissues examined. Isoallergens were not detected. Fel d 4 exhibits a considerable amino acid identity with several mammalian lipocalins; the highest level is observed with Equ c 1, the horse allergen (67%). Among human proteins, epididymal-specific lipocalin-9 shows the highest amino acid identity (38%) with Fel d 4 (SBNS).

Other cat allergens. Cat IgA (Fel d 5w), IgM (Fel d 6w), and IgG (Fel d 7w) have been identified as respiratory allergens. Thirty-eight percent of cat-sensitized patients have IgE to cat IgA (40). It is of interest that the IgE reactivity is mainly directed to the carbohydrates of the α -chain. Further studies are needed to clarify the clinical significance of cat immunoglobulins as aeroallergens.

Dog

Can f 1. The major allergen of dog, Can f 1 (O18873 at SWISS-PROT), formerly called Ag 8, Ag 13, or Ag X, sensitizes 50% to 75% of dog-allergic subjects (41–43). It accounts for about 50% of the IgE-binding capacity of dog hair and dander extract (41) and for 60% to 70% of the IgE-binding capacity of dog saliva preparation (44). Can f 1 belongs to the lipocalin family of proteins (42). Its physicochemical characteristics are shown in Table 2.

Can f 1 is primarily found in dog saliva, but it is also present in dog dander (44). It is absent or in very low concentrations in serum, urine, and feces. The allergen is detected in the hair extracts of all dog breeds examined, with variable amounts among individual dogs within a breed (44,45). Male dogs produce Can f 1 more than female dogs (45). Can f 1 has been cloned from the parotid gland, and it has been produced in a recombinant form (42). It is homologous to human tear lipocalin (see section "Lipocalins"). Can f 1 mRNA is present in the parotid and mandibular glands, tongue epithelial tissue, and skin, but not in the liver or kidney (42,46).

Human cellular immune responses to Can f 1 have been examined (see section "Lipocalins"). In one more study, it was observed that the TCR V β 5.1⁺ CD4⁺ T cells and the DR4-DQ8 haplotype may be protective against allergy to Can f 1 (14). In two studies, no association between the Can f 1-specific IgE response and the HLA class II genotype was observed (35,36).

Can f 2. Can f 2 (O18874 at SWISS-PROT), formerly called dog allergen 2 or Dog 2, is a minor allergen sensitizing 25% to 30% of dog-allergic subjects (42,43). Dog-allergic subjects' average IgE response against Can f 2 is estimated to be 23% of that against dog dander extract (44). Can f 2 belongs to the lipocalin family of proteins (42). Its physicochemical characteristics are illustrated in Table 2.

Can f 2 is found in dog dander and in saliva, whereas urine or feces contain very little of the allergen (44). The amount of Can f 2 in the hair extracts of nine dog breeds varied widely. It has been cloned from the parotid gland and produced as a recombinant protein (42). Can f 2 exhibits the highest level of amino acid identity, 36% with trichosurin, a milk-derived lipocalin from the brush-tailed possum (SBNS). Identities at the level of 30% are observed with rodent urinary proteins (42). Can f 2 mRNA is predominantly expressed in parotid and mandibular glands and to a lesser extent in skin and tongue (42,46). It is not found in the kidney or liver. The immunological properties of Can f 2 have not been studied in detail.

Can f 3. Thirty-five percent of dog-allergic patients have IgE against Can f 3, dog serum albumin (P49822 at SWISS-PROT) (47), although both lower and higher figures are also reported. In individual patients, a major part of dog-specific IgE is directed to Can f 3 (47). Can f 3 has been cloned from dog liver and produced as a recombinant protein (25).

Other dog allergens. Dog can be a source of up to 20 allergens. In an analysis of hair and dander extract with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, a total of 11 allergens in the molecular mass range of 14 to 68 kDa were detected. One of these is an immunoglobulin. In another study, an 18-kDa protein (Can f 4) was recognized by 60% of dog-allergic subjects in IgE immunoblotting (43). The aminoterminal sequencing of Can f 4 (P84494 at SWISS-PROT) suggests that it is a lipocalin.

Horse

Equ c 1. IgE against Equ c 1 (Q95182 at SWISS-PROT), probably previously named Ag 6, is found in 76% of horse-allergic subjects' sera (48). According to one study, Ag 6 accounts for 55% of skin prick test reactivity of horsehair and dandruff extract. The physicochemical characteristics of Equ c 1, a lipocalin allergen (49), are shown in Table 2.

In addition to horse dander (48), Equ c 1 is found in a high concentration in saliva, while urine contains little of the allergen. Equ c 1 mRNA expression is about 100-fold higher in sublingual salivary glands than in submaxillary salivary glands or the liver (49). The allergen has been cloned from sublingual salivary glands and produced in a recombinant form (49). As mentioned above, Equ c 1 exhibits a considerable amino acid identity with cat Fel d 4 (67%). Its amino acid identity with a pig salivary lipocalin is 61% (SBNS) and with rodent major urinary proteins about 50% (49). The amino acid identity with human epididymal-specific lipocalin-9 is 37% (SBNS). There are several isoforms of the allergen. It has been proposed to bind histamine. Unlike some other allergens, such as Equ c 2, Equ c 1 has a surfactant-like property (50).

An analysis of the IgE-binding epitopes of Equ c 1 suggests that the dominant epitopes are localized in a restricted region of the molecule (51). Carbohydrates may have some impact on IgE binding (50,51). Human cellular immune response to Equ c 1 has been examined (see section "Lipocalins").

Equ c 2. The N-terminal sequences of two horse dander allergens, with slightly different isoelectric points (pI), were shown to be identical and the allergens were named Equ c 2.0101 and Equ c 2.0102 (P81216 and P81217 at SWISS-PROT) (52). A 29 amino acid fragment exhibits a 44% identity with bovine Bos d 2 and also contains the highly conserved G-X-W motif of lipocalins. Analyses of the amino acid compositions of the allergens also suggest that they are lipocalins (Table 2). Up to 50% of horse-allergic patients have IgE against Equ c 2 (52).

Equ c 3. As with albumins from other mammals, the significance of horse serum albumin (P35747 at SWISS-PROT) as an allergen is not clear. The prevalence of IgE reactivity against it is between 20% (53) and 50%.

Other horse allergens. Horse dander contains more than 10 IgE-binding proteins (52). Equ c 4 and Equ c 5 are partially characterized (50). Like Equ c 1, these allergens have surfactant-like properties. Equ c 4 was originally described as a 19-kDa glycoprotein with a pI of 3.8 (50). Its partial sequence is 100% identical with the sequence of horse latherin, a surfactant protein (SBNS). About 30% of horse-allergic individuals have IgE against Equ c 4. Equ c 5 is a 17-kDa nonglycosylated protein with a pI of 5.3 (50). Its peptide fragments show almost complete homology with latherin (SBNS). In one study, 77% of horse-allergic patients had IgE against this allergen (50). In the SWISS-PROT database, Equ c 4 and Equ c 5 are contained in a single entry, horse latherin (P82615). It is a 208 amino acid protein with a molecular mass of 24 kDa. The nomenclature of horse allergens is discussed in the publication by Goubran Botros et al. (50).

Cow

Bos d 2. Bos d 2 (Q28133 at SWISS-PROT), a lipocalin allergen (54), also known as Ag 3 or BDA20, is the major respiratory allergen in cow dander (Table 2). About 90% of dairy farmers with asthma of bovine origin react against Bos d 2, as analyzed by IgE immunoblotting (55) or by bronchial allergen challenge. Both Ag 3 (Bos d 2) and Ag 1 account for about 70% of the IgE-binding capacity of cow hair and dander extract. Together they bind about 80% of the IgE.

Bos d 2 is found in cow skin (56), although the same or an immunologically related allergen is present in urine (55). In skin, Bos d 2 is localized in the secretory cells of the apocrine sweat glands and the basement membranes of the epithelium and hair follicles. It is probably a pheromone carrier (56). There are several isoforms of Bos d 2. It has been cloned from cow skin (54) and produced as a recombinant protein (57). Bos d 2 exhibits the highest amino acid identity with a probable bovine odorant-binding protein (68%) from the mammary gland (SBNS). The amino acid identity with other odorant-binding proteins and lipocalins from other species are at the level of 30% to 40%. Sequencing the 15 aminoterminal residues of Cav p 2, a guinea pig allergen, revealed a 69% identity with the bovine allergen (58).

To reduce its IgE-binding capacity, Bos d 2 has been produced in fragments and in mutated forms. IgE binding is highly dependent on an intact three-dimensional structure. The epitopes responsible for IgE binding appear to be localized in the C-terminal part of Bos d 2. Most of the studies on cellular immune response to lipocalin allergens have been performed with Bos d 2 (see section "Lipocalins").

Bos d 3. Bos d 3 (Q28050 at SWISS-PROT), known also as BDA11, is a minor bovine respiratory allergen (59). According to the immunoblotting analysis with recombinant Bos d 3, about 40% of patients with cow dust-induced asthma have IgE against the allergen (59).

Bos d 3 is an 11-kDa protein with a predicted pI of 5.19 (59). In addition to bovine skin, it is found in amniotic fluid. This 101 amino acid long allergen belongs to the S-100 family of proteins and exhibits more than 60% amino acid identity with horse and human psoriasins (SBNS). Human psoriasin (or S100A7) is a calcium-binding keratinocyte protein found in normal skin. It is highly upregulated in psoriatic skin. The calcium-binding motif containing the so-called EF hand is located in psoriasin in the segment that is almost identical to that of Bos d 3. It is probable that Bos d 3 is a bovine homologue of psoriasin. The expression of psoriasin is not limited to psoriasis, and it has chemokine-like properties selective for CD4⁺ T cells and neutrophils. Human psoriasin also has antimicrobial activity against *E. coli* (60).

Other bovine respiratory allergens. Using crossed radioimmuno-electrophoresis, serum proteins, including albumin (Bos d 6; P02769 at SWISS-PROT) and IgG (Bos d 7), have been

found to be allergens in cow hair and dander. By immunoblotting, up to 10 IgE-binding components are detected in the bovine epithelial extract and four in the urine preparation in the molecular mass range of 16 kDa to over 100 kDa (55). Two of the allergens with the molecular masses of 20 kDa (Bos d 2) and 22 kDa are major allergens (55). In another study, an 11-kDa protein showing almost complete homology with the bovine oligomycin sensitivity-conferral protein of the mitochondrial adenosine triphosphate synthase complex (P13621 at SWISS-PROT) was identified as a minor allergen in cow dander.

Mouse

Mus m 1. Mus m 1, a major allergen, known also as Ag 1, prealbumin, or mouse allergen 1 (MA1) (61), is the mouse major urinary protein MUP6 (P02762 at SWISS-PROT). It accounts for the major part of the IgE-binding capacity of the crude male urine (61). Mus m 1 belongs to the lipocalin family of proteins (62). The physicochemical characteristics of Mus m 1 are shown in Table 2.

Mus m 1 is found in mouse urine, serum, pelt, and especially in the liver (61), where it is primarily produced (62). The production of MUPs is under hormonal control and influenced by androgens (62). Forms of MUPs are also expressed constitutively in the exocrine glands of mice and rats (62). Mus m 1 is found in about fourfold higher concentration in male than in female urine (61). Mouse MUPs are encoded by about 35 genes, and 15 forms of MUPs are detected in male urine. Mouse MUP has been produced as a recombinant protein. The amino acid identity between mouse and rat MUPs is about 65% (62). The amino acid identity of Mus m 1 with Fel d 4 is 49%, with Equ c 1, 46%, and with human epididymal-specific lipocalin-9, 39% (SBNS).

Other mouse allergens. The other major allergen of mouse, Ag 3, tentatively named Mus m 2, is a glycoprotein (63). It is found in mouse dander and fur. It is localized in the hair follicles, coating the hairs, and on the skin (63). Mouse albumin is also an allergen.

Rat

Rat n 1. Rat n 1, also known as rat MUP (P02761 at SWISS-PROT), prealbumin, or α_{2u} -globulin (α_2 -euglobulin), is closely related to the major urinary proteins of mouse (see section "Mus m 1") and belongs to the lipocalin group (62). Its physicochemical characteristics are shown in Table 2. Sixty-six percent of laboratory workers with asthma and rhinitis, on exposure to rats, had IgE against Rat n 1 (64). Adult female rats excrete in urine about one-sixth of the amount of MUPs of male rats. As Mus m 1, Rat n 1 exhibits considerable amino acid identities with Fel d 4 (55%), Equ c 1 (47%), and human epididymal-specific lipocalin-9 (42%) (SBNS).

Rat urinary prealbumin and α_{2u} -globulin were considered distinct allergens in the 1980s. Later analyses of these strongly cross-reactive proteins (64) showed that prealbumin is an isoform of α_{2u} -globulin. Therefore, a more appropriate name for prealbumin is Rat n 1.01 and for α_{2u} -globulin, Rat n 1.02 (1). α_{2u} -globulin has been cloned and produced as a recombinant protein. One study suggests that the IgE-binding epitopes of Rat n 1.02 tend to be clustered toward the N- and C-terminal parts of the allergen.

Human cellular immune response to Rat n 1 has been examined (see section "Lipocalins"). In one more study, HLA-DR7 was positively associated and HLA-DR3 negatively associated with sensitization to rat urinary proteins (65).

Other rat allergens. Male rat urine contains a total of eight allergens in the molecular mass range of 17 to 75 kDa. About 20 allergens have been observed in rat fur and in saliva. Rat serum proteins, including albumin, transferrin, and IgG, are allergens.

Guinea Pig

Cav p 1. Cav p 1 (P83507 at SWISS-PROT) is a major guinea pig allergen. The prevalence of guinea pig-allergic subjects' IgE reactivity against Cav p 1 in hair extract is 70% compared with 87% in urine (66). The allergen was purified from the hair extract. The physicochemical characteristics of Cav p 1 are shown in Table 2. Analysis of the 15 aminoterminal residues

shows that Cav p 1 is a lipocalin with a 57% amino acid identity with the major mouse allergen, Mus m 1 (66).

Cav p 2. Cav p 2 (P83508 at SWISS-PROT) is another major guinea pig allergen and belongs to the lipocalin group. About 55% of guinea pig-allergic subjects have IgE against it (66). The physicochemical characteristics of Cav p 2 are shown in Table 2. Analysis of the 15 aminoterminal residues of the allergen shows a 69% amino acid identity with the major cow dander allergen, Bos d 2 (58).

Other guinea pig allergens. In addition to Cav p 1 and 2, guinea pig hair extract and urine contain at least 8 IgE-binding components in the molecular mass range between 8 and 70 kDa (66). Sixty-five percent of guinea pig-allergic individuals have IgE against an 8-kDa allergen, whereas IgE reactivity to the other allergens is below 33%. Guinea pig serum albumin was previously considered a major allergen, but only 8% of guinea pig-allergic patients are found to exhibit IgE reactivity to it (66).

Rabbit

Ory c 1. The major allergen Ory c 1, also known as Ag R1, is found in saliva and, to a slightly lesser extent, in fur (67). It is present in dander in small amounts but not in urine. The physicochemical characteristics of Ory c 1 are shown in Table 2. The sequence of the 20 N-terminal amino acids suggests that the allergen is a lipocalin with a 72% amino acid identity with rabbit odorant-binding protein-II (68).

Other rabbit allergens. Rabbit urine, fur, and saliva extracts contain several allergens with molecular masses from 8 to 80 kDa (68). Saliva, which contains 12 allergens, is the most potent of the extracts, according to radioallergosorbent test (RAST) inhibition experiments (68).

The N-terminus of a 21-kDa allergen exhibits an even higher amino acid identity with the odorant-binding protein-II than Ory c 1 (68). This protein could be the minor allergen Ag2, found in several source materials, and is also referred to as Ory c 2 (Table 2). Rabbit serum albumin is of minor importance, although in individual cases sensitization can be strong (68).

Human Autoallergens

IgE antibodies against human proteins are found in patients with chronic allergic conditions. These are evolutionary conserved proteins, and some of them are homologues of recognized exogenous allergens.

In one study, using extracts from a human epithelial cell line, IgE autoantibodies against a variety of human proteins were found in 43% of patients with atopic dermatitis (69). Recombinant forms of several of the autoantigens induced histamine release from basophils and produced a positive skin prick test. The levels of IgE antibodies to the autoantigens were found to correlate with the severity of atopic dermatitis. However, the role of autoantibodies in the pathogenesis of allergic conditions remains unclear.

Hom s 1. Hom s 1 (O43290 at SWISS-PROT) is one of the five autoallergens listed in the allergen nomenclature. Six out of 65 sera from atopic dermatitis patients had IgE antibodies against Hom s 1 (70). Deduced from the cDNA sequence, it has a molecular mass of 73 kDa. However, a rabbit antiserum against Hom s 1 detected proteins with varied sizes in extracts of human tissues (70). Immunohistochemistry reveals that Hom s 1 is a cytoplasmic protein, although SART-1, a protein with an almost complete sequence identity with Hom s 1, is located in nuclei of normal and malignant cells.

Hom s 2-5 Like Hom s 1, these four autoallergens were found by screening a cDNA library from a human epithelial cell line with IgE antibodies from patients with atopic dermatitis (69). The presence of IgE antibodies was restricted to a few dermatitis patients. The cDNAs of Hom s 2-5 were shown to code for fragments of intracellular proteins. Hom s 2 displays sequence identity with a portion of the α -nascent polypeptide-associated complex (α -NAC) (Q13765 at SWISS-PROT). An isoform of Hom s 2 (Hom s 2.01), containing 21 amino acid exchanges

compared with α -NAC, has been identified (71). Hom s 3 displays sequence identity with the oncoprotein BCL7B (Q13845 at SWISS-PROT). Hom s 4 (O75785 at SWISS-PROT) is a 54-kDa basic protein with a pI of 8.7 (72). It appears to belong to a subfamily of calcium-binding proteins and displays IgE cross-reactivity with exogenous calcium-binding allergens from plants (Phl p 7) and fish (Cyp c 1). Hom s 5 is identical to a portion of cytokeratin type II (P02538 at SWISS-PROT).

Human homologues of exogenous allergens. Several fungal allergens are phylogenetically highly conserved, and the corresponding human proteins react with IgE antibodies from patients with severe fungal allergies. Asp f 6 is a manganese superoxide dismutase (MnSOD) allergen of *Aspergillus fumigatus*. Recombinant human MnSOD, which displays a 48% amino acid identity with Asp f 6 (SBNS), reacts with IgE and stimulates T cells from patients with chronic *A. fumigatus* allergy (73). Similarly, IgE cross-reactivity between several other fungal allergens, such as acidic ribosomal phosphoprotein type 2 (P2 protein) (74), thioredoxin (75), and cyclophilins (76), and their human homologues has been described. Profilins are another group of conserved proteins identified as allergens of several plants, e.g., Bet v 2 of birch. IgE from sera of patients sensitized to plant profilins cross-react with human profilin (77).

ALLERGENIC CROSS-REACTIVITY AMONG MAMMALS

IgE cross-reactivities among mammalian allergens have been characterized to some extent. Earlier analyses were based on inhibiting IgE binding to an allergen extract by another extract. The inhibition was usually found to be individually variable. Moreover, the extracts often showed an unequal inhibitory capacity, which suggests that only a part of the IgE-binding epitopes were common. Such experiments pointed to the possibility that there are IgE-cross-reacting allergens, for example, in cat, dog, and horse allergen extracts (53), including the components representing the major cat and dog allergens (78). Some studies suggest that the taxonomical relationship among animals is probably a factor contributing to the cross-reactivity of IgE antibodies.

More specific results have been obtained by using pure allergen molecules. rFel d 1 (100 μ g/mL) inhibits the binding of IgE from Fel d 1-sensitized cat-allergic patients to a dog allergen preparation by an average of 41% (79). An inhibition of more than 50% was detected with 25% of the sera. The probable IgE-cross-reactive dog allergen of 20 kDa was not characterized further. The IgE cross-reactivity of another cat allergen, Fel d 4, a lipocalin, also has been characterized (39). Allergen extracts from cow (mean 66%), and to a lesser extent, from horse and dog, inhibited IgE binding to rFel d 4. Since cow Bos d 2 shows a low amino acid identity with Fel d 4, the inhibition was supposed to be due to an unidentified bovine homologue. In a study with the guinea pig allergen, Cav p 1, a lipocalin, allergen preparations from cat, mouse, and rat in a hundred-fold excess were able to induce only a weak inhibition (less than 10%) of IgE binding to Cav p 1 (66). A further study suggests that there is IgE cross-reactivity between Cav p 1 and Cav p 2 (58). In another study, a monoclonal antibody raised against Bos d 5 (β -lactoglobulin), a bovine food allergen of the lipocalin family, reacted against human serum retinol-binding protein, another lipocalin (80). The core of the antibody-binding epitope, DTDY, is localized in the second structurally conserved region of lipocalins. The sequence is found, for example, in human glycodelin (SBNS).

Studies on IgE cross-reactivity between pure mammalian allergens are largely missing. One limited study, exploiting *E. coli*-produced rCan f 1 and 2, found IgE cross-reactivity between these two dog allergens (81). This observation has been confirmed by another group, using *Pichia pastoris*-produced rCan f 1 and 2 (82). The cross-reactivity was found, however, to be weaker than that reported by Kamata et al. (81). In the same study, IgE cross-reactivity was also found between Can f 1 and human tear lipocalin and between Equ c 1 and Mus m 1. The cross-reactivities were probably due to sequential and structural similarities of the proteins (82).

The clinical significance of IgE cross-reactivity between mammalian nonserum-derived respiratory allergens is unclear. It seems that the cross-reactivity is mostly weak to moderate. It is possible that IgE cross-reactivity, including to human homologues, could be implicated in the regulation of allergic sensitization.

Animal-allergic patients may have IgE antibodies against a number of albumins from different mammalian species (83). Inhibition experiments show that albumin-specific IgE is often cross-reactive, although patients exhibit individual variation in this respect (53,83). As pointed out for Fel d 2, the primary sensitizer can be difficult to identify (26). A study with three tryptic peptides from horse serum albumin identified regions involved in IgE cross-reactivity with dog albumin (84). Inhibition of a monoclonal antihuman albumin antibody with cat or dog albumin indicated that cat, dog, and human albumins have similar epitopes (25). In another study with monoclonal antibodies specific to cat or dog albumin, antibodies recognized the albumin of both species (85). The study also suggests that the monoclonal antibodies and human IgE recognize identical or closely related epitopes on cat and dog albumin.

Subjects sensitized to cat IgA have IgE cross-reactive with carbohydrate moieties, for example, in cat IgM (40). The IgE cross-reactivity of human autoallergens is discussed above.

ENVIRONMENTAL CONTROL

Exposure to indoor allergens can be reduced by control measures. It is reasonable to assume that the primary prevention of avoiding contact with pets during childhood would restrain sensitization and the clinical manifestations of allergy. This paradigm has been questioned, however, in view of several studies pointing to the protective effect of a high-level exposure to cat- and dog-derived dust (3,86). As a consequence, recommendations about pets and children in the same household, as far as primary sensitization is concerned, may need to be reconsidered.

The guidelines are more straightforward for persons who are already sensitized against mammalian allergens. Avoidance, or reduction of the exposure load when total avoidance is not possible, is the primary strategy to prevent or to alleviate allergic symptoms.

Allergen concentrations in homes with pets are 10 to 1000 times higher than in homes without pets (87). Removing the pet from the household gradually reduces the allergen levels over time (87). In practice, families often try to keep their pets, and various measures have been proposed to reduce the exposure in those circumstances. These include keeping the pet out of the main living area, using vacuum cleaners with high-efficiency particulate air (HEPA) filters, and frequent washings of the pet. Although a reduction in the allergen levels can be achieved, the effect on clinical outcomes is not documented (87).

As the first line of prevention against laboratory animal allergy, persons with an atopic background, especially if they are already allergic to animals, should be discouraged from doing these jobs (88). Within laboratory animal facilities, the aims of preventive measures are to reduce the airborne allergen levels and make use of personal protection against exposure. Ideally, a comprehensive plan should be used, starting from the appropriate designing of the facilities and ventilation system.

The use of curtains in front of cage racks prevents the spread of rodent allergens to the animal room. In one study, individually ventilated cage systems decreased ambient rodent allergen levels 250-fold or more under optimal conditions. To reduce the exposure to persons emptying and cleaning soiled cages, automated cage-handling machines have been developed. Handling animals during experimental procedures in class II ventilated cabinets results in a greater than 10-fold protection factor. The most effective personal protection against airborne allergens is achieved by the use of ventilated, motorized helmets in which inhaled air is pumped through type P2 or P3 filters. Although somewhat inconvenient to use, the helmet allows even asthmatic persons to continue to work with animals.

SALIENT POINTS

- Mammalian respiratory allergens are primarily dispersed in dander, saliva, and urine.
- Exposure to mammalian allergens is not limited to immediate contacts with animals; these allergens are widely present in indoor environments.
- Almost all important mammalian aeroallergens belong to the lipocalin family of proteins. Factors accounting for the allergenicity of lipocalins remain to be identified.

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- Environmental control measures can help symptomatic individuals, although avoidance of exposure is preferable.
- High exposure to pets in early childhood may be protective against sensitization.
- IgE cross-reactivity between animal serum albumins is established. Lipocalin allergens also exhibit IgE cross-reactivity.

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