# Laboratory Animal Allergens

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## Abstract

Allergic sensitivity to laboratory animals can pose a significant occupational hazard to anyone with regular animal contact. Reactions to mice and rats are most common, although all furred animals produce allergens that can lead to sensitization and disease. Most of the relevant allergens of laboratory animals have been defined and characterized, which has revealed that these allergens are typically small, acidic glycoproteins and that many of them are members of a superfamily of extracellular proteins called lipocalins. In addition to understanding their molecular characteristics, the identification of these allergens has also made it possible to measure their distribution in laboratory environments and to relate exposure levels to sensitization and symptoms. These studies have shown that the major laboratory animal allergens are carried on small particles that are both capable of remaining airborne for extended periods and penetrating into the lower airways of exposed workers. These advances in the understanding of these important occupational allergens will allow for the development of better methods of diagnosis and avoidance for affected workers and others who may be at risk for future difficulties.

**Key Words:** allergen; animal allergens; animal allergy; laboratory animal allergy; mouse; rat; rodent

## Introduction

At least 90,000 workers in the United States have direct contact with animals in research or industrial facilities (Eggleston and Wood 1992; Newill et al. 1986). Workers who are in regular contact with furred animals often develop sensitivity to these animals. This sensitivity accounts for the high prevalence of laboratory animal allergy in animal workers, estimated from multiple independent studies to be approximately 21% (Aoyama et al. 1992; Bland et al. 1986; Hunsaker and Fosse 1990; Slovak and Hill 1981). This high prevalence rate has major medical and economic implications. When employees develop laboratory animal allergy, it often results in significant morbidity, at times even necessitating a change in occupation. In addition, it may lead to decreased productivity, increased workloads for others, and increased health and worker's compensation costs for the employer. The major laboratory animal allergens and their environmental distribution are reviewed below.

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## The Allergens

Most of the major laboratory animal allergens have been identified and characterized (Bush et al. 1998; Table 1). The most common causes of laboratory animal allergy are rats and mice, primarily because these animals are used more often than others and not because the other animals are necessarily less allergenic. In fact, in one large epidemiological study of laboratory animal workers in Japan, symptoms were reported in 26% of workers exposed to mice, compared with 25% for rats, 31% for guinea pigs, 30% for rabbits, 26% for hamsters, 30% for cats, 25% for dogs, and 24% for monkeys (Aoyama et al. 1992).

Recent investigations have demonstrated that many of these animal allergens are members of the lipocalin superfamily of small extracellular proteins (Virtanen et al. 1999). Included in this group are Rat n 1A and Rat n 1B (Bayard et al. 1996), Mus m 1 (Robertson et al. 1996), and Can f 1 (Konieczny et al. 1997), as well as Bos d 2 from cattle (Mantyjarvi et al. 1996) and Equ c 1 from horses (Gregoire et al. 1996). Although amino acid sequence homology is not extensive among these allergens, the lipocalins have three highly conserved sequence motifs that lie close to one another on the surface of the molecules and form a common cell surface receptor binding site. The lipocalins are a large, diverse group of at least 50 proteins that serve predominantly to bind or transport small hydrophobic molecules (Flower 1996). With regard to the animal allergens, it has been speculated that many of the lipocalins function as pheremones or pheromone binding proteins.

At least three distinct mouse allergens have been identified and characterized (Price and Longbottom 1990; Robertson et al. 1996; Schumacher 1980; Siraganian and Sandberg 1979). The major mouse allergen, Mus m 1, or mouse urinary protein, is a prealbumin with a molecular weight of 19 kD as determined by dodecylsulfate-polyacrylamide gel electrophoresis. This allergen is found in urine as well as in hair follicles and dander. Mus m 1 is produced in liver cells, and males produce approximately four times more Mus m 1

**Table 1 Laboratory animal allergens**

| Animal | Allergen | MW[a] (kD) | Source | Biological function |
|---|---|---|---|---|
| Mouse | Mus m 1 (prealbumin) | 19 | Hair, dander, urine | Lipocalin-odorant binding protein |
| *(Mus musculus)* | Mus m 2 | 16 | Hair, dander | Unknown |
| | Albumin | | Serum | Serum protein |
| Rat | Rat n 1A/Rat n 1 B | 16-21 | Hair, dander, | Lipocalin-pheromone binding protein |
| (*Rattus norvegicus*) | ($\alpha_{2u}$-globulin) | | urine, saliva | |
| | Albumin | | Serum | Serum protein |
| Guinea pig | Cav p 1 | | Hair, dander, urine | Unknown |
| (*Cavia porcellus*) | Cav p 2 | | Hair, dander, urine | |
| Rabbit | Ory c 1 | 17 | Hair, dander, saliva | Unknown |
| (*Oryctolagus cuniculus*) | Ory c2 | | Hair, dander, urine | |
| Cat | Fel d 1 | 38 | Hair, dander, saliva | Unknown |
| (*Felis domesticus*) | Albumin | | Serum | Serum protein |
| Dog | Can f 1 | 25 | Hair, dander, saliva | Lipocalin cysteine protease inhibitor |
| (*Canis familiaris*) | Can f 2 | 19 | Hair, dander, saliva | Lipocalin |
| | Albumin | | Serum | Serum protein |

[a]MW, molecular weight.

because gene expression is testosterone dependent. A second allergen, Mus m 2, is a 16-kD glycoprotein that is found in hair and dander but not in urine. A final mouse allergen is albumin, which is allergenic in about 30% of mouse-sensitive individuals.

Two rat allergens have been identified in urine, saliva, and pelt (Bayard et al. 1996; Walls and Longbottom 1985). Rat n 1A has a molecular weight of 20 to 21 kD, and Rat n 1B has a molecular weight of 16 to 17 kD. Rat n 1A was originally thought to be a prealbumin, but more recent studies have demonstrated that both allergens are variants of $\alpha_{2u}$-globulin. Rat n 1B is produced in the liver, where it is androgen dependent, as well as in the salivary, mammary, and other exocrine glands, where its production is not androgen dependent (Bayard et al. 1996; Mancini et al. 1989). As in mice, rat albumin also possesses some allergenic activity, with about 24% of rat-allergic individuals manifesting sensitivity to albumin.

Although allergens from guinea pigs have not been fully characterized, two antigenic fragments, designated Cav p 1 and Cav p 2, have been identified. Both of these allergens are found in urine, hair, and dander (Ohman et al. 1975; Swanson et al. 1984; Walls et al. 1985).

Rabbit allergens are also not well characterized, but at least two specific allergens, Ory c 1 and Ory c 2, have been identified (Ohman et al. 1975; Price and Longbottom 1988; Warner and Longbottom 1991). Orc c 1 is a 17-kD glycoprotein that is found in saliva, hair, and dander. Orc c 2 is found in hair, dander, and urine.

Although cats and dogs are more often encountered as domestic pets than as laboratory animals, they are also common in laboratory environments. A total of 12 allergenic cat proteins have been identified; however, the major cat allergen, Fel d 1, is by far the most important (Anderson et al. 1985; Bartholome et al. 1985; Charpin et al. 1991; Leitermann and Ohman 1984). It is a 38-kD tetrameric polypeptide that has been molecularly cloned, and its amino acid sequences and allergenic structure have been elucidated (Morgenstern et al. 1991). However, in spite of the detailed knowledge regarding Fel d 1, its biological function remains unknown.

Fel d 1 is produced primarily in cat sebaceous glands from which it is secreted onto the skin and fur. It is also produced to a lesser extent in salivary glands and thereby excreted into the saliva. Fel d 1 production appears to be under hormonal control inasmuch as males produce higher levels, castration reduces its production, and supplemental testosterone increases its production (Charpin et al. 1994). In addition, approximately 20% of cat-allergic individuals are sensitive to cat albumin and for a few patients this may be the predominant allergen.

The most important dog allergens are Can f 1 and Can f 2, which are produced in hair, dander, and saliva (Konieczny et al. 1997; Larson et al. 1988; Schou et al. 1991; Spitzauer et al. 1993). Can f 1 has a molecular weight of 25 kD, and Can f 2 has a molecular weight of 19 kD. Can f 1 has been shown to be a cysteine protease inhibitor (Virtanen et al. 1999). Dog albumin also has been described as a distinct allergen, and approximately 25% of dog-allergic individuals exhibit sensitivity to this protein (Spitzauer et al. 1993).

Other animals used in laboratories, including gerbils, hamsters, cows, and sheep, may also occasionally cause

reactions. Even though primates are used in research facilities, few cases of sensitivity have been documented. There have been reported cases of allergy to the lesser bush baby (galogo) and the cottontop tamarin monkey (Petry et al. 1985). These allergens were identified in the animals' dander.

## Environmental Distribution

The aerodynamic properties and environmental distribution of many of these allergens have been well characterized. Airborne rodent allergens are found in a wide range of particle sizes, and it has been shown that small and large particles can migrate throughout a facility. For example, previous studies have characterized mouse allergen in public areas of an animal facility and revealed that rooms connected to the animal facility, but not actually containing mice, had detectable allergen on particles ranging in size from 0.4 to 3.3 µm. In free-standing, independently ventilated areas such as a cafeteria not connected to a mouse facility, the allergen was predominantly greater than 10 µm in size (Ohman et al. 1994). This finding suggests that animal allergens can be carried substantial distances in animal facilities so that even workers without direct animal contact could develop problems due to animal allergy.

Airborne rat allergens are carried on particles that range from 1 to 20 µm in mean aerodynamic diameter with the majority on particles less than 7 µm (Platts-Mills et al. 1986). These allergens can remain airborne 60 or more min after disturbance. Allergen levels have been studied in different settings, and the level of exposure has been shown to be primarily dependent on activity, with the highest exposures occurring among cage changers, room cleaners, and animal feeders (Eggleston et al. 1989). Levels of exposure also increase with greater animal density and decreased relative humidity (Gordon et al. 1992; Jones et al. 1995).

Much less is known or understood about the distribution of the other laboratory animal allergens. Guinea pig allergens have been measured in air samples by radioallergosorbent test inhibition, and the high percentage of this allergen found on particles less than 0.8 µm in diameter would be capable of remaining airborne for long periods after disturbance (Swanson et al. 1984).

The best studies of cat and dog allergens have been in home settings. Cat allergen has been well characterized and found to be on particles ranging from 1 to 20 µm in diameter. At least 15% of this allergen is carried on particles less than 5 µm in diameter (Luczynska et al. 1990; Wood et al. 1993). Although less is known about dog allergen, it appears to be distributed much like cat allergen, with approximately 20% of the airborne allergen carried on small particles that may remain airborne for extended periods (Custovic et al. 1997).

### Exposure Levels

It is as still unclear what specific levels of exposure can be expected to induce either sensitization or symptoms. Data on the clinical relevance of airborne allergen levels are currently available only for rat and cat. In one study, rat allergen levels causing nasal symptoms ranged from 1.5 to 310 ng/m$^3$ (Eggleston et al. 1990). In a follow-up study, a dose response was seen with greater symptoms at higher levels, although responses were so variable that it was impossible to determine what level of exposure could be deemed "safe." Likewise, studies on cat allergen have been inconclusive as to what level of allergen is the lowest capable of causing clinical symptoms, with many patients exhibiting significant symptoms at relatively low levels of exposure (Bollinger et al. 1998; Wood et al. 1998).

Epidemiological studies have shown that the greater the exposure to animal allergens, the more likely one will become sensitized and have symptoms related to work (Cockroft et al. 1981; Hollander et al. 1997; Venables et al. 1988). For example, animal handlers and caretakers develop allergic symptoms more frequently than those who do not work in direct contact with the animals (Venables et al. 1988). Hollander et al. (1997) noted a 42-fold higher prevalence of symptomatic rat allergy among heavily exposed atopic individuals. Therefore, identifying individuals with increased exposure is important in estimating risk and implementing measures for prevention.

Different job descriptions are associated with vastly different exposures to animal allergens (Cockroft et al. 1981). The highest exposures typically occur in handlers who are responsible for cage cleaning and feeding of the animals. Users are persons involved in daily experimental use of the animals, such as technicians, students, and investigators. These people have intermittent contact and therefore lower levels of exposure. Unexposed workers are secretaries and administrators who have no direct contact with the animals. When specific tasks are considered, cleaning cages or manipulating active animals are associated with significantly higher levels of airborne rat allergen exposure (Eggleston et al. 1989). Furthermore, it has been shown that symptomatic inflammatory responses in sensitized workers correlated with airborne allergen concentrations, and that more symptoms occurred with active cage cleaning than quiet activity (Eggleston et al. 1989; Rothman et al. 1995).

Interestingly, even those who do not have direct contact with animals can have work-related symptoms. Work-related symptoms were reported in one study in 56% of workers who had no direct contact with animals (Venables et al. 1988). This report suggests that any exposure in environments where animals are present may induce disease, which is not surprising given the data regarding the widespread distribution of these allergens in animal facilities.

## Conclusion

Over the past 2 decades, a great deal has been learned about the major animal allergens and their environmental distribution. Many of the allergens have been extensively characterized, and it has even become clear that most of them belong to a single family of proteins called lipocalins. With this knowledge will come an increased ability to protect the allergic individual through both environmental controls and more specific treatments. In addition, it will allow for the development of better strategies to prevent this affliction in susceptible individuals.

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