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Laboratory Animal Allergy in the Modern Era

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Abstract Laboratory animal workers face a high risk of developing laboratory animal allergy as a consequence of inhaling animal proteins at work; this has serious consequences for their health and future employment. Exposure to animal allergen remains to be the greatest risk factor although the relationship is complex, with attenuation at high allergen exposure. Recent evidence suggests that this may be due to a form of natural immunotolerance. Furthermore, the pattern of exposure to allergen may also be important in determining whether an allergic or a tolerant immune response is initiated. Risk associated with specific tasks in the laboratory need to be determined to provide evidence to devise a code of best practice for working within modern laboratory animal facilities. Recent evidence suggests that members of lipocalin allergens, such as Mus m 1, may act as immunomodulatory proteins, triggering innate immune receptors through toll-like receptors and promoting airway laboratory animal allergy. This highlights the need to understand the relationship between endotoxin, animal allergen and development of laboratory animal allergy to provide a safe working environment for all laboratory animal workers.

Keywords Laboratory animal allergy · Specific IgE · IgG_4 · Exposure · Respiratory protective equipment · Sensitisation · Lipocalin · Endotoxin · Occupational allergy

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Introduction

Laboratory animal allergy (LAA) is an important occupational health issue for the research, pharmaceutical and toxicological sectors. An estimated 12,000 people work with laboratory animals in the UK, most of them research scientists or 'animal technicians' handling mainly mice or rats [1]. The Annual Statistics of Scientific Procedures on Living Animals reported that 4.12 million scientific procedures were carried out in Great Britain in 2013 (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/327854/spanimals13.pdf). Of these procedures, 2.02 million (49 %) were performed for purposes other than to breed animals with either genetically modified or harmful genetic mutation. The total numbers of procedures have increased and are at their highest level since 1995 (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/327854/s panimals13.pdf). There is a large array of species used for scientific procedures; however, the most commonly used were mice (75 %), fish (12 %), rats (6 %) and reptiles/amphibians (0.3 %) (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/327854/spanimals13.pdf).

Laboratory animal workers face the risk of developing an IgE-associated respiratory allergy to airborne animal proteins. The resultant rhinitis, asthma and occasionally anaphylaxis ('laboratory animal allergy' or LAA) are at best disruptive since those affected are advised to reduce or eliminate any future exposure to animals; in the worst cases, the diagnosis is career-ending. In healthcare terms, workers who develop occupational asthma (of which LAA is an important example) generally require long-term treatment with asthma and nasal medications and have rates of emergency and routine hospital care higher than those among the general population of patients with asthma [2]. A recent study of the UK's financial

burden of occupational asthma suggests that each new case costs a total of about £125,000, half of which is met by the employee and most of the remainder by the state, including the NHS [3]. Moreover, a high proportion of laboratory animal researchers have training and experience that is very difficult—and sometimes impossible to replace. Employers are required to carry out routine surveillance of all exposed employees and to report new cases of LAA to the Health and Safety Executive (HSE), triggering an external workplace inspection. Thus, the disease is personally, nationally and institutionally expensive.

Risk Factors for Laboratory Animal Allergy

Our systematic review of the epidemiology of LAA indicates that c. 15 % of exposed employees will develop specific IgE sensitisation and 10 % clinically apparent disease [4]. The risks of LAA are the highest working with male rodents [5] and relate clearly to the intensity of exposure to airborne animal proteins, [6] although the shape of the exposure-response may be complex [6, 7]. Host factors appear also to be important; atopic employees are 3–4 times more likely to develop specific IgE to animal allergens [8], and in those with an HLA-DR7 genotype, the risk is doubled [9]. It is rare for employees to be sensitised to mouse proteins at entry [10].

These host factors are of lesser importance than antigen exposure [9], are unmodifiable and, being common in the general population, are poor predictors of LAA. The absolute (rather than relative) risk of disease is determined primarily by levels of allergen exposure in the workplace, and it is there that the main focus of prevention lies. Exposure control is achieved through a combination of engineering, procedural and personal controls. Only two studies, published in 1995 and 1998, have attempted to examine the success of these approaches. Both concerned employees working with rats in facilities that would now be considered outdated neither included any exposure measurement and both failed to provide a meaningful measure of disease incidence [11, 12].

In the modern era of laboratory animal work, there are two important and recent developments. First is the use of mouse (rather than rat) models in research and second the concomitant drive to improve the facilities in which laboratory mice are kept, driven primarily by the need to maintain gnotobiotic colonies in a microbiologically controlled environment and thus ensure the validity and reproducibility of the scientific data obtained. An important part of this has been, in many facilities, the replacement of standard open cages by individually ventilated cages (IVCs). Personal protective equipment is also increasingly used as a preventative measure for LAA.

Individually Ventilated Cages

IVCs were first developed in the Jackson Laboratory at Bar Harbor (Maine, USA) and became commercially available in the early 1980s. Each IVC has its own air-change system which protects the animals from external infections. In addition, IVCs provide an improved micro-environmental air quality with standardised levels of humidity, ammonia and carbon dioxide, eliminating a further source of environmental variability [13]. Further advantages include a higher stocking density, important where space is limited [13], significant savings in energy use [14] and a reduction in pup mortality [15].

IVCs Reduce Aeroallergen Exposure

A spin-off of these advantages appears to be a reduction in allergen contamination of the working environment. Early comparisons of ambient aeroallergen levels in rooms with open housing or IVCs demonstrated between seven- and 250-fold reductions in animal aeroallergen levels in the latter [16, 17]. It might reasonably be inferred that these reductions would be translated into a lower incidence of allergy in employees working with IVCs. Anecdotal evidence from some IVC-only facilities suggests that this might be the case but it is yet to be tested formally. A study is underway in the Jackson Laboratory where only IVCs are used [18•], but in the absence of a comparator group, it will not be possible to infer cause and effect. We are not aware of any other formal controlled studies anywhere in the world.

Working Practices to Reduce Aeroallergen Exposure

Moreover, it is not necessarily the case that the use of IVCs guarantees low allergen exposures since poor working practices can negate any benefit. For example, during cleaning-out days in a Swedish facility, there was no significant difference between aeroallergen levels in rooms housing either open or IVCs [17]. Handling animals in ventilated cabinets rather than on an open bench, automated cage cleaning and the use of ventilated tables can lead to major reductions in allergen exposures [16, 19]. In contrast, the task of removing used IVC exhaust filters generates increased levels of allergen exposure [19]. These factors may explain why we continue, in our clinical practice, to see employees who have developed LAA in some—but not all—facilities where only IVCs are used.

Safe Workplace Exposure Levels for Laboratory Animal Workers

The Health and Safety Executive in the UK have published a guidance note (EH76) on the health risks of work with laboratory animals, which provides limited advice on the precautions needed to control exposure, but there are currently no occupational exposure limits for any animal allergens. Most animal facilities aim to maintain Mus m 1 levels below 5 ng/m^3, a figure suggested by Gordon and Preece [20], but there is no evidence to suggest that this limit eliminates or reduces the incidence of LAA.

Safe Out-of-Work Exposure Levels for Laboratory Animal Workers and Those Who Work or Live with Them

Krop et al. [21] detected significantly higher levels of mouse allergen in mattresses from the homes of laboratory animal workers than those from non-exposed controls suggesting that there is carry-over of allergen from work to home. Among Scottish technicians, Mus m 1 was detected on their hands and shoes after leaving work and on their car steering wheels and domestic door handles (Semple S—personal communication). These observations may have significant clinical relevance; in Poland, children of laboratory animal workers had a higher prevalence of sensitisation to mouse, rat or hamster than did the children of parents in other occupations [22].

Personal Protective Equipment

A European Respiratory Society Task Force on the management of work-related asthma concluded that the use of personal protective equipment (PPE) can lead to a significant reduction in respiratory symptoms and changes in functional parameters, although it fails to provide complete protection [23]. In the UK, the Health and Safety Executive issued guidance in 2011 on the primary prevention of LAA that effectively requires the use of a full-face-fitted, filtered PPE (FFP3) by all exposed laboratory animal workers [24]. In contrast, a survey of 198 animal facilities revealed a wide variation in the use of PPE [25•].

There is little evidence in the literature regarding the effect of PPE on the risk of sensitisation to laboratory animals. A Swedish study suggested that the use of P2 face masks can decrease the amount of inhaled allergen by 90 %; however, there was no measure of either rat or mouse-specific IgE antibodies or symptoms of asthma [26]. In a UK facility, PPE was introduced as part of a comprehensive exposure reduction programme, and the introduction of PPE was reported to have reduced the incidence of LAA; however, it was difficult to disentangle PPE from the other measures [27]. From a recent survey, 228 laboratory animal workers in the UK pharmaceutical sector, who had been exposed for less than 5 years, were more likely to have used PPE and the use of face masks at first employment was associated with a lower prevalence of sensitisation, irrespective of the intensity of exposure to laboratory animals [28•]. The use of an airstream helmet respirator, often used in sensitised individuals, can be effective in improving symptoms in those with established symptoms of laboratory animal allergy [29].

Current Practices in Laboratory Animal Facilities

An online survey was sent to 1033 facilities within the USA to document current prevention programmes for laboratory animal allergy [25•]. A total of 198 organisations responded who were mostly academic. Three quarters of all organisations use some form of engineering control including biological safety cabinets, local exhaust ventilation, negative pressure environments and filter-top cages. Ventilated cages are used by a third of all facilities, and a third use downdraught tables. Two thirds of facilities limit animal density and use high-efficiency particulate air (HEPA)-filtered vacuums and wet process to clean rooms to minimise aeroallergen exposure. Hand washing is carried out by almost all employers. There was a great variation in the mandated use of personal protective equipment. The most common mandatory PPE in organisations were gloves (88.3 %), uniform/clothing covers (85.7 %), shoe covers (52.6 %), surgical masks (42.9 %) (technically not PPE) and N95 air-purifying respirators (16.6 %), with 63 % of organisations providing optional N95 air-purifying respirators. Perhaps in the modern era of laboratory animal allergy, it is surprising that fitted face masks are not used as a key preventive measure for laboratory animal allergy.

Current Occupational Mouse Allergen Exposure

Two recent studies from the Jackson Laboratory have examined mouse allergen exposure [18•, 30••]. The first study examined 220 new employees (25 % reporting previous exposure to mice) [18•]. The median mouse allergen concentration in the breathing zone was 1.02 ng/m^3 and 0.23 ng/m^3 for the average room. Not surprisingly, mouse handlers had significantly higher concentrations of breathing zone mouse allergen than employees who did not handle mice (medians 4.14 vs. 0.21 ng/m^3). Tasks determined the level of mouse allergen concentration, e.g. animal care (median 8.73 ng/m^3); husbandry (5.83 ng/$m^{3)}$ and laboratory experiments (0.36 ng/m^3), p=0.0001. A

greater frequency of mouse handling was also associated with higher mouse allergen. Perhaps surprisingly, administrative employees who worked in areas where mice were not allowed had detectable breathing zone mouse allergen, with 25–50 % having similar levels to animal caretakers.

The second study examined 179 new employees (43 had previous exposure to mice) with a median follow-up of 23 months [30••]. Twenty-three percent of employees developed a positive mouse skin prick test within 24 months. Intriguingly, the risk of developing a mouse positive skin prick test was non-linear, increasing from low to moderate and peaking at approximately 1.2 ng/m^3 and then decreasing from moderate to high levels of exposure (p=0.04). This observation is significant in that the risk of developing skin prick test is at a level of mouse exposure (1.2 ng/m^3) which is much lower than the level recommended (5 ng/m^3) by Gordon and Preece [20]. The more variable the exposure, the lower the incidence of a positive mouse skin prick test.

Recently, Westall et al. carried out a systematic programme of Mus m 1 allergen monitoring, specifically to assess the risk of allergen exposure, and inform decisions about the risk of exposure to the staff in the facility [31••]. Using this approach, they were able to identify areas with high Mus m 1 concentrations and with the introduction of appropriate controls, were able to demonstrate a significant reduction in Mus m 1 aeroallergen levels [31••].

Allergens

Laboratory animal workers can be exposed to a wide variety of species (Table 1). Rodent allergens can be found in dander, hair, saliva, urine and serum; urine is considered to be the main source of allergenic proteins in rats and mice [32], and the major mouse allergens in urine have been well characterised (Table 1).

Both Mus m 1 and Rat n 1 are produced in the liver under the control of androgenic hormones. Adult male mice produce approximately 5–10 mg of mouse urinary protein per day; female mice produce four times less. Rat n 1 is present in both male and female rats but is found in much larger quantity in the urine of adult male rats. In male rats, it constitutes approximately 30–50 % of the total protein content excreted into the urine and as a result, working with male rodents is an important risk factor for the development of LAA [14]. Mus m 1 and Rat n 1 are also produced in saliva, mammary and other exocrine glands [33, 34].

Mus m 2 is secreted from the hair follicles and coats the stratum corneum and hair shafts [35, 36] but is not found in urine.

Rat and mouse albumin (68 kDa) are also allergenic in 24–30 % of allergic patients [35, 36].

Lipocalins

Rat and mouse urinary proteins seem to play a complex role in chemosensory signalling among rodents. Mus m 1 and Rat n 1 belong to the lipocalin family which is characterised by a common tertiary structure [37•]. They are both odorant- and pheromone-binding lipocalins which bind small odorant molecules in a hydrophobic pocket which are then released slowly to the environment. Mus m 1 and Rat n 1 share 64 % identity in their amino acid structure. Mouse urinary protein has shown IgE cross-reactivity with both Equ c 1, a major horse allergen, [38] and rat urinary protein [39]. Mapping of the identical residues on the surfaces of the 3D structure of Rat n 1 and Mus m 1 demonstrates a substantial area in which several identical amino acids in the primary sequence, located primarily at the terminal ends of the proteins, join together to form a large accessible site for potential IgE binding. It is likely that this identical conformational epitope is predominantly responsible for the cross-reactivity observed between the two proteins.

The T cell epitopes have been identified for both the major cow (Bos d 2) and rat (Rat n 1) allergens [40•]. The cellular response to both allergens was weak, and there was a significant overlap in the T cell epitopes between both molecules [40•, 41].

Aeroallergens

Animal allergens are carried on a wide range of particle sizes, which can remain airborne for extended periods. Mouse allergen has been detected on particles ranging from 0.4 μm to those greater than 10 μm [42] with the majority in mouse-containing rooms being in the mid-range 3.3–10 μm. Similarly, rat allergens are carried on particles that range from 1 to 20 μm in mean aerodynamic diameter with the majority on particles <7 μm [43]. Total Mus m 1 levels ranged from 1.2 to 1.5 ng/m^3 in rooms without mice and 0.5 to 15.1 ng/m^3 in rooms with mice [42].

Whilst there are similarities between the amino acid sequences of rat and mouse allergens, and in the size of the particles on which they are found, it is not known whether their allergenicity is similar. Interestingly, whilst two major dog allergens, Can f 1 and Can f 4, have a similar capacity to sensitise, there were 200 times more Can f 1 in dog dander than Can f 4, suggesting that the quantity in the animal source is not necessarily related to the capacity to sensitise [40•].

Aeroallergen Exposure and Immune Response

Aeroallergen exposure is believed to be the most important risk factor for LAA, although the dose-response

Table 1 Major laboratory animal allergens

Animal	Allergen	Molecular mass (kDa)	Allergen production
Mammalian			
Mouse (*Mus musculus*)	Mus m 1 (prealbumin)	18–21	Hair, dander, urine
	Mus m 2	16	Hair, dander
	Albumin	68	Serum
Rat (*Rattus norvegicus*)	Rat n 1.01	21	Hair, dander, saliva
	Rat n 1.02	17	Hair, dander, saliva
	Albumin	67	Serum
Guinea pig (*Cavia porcellus*)	Cav p 1	20	Hair, dander, urine
	Cav p 2	17	Hair, dander, urine
	Cav p 3	19	Hair
Hamster (*Cricetus cricetus*)	Pho s 21	18, 21, 23	Hair, dander, urine, serum, saliva
	Cri c 4	69	Serum, urine
Ferret (*Mustela putorius furo*)	Unknown	17	Urine
Rabbit (*Oryctolagus cuniculus*)	Ory c 1	18	Hair, dander, saliva, urine
	Ory c 2	21	Hair, dander, urine
Cat (*Felis domesticus*)	Fel d 1	38	Hair, dander, saliva
	Fel d 4	19.7	Saliva
	Fel d 7	18–20	Saliva, hair
	Fel d 2	67	Serum
Dog (*Canis familiaris*)	Can f 1	22–24	Hair, dander, saliva
	Can f 2	19	Hair, dander, saliva
	Can f 3	65	Dander, saliva, serum
	Can f 4	16	Dander
	Can f 6	27–29	Dander
Pig (*Sus domesticus*)	Unknown	–	–
Sheep (*Ovis aries*)	Unknown	–	–
Goat (*Capra hircus*)	Unknown	–	–
Other			
Fruit fly (*Drosophila melanogaster*)	Unknown	–	–
Locust (several species)	Unknown	–	–
Cockroach (several species)	Bla g 2	36	Faeces, saliva, body of cockroach
	Bla g 4	20	
	Bla g 5	23	
	Bla g 7	35	

relationship may be non-linear [44–48] with attenuation of sensitisation and symptoms to rats at high allergen exposure. This has been ascribed to a healthy worker effect, but the recent demonstration of specific immune responses has provided a possible but controversial alternative explanation. Ratios of specific IgG_4:IgE antibodies were significantly increased in workers with the highest exposure to rats [45]. Furthermore, there was evidence that rat-specific IgG_4 provided some protection against symptoms in sensitised individuals since laboratory animal workers producing both specific IgG_4 and IgE had an almost twofold reduction in work-related symptoms compared with those producing IgE only [45]. The role of allergen-specific immunoglobulin IgG_4 antibodies at high allergen exposures in LAA is controversial with some studies suggesting a protective effect [30••, 49] whilst others claim no protection against the development of either sensitisation or symptoms [50, 51]. In support of natural tolerance, a functional role for rat-specific IgG/IgG_4 antibodies in laboratory animal workers was similar to that in patients treated with high-dose immunotherapy who become clinically tolerant [52••]. Rat-specific IgG and IgG_4 antibodies were shown to significantly decrease the binding of IgE-allergen complex binding to CD23

receptors on B cells which has previously been shown to result in downregulation of both the T cell response and allergic symptoms [53, 54]. Peng et al. suggested that having a higher variability of mouse exposure was more likely to lead to tolerance, perhaps because they are likely to have 'peaks' of high allergen exposure [30••]. Currently, there are no published studies examining the functional role of mouse-specific IgG and IgG_4 antibodies.

Innate Immune Response

Recent studies have suggested that allergens may act as immunomodulatory proteins, triggering innate immune receptors and promoting airway hypersensitivity reactions in diseases such as asthma [55••, 56]. Der p 2, a lipid-binding protein, can activate innate immune signalling through the toll-like receptor 4 (TLR4), since it mimics MD-2 [55••, 56]. The MD-2 protein appears to associate with TLR4 on the cell surface and confers responsiveness to lipopolysaccharide (LPS), thus providing a link between the receptor and LPS signalling. Nickel, on the other hand, activates the innate immune response through a direct, lipid-independent activation of TLR4, dependent on specific amino acids present in nickel [57, 58]. More recently, Fel d 1 and Can f 6 (members of the lipocalin family) and lipid-binding proteins have been reported to bind LPS to form larger complexes which promote greater clustering of TLR4-bearing lipid rafts, leading to increased receptor activation and increased pro-inflammatory cytokine production [55••].

Functional genetic variants of cell surface receptors for endotoxin such as TLR4 and CD14 have been investigated in laboratory animal allergy. Carriers of one or two copies of the TLR4/8551 minor G allele, which is less responsive to endotoxin, were significantly more likely to be sensitised to laboratory, pet and environmental allergens [59]. An association was observed for the CD14/1619G allele for which carriers who experienced higher endotoxin exposure had a significantly lower FEV1 than those with CD14/1619AA alleles. TLR4/8551 alleles have little effect on rat-related sensitisation and work-related chest symptoms; however, TLR4/8551 was related to rat exposure, and the risk of sensitisation and work-related symptoms was much greater in those with one or two copies of TLR4/8551G alleles than in those expressing TLR4/8551 AA alleles (16 vs. 6 %, $p=0.09$) (personal communication M Jones).

Endotoxin

The possibility that allergens may somehow immunomodulate the innate immune response through activation of the toll-like receptors is intriguing. Modest levels of airborne lipopolysaccharides (also known as endotoxin) have been reported in laboratory animal facilities [60, 61] compared with other occupational settings [62]. Some studies suggest that endotoxin is associated with direct mouse work [60] whereas others suggest that it is more likely to be associated with dirty bedding and animal feed [61]. Pacheco et al. suggest that endotoxin can elicit symptoms in non-sensitised laboratory animal workers [63]. Further work is required to assess whether there is an interaction between endotoxin, Mus m 1 and development of sensitisation and laboratory animal allergy.

Conclusions

Laboratory animal allergy remains a significant problem for laboratory animal workers despite a significant drive to reduce allergen exposure through a combination of engineering, procedural and personal controls. Exposure remains to be the most important risk factor. Rather surprisingly, there has been little work undertaken to assess the risks associated with specific work procedures. Furthermore, there is limited evidence on the effect of interventions on aeroallergen levels and whether this translates to a reduction in laboratory animal allergy.

The exposure-response relationship in laboratory animal allergy is complex with attenuation at high allergen exposure. This has been ascribed to a healthy worker effect, but recent evidence seems to suggest a form of natural immunotolerance at high allergen exposure. However, the relationship between patterns of allergen exposure and development of sensitisation and work-related symptoms also appears complex; some evidence suggest that the pattern of exposure can determine whether laboratory animal workers become sensitised or develop natural immunotolerance to the allergen. Further work is required to understand what drives immunotolerance in the work environment so that this can be applied to ensure a safe work environment for everyone.

Recent evidence suggesting that lipocalins such as Mus m 1 could act as immunomodulatory molecules triggering the innate immune response proposes to be a new form of mechanism which could initiate laboratory animal allergy. This finding warrants further investigation into the interaction between Mus m 1 (or other animal allergen), endotoxin, innate immune response and the development of laboratory animal allergy.

There are several challenges ahead to understand and prevent laboratory animal allergy; however, as described within this chapter, there are clear areas where further research should yield positive results.

Compliance with Ethical Standards

Conflict of Interest Dr. Jones declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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