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Allergy to Laboratory Animals

Risk Factors for Development of Allergy and Methods for Measuring Airborne Rodent Allergens

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You are
never given a wish
without also being given
the power to make it true.

You may
have to work for it,
however.

Richard Bach, Illusions

To my family, with love

List of publications

I. Renström A, Malmberg P, Larsson K, Sundblad B-M, Larsson PH. Prospective study of laboratory-animal allergy: factors predisposing to sensitization and development of allergic symptoms. *Allergy* 1994; 49: 548-552.

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IV. Renström A, Larsson PH, Malmberg P, Bayard C. A new amplified monoclonal rat allergen assay used for evaluation of ventilation improvements in animal rooms. *J Allergy Clin Immunol* 1997; 100; 649-655 .

V. Renström A, Gordon S, Larsson PH, Tee RD, Newman Taylor AJ, Malmberg P. Comparison of a radioallergosorbent (RAST) inhibition method and a monoclonal enzyme linked immunosorbent assay (ELISA) for aeroallergen measurement. *Clin Exp Allergy* 1997; 27: 1314-1321.

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Abbreviations

LAA	Laboratory animal allergy
MUA	Mouse urinary allergen
RUA	Rat urinary allergen
FEV_1	Forced expiratory volume in 1 second
PD20	Provocative dose of methacholine to cause a 20% decrease in FEV_1
VC	Vital capacity, maximum expired volume
SPT	Skin prick test
Ab	Antibody
MAb	Monoclonal antibody
EIA	Enzyme immunoassay, used interchangedly with
ELISA	Enzyme-linked immunosorbent assay
RAST	Radioallergosorbent test
RIA	Radioimmunoassay
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
BHBH	N-biotinyl-4-hydroxybenzohydrazone, amplifying agent
BSA	Bovine serum albumin
HSA	Human serum albumin
kd	KiloDalton
PBS	Phosphate buffered saline
PC	Polycarbonate
PTFE	Polytetraflouroethylene (teflon)
NHLI	National Heart and Lung Institute, London, UK
NIWL	National Institute for Working Life, Solna, Sweden
WAU	Wageningen Agricultural University, Wageningen, The Netherlands

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Introduction

Working with laboratory animals

Animals have been used in scientific study since antique times. Alkmaion from Kroton, a pupil of Pythagoras, is mentioned as the first to perform scientific dissections, as early as 500 B.C. (96). The animals most commonly studied in biological and medical education, research and industry today are rats and mice. In Sweden, by law (The Animal Protection Act 1988:534 and the Animal Protection Ordinance 1988:539), records are kept on animal research, work with animals is regulated by strict rules, and all personnel working with animals are required to participate in educational programmes. Intended use of animals must be approved of by the local ethical review committees on animal experiments, of which half are scientists and half are laymen. Between 160 000 to 190 000 mice and rats, respectively, are used yearly according to the Swedish definition of experimental animal usage. Table 1 shows the number and species of animals used between 1990 - 1995 (77, 78). According to the definition in "Council of Europe convention for the protection for vertebrate animals used for experimental and other scientific purposes" (ETS 123), animals that are used in for instance studies of caring or feeding systems or behavioral studies without infliction of suffering, or animals bred for post-mortem extraction of organs, are not defined as experimental animals. Thus, the latter statistics provide an underestimation of the numbers of animals to which personnel are exposed.

According to estimations, about 90 000 people work with laboratory animals in the USA (71), 32 000 in the UK (22) and 4600 in the Netherlands (45). In Sweden, in 1995, 4000 had undergone the mandatory course for work with laboratory animals. Furthermore, 1500 veterinarians may have occupational small animal exposure (107), as most pet shop staff.

Rodents are also common as pets. In a study of about 2000 Swedish school children, 19% answered that they owned small fur animals (of which about half were rabbits), to which other family members are presumably also exposed (5).

Allergy to laboratory animals

Laboratory animal allergy (LAA) is today a well-documented world-wide health hazard among animal exposed personnel (51, 66). Allergy is defined as hypersensitivity due to an incongruous immune reaction to a harmless substance (as opposed to appropriate immune reactions to for instance harmful bacteria). John Bostock found and described, in an inventory of London clinics in 1828, 27 subjects with hay fever (57). In 1873, Blackley proved the connection between symptoms and pollen exposure. Pollen was captured on sticky surfaces on a kite, flown at different hights, and skin and nasal provocation tests were performed out of the pollen season, not much differently than today (57). The first descriptions of allergy to laboratory animals are case studies (87, 106). In these and subsequent studies, LAA is characterised as an immediate type I IgE mediated allergy, causing symptoms at contact with laboratory animals, such as rodents.

Table 1. Number of experimental animals used in Sweden according to the Swedish definition 1990-1995 (77, 78).

Species	1990	1991	1992	1993	1994	1995
Mice	153 000	160 732	171 099	193 560	195 195	185 543
Rats	188 000	182 686	178 039	175 438	171 029	160 627
Guinea pigs	15 100	16 014	16 294	19 581	16 864	15 681
Other rodents	3 770	3 383	3 625	2 785	2 728	1 299
Rabbits	9 870	8 964	8 547	7 614	7 578	8 006
Primates	690	539	621	300	203	169
Dogs	670	643	873	558	700	797
Cats	690	625	547	471	394	272
Other carnivores	3 360	116	158	122	388	220
Horses, donkeys and mules	110	11	23	41	76	30
Swine	5 380	6 345	5 363	6 057	6 473	5 761
Goats and sheep	500	246	672	421	246	139
Cattle	540	593	1 151	762	929	945
Other mammals	170	68	87	306	757	642
Birds[1]	98 000	103 952	140 298	180 490	200 727	153 651
Reptiles	0	180	15	55	72	8
Amphibians	3 090	2 866	2 279	2 873	1 892	1 104
Fish	10 700	14 983	22 446	25 403	22 627	23 096
Cyclostomes	290	467	630	532	708	620
Total (Swedish definition)	494 100	503 763	553 017	617 619	629 586	558 610
Total (ETS definition)	338 343	347 732	349 235	351 150	351 615	331 201

[1] of which about 90% were used in either behavioral studies, primarily for the development of alternatives to cage-keeping of hens, or for the extraction of hyaluronic acid from cocks combs.

Symptoms to laboratory animals

Symptoms to laboratory animals usually appear immediately at exposure; Lutsky found that 93% of 191 patients experienced symptoms within 10 minutes (68). The first symptoms of LAA to appear are usually sneezing, runny or blocked nose (rhinitis) (2, 27). These symptoms are also the most common, often in combination with swollen, itchy or runny eyes (conjuctivitis) (51). Up to half of the symptomatics also develop asthma symptoms; cough, wheezing, tightness of the chest. Some asthmatic subjects also have a late phase bronchocontriction (42, 73). Skin symptoms are common, especially contact urticaria (hives) provoked by contact with the tail or urine, or giving wealing of the skin if lightly scratched by the animal; more infrequently angioedema (3, 51). Sometimes a more persistant eczema is seen, however, some cases might be attributed to for instance use of gloves and latex allergy, which is fairly common among

glove users (124). Also, itching of the palate has been described and occurred in 38% of symptomatics in one study (68). Fortunately, anaphylaxis, with generalised swelling and severe systemic effects is rare, but has been described in connection with for instance animal bites (44, 62, 114, 122). About half of the patients have two or more symptoms (6, 46, 51).

LAA prevalence and incidence

A large number of epidemiological studies have been carried out since the mid-70's, see table 2. The subjects under study have differed as have the definitions of LAA, but the reported prevalence of any symptoms has been 11-56%, and 3-13% had laboratory animal work associated asthma (32). Many studies have included medical examination to verify reported symptoms, for instance skin prick tests or measurements of specific IgE in serum. Sensitization rates and how well they correlate with reported symptoms have varied, partially depending on the methods, cut-off values and extracts used. Nevertheless, prevalence of sensitization to laboratory animal allergens in cross-sectional studies are between 10-62%, see table 2. Rats and mice, to which most laboratory animal workers are exposed, are also responsible for the majority of laboratory animal allergy (LAA) cases.

A small number of prospective studies have been carried out, and present one-year cumulative incidences of 9-37% (16, 17, 27) and 2-year cumulative incidences of 12% (17) and 13% (54). The yearly incidence of LAA asthma development is about 2% in exposed subjects (27). Lincoln et al (62) also noted that several subjects did not develop seasonal hay fever until they experienced symptoms related to their work with animals, and in some subjects, existing pollen allergy was exacerbated by exposure to laboratory animals.

Most LAA subjects develop their symptoms during the first three years of occupational exposure, but it may take only weeks or up to decades. The mean or median time for development of allergic symptoms is between 0.5-3 years (6, 16, 25, 42, 68, 102). However, in one retrospective study, the median time until development of LAA was approximately 8.2 years in non-atopics and 2.2 years in atopics (58). Thus the presence of atopy will influence the development of sensitization and symptoms.

The role of atopy and other host factors

Atopy is usually defined as a (genetic) predisposition to develop allergic symptoms to common environmental allergens. The operative definitions of atopy used in studies of allergy vary, and several LAA studies have used or compared more than one definition, for instance presence of allergy in the family, personal allergy or skin prick test positivity to common aeroallergens (103). Family allergy is in some studies significantly associated with LAA (6, 99), but not in others (103, 113). Having a personal history of allergy prior to occupational laboratory animal exposure seems a better risk indicator, especially for development of LAA asthma (12, 42, 62, 92, 103). Many cross-sectional studies have shown significant correlations between skin prick test positivity to a panel of common aeroallergens and LAA (22, 33, 67, 102, 103, 117), especially if the skin prick tests are positive to other fur animals, such as dog or cat (46, 123).

Total IgE has been measured in a few studies, which have found an association between elevated total IgE levels in serum and LAA (46, 99). However, the ongoing

activation of the immune system because of LAA might influence skin prick test and total IgE results. Therefore to ascertain to what extent these constitute predisposing factors, prospective studies with pre-exposure tests are needed. In the two studies found presenting pre-employment skin prick test data, atopy by this criterion was indeed correlated with subsequent LAA development (16, 17); in one study, the increased relative risk was between 4-8 after 1-3 years of exposure (17). Also, as mentioned, atopics might develop symptoms earlier (16, 58) and are more likely to progress to asthma than nonatopics (27, 74, 99).

Hereditory factors might predispose subjects to LAA. Indeed a few studies of genetic markers have found HLA-linked (human leucocyte antigen) factors suggested to protect from (HLA-B16) (101) or increase the risk for LAA (HLA-DR4) (79).

Other host factors have been shown to exert at most a mild influence over the risk to develop LAA. Smoking has in some studies been shown to be a significant effect modifier (25, 33, 118), but not in others (2, 12, 22, 46, 102). No significant correlations have been found between gender and LAA (12, 33, 46).

Allergens from rats and mice

Personnel working with animals are exposed to animal urine, hair, dander, saliva and blood, depending on the work. By sheer volume, urine is the most important potential allergen source in laboratory rodents; a mature rat excretes 10-20 ml of urine/day. In an average rat room with a few hundred animals, thus several litres of urine are being produced per day, some of which will dry out and become airborne. Both rat and mouse urine has been shown to contain high amounts of protein, especially in males, and these proteins have been shown to constitute important allergens (39, 97).

The most important rat and mouse allergens are pheromone-binding proteins, members of the lipocalin superfamily, α_{2u}-globulins in rat and Mus m 1 isoallergens (or MUPs, major urinary proteins) in mouse (19). These proteins are related, showing 66% sequence homology between rat and mouse (43). The bulk of these proteins are synthesised in the liver and excreted in urine (63). Mature male rodents excrete up to 300-fold higher amounts than female rodents (64, 94, 116). Several studies describe two cross-reacting proteins in rat urine with slightly different molecular weights in SDS-PAGE, α_{2u}-globulin (17 kd) and prealbumin (21 kd) (39). However, Bayard et al (8) could show that these were in fact isoallergens. It has therefore recently been proposed that they be termed Rat n 1.01 (prealbumin) and Rat n 1.02 (α_{2u}-globulin) (108). Half or more of the protein in male rat urine consists of these isoallergens. In the mouse, the MUP complex comprises about 90% of the protein in the urine (73). However, varieties of these rat and mouse isoallergens are also excreted by various glands by both sexes, and can also be found in saliva (59, 95). Perhaps the allergen termed Ag 3 or Mus m 2 and found in mouse hair (86), is a member of the MUP complex. About 60-90% of rat allergic patients react to the α_{2u}-globulin isoallergens, and most mouse allergics react to the Mus m 1 complex (39, 49, 63, 91).

Albumin (68kd) is another allergen present in rat urine and serum to which about 30% of rat allergics react (37, 39, 120).

Table 2. Sensitization and symptoms among laboratory animal workers (exposed to mostly rats and/or mice).

Reference	No. subjects	% sensitized (spec IgE)	% symptomatic	Nasal/eye symptoms, % of symptomatic	Chest symptoms, % of symptomatic	Skin symptoms, % of symptomatic
Lincoln et al -74 (62)	238	-	11	81	48	56
Lutsky & Neuman -75 (68)	1 293	-	15	100	71	58
Taylor et al -76 (113)	474	-	23	74	39	47
Cockcroft et al -81 (22)	179	16	27	89	43	51
Davies & McArdle -81 (26)	585	-	20	56	16	29
Newman Taylor et al -81 (74)	145	-	19	85	41	15
Schumacher et al -81 (92)	121 (mouse)	32 (SPT) 22 (EIA)	32	74 (nasal) 36 (eye)	13	41
Slovak & Hill -81 (102)	146	15	30	67	32	-
Beeson et al -83 (9)	69	-	22	67	20	13
Venables et al -88 (117)	124	14 (SPT) 40 (RAST)	33	85	25	42
Aoyama et al -92 (6)	5 641	ND	23	82	47	45
Cullinan et al -94 (25)	238	10	31	71	32	48
Bryant et al -95 (18)	130	62	56	100 (nasal) 63 (eye)	46	41
Fuortes et al -96 (33)	103	19 (rat) 11 (mouse)	33	85	42	30
Hollander et al -96, rat (46)	458	18	19	90	32	57
Hollander et al -96, mouse (46)	377	10	10	90	32	42

SPT = skin prick test; RAST = radio-allergosorbent test; EIA = enzyme immunoassay

Allergens from other laboratory animals

The main allergen from rabbit is present in saliva and fur, Ory c 1 (or Ag R1) (84); also a rabbit immunoglobulin light chain has shown some allergenic potency (83). Among work exposed, allergy to guinea pig is common (68). Four major allergens (to which ≥50% patients have IgE), were found in guinea pig room dust, all of which were also present in both dander, fur, saliva and urine (121). Urinary allergens were found to dominate in air samples (111).

Measuring airborne allergens

Many methods have been devised to quantify levels of airborne allergens, for the estimation of exposure and to evaluate ventilation improvements (1, 24, 28, 29, 35, 41, 49, 61, 81, 90, 110, 111, 112, 115). The values reported using these methods may differ by several orders of magnitude for one and the same species. Although this might reflect actual environmental differences to some extent, these methods differ in many ways, most of which are likely to influence the allergen values;

Sampling and elution:
- Pumps for air sampling, air flow, filter holders
- Filter types, e g fibre glass, polycarbonate or polytetraflouroethylene, and pore sizes
- Methods to elute allergens from the filters, e g shaking or homogenisation, use of Tween 20 or not

Immunological analysis:
- Assay set-up, e g inhibition or sandwich assay
- Source of detection antibodies, patient serum, polyclonal, or monoclonal antibodies
- Specificity, measuring one or several antigens
- Visualisation method, e g radioactive, fluorometric, or enzymatic

Although sampling and elution methods have been compared in a few papers (1, 38), only one presents a comparison between different immunological assays (112). In order to be able to compare values derived from different studies or laboratories, and certainly before any exposure limits can be proposed, a thorough standardization of methods must take place.

However, conclusions drawn from studies with allergen measurements can often be generalised. For instance that increasing air changes or reducing stock density in rat rooms (29, 36) or housing rodents in ventilated cages will diminish allergen levels (41), or that certain tasks expose workers to higher levels of aeroallergen than others (75).

The role of exposure for development of LAA

In several studies exposure as a risk factor has been assessed. Exposure variables defined according to job title, numbers of years employed, hours of work with rodents/week or by measured allergen load have been used. Exposure intensity defined by job title or exposure years, have not been proven to be related to LAA (2, 6, 12). Indeed, some have found an inverse relationship between exposure intensity by job title or degree and LAA (62, 117), possibly because of healthy worker selection. Hours of exposure/week has been shown to correlate significantly with LAA in some studies (6, 12), but not in others (22, 92).

Using allergen measurement methods to estimate aeroallergen load can provide further and more detailed information on exposure-response relationships. In a study by Cullinan et al, full-shift personal samples were collected and allergen measurement results distinguished between exposure categories. However, only a connection with skin symptoms was shown; neither upper nor lower airway symptoms correlated to the aeroallergen levels (25). In another study (54), tasks were assigned values and weighted, and exposure intensity was found to significantly correlate with LAA prevalence, although atopy gave a better correlation with LAA. In another recently published study, Hollander et al found no exposure-response relationships in the whole group of exposed workers. However, when subjects exposed <4 years were analyzed, strong dose-response relationships were found to time-multiplied exposure if combined with evidence of atopy (48).

Aims of the thesis

There were two major aims for this thesis:

1. To study the development of symptoms and sensitization against laboratory animals, and assess host and exposure related risk factors with particular emphasis on work in relatively clean environments.
2. To develop sensitive methods to quantify airborne rodent allergens and compare these methods with those developed in other laboratories.

Materials and Methods

All studies were approved of by the local ethical committees. All subjects gave their informed consent to participation and received information on their personal test results. The subjects from the university cohort were also given lectures on laboratory animal allergy and on prevention and management. This chapter presents an outline of the studies and methods. Detailed descriptions of methods are provided in papers I-VII.

Prospective study (I, II) and cross-sectional study (III)

Prospective study aims and design (I, II)

A prospective study was designed in which laboratory technicians were examined with regard to lung function and immunology during their education. Those who according to a postal follow-up questionnaire had subsequently worked as laboratory technicians and were exposed to laboratory animals were re-examined two years after work start together with matched unexposed referents (figure 1).

Those who had graduated and worked as technicians did not differ in allergy, atopy, lung function etc. from those who had not. Thirteen subjects were excluded: they had been only sporadically exposed at work or had one of these animals at home. Work exposure to dog or cat was disregarded, as a majority had been exposed to one or the other at home, often for a long time prior to the investigation.

To study selection to animal work, the pre-exposure values from all 43 laboratory animal workers were compared to those of the 112 unexposed.

To study potential risk factors and their role in the development of sensitization and symptoms against the animals, all exposed subjects were invited for a follow-up, and were examined with the same tests and equipment as prior to exposure.

To compare symptom development among laboratory technicians with and without animal exposure, non-exposed subjects were matched with exposed on 1) pre-exposure atopy (skin prick test and/or Phadiatop positivity), 2) smoking/non-smoking, 3) age, 4) gender.

Cross-sectional study aims and design (III)

Subjects (n=80, 21-53 years old, 68% women) who had worked ≥5 months with rats and/or mice in research departments at a university (n=48) and from the prospective study cohort (n=32), were included in a cross-sectional study. Subjects with a rodent at home were excluded from the study. The aims were to assess the possibility to avoid development of allergy and sensitization to animals by working in a clean environment, and to study potential risk factors (both host related and environmental) in a study group with longer exposure time. Air samples were collected in order to assess aeroallergen levels in research departments compared to animal house levels.

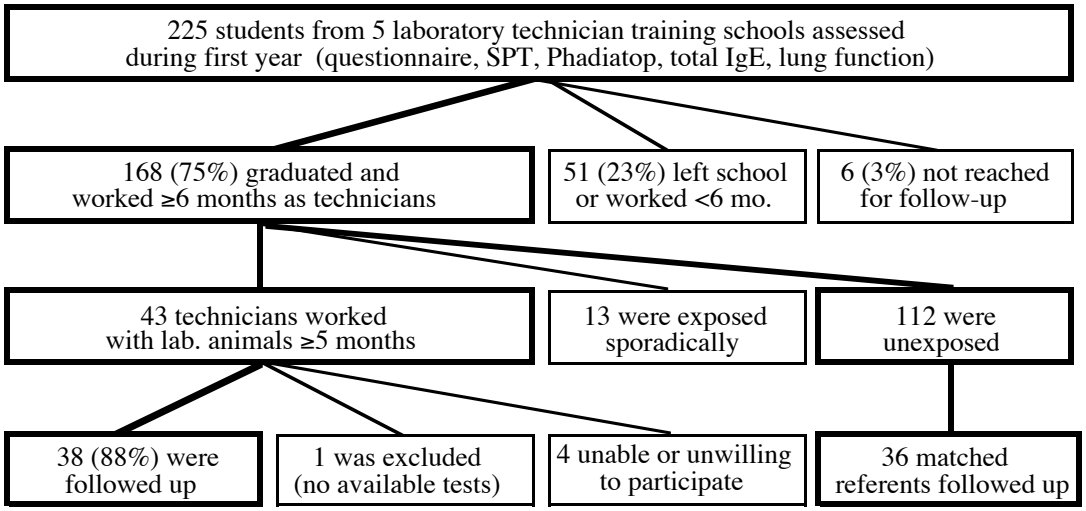

Figure 1. Study design of the prospective study.

Questionnaires (I-III)

All subjects answered extensive questionnaires on allergy in the family, personal allergic and medical history, exposure to irritants, smoking habits and animal contact. In the postal follow-up questionnaire sent to laboratory technicians, subjects answered whether they had graduated, worked as technicians etc. Laboratory animal exposed subjects also answered a questionnaire on extent of animal exposure, sex of the animals used, protective measures etc.

Lung function, methacholine provocation (I, II)

Spirometry was performed with a wedge spirometer (Vitalograph, Buckingham, UK) at least 20 minutes prior to the bronchial provocation. The highest value of three forced expirations was chosen as FEV_1 (forced expiratory volume during 1 second) and the highest of three forced and three slow maximal expirations as VC (vital capacity) for each patient.

Bronchial provocation was performed by inhalation of increasing concentrations of methacholine (0.5, 2, 8, and 32 mg/ml). The nebulisate passed through a drying device before inhalation to achieve maximum deposition in the lungs (69). The test was stopped at an FEV_1 decrease of ≥20% compared to the value measured after inhalation of diluent, or after inhalation of the highest concentration. The cumulative methacholine dose which caused a 20% decrease in FEV_1 was calculated by interpolation on a log cumulative dose scale (PD20). In cases where FEV_1 did not decrease by >20% at the highest concentration, PD20 was assigned a value of >10 mg. The average percent decrease in per mg of inhaled methacholine (cumulative dose, linear scale; "slope") was calculated by linear regression (23, 76).

Skin prick tests (I, II)

Extracts from eight common aeroallergens were tested on the volar aspects of both forearms: birch, timothy, mugwort, dog, cat, two types of moulds (*Alternaria* and *Cladosporium*) and house dust mite (*Dermatophagoides pteronyssinus*). Histamine di-

hydrochloride, 10 mg/ml, was used as a positive reference and dilution solution as negative control. Reactions ≥2 mm in diameter larger than the negative control were considered positive. A weal size equal to the histamine weal was defined as +++ in accordance with Agrup et al (2). A person with at least one +++-reaction or two ++ (weal area=half of histamine weal area) reactions was defined as atopic. Sensitivity to laboratory animals was determined by skin prick tests using hair extracts from guinea pig, mouse, rat, rabbit and hamster (1:20 w/v, ALK, Copenhagen, Denmark).

Serum tests (I-III)

Phadiatop and total-IgE Sera were stored at -70°C until analyzed. Total-IgE was analyzed using an in-house ELISA (Enzyme Linked Immuno-Sorbent Assay) using alkaline phosphatase conjugated rabbit anti-IgE antibodies for detection (I and II). Phadiatop analysis (Pharmacia) (I-III) and total IgE (III) was performed at the accredited Department of Clinical Immunology, Karolinska Hospital.

Specific IgE against laboratory animals Post-exposure sera from laboratory technicians and university cohort sera were tested for IgE against rat urinary proteins and Mus m 1, respectively, using in-house ELISAs. Sera from laboratory technicians exposed to other species were tested with Phadebas RAST (by Pharmacia, Uppsala), for specific IgE against these animals (rabbit, hamster, horse, pig and chicken).

Methods to measure rat and mouse urinary aeroallergens (III, IV)

To enable quantification of airborne allergens from rats and mice in different environments, for the evaluation of ventilation solutions and subject exposure, sensitive methods were developed.

Filter sampling and elution

Air samples were collected at 2 l/min air flow using 25 mm filters (different types were tested) in IOM filter cassettes (SKC Inc. Valley View, Pa. USA). Filters were eluted within hours of sampling in 1 ml PBS, 0.5% Tween 20, 0.15% Kathon (Rohmand Haas, Hydrosupra Kemiservice AB, Helsingborg, Sweden) during rotation for 1 hour. The filters were discarded and the eluates stored at -20˚C until analysis.

Quantification of rat urinary allergen

Two murine monoclonal antibodies (MAbs) against rat urinary allergen were developed using hybridoma techniques (34) (Mabtech AB, Stockholm, Sweden). Epitope map analysis was performed using a BIAcore Biosensor (53) (Pharmacia, Uppsala, Sweden). Specificity of MAb(1) and Mab(6), respectively, was determined using Western blot analysis. ELISA was used to study binding to purified Rat n 1 isoallergens prealbumin and α_{2u}-globulin.

A Rat n 1 standard extract was prepared from male post-pubertal Sprague-Dawley (S-D) rat urine, which was concentrated and dialyzed (12-14 kd cut-off), and thereafter affinity purified using Mab(6). A sandwich ELISA was developed in two versions as shown in figure 2, with standard or amplified sensitivity.

To achieve amplification, a novel compound, N-biotinyl-4-hydroxybenzohydrazone (or BHBH) was synthesized (by Per Larsson) and used according to the CARD (catalyzed reported deposition) concept (13).

Specificity of the assay was assessed by studying binding to a number of proteins derived from both rodents (S-D and Wistar urine, purified rat serum albumin, fur extract, rat room dust) and other animals (Mus m 1, mouse urine, mouse serum, guinea pig urine, or sera from rabbit, cat, dog or horse).

Quantification of mouse urinary allergen

Urine from post-pubertal male NMRI mice was concentrated, and the proteins were size separated through FPLC gel filtration on Superose 12 columns (Pharmacia). Fractions from the protein peak (Mus m 1) were collected and freeze dried. New Zealand White rabbits were immunized according to Hudson and Hay (50) and boostered twice. The rabbit serum was Na_2SO_4 precipitated for IgG enrichment and thereafter dialyzed, after which the antibodies were affinity purified using Mus m 1.
Antibody specificity tests were performed on a panel of antigens (mouse serum, rat urine and serum, guinea pig urine, serum and IgG, sheep serum and IgE, sera from goat, cat and cattle, IgG from pig, horse, dog and monkey, and human urine and serum).

A polyclonal Ab sandwich ELISA against Mus m 1 was developed, similar to the standard RUA assay (figure 2). Microtiter plates were coated with rabbit antibodies over night. After washing, Mus m 1 (50-1500 pg/ml) and filter eluates diluted at least twofold were added to the plates and incubated. Bound antigen was detected with biotinylated anti-Mus m 1 followed by streptavidin-horse radish peroxidase incubation, and visualised with TM-Blue (soluble form, TSI-CDP, Milford, MA, USA). The colour reaction was stopped with 1M H_2SO_4 and read at 450 nm, and background absorbance at 650 nm was subtracted.

Step	Standard RUA assay	Time
1.	Coating with capture antibody MAb(6) in PBS	over night
2.	Incubation with standard and sample eluate	90 min
3.	Incubation with biotinylated detection Ab, MAb(6)	60 min
4.	Incubation with straptavidin-horseradish peroxidase	60 min
5.	Incubation with substrate	15 min
6.	Colour development stopped	
7.	Plate read at 450 nm	

Step	Amplified RUA assay	Time
1.	Coating with capture antibody MAb(6) in PBS	over night
2.	Post-coating with 1% BSA	60 min
3.	Incubation with standard and sample eluate	90 min
4.	Incubation with biotinylated detection Ab, MAb(6)	60 min
5.	Incubation with straptavidin-horseradish peroxidase	30 min
6.	Incubation with amplifier	15 min
7.	Incubation with straptavidin-horseradish peroxidase	30 min
8.	Incubation with substrate	15 min
9.	Colour development stopped	
10.	Plate read at 450 nm	

Figure 2. Schematic description of the RUA assay. In the standard version, the plates are washed 3 x prior to the incubations, which were carried out at 37°C (until step 5). In the amplified version, the plates are washed 4 x prior to the incubations, which were carried out at room temperature.

Comparison of methods to measure rodent aeroallergens (V-VII)

Comparison of a RAST inhibition method and a monoclonal ELISA assay, aims and study design (V)

This study was initiated as we noted that airborne RUA sample values differed greatly between those measured in another laboratory (National Heart and Lund Institute, UK) and in our laboratory (National Institute for Working Life, Sweden). To investigate whether this was due to actual differences in the occupational environment or was a result of methodological differences, 40 samples (15 in the UK, and 25 in Sweden) were collected in animal facilities containing mainly rats. The samples were eluted and aliquoted for analysis in each laboratory. Thus each laboratory analyzed all 40 samples by its own method, the RAST inhibition method (see table 3 and Gordon et al (36)) and the unamplified monoclonal sandwich ELISA, respectively.

Investigation of some sources of assay variation (V)

Since sample values are interpolated from a standard curve, the binding of the standard extracts in the assays will influence the resulting sample values. To assess the immunological similarity of the standards (derived from Wistar rats in the UK assay, and from S-D rats in Sweden), they were assayed in serial dilutions in parallel with each laboratory's own standard extract.

Also, the albumin content of the samples was estimated (by RAST inhibition) because

1) the Swedish rats were on average older than the UK rats in the rooms in which samples were collected (and rats excrete increasing amounts of albumin with age (39)), which could increase the proportion of airborne albumin, and

2) should albumin be present it would be detected in the RAST inhibition assay, but not in the monoclonal assay, contributing to the differences in values.

Comparison of methods to assess airborne RUA and MUA levels, aims and study design (VI, VII)

In a European Concerted Action programme ("Epidemiology of occupational allergic asthma and exposure to bio-aerosols"), the need for comparison and evaluation of current methods to measure aeroallergens was recognized. The ultimate objective was to give an informed recommendation for future aeroallergen measurement, and standardization of methods. Thus a three-country study was designed, in which air samples were taken in triplicate in three countries, were divided among the participants and then eluted and analyzed by the rat and mouse urinary allergen measurement methods of each participating laboratory, as shown in figure 3.

Factors influencing RUA and MUA antigen detection, aims and study design (VII)

In the air filter analysis we noted a large variation in nominal allergen levels between the methods, the greatest being between RUA RAST inhibition and sandwich EIAs. Further investigations were performed in order to evaluate the relative importance of assay set-up, antibody specificities, standard extracts and antigen decay, on the abilities of the assays to detect antigen. Thus the following studies were performed:

1) The quantification of protein concentrations of the respective assay standard extracts will ultimately determine the value of the air samples. As we also wished to perform the following studies in all three institutes using the same protein concentrations, the extracts were distributed and protein quantified according to the methods of each institute.

2) The importance of the assay set-up (inhibition or sandwich) was assayed using identical standard extract and antibodies, and analysing 25 air samples for RUA.

3) To compare the RUA and MUA standard extracts, and study the specificitites of the antibodies to these and to rat and mouse room dust samples, Western blotting was performed.

4) To compare the antigen detection of the respective RUA and MUA assays, all standard extracts, rat and mouse room dust extracts, an animal food extract and dust from a home with cats and a home without cats, were assayed.

5) To study the influence of antigen decay, RUA and MUA were subjected to an accelerated degradation protocol and analyzed in parallel to fresh antigen in serial dilutions in all assays.

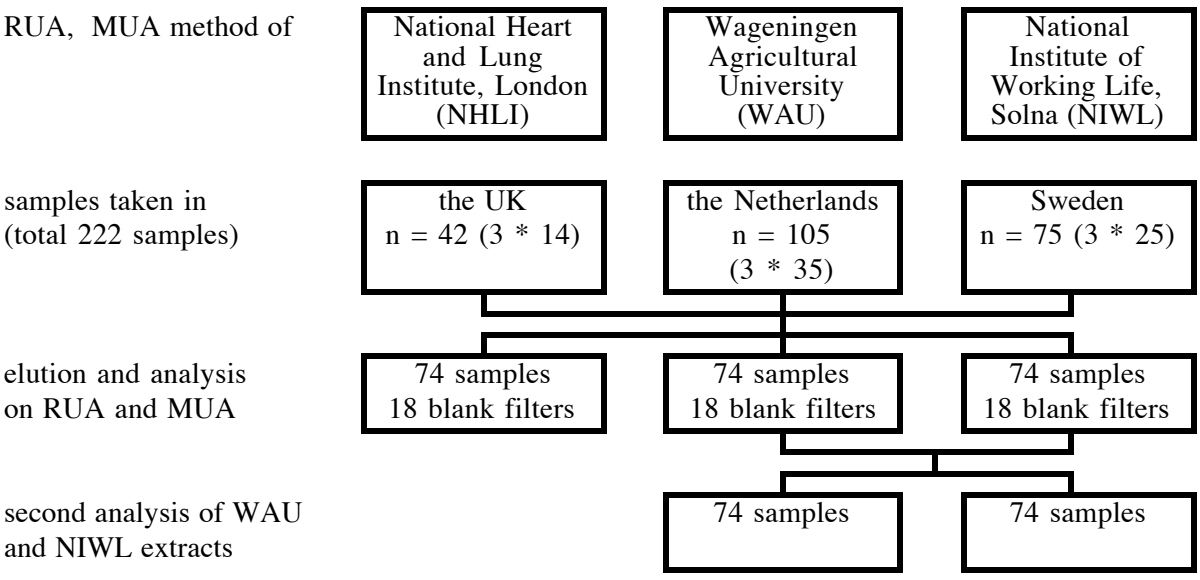

Figure 3. Study design for comparison between RUA and MUA air sample values measured in three laboratories. Submitted.

Table 3. Essential features and differences between the methods developed by the three institutes to measure airborne rat and mouse urinary allergen levels. Submitted.

	Institute		
	NHLI	WAU	NIWL
Sampling method			
inhalable dust sampler	seven-hole	IOM	IOM
PTFE filter	pore size 1.2 mm	pore size 1.0 mm	pore size 1.0 mm
Elution method			
buffer	2 ml 0.1 M NH_4HCO_3 + 0.5% Tween 20	2 ml 0.15 M PBS	1 ml 0.15 M PBS + 0.5% Tween 20
method (extracts were all stored at -20°C)	vortexed, centrifuged, and lyophilised. Reconstituted in PBS + 0.3% w/v HSA before assay to get 10 fold concentrated extract	vortexing 2 min, sonicating 2 min, vortexing 5 min, sonicating 2 min and centrifuged	rotation 1 hour, filter was discarded and 1% w/v BSA was added
RUA immunoassay			
immunoassay	competitive inhibition radioimmunoassay (RIA)	enzyme immunoassay (EIA) sandwich	EIA-sandwich
rat standard preparation (urinary proteins)	from male, post-pubertal Wistar rats	from young/old and male/female Wistar rats	*Rat n* I from 3-4 month male Sprague Dawley rats
antibodies	IgE pool of 8 rat allergic workers	polyclonal antibodies against RUA	monoclonal antibodies against *Rat n* I
detection limit assay	50 ng dry weight/ml	0.075 ng protein/ml	0.10 ng protein/ml
detection limit method	10 ng per filter (10.9 ng/m^3)	0.15 ng per filter (0.16 ng/m^3)	0.10 ng per filter (0.11 ng/m^3)
MUA immunoassay			
immunoassay	competitive inhibition RIA	EIA-sandwich	EIA-sandwich
mouse standard preparation (urinary proteins)	from male, post-pubertal mice	from young/old and male/female Balb/c mice	*Mus m* I from post pubertal male NMRI mice
antibodies	polyclonal antibodies against MUA	polyclonal antibodies against MUA	polyclonal antibodies against *Mus m* I
detection limit assay	0.5 ng dry weight/ml	0.075 ng protein/ml	0.10 ng protein/ml
detection limit method	4.0 ng per filter (4.3 ng/m^3)	0.15 ng per filter (0.16 ng/m^3)	0.10 ng per filter (0.11 ng/m^3)

Statistics (I- VII)

Calculations and statistical analysis were performed using Microsoft® Excel (Microsoft Corp, Redmond, WA, USA) and Statview® (Abacus Concepts, Berkeley, CA, USA), with the addition of EpiInfo, version 5 (USD Inc, Stone Mountain, GA, USA) (in paper III) or Minitab (Minitab Inc, State College, PA, USA) software (in paper IV). In paper VI, SAS (version 6.09; SAS Institute, Cary. NC; USA) was used.

For variables with skewed distribution, significance was tested with the non-parametric Wilcoxon signed rank test, Kruskal-Wallis or Mann-Whitney U-test as appropriate, otherwise Student's t-test. Discrete variables were tested with $\chi 2$-test (or Fisher's exact test). Skewed continuous variables are presented as median values (with 25th to 75th percentiles or range) or geometric mean, and normally distributed variables with mean value. To study correlation and agreement between methods, linear regression and Bland-Altman (11) plot analysis was performed. In addition, 95% confidence intervals were calculated in papers III and VI. A *P*-value <0.05 was considered significant.

Results

Prospective study (I, II)

Comparison between exposed and matched non-exposed subjects

The prevalence of atopy and allergic symptoms had increased in both the exposed and unexposed groups at follow-up. The increase in reported asthma symptoms among exposed was statistically significant (from 1 person to 7, $P<0.05$). At follow-up, FEV_1 had decreased significantly, by an average 28 ml/year and VC by 12 ml/ year ($P<0.01$, paired t-test, combined groups). There were no significant differences between the groups in any of the tested allergy or lung function variables at follow-up.

Thus, in two matched groups of subjects with the same profession, exposure to laboratory animals *per se* did not elicit significant differences between the exposed/non-exposed groups during the follow-up time.

Sensitization and symptom development

Forty-three subjects had worked with laboratory animals ≥5 months at follow-up and were invited to participate. One 23 year-old female was investigated, but was subsequently excluded from the analyses, since objective tests for sensitization against the animal to which she was exposed (vole) were not available [1]. Two declined to participate, one had moved abroad and changed profession, and one could not participate for medical reasons.

All of the remaining 38 exposed subjects who participated in the follow-up were SPT negative to the laboratory animal hair extracts prior to employment. After a median laboratory animal exposure time of 18 months (mean 19 months, range 5-33), seven had developed a positive SPT against one or more of these extracts. Among the 30 technicians exposed to rats, six were skin prick test positive against rat (20%), and among the 23 exposed to mice, two were positive against mouse. Four SPT positive subjects were found to have measurable specific IgE in serum against rat and/or mouse urinary allergens. All RAST tests to other animals to which subjects were work exposed, were negative.

Six the 7 laboratory rodent sensitized, and another 2 subjects (altogether 8/38) reported at least one allergic symptom related to laboratory rodent exposure. Six had experienced nasal symptoms, 5 eye, 3 chest and 4 had skin symptoms. One SPT positive subject had rhinitis but was uncertain of the relationship to animal work. Most symptoms were mild. Of the three who reported chest symptoms (wheezing, tightness of the chest or coughing) during animal work, one had developed physician diagnosed laboratory animal asthma. The other two had not sought medical help.

The subject with the most hyperresponsive airways had experienced wheezing, but reported only urticaria at laboratory animal contact. She kept a laboratory rat at home as a pet and had high levels of rat specific serum IgE. She was subsequently put on asthma medication.

To summarise, 9/38 (24%) had developed animal work related symptoms (n=8), and/or specific IgE to the animals (n=7). The incidence of symptoms and sensitization was thus 13 and 12, respectively, in 100 person years in the first average 19 months of exposure.

Lung function and bronchial responsiveness

FEV_1 and VC values did not differ between the nine subjects with sensitization and/or symptoms against laboratory animals and the 29 subjects without, neither before first exposure, nor at follow-up. Neither was there any difference between the groups regarding change in FEV1 or VC from before first exposure to follow-up.

[1]Prior to exposure, she was non-atopic by both SPT (skin prick test) and Phadiatop, had no allergic symptoms, had an elevated total IgE level, and a PD20 value of 2.88 mg. After 8 months of 20 hours/month exposure in animal confinement facilities, she reported eye and nose symptoms, but only at contact with vole. She had developed a 3+ SPT reaction to birch pollen, yet Phadiatop remained negative, and her total IgE was elevated. The PD20 value was 0.53 mg.

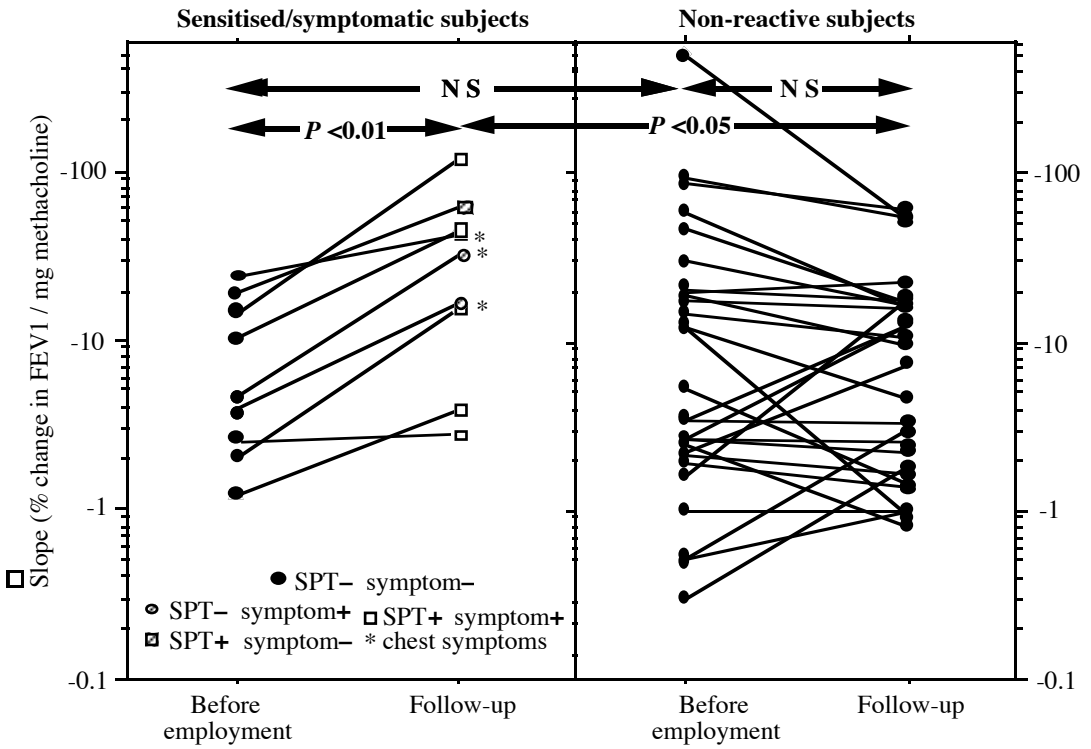

Figure 4. Slope values (% change in FEV1 per mg methacholine (cumulative dose) before employment and after follow-up in 38 laboratory animal exposed subjects. Modified from Renström et al, Eur Resp J 1995; 8: 1514-1519, European Respiratory Society Journals Ltd.

There were no differences before exposure between those who developed sensitization and/or symptoms against the animals and those who did not, with regard to bronchial responsiveness. However, at follow-up, sensitized/symptomatic subjects had significantly more reactive airways (PD20 or slope) than non-reactive subjects (P<0.05).

The sensitized/symptomatic subjects had lower PD values (P<0.01) and a steeper slope (P<0.01, figure 4) at follow up than before first exposure. Six of the 9 had a more than 3-fold increase in bronchial responsiveness.Bronchial responsiveness among non-LAA subjects was by average unchanged.

Thus early LAA was associated with increased bronchial responsiveness in most subjects. The level of pre-employment bronchial responsiveness did not influence the magnitude of change in responsiveness.

Predisposing factors for sensitization and symptoms

Several potential risk factors from the investigation prior to exposure were compared between the seven subsequently sensitized and the 31 non-sensitized. Neither pre-exposure SPT or Phadiatop positivity, total IgE, allergic symptoms, allergy in the family, smoking, exposure to fur pets, or lung function data were significantly different between sensitized/non-sensitized.

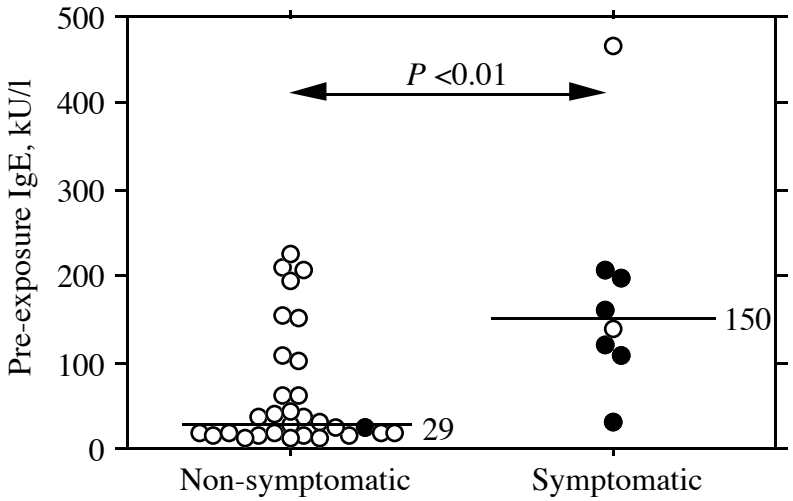

Figure 5. Pre-exposure total IgE levels in subjects with or without laboratory animal related symptoms at follow-up. Bars indicate medians. Filled circles = laboratory animal sensitized subjects. Modified from Renström et al, Allergy 1994; 49: 548-552, Munksgaard International Publishers Ltd.

There was a tendency of association between some indicators and subsequent sensitization, for instance total IgE (p=0.09, Mann-Whitney U-test). Total IgE was however significantly higher before exposure among the eight who developed symptoms against the laboratory animals compared to the 30 non-symptomatics, see figure 5. Of those who reported symptoms or were sensitized against laboratory animals, 7 out of 9 had pre-exposure total-IgE >100 kU/l. Among non-LAA subjects the number was 8 of 29 (P<0.01); relative risk for those with elevated total IgE was 5.4 (95% confidence interval 1.3-22). Paper II erroneously states other values (page 1518, second paragraph), which however does not influence the conclusions.
Positive predictive values were calculated (4) for development of symptoms. The positive predictive value (4, 103)was 0.44 for total IgE >100 kU/l for development of symptoms. For development of sensitization, the predictive values were 0.33 for total-IgE and 0.40 for previous rhinitis/conjunctivitis. The predictive value of family allergy was 0.17.

Table 4. Potential exposure risk or prevention factors for matched rat and/or mouse exposed sensitized/symptomatic ("LAA") and non-LAA subjects.

Risk/prevention factor	LAA n=9	Matched non-LAA, n=9	Significance P -value
Exposure, months	18 (15.5-25)#	22 (15-24)	N S
Exposure, hours per month	80 (20-160)	9 (4-36)	<0.05
≥60% male rodents	8	1¤	<0.01
Gloves often/always	5	6¤	N S
Mask often/always	2	1¤	N S
Use of vent. bench	3	4¤	N S
Organic solvent exposure	5	3	N S

median (25th-75th percentile) N S not significant ¤ n=8, due to missing answers

Exposure as a risk factor for sensitization and symptoms

The laboratory animal workers who developed sensitization or symptoms "LAA" (n=9) were not exposed for more months than those who did not (n=29). However, they had been exposed for more hours/month (median 80 and 18, respectively, $P<0.05$). Almost all sensitized/symptomatic subjects had an elevated total-IgE.

However, we wished to study potential exposure risk or protective factors *per se*, therefore the 9 were matched with non-LAA subjects with regards to pre-exposure smoking and elevated total IgE and/or positive Phadiatop.

Although this was a small study, we found that sensitized/symptomatic subjects worked for significantly more hours/month, and mostly with male rodents, see table 4. These factors were also associated ($P<0.01$). However, we can not exclude that factors not significant in this study, might be shown to be of importance in a larger study.
In the cohort of 38 laboratory animal exposed, 7 subjects had a combination of elevated pre-employment total IgE and exposure to mainly male rodents for more than 20 hrs/month. Of these, 6 had developed sensitization and/or symptoms, compared to none of the 8 with neither risk factor.

The results of the prospective study might be phrased thus: in workers newly exposed to laboratory animals, total IgE increased the risk to develop sensitization/symptoms; in combination with high exposure (many hours work with male rodents), most subjects were sensitized and/or symptomatic.

Selection

The 155 (139 women) laboratory technicians had at pre-exposure a median age of 21 (range 18-51) years. No significant differences are found between the prevalence of different indicators of allergic disposition among those who subsequently worked with laboratory animals (n=43) and those who did not (n=112). However, there was a tendency towards lower prevalence of positive SPT against common allergens among subsequently exposed compared to non-exposed subjects (21% vs 37%, $P=0.06$).

Cross-sectional study (III)

Subjects, and sensitization and symptoms to laboratory rodents

Of the 80 subjects (university staff subjects, n=48, and laboratory technician cohort, n=32), 70 had worked with rats an average 6.8 years (median 2.4 years) and 44 with mice for an average 5.8 years (median 1.9). All subjects worked with animals only in research department laboratories. Among the rat exposed subjects, 16 (23%) were sensitized to rat urine (spec IgE ≥0.2 kU/l). Of the mouse exposed, 5 (11%) were sensitized to mouse urine. Sensitization to one rodent was significantly related to sensitization to the other ($P<0.0001$), even without exposure to the other. Since there seemed to be immunological cross-reactivity between the species, rat and mouse exposure, sensitization or symptoms were pooled in some analyses.

Symptoms to rats were reported by 22 (31%) of rat exposed and symptoms to mice by 9 (20%) of mouse exposed. Sensitization to the rodents was significantly associated with expression of LAA symptoms (P<0.0001).

Aeroallergen measurements

The sample eluate detection limit for the amplified RUA assay (twofold diluted samples) was 40 pg/ml. For air filter eluates with undetectable RUA levels, the mean detection limit was 0.26 ng/m^3. Samples were collected in the animal facility for comparison with research department levels. RUA levels in personal animal work samples from the animal house (median (range) 1.5 (<0.26 - 5.3) ng/m^3, n=11) were higher than the personal animal work samples in the research departments (<0.26 (<0.26-0.52) ng/m^3, n=7), P=0.01. Also static samples were significantly higher in the animal house (0.25 (<0.26 - 13) ng/m^3, n=26) than in the research departments (<0.26 (<0.26 - 0.32) ng/m^3, n=19), P<0.001. The detection limit for the MUA assay was 0.1 ng/ml (two-fold diluted samples). For air filter samples with undetectable MUA levels, the mean detection limit was 0.8 ng/m^3. The differences between MUA levels in the animal house (median (range) <0.8 (<0.8 - 26) ng/m^3, n=21) and research department (<0.8 (<0.8 - 1.1) ng/m^3, n=13) did not reach statistical significance.

Thus the rodent aeroallergen levels in the research departments were very low, and few research department samples were measurable.

Sensitization and symptoms at low exposure

The possibility to avoid sensitization was studied among staff with ≤4 years exposure, working in research department laboratories with special ventilation solutions, such as ventilated benches or cabinets (26 of totally 48 with ≤4 years rodent exposure). Three subjects had developed specific IgE to rats: all 3 had worked with mostly or exclusively male rodents and had a total IgE ≥100kU/l and/or a positive Phadiatop test. Two were from the prospective study, and were skin prick test negative to rodents prior to first exposure. Six of the 26 reported symptoms to rodents - all worked with ≥60 % male rodents and/or ≥10 hours/week and/or had a total IgE ≥100kU/l and/or a positive Phadiatop test.

Sensitization and symptoms were studied in research department subjects with long exposure who presently worked with rodents using ventilated cabinets or benches (24 of totally 32 exposed >4 years). Of these, 10 (42%) had specific IgE to rodents, some with very high levels. Twelve had experienced symptoms during animal work. Ten subjects with >4 years rodent exposure reported to have been rodent positive by skin prick test or RAST prior to this study, some more than a decade previously. Eight of these were found to be have rodent specific IgE in our tests, 7 of whom worked with ventilated benches.

Those with >4 years of exposure worked significantly more with male rodents (75% vs 48%, P<0.05), but not more hours/week with either rats or mice, than subjects exposed ≤4 years. Furthermore, a higher proportion was symptomatic (50% vs 21%, P<0.01) and sensitized (39% vs 10%, P<0.01) against the animals.

Table 5. Risk factors for sensitization (specific IgE ≥0.2 kU/l to rat and/or mouse urine) and allergic symptoms when working with laboratory rodents.

Risk factor	Sensitized ¤				Symptomatic			
	N and (%) sensitized **without** risk factor	N and (%) sensitized **with** risk factor	Prevalence Rate Ratio	95% Confidence Interval	N and (%) symptomatic **without** risk factor	N and (%) symptomatic **with** risk factor	Prevalence Rate Ratio	95% Confidence Interval
Smoking	16/63 (**25**)	3/16 (**19**)	0.74	0.24-2.2	20/64 (**31**)	6/16 (**38**)	1.2	0.58-2.5
Total IgE >100 kU/l#	7/59 (**12**)	10/20 (**50**)	4.2	1.8-9.6	13/59 (**22**)	12/20 (**60**)	2.7	1.5-5.0
Positive Phadiatop#	8/58 (**14**)	9/21 (**43**)	3.3	1.5-7.3	13/58 (**22**)	12/21 (**57**)	2.6	1.4-4.7
Total IgE >100 or pos Phadiatop #	4/48 (**8.3**)	13/31 (**42**)	5.0	1.8-14	8/48 (**17**)	17/31 (**55**)	3.3	1.6-6.7
Allergy in parents/ siblings¤	7/47 (**15**)	10/31 (**32**)	2.2	0.92-5.1	14/48 (**29**)	11/31 (**35**)	1.2	0.64-2.3
Allergy to pollen/dust¤	9/61 (**15**)	8/18 (**44**)	3.0	1.4-6.7	17/62 (**27**)	9/18 (**50**)	1.8	0.99-3.4
Allergy to other animals¤	8/65 (**12**)	9/14 (**64**)	5.2	2.4-11	17/66 (**26**)	9/14 (**64**)	2.5	1.4-4.4
Exposure ≥10 h/week¤	10/49 (**20**)	6/27 (**22**)	1.1	0.44-2.7	12/49 (**24**)	13/28 (**46**)	1.9	1.0-3.6
≥60% male rodents¤	3/30 (**10**)	13/44 (**30**)	3.0	0.92-9.5	5/30 (**17**)	21/45 (**47**)	2.8	1.2-6.6
No use of gloves¤	8/31 (**26**)	9/43 (**21**)	0.81	0.35-1.9	10/31 (**32**)	16/44 (**36**)	1.1	0.59-2.1
No ventilated bench¤	13/50 (**26**)	4/25 (**16**)	0.62	0.22-1.7	18/50 (**36**)	8/26 (**31**)	0.85	0.43-1.7

= 1 missing blood sample

¤ = 1 or more subjects have answered “don’t know” or not answered question

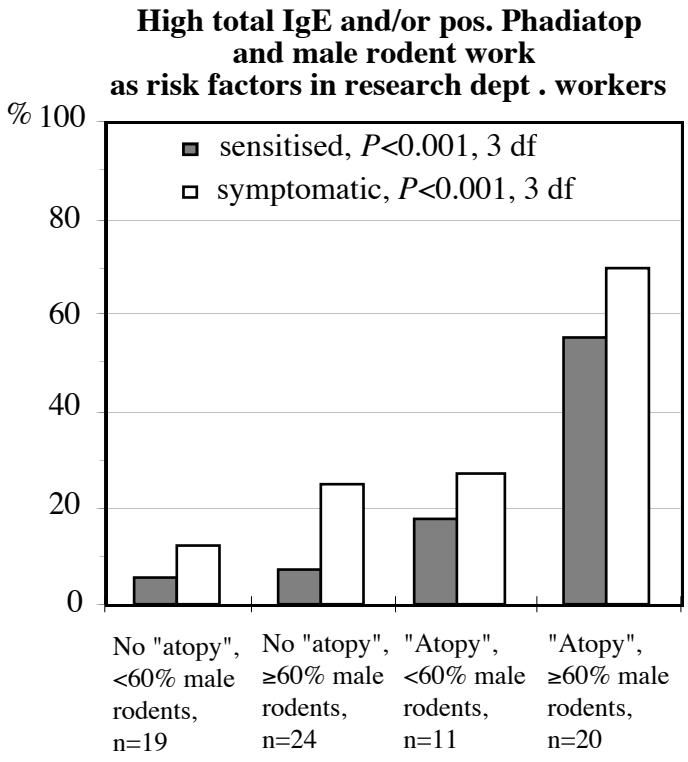

Figure 6. Elevated total IgE and/or positive Phadiatop ("atopy") combined with working with ≥60% male rodents as risk factors for sensitization and symptoms against laboratory rodents.

Relationship between atopy, exposure and sensitization

We found significantly more subjects who were sensitized or had experienced symptoms among those who were Phadiatop positive, or had an elevated total IgE or were allergic to other fur animals, but not for those with allergy in the family (table 5). Those working with mainly male rodents (≥ 60% male animals), had a higher symptom prevalence rate. However, working ≥10h/week was not significantly associated with rodent sensitization. Working ≥10 h/week and with male rodents was correlated ($P<0.01$). Research department personnel with a combination of elevated total IgE and working with male rodents had an 11-fold increased prevalence of sensitization and 6-fold increased prevalence of symptoms compared to subjects with neither risk factor (figure 6). All 7 subjects with a combination of elevated total IgE and/or positive Phadiatop, allergy to other fur animals and >60% male rodent exposure were sensitized, whereas only 1/21 with neither risk factor was sensitized. Use of gloves and/or ventilated benches were not, however, significantly associated with lower sensitization or symptom prevalence (table 5).

Aeroallergen measurement methods (III, IV)

Monoclonal RUA sandwich ELISA and measurements in a refurbished rat room (IV)

The properties of the monoclonal antibodies are presented in table 6. The MAbs were shown to be highly specific, and did not react to proteins from mouse urine (although they are 66% homologous (43)), nor to any of the other tested extracts derived from other than rats. The MAbs did recognise urinary antigens from other rat strains, and detected antigens in other rat extracts, such as rat room dust.

The detection limit for the sample eluates (diluted twofold) was 10 pg/ml, or 0.08 ng/m^3 for 1-hour samples collected at 2 l/min. In the unamplified assay, the detection limit was ten-fold higher, 0.1 ng/ml.

Samples were collected in a refurbished rat room in which a perspex screen had been installed, behind which were the cage racks. This had pores through which air was drawn to the outlet behind the screens. When the screens were closed, the RUA levels were very low, median 0.2 ng/m^3 (similar to corridor levels), significantly lower than if the screens were open (0.9 ng/m^3), or behind the screens (0.9 ng/m^3). During cage changing or cleaning the levels were however high (18 ng/m^3).

Polyclonal MUA sandwich ELISA (III)

The properties of the polyclonal antibodies and the MUA sandwich ELISA are summarized in table 6. Native SDS-PAGE showed that the standard consisted of Mus m 1, showing one strong band at 19 kd, and isoelectric focusing showed a group of proteins with pI values between 4.2 - 4.6. Antibody specificity tests showed strong binding to mouse serum (which contains Mus m 1), very weak binding to goat and sheep serum, and none to the other antigens.

The detection limit for the sample eluates (diluted twofold) was 0.1 ng/ml, or 0.8 ng/m^3 for 1-hour samples collected at 2 l/min.

Table 6. Properties of the murine monoclonal antibodies developed for use in the sandwich ELISA assay against RUA.

	MAb(6)	MAb(1)
Isotype	IgG1	IgG1
Specificity	Rat n 1.02	Rat n 1.02
	Rat n 1.01	Rat n 1.01 weak
Ab affinity constant to rat urinary protein	5.3 x 10^9	2.6 x 10^9
Epitope	Unique, present on Rat n 1 isoallergens	Unique (other), on Rat n 1 isoallergens
RUA sandwich ELISA role	”capture” Ab	”detection” Ab

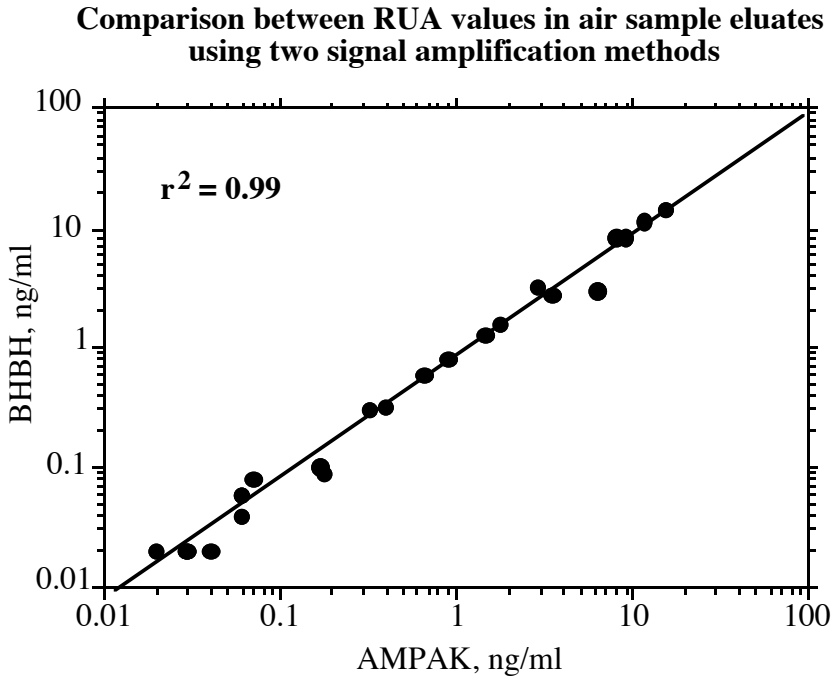

Figure 7. Comparison of RUA values obtained between the in-house amplification method (BHBH) and a commercial amplification method (AMPAK, Dakopatts).

Comparison between some different filters

Values obtained using different filters were tested in parallel samples (i e collected with pumps placed 15 cm apart). RUA or MUA values were found to correlate between 0.8 μm polycarbonate (PC) and polytetraflouroethylene (PTFE) filters: the median ratio PC/PTFE was 1.1, r^2=0.69 (n=15 pairs). PC filters were also compared with 0.3 μm Gortec filters (n=9 pairs) (Quan-tec-air Inc, Rochester, MN, USA) and although the values correlated well (r^2=0.98), Gortec RUA values were a median 1.9-fold higher. Different pore sizes of PTFE filters (0.5 and 1.0 μm, n=11 pairs) were compared; the median ratio between values obtained with 0.5 μm to 1.0 μm filters was 1.0 and the values correlated (r^2=0.90).

Comparison between values obtained using the in-house and a commercial signal amplification system

To compare the values obtained using the developed amplification system (BHBH), with values using a commercially available method, AMPAK (Dakopatts, Älvsjö, Sweden) (93), RUA concentrations were determined using both in 23 sample eluates. The values obtained were very similar, r^2=0.99, figure 7. However, in samples with high concentrations, the in-house method appears to be inhibited, resulting in low OD values. For this reason, and to avoid the need for dilution series to save reagent, samples are routinely first run in the unamplified method.

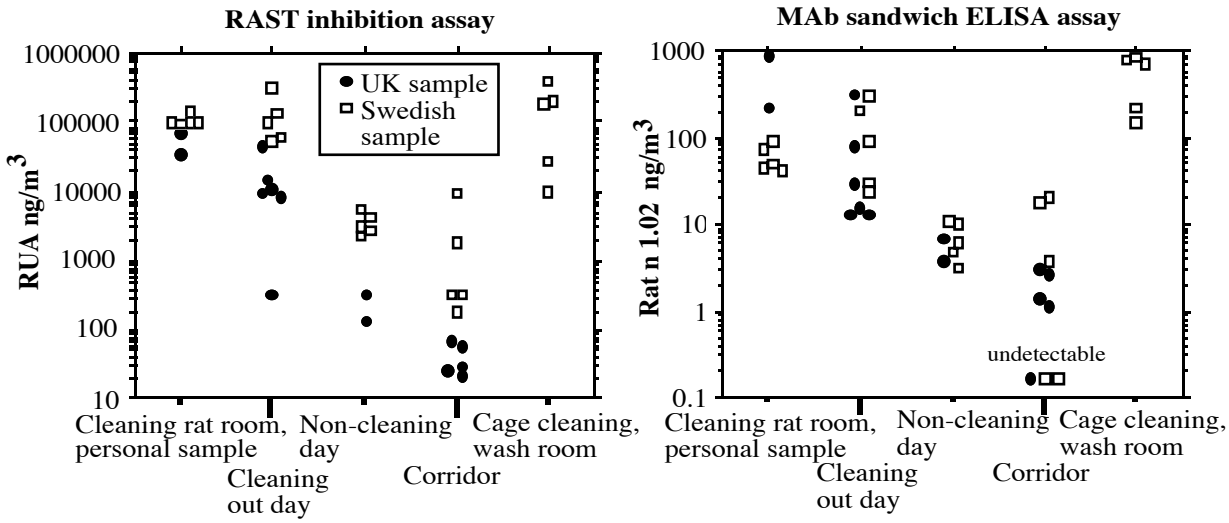

Figure 8. Nominal aeroallergen values using RAST inhibition and MAb sandwich ELISA, respectively. Modified from Clin Exp Allergy 1997; 27: 1314-1321, Blackwell Science Ltd.

Comparisons of methods to measure RUA and MUA (V-VII)

Comparison between RAST inhibition and monoclonal sandwich ELISA for RUA measurement (V)

Log RUA values obtained using RAST inhibition and MAb ELISA, respectively, were correlated (r^2=0.72, p<0.0001). However, the RAST inhibition values were several and varying orders of magnitude higher than the ELISA values; median (range) ratio RAST/ELISA for 37 eluates with measurable RUA levels, was 316 (7 - 2676). RAST inhibiton median (range) values were 9670 (21.6 - 384 000) ng/m^3 and ELISA median (range) values 22.2 (<0.8 - 861) ng/m^3 for all 40 samples.

Moreover, comparing samples collected in Sweden with samples collected in similar situations in the UK, the Swedish samples contained higher levels of RUA, if analyzed by RAST inhibition (median Swedish samples 31 700 ng/m^3, median UK samples 333 ng/m^3, p=0.01), but similar levels according to the monoclonal sandwich ELISA (median Swedish samples 27.1 ng/m^3, median UK samples 12.9 ng/m^3).

Albumin quantification of the sample eluates showed that RUA and albumin content were associated. However, in the samples with high albumin content, the RAST inhibition and the ELISA values were not correlated.

The rat urine standard extracts (concentrated, dialyzed Wistar or S-D rat urine, respectively) were antigenically similar. Both gave parallel curves in the assays, and both assays had a slightly stronger binding to its “own” standard extract. Using the other extract would result in a 2.8 or 1.3-fold shift in nominal allergen levels in the RAST inhibition and in the ELISA assay, respectively.

Table 7. Comparison between nominal values obtained by the RUA and MUA methods of the three institutes.

Compared methods	No. air samples#	Ratio, geom mean (95% confidence interval)	Correlation coefficient, r^2
RUA			
NHLI/WAU	40	3 000 (1 900 - 4 900)	0.31
NHLI/NIWL	56	1 700 (1 200 - 2 500)	0.35
WAU/NIWL	38	2.2 (1.6 - 3.1)	0.45
MUA			
NHLI/WAU	21	4.6 (2.3 - 9.1)	0.68
NHLI/NIWL	34	5.9 (3.5 - 9.8)	0.64
WAU/NIWL	32	1.6 (1.0 - 2.5)	0.80

samples with measurable levels by the methods of both institutes were compared.

Comparison between airborne RUA or MUA levels in filter eluates (VI)

The RUA concentrations in filter eluates found by the RAST inhibition (NHLI) was several orders of magnitude higher than those of the polyclonal (WAU) and monoclonal (NIWL) sandwich ELISA methods. The MUA levels of the polyclonal competitive inhibition RIA (NHLI) and sandwich ELISAs (WAU and NIWL) were more similar, as shown in table 7. The MUA method of the NHLI gave relatively higher values at low MUA levels and vice versa at high MUA levels when compared with the other two MUA methods. This suggests that the relationship between the MUA method of the NHLI and the MUA methods of the other two institutes is concentration dependent.

To test assay performance, 38 rat room filters and 22 mouse rooms filters were analyzed with respect to both RUA and MUA together with 18 blank filters (table 8). The NHLI and the NIWL RUA and MUA methods were more sensitive than the WAU method; however the NHLI method also detected allergen in several of the blank filters.

In order to study the influence of the WAU and NIWL elution methods and of the immunoassays seperately, the aliquots of eluates from the parallell filters were exchanged and analyzed. In order to account for reproducibility after storage (about 9 months), the “own” extracts were re-analyzed simultaneously. Re-analysis showed that the WAU RUA and MUA levels were 63 and 38%, respectively, of their previous levels. The NIWL RUA and MUA values were 77 and 109% of the previous values.

Comparison of elution methods (parallel filters extracted with WAU method compared to NIWL method) showed that using the NIWL elution (gentle rotation with 0.5% Tween 20) gave 10 and 5 times higher RUA and MUA levels, respectively, than WAU elution (vortexing, sonication, no Tween 20).

Comparison of immunoassays (by measuring the same eluate with both WAU and NIWL methods) showed that the polyclonal WAU RUA assay gave 4-fold higher levels than the monoclonal NIWL RUA assay. The two polyclonal MUA methods were again more similar, the ratio between WAU to NIWL immunoassay values was 0.9.

Study of potential factors influencing antigen detection in the RUA and MUA immunoassays (VII)

Initial comparisons of protein quantification methods showed that although values for dust extracts differed between Bradford (NHLI) and bicinchoninic (BCA) methods (WAU and NIWL), the rat and mouse urinary standard extract values were similar.

Table 8. RUA and MUA levels in filters from rat rooms and mouse rooms and blank filters, according to the methods of the three institutes. Modified from VI, submitted.

	Rat rooms			Mouse rooms			Blank filters		
Method	N (n.d.#)	median ng/m^3	pos. test %	N (n.d.)	median ng/m^3	pos. test %	N (n.d.)	range ng/m^3	pos. test %
RUA									
NHLI	38 (1)	6 960	97	21 (2)	330	90	18 (10)	<10.9 - 1290	44
WAU	38 (9)	0.62	76	22(20)	<0.16	9	17 (16)	<0.16 - 0.42	6
NIWL	38 (1)	2.1	97	22(17)	<0.11	23	18 (15)	<0.11 - 0.72	17
MUA									
NHLI	29 (11)	2.9	62	13 (1)	17.0	92	10 (7)	<4.3 - 5.6	30
WAU	38 (27)	<0.16	29	21(7)	3.0	67	16 (15)	<0.16 - 0.43	6
NIWL	38 (12)	0.29	68	22 (3)	17.3	86	18 (17)	<0.11 - 0.18	6

not detectable

The comparison between sample values obtained using an inhibition assay set-up and a sandwich ELISA set-up showed, that despite using identical reagents, inhibition gave values 7-fold higher than ELISA. Just as was observed in the MUA inhibition RIA vs sandwich ELISA sample values, the ratio between inhibition/sandwich decreased as the allergen level increased.

Immunoblotting of the RUA and MUA standards and the rat and mouse room dust samples, detected by the antibodies of the methods, showed that both the standard antigens and the antibody specificities differed slightly. Unfortunately, MAb specificities could not be demonstrated by the used methods. Pooled patient anti-RUA IgE bound to a 21 kd protein in the urine standards, which was not detected by the anti-RUA polyclonal rabbit Ab. Also, whereas the patient IgE bound diffusely to the rat room dust, the rabbit Ab gave distinct bands. Although the mouse urinary standards, which were also used to immunize rabbits, originated from different mouse strains of different ages, all Ab bound strongly with similar pattern to the 15-18 kd MUA antigens, and the WAU and NIWL Ab (which gave stronger binding) detected antigens at 44-50 kd.

The comparisons of RUA assay binding to reference standards and dust extracts showed that, generally, the different standards were detected similarly, as shown in figure 9. However, the Rat n 1 (NIWL standard) was not an efficient inhibitor in the NHLI RAST inhibition assay. Whereas rat room dust was detected similarly to urine in the RAST inhibition assay, it was detected with 690 and 760-fold less sensitivity than the urine standards in the polyclonal WAU and the monoclonal NIWL sandwich ELISA assays, respectively. The MUA assays also detected the urinary standards similarly, and in contrast to the RUA assays, mouse room dust was detected in all three assays with 30-52-fold less sensitivity than the urinary standards, see figure 10. All RUA and MUA assays were specific, and cross-reactivity to other extracts was weak enough so as to be of little, if any, practical importance.

Antigen aging tests showed that binding of artificially decayed urinary antigen was only slighly less (90-99% of the inhibition or optical density values) than that of “fresh” urinary antigen in the RUA or MUA assays.

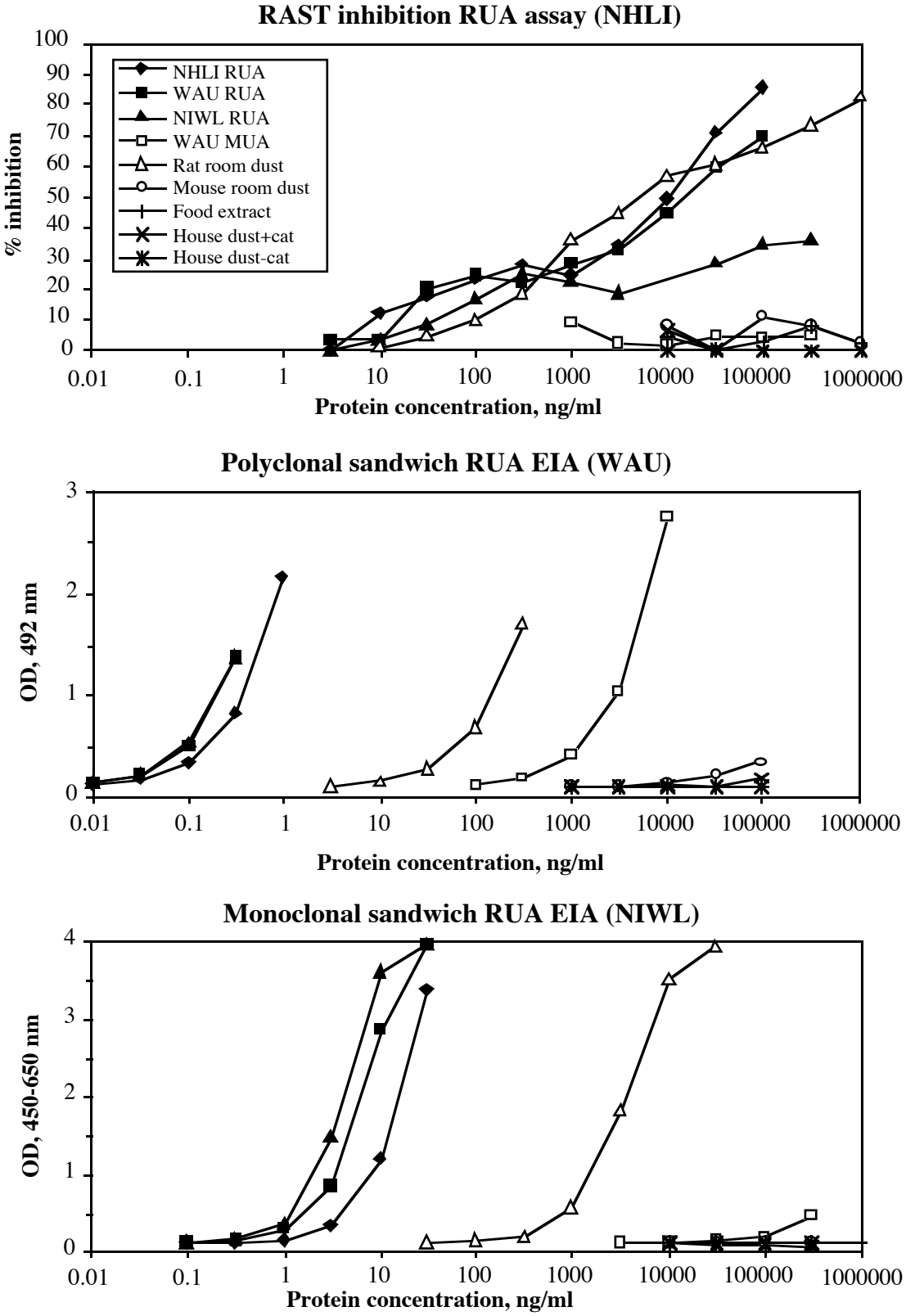

Figure 9. Antigen detection of different extracts in the 3 RUA assays. Submitted.

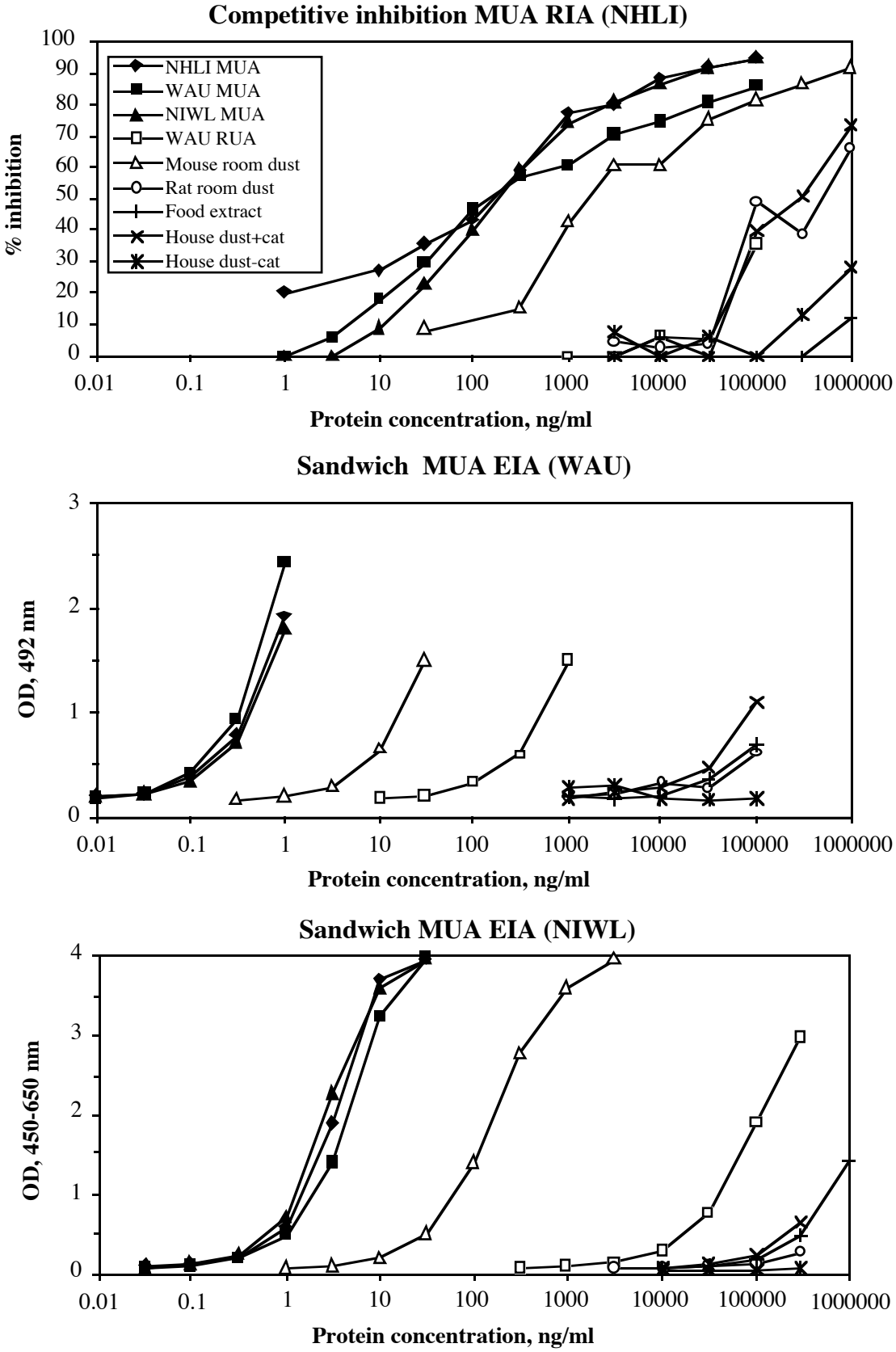

Figure 10. Antigen detection of different extracts in the 3 MUA assays. Submitted.

Discussion

Prospective study

A great number of cross-sectional studies on laboratory animal allergy have been published, presenting prevalence data. Only few prospective or follow-up studies have been undertaken (16, 17, 27, 54, 98, 100). In these studies, subjects with previous occupational or home exposure to these animals have been included in the analysis - Davies et al (27) mention that 54% of their subjects had such prior contact with animals. Thus these studies have followed a number of subjects over a specified amount of time, irrespective of previous duration of exposure, and recorded new cases of LAA.

One study involved 238 workers unexposed occupationally prior to current employment (25), but does not present information on pet ownership. The median exposure duration was 21 months, (range 1 month-less than 4 years), and 21% had developed work related symptoms, after a median time of 1 year, similar to our study. Pre-employment atopy and sensitization to laboratory animals were presented in one study, in which work or home exposure prior to employment in the company was disregarded. At 2 years of follow-up, the increased relative risk for atopics to develop sensitization or symptoms to animals was 7.5, and the relative risk was 7.6 to develop symptoms for subjects previously sensitized to animals (17).

In conclusion, to our knowledge no prospective study has been published, in which subjects have been investigated, presenting changes from pre-exposure to follow-up after the first few years of exposure. In order to obtain never-exposed subjects for the prospective study of laboratory technicians, the pre-investigation was carried out at an early stage of their education, since many students are exposed to laboratory animals during their trainee periods. Students from five out of six training schools in Sweden were invited, and 225 students participated in the pre-employment investigation. Of these, 168 completed their education and worked as technicians, 43 with laboratory animals. While most of the 38 who ultimately participated in the follow-up study had had dogs or cats, and a few had rabbits as pets as children, none had owned a pet rat or mouse, with the exception of one woman, who had a mouse for one year more than 20 years previously.

The design allowed a study of selection into laboratory animal work. Allergic diagnosis and SPT positivity were known to the subjects (the latter as a result of participating in our pre-exposure investigation), but they were unaware of their Phadiatop and total IgE status before starting to work. Interestingly there was a roughly doubled prevalence of pre-exposure SPT positives and atopics among non-animal workers (not significant).

A second consequence of the design was that it allowed longitudinal comparison with a well-matched unexposed group. This has to our knowledge not been reported before. The point was to investigate to what extent laboratory animal exposure *per se*, in a group of subjects with certain characteristics, would provoke allergy, immunology or lung function changes among the exposed, and among matched unexposed. We did find a tendency of increased general prevalence of allergic symptoms (to any allergens) in the exposed group (reported asthma symptoms were significantly increased). However, also non-exposed subjects showed similar increases in allergic symptoms.

The cause of this is enigmatic. Possibly the increase in symptoms reported by both groups at follow-up is partly a result of increased awareness as a result of participating in the study. However, environmental exposures other than laboratory animals (occupational or not) may also have contributed to the increase.

Development of sensitization and symptoms

All of the exposed subjects were SPT negative to laboratory animals prior to exposure. Only 16% were atopic according to Phadiatop, 10% had rhinitis or conjuctivitis and only one subject had experienced asthma symptoms, on the whole a healthy group of mostly young female students, rather less allergic or atopic than those of several other studies (16, 18, 25). Two years after graduation, they were followed up, and found to have worked with laboratory animals, mostly rodents, for about 1.5 years. In this short time, 18% had developed STP positivity to rodents, and 21% had experienced symptoms, mostly mild, during animal work. One individual, however, had developed symptoms such that she left after 14 months to pursue another career. Although some subjects were exposed to other animals, we found no indications of sensitization or symptoms against them. In accordance with Hollander et al (46), rats seemed to be more allergenic than mice.

Three of the technicians reported chest symptoms related to exposure to laboratory animals. The symptoms had been identified and treated as asthma in one subject at the time of investigation, and another subject, with high levels of specific IgE against rat, subsequently needed asthma medication.

Changes in lung function

The prospective design, with measurements prior to first exposure, allowed a more detailed description of changes induced by the animal allergens than would have been possible in a cross-sectional study. In six of nine sensitized and/or symptomatic subjects, a more than threefold decrease in PD20 was observed. This suggests that a majority of sensitized subjects with early reactions to laboratory animal allergens, develop increased bronchial responsiveness after a short time of work exposure. This has not been demonstrated earlier. Newill et al (72) reported an association between LAA and bronchial hyperresponsiveness, in a study where subjects who had reported chest symptoms at animal contact were excluded from the analysis. A significantly higher percentage of symptomatic subjects were defined as having bronchial hyperresponsiveness compared to non-symptomatic animal exposed. In the present study, it was confirmed that bronchial hyperresponsiveness can be demonstrated also in subjects who do not report chest symptoms. In a cross-sectional study by Hollander et al (47), peak-flow (PEF) measurements measurements were performed in subjects on days working with animals, and on non-animal working days in 209 subjects. No difference in PEF variability was found between days with and without animal contact, even among subjects with chest symptoms to rats (n=14), although PEF variability in these subjects was higher ($p<0.05$) than in subjects without chest symptoms. However, significant differences were found, with slightly higher PEF amplitudes (PEF max-PEF min) in rat sensitized (n=38) compared to non-sensitized subjects (n=169).

In a cross-sectional study by Willers et al (123), methacholine provocation was performed. The volume of trapped gas (a measure of small airways function) was found to be significantly increased in 7 workers highly sensitized (≥3+) to laboratory

animals compared to 30 non-sensitized laboratory animal exposed. In a 7-year follow-up study of subjects exposed 1-31 years at first examination (98), VC and FEV1 were, as in our study, no different between sensitized/non-sensitized, but bronchial responsiveness by methacholine provocation was significantly higher among STP positive subjects at follow-up, (also SPT positive at first examination), while unchanged among non-sensitized. However, the majority of sensitized (of which 5 asthmatics) had discontinued their exposure during the follow-up time. None of these studies, however, demonstrate an increase in responsiveness over time associated with sensitization to laboratory animals.

Altogether, the above results and ours suggest that bronchial responsiveness increases in most laboratory animal sensitized subjects, even before any lower airway symptoms appear. Possibly sensitization against laboratory animals, and the accompanying changes of the airways, may facilitate reactions to other airborne allergens, and induce non-specific bronchial hyperreactivity.

Exposure and LAA

Those who developed sensitization or symptoms worked significantly more hours/month with rodents. Nine sensitized/symptomatic ("LAA" subjects), were matched for atopy with exposed non-LAA subjects. Eight of the 9 LAA subjects had worked with mainly or exclusively male rodents, compared to only 1/8 non-LAA subjects. In the present literature of allergy to laboratory animals, one paper mentions rodent gender in the context that of 15 allergic to rats, 14 had been working with male animals (2). However, it was not stated whether that implied exclusive, main, or some exposure to male animals. We wished to further investigate whether exposure to male rodents constituted an overlooked risk factor in a larger group of subjects, with longer exposure time.

The fact that mature male rodents excrete potent urinary allergens in up to hundreds of times higher amounts than female rodents (39, 63, 64, 70, 73, 91, 94, 116) hinted strongly that male rodents could be more allergenic, but the role of animal gender as a risk factor has not been studied previously.

The working environment for laboratory animal exposed personnel had progressively been improved over the last decade in the university facility visited in the cross-sectional study, as in other facilities, with main ventilation improvements, animal facility refurbishments, the gradual installation of ventilated benches and cabinets, as well as with improvements in work routines and use of personal protective devices. The work allergen load is thus likely to have decreased over the years. As most air samples from research laboratories were below detection level (<0.26 ng/m^3 for RUA, <0.8 ng/m^3 for MUA, respectively), research staff working with special ventilation solutions, such as ventilated benches or cabinets, should be exposed to minimal allergen amounts. Furthermore, our aeroallergen methods were found to be at least as sensitive as those of other laboratories, especially when using amplification (papers IV and VI). Correcting for methodological differences, other laboratories have found higher allergen levels in both animal confinement buildings and research laboratories (49, 75), which seems to indicate that the levels found in the study were indeed low.

We studied whether it was possible to avoid sensitization among staff who had worked less than 4 years in research laboratories, using ventilated benches or similar solutions (n=26). The division at 4 years of exposure was chosen to conform with other studies (25, 48). We found that 3 (12%) were sensitized (all of whom were atopic

or had an elevated total IgE, and had worked with male rodents) and 6 (23%) reported symptoms .

Among all those exposed ≤4 years (n=48), 23% were either sensitized or symptomatic against the rodents, and 8% were both. Among those exposed >4 years (n=31) 48% were either/or and 39% were both sensitized and symptomatic. Possibly a number of the shorter exposed either sensitized or symptomatic, will eventually develop both, in accordance with one study (17), in which a positive SPT predicted a subsequent development of LAA with more than 80% accuracy.

There are several possible explanations for the higher prevalence of sensitization and symptoms against rodents noted among the longer exposed subjects. The low exposure may have lead to a delayed onset of sensitization, instead of the 1-3 years usually found (51). Additionally, although these subjects did not work more hours/week with rodents, they worked to a greater degree with male rodents. Also, this group may have had a higher exposure in the past. Interestingly, most of the long exposed LAA subjects had been skin prick test or RAST positive to rodents previously. Instead of healthy worker selection, which has been offered as the possible cause for fewer LAA subjects in high exposure groups (62, 117), these research department workers remained at work, despite having experienced symptoms and being aware of their sensitization (”stubborn workers”). Thus the exposure levels were perhaps low enough to continue working, with managable symptoms, yet high enough maintain sensitization. It is of course possible that additional staff have left because of LAA. However, in the follow-up study of Kibby et al (54), allergic subjects (especially college educated) were as likely as non-allergics to participate in the follow-up.

We did find indications that working with male rodents was an independant risk factor for LAA sensitization and symptoms. Subjects with both atopy and male rodent work had an 11-fold higher rodent sensitization prevalence compared to those with neither risk factor. Among these low exposed subjects, working with mainly male rodents appeared to be better correlated to LAA than working many hours/week with rodents. However, both factors were correlated ($P<0.01$). Similarly to a previous study (54), use of gloves and/or ventilated benches in this environment did not appear to protect from sensitization or symptoms. Conclusions are however uncertain, since LAA subjects might be more inclined to use such protection.

We found that sensitization to one of the rodents was significantly associated with sensitization to the other ($P<0.0001$). The reasons for this immunological cross-reactivity might be that the rodent allergens, both Mus m 1 and Rat n 1 and rodent albumins, are closely related (21, 120). Skin prick test cross-reactivity between rat and mouse allergens and between guinea pig and hamster allergens has been noted in another study (2).The observation that subjects allergic to other fur animals were at higher risk to be sensitized or symptomatic, is entirely in accordance with the results of another cross-sectional study (46).

In conclusion, exposure to male rodents seems to be an overlooked risk factor. We suggest that to further diminish allergen load, and LAA development, female rodents should be used when possible. Botham et al showed, in an improved working environment, that first year LAA symptom incidence decreased (but was not entirely abolished), yet LAA sensitization was high (16, 17). It is possible, that among our cross-sectional subjects with ≤4 years work in these low exposure surroundings, fewer will develop both sensitization and symptoms against the animals. However, the allergen level threshold for sensitization and maintenance of specific IgE appears low indeed.

Prediction of LAA

Elevated pre-exposure total-IgE levels gave the best predictive values for development of LAA in our prospective study. This was also indicated in both LAA asthma and test positive rhinitis groups compared to non-symptomatics in another of the few studies that have measured total IgE (100). Predictive values were generally low for the other parameters studied from before exposure. In earlier reports comparing several criteria of atopy (99, 103) results were similar. The presence of atopy (by any definition) or of elevated total IgE, correctly predict LAA to no more than, at best, 50%. Risk indicators must be highly sensitive and specific to result in useful prediction, even in a common occupational disease such as LAA. Thus if atopy or total IgE were to be used as a screening instrument prior to employment, many competent individuals would be refused employment, entirely unneccessarily. Therefore, judging from our results and others, both atopy and total IgE are too blunt instruments for personnel selection into general laboratory animal work.

We found high risks to develop LAA when host factors and exposure factors were combined. Hollander et al (48) showed that exposure intensity is of importance for LAA, with atopy as a strong effect modifier, resulting in steeper exposure-response curves. For example, an atopic individual may manage fine in a laboratory setting, with a few hours of work per week on female rodents, but may increase the risk to develop allergy considerably, if instead working many hours per week with male rats, or if working in animal rooms. The risk to develop LAA would probably increase further, if he/she was already allergic to other fur animals. An attempt to rank several risk factors and estimate predictive values for them (based on many studies, both cross-sectional and follow-up) is found in table 9.

Table 9. Estimated predictive values of some potential risk factors.

Risk factor	Predictive value (of +++++)
Smoking	(+)
Lung function, VC or FEV1	(LAA asthma +)
Bronchial hyperresponsiveness	+ (LAA asthma ++)
Family history of allergy	+(+) (allergic mother ++)
Personal history of allergy	++
Personal history of asthma	+++
Personal history of allergy to pets	+++
SPT positivity or spec. IgE to common aeroallergens	++
SPT positivity or spec. IgE to pets	+++
SPT positivity or spec. IgE to laboratory animals	++++
Phadiatop positivity	++
Total IgE ≥ 100 kU/l	++(+)
High allergen exposure (many hours/week and/or work with male rodents)	++
Total IgE ≥ 100 kU/l and high exposure to allergen	+++(+)
Allergy to pets and high exposure to allergen	+++(+)
Total IgE ≥ 100 kU/l and asthma to pets and high exposure to allergen	++++(+)

In conclusion, 1) positive IgE tests to laboratory animals predicts LAA with high accuracy; 2) different markers for atopy (Phadiatop positivity, positive SPT to common aeroallergens) or high total IgE are not sufficiently good predictors to be use dfor selection. However sensitization or allergy to other fur animals appears to be well correlated with LAA. Atopy and elevated total IgE appears to be a good predictor of senstitisation to laboratory animals in environments with high exposure to male rodents.

Development of methods to measure airborne allergens

Although exposure proxies, such as job title, or hours/week of animal work, provide valuable information, exposure measurements are also important in the investigation of laboratory animal allergy. Quantification of aeroallergens is also necessary to enable exposure surveillance or for the evaluation of ventilation improvements.

One of the aims was possibility of standardization. Therefore efforts were made to develop monoclonal antibodies against the major rodent allergens. MAbs against the highly allergenic Rat n 1 isoallergens were successfully produced, making it possible to establish an assay against primarily Rat n 1.02 (α_{2u}-globulin). The MAb sandwich ELISA thus captures a marker for allergen load, similarly to using monoclonals against Fel d 1 or Can f 1 to determine airborne cat and dog allergen levels (55). Utilising monoclonal antibodies in the detection of airborne rat allergens has to our knowledge not been described previously. Unfortunately, efforts to produce MAbs against Mus m 1 were not successful. However, as it transpired from the European comparison between methods to measure mouse urinary allergens, Mus m 1 has a dominant role in both urine, dust, and as an antigen. Therefore, polyclonal methods, even if based on different mouse strains, performed with different set-ups and methods of detection, gave similar values.

Some studies indicated that low exposure to allergens might be of clinical importance: Eggleston had showed that low Rat n 1 levels may provoke immediate reactions in sensitized subjects (30), and in another study, repeated inhalation of very low (subclinical) doses of allergen gave increased airway responsiveness in allergics (52). Therefore, another goal was to devise sensitive methods. An in-house method of signal amplification was developed using a newly synthesised amplifier, which further increased sensitivity tenfold. This amplifier is also used in this laboratory for the measurement of cat aeroallergens in schools, sampled with personal pumps at 2 l/min (60). The amplification method was found to give values which corresponded very well with those obtained when using a commercial amplification kit. However, despite using amplification to assess RUA in the cross-sectional study, most samples from laboratories were below detection limit.

Comparisons between different aeroallergen measurement methods

The first comparison between measurement methods was initiated since RUA levels in animal confinement buildings reported by another laboratory, using RAST inhibition, differed greatly from the values found in our laboratory. The study, in which 40 samples were collected, aliquoted and analyzed by both methods, gave a 300-fold difference between values in one and the same extract, although the correlation was good (except in samples with high albumin content). Furthermore, in the RAST inhibition assay, the samples collected and eluted in Sweden contained higher RUA

levels than the UK samples, whereas according to the MAb ELISA method, the levels of Rat n 1.02 (α_{2u}-globulin) were similar.

The variations between methods in sampling and elution (buffers, types of filter, freeze-drying etc) might account for some of the difference in the levels observed between UK and Swedish samples. However, the factor thought to be of most importance for the RUA elution, the concentration of Tween 20 (38) in the elution buffer, was identical in both protocols.

The main explanation of the great difference in values must thus lie in the specificities of the antibodies, and the fundamental differences in assay design.

One method used patient serum and the other monoclonal antibodies detecting Rat n 1.02; other allergenic proteins deriving from for instance fur or saliva may be present. However, Rat n 1.02 is a dominant urinary protein and was shown to be present and stable in both airborne and accumulated animal house dust (40), so this difference was estimated to influence the resulting values to a minor extent.

The methods might detect dust and urine differently, since dust was a fivefold more potent inhibitor than rat urine in the RAST inhibition assay, in a prior observation made by Gordon et al (40), and this would result in relatively higher nominal RUA values. In the sandwich ELISA assay, for detection of rat urinary antigen, the protein must contain two conserved epitopes orientated spatially so that each MAb may bind. Little is known about the stability of these epitopes in the occupational environment, or during the collection, elution and assay procedures. Degradation of the proteins might potentially result in a relative increase of RUA in an inhibition style assay, and a relative decrease in a 2-site binding assay. These queries were further investigated in the 3-country study.

In the RAST inhibition, the presence of large amounts of IgG, some of which is specific for RUA (15, 82), might influence the values by competing with the specific IgE for available allergen. It can be estimated that in the standard curve wells, each IgE antibody is surrounded by from a few to half a million urinary antigen molecules, and thousands of IgG molecules. In another RAST inhibition assay described by Eggleston (31), IgG was removed by precipitation with goat-antihuman IgG, and Rat n 1 was used as the standard extract. By this method, levels were more similar to those found in our laboratory.

To summarise, we demonstrated that it was difficult to compare air sampling values from different laboratories, and that several factors might influence the nominal values.

In the European Concerted Action project, the need for further study was recognized and both RUA and MUA methods were compared. These studies provided additional insight about the factors influencing antigen detection and resulting sample values, and a tentative recommendation for future aeroallergen sampling and analysis could be suggested.

Filters were collected in all three countries in triplicate, each country received one set of filters (including blank filters), which were eluted and aliquoted, so that also aliquots could be exchanged and analyzed by another country's method. The analysis of parallel filters verified the observation that the RAST inhibition method generated values several orders of magnitude higher than those obtained by sandwich ELISA: RUA values were 3000-fold and 1700-fold higher than those in the polyclonal and monoclonal ELISA, respectively. MUA levels, measured using polyclonal rabbit antibodies, were less different; inhibition RIA gave values 5-6-fold higher than sandwich ELISA. The NIWL RUA and MUA sandwich ELISAs gave values about two-fold higher than the WAU methods. The methods of the WAU and NIWL gave only a few false positive values,

i.e. RUA and MUA were detected in only a few blank filters, whereas the RUA method of the NHLI detected RUA in 44% of the blank filters. The reason for this might be the high variability of the inhibition assays. Furthermore, the detection limit of the WAU assay was determined by analysing extracts of 126 blank filters, and setting a cutoff value at the mean value obtained for the blanks +2 SD. The NIWL methods used the lowest standard point as detection limit, but the methods were highly reproducible also in the low concentration range. However, it is possible that not only the immunoassays, but also handling of filters and extracts or contamination between tubes or wells may give a positive blank.

The set-up of the sampling allowed for conclusions to be drawn about the importance of elution methods, and storage stability. The elution method of the NIWL gave approximately 10 and 5 times higher RUA and MUA levels than the elution method of the WAU, respectively. The major differences between the methods were agitation technique and the addition of 0.5% Tween, previously shown to increase RUA yield (38). One study on proteins derived from potatoes (125), found that sonication plus vortexing gave a 13% higher yield than gentle shaking. In our study the differences in elution between the WAU and NIWL methods are therefore probably due to the addition of Tween in the elution buffer of the NIWL.

The polyclonal RUA immunoassay of the WAU gave approximately 4 times higher values than the monoclonal NIWL method. These differences could not be explained by type of immunoassay used, because both institutes used a sandwich immunoassay. In contrast to the RUA immunoassays, the polyclonal MUA immunoassays showed no significant difference in allergens levels between the WAU and NIWL assay. The differences between the RUA levels derived from the WAU and NIWL immunoassays are therefore probably due to the type of antibodies used.

Furthermore, the methods of the WAU showed a large decrease in allergens level after a storage period of the extract of 9 months at - 20°C. This decrease was not present in the extracts of the NIWL, which was probably due to the addition of BSA and Tween to the elution buffer (119).

In the further investigation of factors influencing antigen detection in the assays, the protein quantification methods of the laboratories were compared, the Bio-Rad Bradford and the Pierce BCA method. The latter gave higher values, especially for undialyzed dust extracts, which contained partially degraded proteins, peptides or uric acid etc, that may also be detected or interfere in the BCA assay (105) (56). Moreover, in Bradford assays, the standard used, BSA, gives about twofold higher values than many other proteins (80). For experiment consistency, protein was quantified using the Pierce BCA kit, by one individual in one laboratory.

When using identical antibodies and standard extracts in a sandwich EIA and an inhibition EIA, respectively, the set-up of the assay contributed to a 7-fold difference between nominal RUA values. One reason for this phenomenon could be that antigen epitopes may be presented differently in solution compared to if bound. Binding to a plate or disk changes the three-dimensional structure of the molecules (14).

The Western blots showed that the specificities of the anti-RUA antibodies differed, whereas the MUA antibodies bound with similar pattern to the antigens. We speculate that the ability of the Mus m 1 affinity purified antibodies (NIWL) to bind to the 44-50 kd allergens, suggests that these might be dimer forms of Mus m 1.

In agreement with the previous comparison, all concentrated, dialyzed urine standard extracts were detected similarly in the MUA and RUA assays. However, the Rat n 1 standard used in the MAb assay was inefficient as an inhibitor in the RAST assay

compared to the other urine standards, indicating that a significant amount of the patient pool Ab were specific for other rat urinary proteins, which was confirmed in the Western blots.

However, the major difference between RUA RAST inhibition and sandwich EIA assays, was in the detection of rat room dust. Whereas the sandwich methods were 700-800-fold less sensitive to this extract, it was detected with the same potency as rat urine by the patient serum pool. If the dust extract closely approximates air sample eluates, this could explain a major part of RAST inhibition sample values being higher than EIA values. The dust extract (containing dust from bedding, food, faeces, hair, etc) was as potent an inhibitor in the RAST assay as rat urinary allergen by protein content (as measured with Pierce BCA method). Food and mouse room dust extracts did not react in the RAST inhibition, suggesting that the antigens are rat-derived, perhaps rat dander or hair play a part. A further explanation is that urinary antigens from dust might be presented in a form more readily recognized by the patient pool IgE, since the patients' immune system developed Ab to inhaled dust, rather than dialyzed urine. Conversely, the rabbit or mouse Ab were from animals actively immunized with mouse and rat urinary proteins. Although the EIA methods may measure the present rat urinary allergens in air samples, they may underestimate the clinical allergenic potency of an air sample.

In the rabbit polyclonal MUA assays, detection of urine standards was similar. In contrast to the RUA assays, all bound to mouse room dust with 30-50-fold less sensitivity than to mouse urine. All RUA and MUA assays were specific; binding to other allergen extracts was weak enough so as to be of very limited, if any, practical importance for the measurement of RUA or MUA in air samples.

Swanson et al (112), measuring airborne mite allergens in samples using monoclonals, rabbit polyclonals or human sera as detection antibodies, found that human sera gave about twentyfold higher values than the others. The antibody source thus affects the dynamics of an immunoassay. A MAb has a specific affinity to one epitope, whereas rabbit polyclonal or patient serum antibodies display a spectrum of affinities and epitope specificities. The antigen-antibody curve in a polyclonal assay is thus the sum of several simultaneous dynamic equilibriums.

The inhibition standard curves are considerably less steep than the sandwich EIA curves. As a result, a small difference in inhibition will give a large difference in nominal allergen level, and a larger measurement variability.

In the "aging" experiment, all RUA or MUA assays detected decayed antigen marginally less than fresh antigen. Aging is thus unlikely to greatly affect the nominal values. It is, however, possible that the accelerated degradation protocol used is not representative of natural antigen breakdown.

In conclusion, large differences can be found between the various methods to measure aeroallergens. For estimation of total allergen exposure, for clinical purposes, polyclonal assays may be useful. However, to enable comparison between epidemiological or allergen control studies, and certainly before any meaningful occupational exposure limits can be proposed, standardization is necessary. We found several factors contributing to differences in nominal levels of allergen measured in air samples. The type and source of the antibodies accounted for the greatest discrepancy between the RUA methods, because of their different specificities and affinities. Assay set-up also contributed, with inhibition giving about 7-fold higher values. The use of Tween and BSA enhanced extraction efficiency up to about tenfold and improved stability during storage.

However, what is measured by any immunological method is a marker of allergen load: the amount of a certain molecule or molecules detected by a set of antibodies in an extract of what has been eluted from a filter. It is not the actual dose of airborne allergen inhaled and potentially reacting with the immune system of an individual.

Suggestions for standardization of assays

The comparison of assays performed in these studies were the first steps towards standardization. In general, the differences between the methods were systematic and conversion factors may be used to compare or exchange data of already performed exposure measurements. However, there are several limitations of the use of conversion factors. For instance, inhibition gave a deviation from a linear relationship when compared with sandwich ELISA, giving a higher inhibition to sandwich value ratio at lower concentrations and vice versa, the reasons for which are yet unclear. Additional limitations are differences in sampling strategy, and definitions of job titles and tasks. For future sampling, standardization is preferred.

The ideal assay would combine simplicity, sensitivity and reproducibility over time with a good correspondance to clinical symptoms provoked by the environment. Standardization has in the last decade or so been achieved by the use of monoclonal antibody based assays, such as the commercially available airborne allergen ELISA assays for cat allergen Fel d 1 (20). Unlike the cat where there is thought to be one major allergen, Fel d 1 (65), the rat is more complex and three major allergens have been identified (40). Rat n 1.02 is however an ideal candidate for a rat allergen marker protein as 1) it is a major allergen for nearly all rat allergic subjects (39, 49) 2) it is present in both airborne and accumulated animal house dust (40) and 3) there is knowledge of some of the factors which may influence its excretion, such as the age and sex of the rats (39). In the case of RUA, the described MAb assay is specific to this allergen and it is possible to standardize both antibodies and standard extract. The disadvantage is that it does not detect several allergens present in rat urine or dust, thus potentially underestimating the clinical allergenic load. To our knowledge, no-one has produced MAbs against mouse urinary antigen. However, mouse urine has one dominant allergen, the Mus m 1 complex, and the three different polyclonal assays were found to give values within the same order of magnitude. Further standardization can be achieved by the aquisition of large amounts of both antibodies, standard extracts and controls, which should be well characterised, and using them in a sandwich ELISA. Furthermore, when necessary, sensitivity may be enhanced using commercially available or in-house signal amplification methods.

To conclude, to enable comparisons between studies, for allergen control or to establish thresholds for sensitization/symptoms, we recommend sandwich ELISAs against major allergens, preferrably using monoclonal antibodies. Standardization of polyclonal sandwich assays is possible using purified polyclonal antibodies and specified allergen extracts. Since the addition of Tween and BSA significantly increased elution and eluate stability we recommend its use in future aeroallergen filter sampling.

Prevention and legislation

According to the Swedish Work Environment Act (1977:1160, reprinted 1991:677), the employer bears the main responsibility for providing a safe workplace. Thus: “working conditions shall be adapted to people’s differing physical and mental aptitudes” (chapter

2, section 1) and “work shall be planned and arranged in such a way that it can be carried out in healthy and safe surroundings” (chapter 2, section 2). Furthermore, “the employer shall ensure that the employee acquires a sound knowledge of the conditions in which work is conducted and that he is informed of the hazards which the work may entail. The employer shall make sure that the employee has received the training necessary and that he knows what measures must be taken for the avoidance of risks in the work” (chapter 3, section 3). This is also prescribed by the National Board of Occupational Safety and Health (Arbetarskyddsstyrelsen) (7). Up to 1995, 4000 Swedish laboratory animal exposed personnel had undergone such education, in which information on laboratory animal allergy should be a natural part.

However, also the employee has responsibilities: according to the Act, the employee is under obligation to “take part in the implementation of the measures needed in order to achieve a good working environment. He shall comply with directions issued and use the safety devices and exercise such other precautions as are needed for the prevention of ill health or accidents” (chapter 3, section 4).

In AFS 1990:11, §12 states: “The employer shall offer employees, who routinely will work with allergy causing laboratory animals, medical examination prior to commencement of work. Also, the Swedish Work Environment Act, chapter 4, section 5, states “A prohibition may also be issued against the use in such work of any person whom medical examination has shown to be suffering from a disease of weakness rendering him particularly vulnerable to such a risk”. As indicated by the predictive values, host factors such as various indicators of atopy, are of little value as selection instruments. Selection of staff based upon prior atopy or allergy will not prevent the occurence of LAA, but would exclude a large proportion of subjects who will not develop LAA, from the workforce. The medical examination should instead be used to inform the worker of his/her particular risks, and encourage suitable preventive measures. However, individuals with pre-exposure sensitization to laboratory rodents, combined with a history of personal allergy or asthma against fur animals, should be discouraged from employment with high exposure to rodents, since they will be highly susceptible to developing LAA, which might also further exacerbate the prior allergy/asthma. A medical investigation should include tests such as skin prick tests or Phadiatop, total IgE and specific IgE to the laboratory animals in question. Also, if possible, bronchial provocation should be carried out.

The primary instrument for prevention of LAA should not be selection of personnel, but protection by minimizing exposure. In general, employers now seek to reduce environmental allergen exposure by installing efficient ventilation systems and ventilated laboratory benches, use of better cage cleaning practices and bedding material (36, 88, 109). One example is the sliding curtain system (described in paper IV), which improved the environment in the aisle of the animal rooms. Several other solutions are being evaluated, such as vacuum removal of litter, or ventilated cage systems (41, 90). We also showed that working with male rodents appears to constitute a higher risk than females (paper III), and recommend increased use of females, whenever possible.

Although we could not determine any preventive effects of glove use (possibly because allergics might be more inclined to use gloves), it is logical to assume that a consistent use of gloves (and long-sleeved laboratory coats) at least should prevent the appearance of LAA skin symptoms. Furthermore, sensitization via skin might facilitate the appearance of subsequent airway symptoms. In high-allergen load situations, and for subjects at high risk to develop LAA, use of filtered-air helmets is recommended (89, 104). Also cleaning and maintenance personnel must be informed of risks and

proper work routines. A study by Botham et al (16), showed that education, a medical examination, and improved work routines, indeed did reduce the percentage of subjects developing symptoms against laboratory animals in the first year of employment, from previously close to 40%, to about 10%.

Management of LAA

According to AFS 1990:11, "If an employee working with laboratory animals develops symptoms indicative of allergy or other hypersensitivity, the employer shall furthermore offer medical examination....The employee shall receive the information and the advice motivated by the result of the medical investigation".

If an individual has developed LAA, to prevent early, mild symptoms from developing into more severe symptoms or LAA asthma, several courses of action should be taken. The first priority would be to diminish allergen exposure, by increasing the use of personal protection and decreasing the amount of time in contact with animals as much as possible. If necessary, medical therapy may include antihistamines, steroids, decongestants or bronchodilators.

Also, the employer should be informed, and an inspection of the workplace might reveal flaws in animal handling routines which might be rectified. In Sweden, employees are required to report occupational injuries and diseases to the employer, and national statistics are collected at the National Board of Occupational Safety and Health, at the Occupational Injury Statistics Division. Between 1990-1996, 22 subjects involved with research reported LAA, and between 1992-1996 only one case of suspected anaphylaxis due to a mouse bite was reported (Börje Bengtsson, personal communication). Since investigations on LAA performed in Sweden have showed a prevalence of between 20-50% of sensitization or symptoms [(2, 10), Krister Iwarsson and Per-Åke Öhrsten, personal communication], this disease is seriously underreported.

Some LAA subjects cannot continue working with animals. In a follow-up study by Sjöstedt et al (98), 9/11 SPT positive subjects had stopped working with animals (including all who had developed asthma) compared to 7/24 SPT negative subjects.

A summary of recommendations for the prevention and management of LAA is provided on next page.

Recommendations
for management of allergy against laboratory animals

Preventive measures for new personnel

1. Information: regulations, risk factors, symptoms, treatment
2. Demonstration of general and personal protective equipment
3. Information on local laboratory and animal handling routines
4. Medical examination, which should include questionnaire, Phadiatop or skin prick tests, specific IgE to fur animals including the laboratory animals in question, total IgE, bronchial provocation if possible. This should be annually repeated.

Management of LAA

For the individual with LAA:

1. Decrease of exposure through
   - increased use of personal and general protective equipment
   - decreased exposure time
2. Medical examination, medication if necessary. Follow-up to assess improvement
3. Report to nearest superior, safety officer and to employer

(4. If necessary: relocation.)

For the employer:

1. Report occupational disease/injury to (in Sweden) the Occupational Injury Statistics Division at the National Board of Occupational Safety and Health
2. Investigate work routines, ensure availability of safety equipment
3. Long-term investment in improved working environment, e g of general ventilation, installation of automated cage washing and ventilated benches. Transport of animals should be performed with minimal allergen spread. We recommend 3 seperated work zones:
   Research laboratories and offices (no animal work)
   Animal research laboratories (restricted access, clothes change)
   Animal confinement area (animal caretakers only, clothes change)

Conclusions

- An elevated total IgE predicts the early development of sensitization or symptoms against laboratory rodents.

- Most subjects who are sensitized against laboratory rodents develop increased bronchial responsiveness.

- Subjects with intense exposure to male rodents (who excrete much higher levels of urinary allergen) are at increased risk to develop LAA

- Subjects with a combination of atopy or elevated total IgE, and relatively high exposure to rodents, highly risk to be symptomatic and/or sensitized against laboratory animals, even in “clean” laboratory environments.

- Immunological methods to measure rodent aeroallergen exposure may give nominal allergen levels that differ widely, yet are correlated, allowing conclusions to be generalised.

- The reasons for differences in measured levels are mainly due to assay set-up (inhibition/sandwich) and choice of allergen detecting antibodies.

- Use of Tween and BSA in the elution buffer improves elution and extract stability. If needed, assay sensitivity may be enhanced using in-house or commercially available signal amplifiers.

- Standardization of aeroallergen measurement methods may be achieved by the use of sandwich ELISAs with defined and sensitized antibodies and extracts.

Suggestions for future research

Longitudinal studies with repeated investigation and extended follow-up time are required to further explore several risk factors that have been suggested by these and other studies on LAA. One question is whether allergy or sensitization to other fur animals precedes LAA development, or is coincidental with LAA.

Atopics might differ from non-atopics with respect to exposure threshold for developing LAA. Furthermore, the threshold for sensitization or symptom development might differ, and additional information is needed about which levels of exposure may maintain already developed sensitization and symptoms.

The protective value of routinely using gloves or other personal protective equipment from the beginning of exposure have not been ascertained.

The pattern of exposure may perhaps be important, and subjects occasionally report development of symptoms at returning to exposure after a period of absence. This observation might indicate an effect on the immune system resembling development of symptoms to seasonal aeroallergens, and requires further study.

Another point of interest which might be adressed in longitudinal studies, is whether some individuals - and if so, which individuals - might initially react to animals but subsequently adapt through down-regulation of the immune response.

A number of studies have shown that, although being constantly exposed to high levels of allergen, fewer among animal department staff have LAA compared to low exposed research staff. This is usually attributed to healthy worker selection. However, it is conceivable, that animal house exposure is different than laboratory exposure, in that dust levels are higher, and that the dust may contain endotoxin or other agents which might induce immune responses of the TH1-type (with IFN-γ and IL-2 production) rather than TH2 (with production of IL-4 and IgE).

Initial genetic studies have suggested that certain patterns of genetic setup might decrease or increase susceptibility to LAA development, however, more research is needed in this area. Also, studies of the allergenic epitopes present on the rodent allergens, for instance the Rat n 1 or Mus m 1 isoallergens, may provide interesting information.

Summary

Anne Renström. Allergy to laboratory animals. Risk factors for development of allergy and methods for measuring airborne rodent allergens. *Arbete och Hälsa 1997; 26.*

Between 10-50% of workers exposed to laboratory animals, mainly rats or mice, develop laboratory animal allergy (LAA) with symptoms of rhinitis, conjuctivitis, asthma, or urticaria, and develop IgE against animal allergens. Symptoms often arise within the first years of animal work. Up to half of the symptomatic subjects have asthma. One aim of the thesis was to determine risk factors for LAA, especially in laboratory environments. A second aim was to develop methods to quantify aeroallergen exposure.

In a prospective study, 225 laboratory technician students were investigated. Two years after graduation, those who were laboratory animal exposed (median exposure 18 months) were re-examined (n=38). We found indications that atopics were under-represented among those who subsequently worked with animals. Seven exposed (18%) had skin prick test positivity (sensitization) to laboratory rodents and 8 (21%) had experienced allergic symptoms at animal work. Those sensitized and/or symptomatic (n=9) against laboratory animals had an increased bronchial responsiveness compared to at pre-exposure ($P<0.01$), and compared to exposed without sensitization or symptoms ($P<0.05$). Elevated total IgE ($P<0.01$) and hours/month of exposure ($P<0.05$), were both risk factors for LAA.

The risk of developing LAA in research departments with low exposure was investigated in a cross-sectional study (n=80). A fourth of those with ≤4 years of rodent exposure were sensitized or symptomatic whereas half of those with >4 years of exposure were sensitized or symptomatic. Risk factors for LAA were Phadiatop positivity, elevated total IgE, allergy to other fur animals and exposure to mainly male rodents. For subjects with both exposure to male rodents and elevated total IgE or positive Phadiatop, the prevalence of sensitization was 11-fold higher compared to subjects with neither risk factor. Thus, exposure to male rodents, who excrete up to hundreds of times higher levels of urinary allergen than females, may constitute an overlooked riskfactor. Even low exposure to allergen seems to maintain allergic symptoms and specific IgE.

To measure airborne rat urinary allergens (RUA), a two-monoclonal sandwich ELISA was developed, specific for the potent Rat n 1 isoallergens. An amplification method was developed, which increased sensitivity tenfold, to 0.1 ng/m^3 in one-hour air samples collected at 2 l/min. A polyclonal sandwich ELISA for mouse urinary allergen (MUA) measurement was developed against the main allergen complex Mus m 1, with detection limit 1 ng/m^3.

The ELISA RUA method was compared with a RAST inhibition method developed by a laboratory in the UK. Samples were collected in each country, and analyzed in both laboratories. The values were correlated (r^2 of log values =0.72, $P<0.001$) but the RAST inhibition values were about 300-fold higher than the sandwich ELISA values.

In a European Community study, methods to measure rat and mouse aeroallergens used in laboratories in the Netherlands (NL), UK, and Sweden were compared. Samples were collected in triplicate and were divided between the laboratories, where they were eluted and analyzed. RAST inhibition using patient serum gave RUA values

several orders of magnitude higher than those obtained using the sandwich ELISA methods. Polyclonal rabbit antibodies against MUA used in an inhibition RIA gave values 5-6 times higher than in the sandwich ELISAs. The NL polyclonal ELISAs were the least sensitive and the inhibition assays were the least specific of the assays. Use of polyclonal antibodies gave higher values than monoclonal antibodies. Addition of Tween and BSA increased elution efficiency and storage stability. A major difference between the assays was the reaction to rodent room extracts: whereas the RUA RAST inhibition detected rat room ventilator dust similarly to concentrated urine, dust was detected with 700-800-fold less sensitivity in the ELISA assays. The polyclonal MUA assays all detected mouse room dust with 30-50-fold less sensitivity to mouse urine.

We concluded that assay set-up and antibody specificity are the most important factors influencing antigen binding. A thorough standardization of methods to measure airborne allergen is necessary and may be achieved using a sandwich ELISA assay with defined and purified antibodies and standard antigens.

Key words: laboratory animal allergy, atopy, IgE, methacholine provocation, aeroallergen measurement, ELISA, monoclonal antibody, Mus m 1, Rat n 1

Sammanfattning

Anne Renström. Allergy to laboratory animals. Risk factors for development of allergy and methods for measuring airborne rodent allergens. *Arbete och Hälsa 1997; 26.*

Mellan 10-50% av personal som arbetar med försöksdjur, främst råttor och möss, utvecklar försöksdjursallergi (LAA), med symptom som rinit, ögonkonjunktivit, astma eller urtikaria (nässelutslag), och utvecklar IgE mot djurallergener. Symptomen uppkommer ofta under de första åren med arbete med djur och uppemot hälften av personerna med symptom har astma. En målsättning med studien var att bestämma riskfaktorer för försöksdjursallergi, särskilt i laboratoriemiljöer. Ett andra mål var att utveckla metoder att mäta halter av luftburna allergener.

I en prospektiv studie undersöktes 225 laboratorieassistenter under deras studietid. Två år efter examen undersöktes de som arbetat med försöksdjur igen (n=38, mediantid för exponering = 18 månader). Det fanns indikationer på att atopiker var underrepresenterade bland de som kom att arbeta med djur. Sju av de exponerade (18%) hade utvecklat pricktestpositivitet (sensibiliserats) mot gnagare och 8 (21%) hade haft allergiska symptom i samband med arbete med djuren. De sensibiliserade och/eller symptomatiska (n=9) hade fått ökad bronkiell reaktivitet jämfört med vid den första undersökningen ($P<0.01$), och jämfört med icke överkänsliga exponerade ($P<0.05$). Förhöjt total-IgE ($P<0.01$) och antal timmar per månad med exponering ($P<0.05$) var riskfaktorer för LAA.

Risken att utveckla LAA i laboratorier med låga exponeringsnivåer undersöktes i en tvärsnittsstudie (n=80). En fjärdedel av personal med ≤4 års exponering för råttor eller möss var sensibiliserade eller symptomatiska jämfört med hälften av de med >4 års exponering. Riskfaktorerer för LAA var Phadiatop-positivitet, förhöjd total-IgE-nivå, allergi mot andra pälsdjur, och arbete med mestadels hanråttor eller hanmöss. Bland personal med både exponering för handjur och förhöjt total-IgE eller positiv Phadiatop påvisades en 11 gånger så hög förekomst av sensibilisering mot djuren, jämfört med personer utan dessa riskfaktorer. Exponering för handjur, som utsöndrar flera hundra gånger högre allergenmängder än hondjuren bland gnagare, tycks vara en förbisedd riskfaktor. Även mycket låga allergenhalter kan vidmakthålla allergiska symptom och specifikt IgE mot djuren.

För att kunna mäta luftburna allergener från råtturin (RUA), utvecklades en monoklonal sandwich-ELISA, där de två monoklonalerna var specifika för de potenta Rat n 1-isoallergenerna. En amplifieringsmetod utvecklades, som ökade känsligheten i testet tio gånger, till 0.1 ng/m^3 i en-timmes prover insamlade med ett luftflöde på 2 l/min. En polyklonal sandwich-ELISA för att mäta musurinallergen (MUA) utvecklades mot det huvudsakliga allergenkomplexet Mus m 1, med en detektionsnivå på 1 ng/m^3.

Metoden att mäta RUA jämfördes med en RAST inhibitionsmetod utvecklad i ett laboratorium i Storbritannien. Luftprover samlades in i båda länderna, och analyzerades av båda laboratorierna. Värdena var korrelerade (r^2 för logaritmerade värden = 0.72, $P<0.001$), men RAST-inhibitions-värdena låg 300 gånger högre än sandwich ELISA värdena.

I en EG-studie jämfördes metoder att mäta luftburna allergener från råtta och mus utvecklade i laboratorier i Nederländerna, Storbritannien och Sverige. Luftprover samlades in i triplikat och delades upp mellan laboratorierna där de extraherades och

analyzerades. RAST inhibition, där man använder patientserum, gav RUA-värden som var flera tiopotenser högre än de som erhölls med sandwich-ELISA metoderna. Polyklonala kaninantikroppar mot MUA som användes i en inhibitions-RIA gav värden 5-6 gånger högre än i sandwich-ELISA-metoderna. Nederländernas polyklonala ELISA-metoder var de minst känsliga och inhibitions-metoderna de minst specifika av metoderna. Användning av polyklonala antikroppar gav högre värden än monoklonala antikroppar. Tillsats av Tween och BSA ökade extraktionseffektivitet och lagringsstabilitet. En stor skillnad mellan metoderna visade sig i reaktionen mot extrakt från djurrum: RAST-inhibitionsmetoden påvisade råttrumsdamm och koncentrerat råtturin på likartat sätt, medan dammextraktet detekterades med 700-800 gångers mindre känslighet än råtturin i sandwich ELISA-metoderna. De tre polyklonala MUA-metoderna påvisade musrumsdamm med 30-50 gångers mindre känslighet än musurin. Vi drog slutsatsen att val av metod (inhibition eller sandwich), samt specificiteten hos antikropparna, är de viktigaste faktorerna som påverkar antigensbindningen. En genomgripande standardizering av metoder är nödvändig och kan åstadkommas genom användning av en sandwich-ELISA med definierade och renade antikroppar och standardextrakt.

Nyckelord: försöksdjursallergi, atopi, IgE, metakolinprovokation, luftprovtagning, luftburna allergener, ELISA, monoklonala antikroppar, Mus m 1, Rat n 1

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12 **R-M Högström, M Tesarz, T Lindh, F Gamberale och A Kjellberg.** Buller – exponering och hälsoeffekter inom kraftindustrin.

13 **G Lidén, L Kenny, D Mark och C Chalmers.** Provtagningseffektivitet för den svenska metoden för mätning av totaldamm.

14 **B Lindell.** DECOS and NEG Basis for an Occupational Standard. Platinum.

15 **A Iregren, B Sjögren, M Andersson, W Frech, M Hagman, L Johansson och A Wennberg.** Exponering för aluminium i smältverk. Effekter på nervsystemet.

16 **L Punnett and U Bergqvist.** National Institute for Working Life – Ergonomic Expert Committee Document No 1. Visual Display Unit Work and Upper Extremity Musculoskeletal Disorders. A Review of Epidemiological Findings.

17 **M Sundström.** Arbetsskadeförsäkringen – bedömningen i domstol av belastningsskador hos kontorister och sjuksköterskor.

18 **E Åhsberg.** Upplevd trötthet efter mentalt arbete – en fältstudie.

19 **U Bergqvist and E Vogel (eds), L Aringer, J Cunningham, F Gobba, N Leitgeb, L Miro, G Neubauer, I Ruppe, P Vecchia and C Wadman.** Possible health implications of subjective symptoms and electromagnetic fields. A report prepared by a European group of experts for the European Commission, DG V.

20 **B Beije and P Lundberg.** DECOS and NEG Basis for an Occupational Standard. Glutaraldehyde.

21 **G Aronsson och L Svensson.** Nedvarvning, återhämtning och hälsa bland lärare i grund- och gymnasieskolan.

23 **Z Wang**. Acute Cytokine Responses to Inhaled Swine Confinement Building Dust.

24 **Kriteriegruppen för hygieniska gränsvärden. Ed. P Lundberg.** Vetenskapligt Underlag för Hygieniska Gränsvärden 18.

25 **Criteria Group for Occupational Standards. Ed. P Lundberg.** Scientific Basis for Swedish Occupational Standards XVIII.

26 **A Renström.** Allergy to Laboratory Animals. Risk Factors for Development of Allergy and Methods for Measuring Airborne Rodent Allergens.

27 **C Sconfienza och F Gamberale.** Unga mäns och kvinnors arbetssiuation.