

A new amplified monoclonal rat allergen assay used for evaluation of ventilation improvements in animal rooms

Anne Renström, BSc, Per H. Larsson, MSc, Per Malmberg, MD, PhD, and
Cecilia Bayard, BSc Solna, Sweden

Background: Available methods for the measurement of airborne laboratory animal allergens are not standardized and are often insufficiently sensitive for measurements in laboratories or in undisturbed animal rooms. Although low, the levels may be clinically relevant, because many scientists not involved in cleaning out cages or handling animals have rodent allergies.

Objective: The purpose of this study was to develop a sensitive monoclonal assay to determine airborne rat allergen and test it in the evaluation of a sliding curtain system installed in refurbished rat rooms, with perforated, transparent polycarbonate screens, behind which were the cage racks.

Methods: Monoclonal antibodies were produced against male rat urine by immunization in mice and fusion with mouse cell line Sp2/0. A novel biotinylated phenol compound was synthesized for immunoassay signal amplification in conjunction with horseradish peroxidase. Air filter samples were collected at a rate of 2 L/min, and allergen content in the filter eluates was determined.

Results: Two monoclonal antibodies were produced and used in a sandwich ELISA, which bound α_{2u} -globulin (Rat n 1.02). The assay detection limit was 3.2 pg/ml, about tenfold increased sensitivity compared with the unamplified assay. Allergen levels were lower in rooms when curtains were closed (median, 0.2 ng/m³) than behind the curtains (0.9 ng/m³, $p = 0.01$) or if the curtains were open (0.9 ng/m³, $p = 0.001$). However, allergen levels during cage cleaning, when curtains were drawn apart, were high (18 ng/m³).

Conclusion: We have developed a method for measurement of airborne rat allergen that can be standardized, measures an important allergen, and is sufficiently sensitive to measure low allergen levels with personal samplers. (*J Allergy Clin Immunol* 1997;100:649-55.)

Key words: Air sampling, ELISA, laboratory animal allergy, monoclonal antibody, rat urinary allergen, α_{2u} -globulin

Exposure to laboratory animals is common among personnel in the fields of Medicine and Biology; an estimated 100,000 persons in the United States,¹ 32,000 persons in the United Kingdom,² and 6000 persons in

Abbreviations used

CV:	Coefficient of variation
mAb:	Monoclonal antibody
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Sweden³ work with laboratory animals. Antigens from the animals, of which rats and mice are most common, are potent inducers of allergic symptoms. Rodent urinary allergen levels as low as nanograms per cubic meter of inhaled air may produce symptoms in sensitized subjects.⁴ Among exposed personnel, often 10% to 30% are allergic and experience rhinitis, conjunctivitis, urticaria, or asthma when working with the animals.⁵⁻⁹ Protective devices (e.g., ventilated helmets, gloves, and ventilated laboratory benches) can alleviate or even abolish symptoms of allergy.^{10,11} A few studies have evaluated changing of materials or ventilation on the effect of airborne laboratory animal allergen levels.¹²⁻¹⁴

The sensitivity of most methods for measurement of allergens has been insufficient to determine concentrations in laboratories with personal samplers or to evaluate protective devices or ventilation improvements. Some investigators have used pooled samples or high-volume air samplers.¹⁵⁻¹⁷ The detection limits vary widely among assays; furthermore, the levels of allergen reported by different laboratories vary to a great extent, for example, from nanogram to microgram mean amounts in rat rooms.^{14-16, 18, 19} Therefore a sensitive, standardized assay is required to evaluate work improvements or sampling situations in which allergen load may be low or to make interlaboratory comparisons. Monoclonal antibodies (mAbs) against rat urinary allergen were developed and used together with an affinity-purified standard solution and a biotinylated amplifying compound to set up a sensitive enzyme immunoassay.

The new method was tested on air samples from the animal rooms of a research department that uses mostly rats. In a health survey performed by the Swedish Foundation for Occupational Health and Safety for State Employees at the Karolinska Institute, the department was shown to have clinically verified laboratory animal allergy in 22 of 46 employees. A program was developed to improve the working conditions including

From the Department of Occupational Health, Division of Allergy and Immunology, National Institute for Working Life, Sweden.

Supported by the Swedish Work Environment Fund (research grants AMFO 89-0098 and 93:0450).

Received for publication Apr. 2, 1996; revised June 5, 1997; accepted for publication June 6, 1997.

Reprint requests: Anne Renström, BSc, Department of Occupational Health, Division of Allergy and Immunology, National Institute for Working Life, S-171 84 Solna, Sweden.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/84004

TABLE I. BIAcore Biosensor binding matrix for pairwise map analysis: Binding of molecules detected by changes in resonance units

1. First mAb (mean RU)	2. RUP (mean RU)	3. mAb(1) (RU)	mAb(6), RU
mAb(1) 1671	164	-223	316
mAb(6) 2368	398	381	-164

RU, Resonance units; RUP, rat urinary protein.

Values represent increase/decrease in RU at each step.

1. First mAb bound to the solid phase.

2. Addition of rat urinary protein.

3. Addition of second mAb.

ventilation improvements, changes in work routines, and use of personal protective equipment. In an attempt to reduce allergen levels in the animal rooms, a sliding Perspex curtain system (Energo AB, Nacka Strand, Sweden) was installed.²⁰ To evaluate the efficacy of this system, air samples were collected on filters, extracted, and analyzed for rat urinary allergen content.

METHODS

Protein concentrations were determined by using the Pierce bicinchoninic acid method according to the manufacturer's recommendations, with bovine serum albumin as reference (Pierce Europe B V, Oud-Beijerland, The Netherlands).

mAbs

The mAbs were produced by four intraperitoneal immunizations in BALB/c mice of concentrated, dialyzed urine from 3- to 4-month-old male Sprague-Dawley rats. After fusing mouse spleen cells with mouse cell line Sp2/0 as described by Galfre et al.²¹ two hybridoma clones were selected and subcloned twice: MsMcAb(1) α RUP, mAb(1), and MsMcAb(6) α RUP, mAb(6) (Mabtech AB, Stockholm, Sweden). Immunoglobulin subclass of the mAbs was determined by using ELISA.

The mAbs were tested by pairwise epitope map analysis with a BIAcore (Biospecific Interaction Analysis) Biosensor²² (Pharmacia, Uppsala, Sweden). Detection is through surface plasmon resonance in which real-time interaction between molecules is monitored as shifts in the reflection angle of a light beam over a gold-plated chip onto which the studied molecules are bound. The shift is recorded as resonance signal in resonance units over time in a sensorgram. The analysis was performed as follows. Rabbit anti-mouse IgG₁ was bound to the dextran surface of the sensor chip. Monoclonal antibody(1) or mAb(6) was added, followed by blocking of unoccupied sites with a nonspecific IgG₁ antibody. Concentrated rat urinary protein was added, and thereafter a second antibody, mAb(1) or mAb(6). If the second antibody can bind to the antigen, this indicates that the antibodies have different epitope specificities on the same protein or that the epitope is repeated. The chip was regenerated by adding HCl, removing all but the rabbit anti-mouse IgG₁.

Antibody affinity constants were determined as described in detail by Beatty et al.²³ The antibodies were tested for binding to the isoallergens α_{2u} -globulin (Rat n 1.02, 17 kd) and prealbumin (Rat n 1.01, 20 kd)^{9,24,25} purified by fast protein liquid chromatography gel filtration and ion-exchange chromatography as described by Bayard et al.²⁶ α_{2u} -Globulin and prealbumin were coated to microtiter plates at 5 μ g/ml, and biotinyl-

ated mAb(1) and mAb(6), respectively, were added at semilogarithm concentration dilutions (0.1 to 31.6 ng/ml). The bound mAbs were visualized after incubation with streptavidin-alkaline phosphatase by using substrate p-nitrophenyl phosphate. The plates were read at 405 to 650 nm by using a Spectramax 250 reader (Molecular Devices, Sunnyvale, Calif.) and analyzed with Softmax Pro (Molecular Devices). To study the effect of aging, α_{2u} -globulin and prealbumin stored for 1 year at +4°C at a concentration of 10 μ g/ml and another preparation, repeatedly frozen and thawed, were similarly analyzed.

The mAbs were studied by Western blotting. Briefly, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 1.5 mm thick slab gels (10% to 20% linear gradient, 0.1% SDS) under reducing (mercaptoethanol) and nonreducing conditions. The separated rat urinary proteins were transferred to a 0.22 μ m polyvinylidene difluoride membrane (PVDF; BioRad, Hercules, Calif.) by electroblotting. Rat serum albumin (fatty acid- and globulin-free; Sigma, St. Louis, Mo.) was used as negative control. Blotting and membrane blocking were performed as described by Vesterberg et al.,²⁷ except that blocking was prolonged and carried out at room temperature. Membrane incubation was at room temperature overnight with 10 ng/ml biotinylated mAb(1) or mAb(6). After washing, bound mAb was detected with streptavidin-alkaline phosphatase diluted 1:2000. The membrane was washed, followed by equilibration in substrate buffer (0.1 mol/L NaCl, 5 mmol/L MgCl₂, 0.1 mol/L Tris-HCl, pH 9.5). Nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (BioRad) was used for color development. AuroDye forte (Amersham, Little Chalfont, U.K.) was used for total protein staining of rat urinary proteins and rat serum albumin transferred to a polyvinylidene difluoride membrane according to the manufacturer's instructions.

Standard extract

Urine from 3- to 4-month-old Sprague-Dawley male rats was concentrated by YM-2 (Amicon, Danvers, Mass.) ultrafiltration and dialyzed against phosphate-buffered saline (PBS) with a Spectra/Por membrane with a molecular weight cutoff of 12 to 14 kd (Spectrum Medical Industries, Inc., Los Angeles, Calif.). A standard extract was produced by affinity purification of the urine extract by using the Pierce ImmunoPure Ag/Ab Immobilisation Kit with mAb(6) bound to its AminoLink column (Pierce Europe B V), as described in the kit instructions.

The purified allergen extract was analyzed by SDS-PAGE under reducing conditions with ready-made Phast gradient gels (10% to 15%) in a Phast system (Pharmacia Biotech, Uppsala, Sweden). SDS-PAGE and silver staining of the gel were performed as recommended by the manufacturers.

Amplified ELISA protocol

Levels of rat urinary allergens were measured in the samples by using a sandwich ELISA. Throughout the assay, 100 μ l volumes were used. Monoclonal antibody(6) in PBS, 0.15% Kathon CG (Rohmand Haas; Hydrosupra Kemiservice, Helsingborg, Sweden) at 10 μ g/ml was bound to 96-well Maxisorp microtiter plates (Nunc InterMed, Roskilde, Denmark) overnight at room temperature. Plates were postcoated for 60 minutes with PBS, 0.15% Kathon, and 1% bovine serum albumin. After washing four times, standard dilutions (5 to 200 pg/ml) of affinity-purified rat urinary allergen and sample extracts diluted at least twice in PBS, 0.1% Tween-20, 0.15% Kathon, and 1% bovine serum albumin (dilution buffer) were

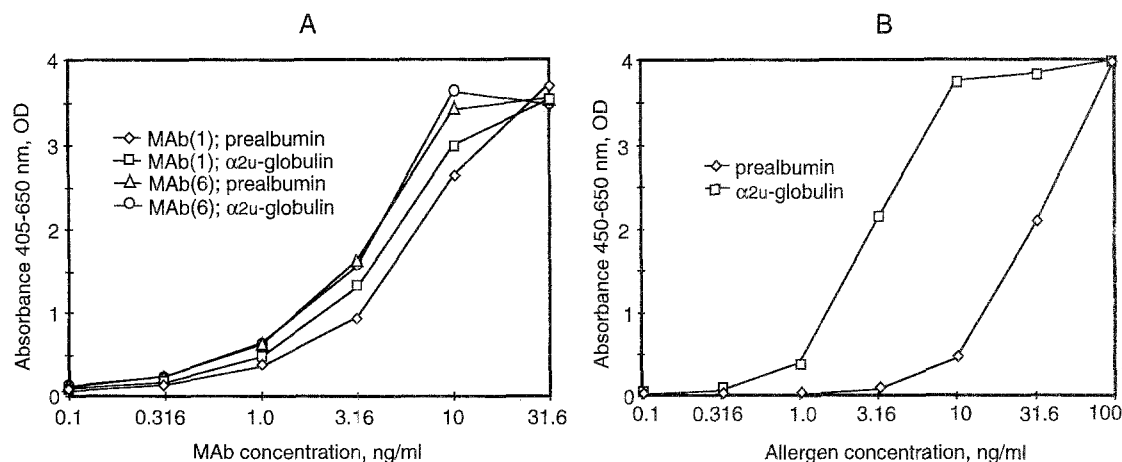


FIG. 1. A, Monoclonal antibody(1) and mAb(6) detection of α _{2u}-globulin and prealbumin coated to microtiter plates at 5 μ g/ml. B, Sandwich ELISA detection of α _{2u}-globulin and prealbumin (nonamplified method). Capture antibody was mAb(6), and detection antibody was mAb(1). OD, Optical density.

added to the wells in duplicate and incubated for 90 minutes at room temperature. Biotinylated mAb(1) (0.3 μ g/ml) in dilution buffer was added after washing, and the plate was incubated for 60 minutes at room temperature. After another wash, streptavidin-horseradish peroxidase was added, and the plate was incubated for 30 minutes. After washing, a new compound, N-biotinyl-4-hydroxybenzohydrazide (13.5 μ g/ml; molecular weight, 362 d; synthesized by P.H. Larsson) in 0.1 mol/L borate/0.003% H₂O₂, pH 8.5, in accordance with the CARD (catalyzed reporter deposition) concept²⁸ was added and incubated for 15 minutes. Briefly, bound horseradish peroxidase in the presence of peroxide is believed to induce a radical of the biotinylated amplifier, leading to its covalent binding with electron-rich parts (e.g., tryptophan or tyrosine) of the solid-phase protein molecules, thus increasing the amount of bound biotin. Streptavidin-horseradish peroxidase was added again after washing and incubated for 30 minutes. After washing, substrate TMBblue (soluble form; TSI-CDP, Milford, Mass.) was added, the reaction was terminated after 15 minutes by using 1 mol/L H₂SO₄, and absorbance was read at 450 nm (minus background absorbance at 650 nm) and analyzed by Softmax (Molecular Devices). Background optical density was subtracted, and a coefficient of variation (CV) of 15% was accepted for duplicate standard or eluate wells. Samples with higher CV% were reassayed. The efficiency of the amplified ELISA was compared with that of the unamplified version. In the latter each washing was threefold instead of fourfold; the blocking and the biotinylated amplifier steps were excluded. Incubations were carried out at 37°C, and the streptavidin-horseradish peroxidase incubation step was 1 hour. The unamplified standard curve range was 0.05 to 5 ng/ml.

A panel of antigens was tested for binding in the unamplified sandwich ELISA: α _{2u}-globulin and prealbumin; rat serum albumin (Sigma); concentrated and dialyzed rat urine from Sprague-Dawley and Wistar rats; concentrated and dialyzed rat epithelium extract prepared by PBS-Tween extraction of rat pelt; mouse urinary protein Mus m 1 (purified by fast protein liquid chromatography gel filtration on Pharmacia Superose-12 columns); concentrated and dialyzed mouse and guinea pig urine; and normal sera from mouse, rabbit, cat, dog, and horse. Rat and mouse antigens were tested at 1 and 100 ng/ml; other antigens were tested at 100 ng/ml.

Animal rooms and sliding curtain system

The 6 $\times$ 3 $\times$ 3 m (length $\times$ width $\times$ height) = 54 m³ rooms with an air exchange rate of 15 times/hour housed about 300 rats each. Six sliding ceiling-to-floor screens, of 3 mm thick transparent polycarbonate (Perspex), with eight rows of 40 mm holes 150 mm apart, were fitted along each side of the room. Behind these were stainless steel cage racks (1260 $\times$ 595 $\times$ 1700 mm [length $\times$ width $\times$ height]) on wheels (Stålstandard, type 01.1030-4; B&K Universal, Nittedal, Norway). One to four mature male or female rats, mostly Sprague-Dawley, were kept in Makrolon type IV cages (Ehret, Emmerdingen, Germany) with hardwood chip bedding (B&K Universal). Details of the ventilation solution are described elsewhere.²⁰

Air sampling

Air samples of inhalable dust were collected on 25 mm diameter (0.8 μ m pore size) polycarbonate ($n = 71$) or polytetrafluoroethylene ($n = 7$) filters (Millipore, Sundbyberg, Sweden) in IOM filter cassettes meeting the American Conference of Governmental Industrial Hygienists' sampling criteria (SKC Inc., Valley View, Pa.) by using small portable pumps at airflow of 2 L/min. Parallel samples (sampling heads placed 15 cm apart) were collected on 31 of 38 total sampling occasions (25 duplicate and 6 triplicate samples). Airflow was measured before and after sampling, and the sampling time was recorded. Static samples were collected in undisturbed rat rooms, either in the center aisle of the room or behind the sliding curtains on the animal shelves. Samples were also collected during changing of the animal cages in the rat rooms, during cage cleaning in the wash room, and in the animal house corridor. Personal samples were collected during cage changing and cleaning, during which animal technicians carried portable pumps in a belt, with the filter samplers positioned in the breathing zone.

Allergen extraction

Allergens were extracted from the filters within hours of sampling in 1 ml of PBS, 0.5% Tween-20 (for improved protein stability and extraction efficiency¹⁹), and 0.15% Kathon in Minisorp tubes (Nunc, InterMed, Roskilde, Denmark) during rotation for 1 hour, after which the filter was discarded; the extracts were stored in 250 μ l aliquots and frozen at -20°C until analysis.

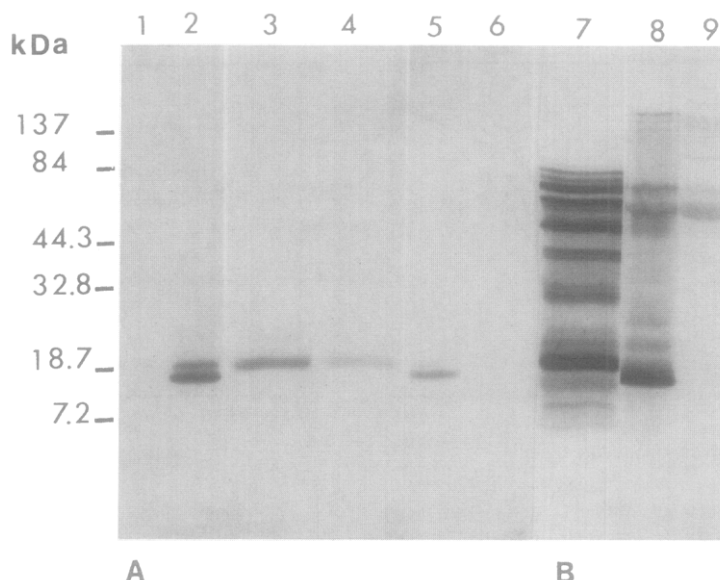


FIG. 2. **A,** Binding of biotinylated mAb(1) or mAb(6) (10 μ g/ml) to rat proteins (3 μ g/lane) in Western blot after separation by SDS-PAGE. Detection with streptavidin-alkaline phosphatase, followed by nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate substrate. *Lane 1*, Nonreducing conditions, rat serum albumin (RSA) (negative control), mAb(6); *lane 2*, nonreducing conditions, male Sprague-Dawley rat urinary proteins (RUP), mAb(6); *lane 3*, reducing conditions (mercaptoethanol), RUP, mAb(6); *lane 4*, reducing conditions, RUP, mAb(1); *lane 5*, nonreducing conditions, RUP, mAb(1); *lane 6*, nonreducing conditions, RSA, mAb(1). **B,** Colloidal gold total protein staining of RUP and RSA (3 μ g/lane) after Western blotting. *lane 7*, Reducing conditions, RUP; *lane 8*, nonreducing conditions, RUP; *lane 9*, nonreducing conditions, RSA.

Statistics

Calculations were performed by using Statview (Abacus Concepts, Berkeley, Calif.) or Microsoft Excel (Microsoft Corp., Redmond, Wash.) software. Significance ($p < 0.05$) was tested with the Mann-Whitney U test. Sample eluates diluted twofold with absorbance values below the lowest standard point (5 pg/ml) were assigned the value 5 pg/ml for calculation purposes.

RESULTS

Properties of mAbs MsMcAb(1) α RUP and MsMcAb(6) α RUP

Both mAbs were of the IgG₁ subclass. BIAcore Biosensor epitope map analysis showed that the antibodies mAb(1) and mAb(6) bound to two different unique epitopes on the same protein. Thus binding could take place when one mAb was used as antigen capture and the other as detection antibody, but binding was abolished when the same mAb was used twice (Table I). Antibody affinity constant was calculated to be 2.6×10^9 for mAb(1) and 5.3×10^9 for mAb(6).

Monoclonal antibody(6) detected the isoallergens equally when coated to microtiter plate wells, and mAb(1) detected α_{2u} -globulin slightly better than prealbumin (Fig. 1, A). When allergens that had been aged 1 year were compared, mAb(6) still detected both equally, whereas mAb(1) detection of aged α_{2u} -globulin was seven- to tenfold greater compared with aged prealbumin. Another preparation of prealbumin, repeatedly frozen and thawed, was not detected at all by mAb(1) (data not shown). In the two-mAb sandwich ELISA

assay, α_{2u} -globulin was detected with approximately 10 times greater sensitivity than prealbumin (Fig. 1, B). In the cross-reactivity tests, all rat-derived preparations (concentrated rat urine from Sprague-Dawley rats and Wistar rats and rat fur extract) except pure rat serum albumin reacted as antigens in the sandwich ELISA. Concentrated mouse urine; purified mouse urinary protein (Mus m 1); mouse serum; guinea pig urine; and sera from rabbit, cat, dog, or horse were not bound at all in the ELISA (data not shown).

In Western blotting after electrophoretic separation of rat urinary proteins, mAb(6) bound to both α_{2u} -globulin and prealbumin, whereas mAb(1) only bound α_{2u} -globulin (Fig. 2, A). The protein bands detected with mAb(6) were more intense than those detected with mAb(1) at the same antibody concentration. Under reducing conditions both mAbs bound with an identical pattern to a single component with an approximate molecular weight of 19 kd. Neither mAb(1) nor mAb(6) bound rat serum albumin. Total protein staining showed that the dominating proteins in male rat urine were the isoallergens (Fig. 2, B). The standard allergen preparation, affinity-purified by using mAb(6), contained components only in the molecular weight range of 14.4 to 20.1 kd, as determined by SDS-PAGE (Fig. 3).

Amplified monoclonal rat urinary allergen ELISA

The detection limit of the amplified assay was 3.2 pg/ml, and in the unamplified assay it was 26 pg/ml,

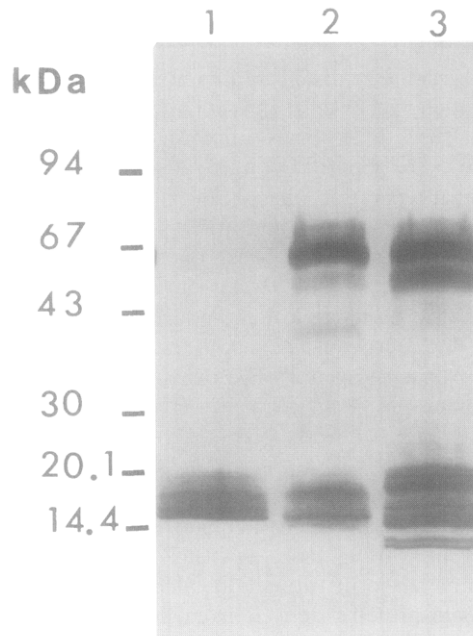


FIG. 3. Electrophoresis of rat urinary extracts. Silver-stained Phast gradient gel, 10% to 15%. Lane 1, Standard extract: mAb(6) affinity-purified from concentrated, dialyzed male Sprague-Dawley rat urine, 100 ng; lane 2, concentrated, dialyzed male Sprague-Dawley rat urine, 80 ng; lane 3, metabolic cage urine, 4-month-old Sprague-Dawley male rats, 100 ng.

calculated on the mean values of five consecutive runs each, determined as in the study by Ekins et al.²⁹ At an optical density of 1, the concentration of allergen was 75 pg/ml in the amplified assay, compared with 800 pg/ml in the unamplified assay (Fig. 4). In 1-hour air samples with pumps at 2 L/min, the detection limit in twofold diluted eluates (lowest standard point = 5 pg/ml) was 0.083 ng/m³. At high allergen concentrations, the binding or signal may be blocked, resulting in low optical density values. Among 120 duplicate eluates within the standard curve range, the median CV was 4.0% (25th to 75th percentile, 1.7% to 8.1%) for duplicate wells; 13 (11%) had a CV greater than 15%.

Air sampling results

Rat urinary allergen levels in air filter eluates ($n = 75$) from 38 different samplings are shown in Fig. 5. Mean values are shown for parallel samples. One filter extract with a duplicate well CV of 18% was included. Four filter eluates were below the detection limit. Theoretically, 27 of the eluates would have been undetectable in the unamplified assay. The three control filter samples (eluted and assayed in the same way) were below the lowest standard point (5 pg/ml). For estimation of variability caused by differences in air sampling, handling, and assaying, the CV was calculated for each parallel sample pair (among triplicates one sample was randomly excluded). The median CV was 22.9% (25th to 75th percentile, 16.1% to 34.1%; $n = 28$). Allergen levels were lower in the rat room aisle when

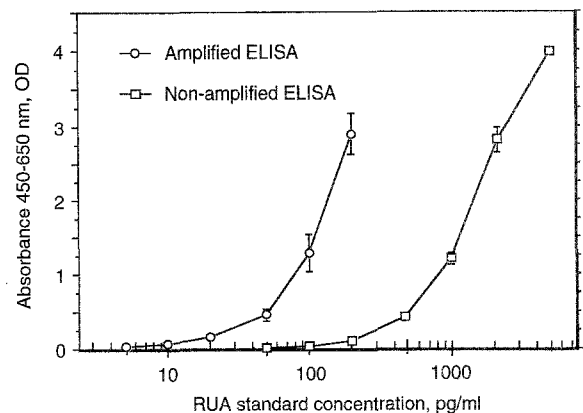


FIG. 4. Comparison between standard curves obtained with amplified and nonamplified ELISA methods, respectively, to detect mAb(6) affinity-purified rat urinary allergen (standard extract). Mean values from five consecutive plates of each. Error bars represent standard error of the mean. OD, Optical density; RUA, rat urinary allergen.

curtains were closed (median, 0.2 ng/m³) than behind the curtains (median, 0.9 ng/m³, $p = 0.01$) or if the curtains were open (median, 0.9 ng/m³, $p = 0.001$). Median rat room allergen levels were 18 ng/m³ both during cage changing, when curtains were drawn apart, and when cleaning personnel were carrying samplers.

DISCUSSION

Dose-dependent relationships seem to exist for sensitization and symptom expression among personnel exposed to laboratory animals.^{6, 8, 30} However, the thresholds for these immunologic reactions may be low. Many subjects with allergy are scientists and assistants working only intermittently with animals, and then in relatively "clean" laboratory settings using ventilated benches, in contrast to animal caretakers working in animal confinement buildings, performing cage cleaning and other activities that generate much higher allergen levels.^{9, 30} Eggleston et al.⁴ showed that α_{2u} -globulin (Rat n 1.02) levels of only nanograms per cubic meter of inhaled air may produce immediate symptoms in sensitized subjects. Interestingly, Ihre and Zetterström³¹ also showed that inhalation of very low doses of allergen (insufficient to result in an immediate or late bronchial response in sensitized subjects), given repeatedly for 5 to 7 days, resulted in increased airway reactivity in histamine provocation testing.

Hence, we believed quantification of allergen in area-related or personal task-related sampling in relatively clean environments to be of interest. The described method uses, in addition to biotin-streptavidin (commonly used to increase the sensitivity in ELISA systems), a novel amplifier, N-biotinyl-4-hydroxybenzohydrozone. This amplifier is used in conjunction with peroxidase, essentially according to the CARD (catalyzed reporter deposition) concept.²⁸ The detection limit in the amplified assay was 0.083 ng/m³ in samples

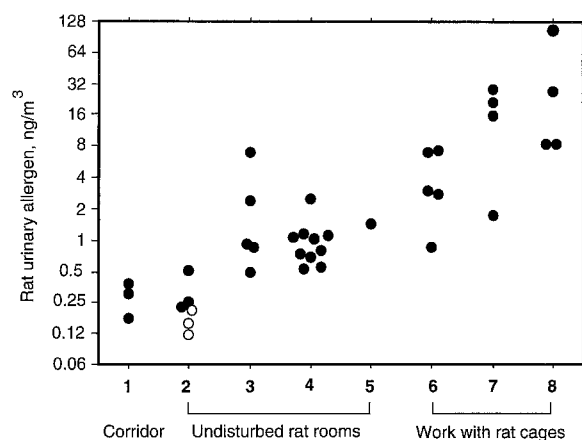


FIG. 5. Amplified ELISA rat urinary allergen values in filter eluates from 38 samplings (mean sampling measurements). *Open circles* represent parallel samples in which there were samples below detection limit. 1, Corridor in animal house, sampling time 61 to 145 minutes; 2, rat room aisle, closed curtains, 60 to 62 minutes; 3, rat room, behind closed curtains, 60 to 82 minutes; 4, rat room, open curtains, 60 to 116 minutes; 5, rat room without curtains, 63 minutes; 6, wash room, cage cleaning, 79 to 126 minutes; 7, rat room, cage changing, 30 to 112 minutes; 8, cage changing and cleaning, personal sampling, 33 to 185 minutes.

collected at 2 L/min during 1 hour, about one tenth of the detection limit in the unamplified ELISA. The allergen levels measured in the undisturbed animal room were very low; without amplification, many of the eluate measurements would have been below the detection level. The amplifier is also used by this laboratory to measure airborne cat allergen in schools.³²

The urinary allergens α_{2u} -globulin (Rat n 1.02, 17 kd) and prealbumin (Rat n 1.01, 20 kd) are described as major allergens encountered when working with laboratory rats.^{24, 33, 34} α_{2u} -globulin, a member of the lipocalin superfamily, has pheromone-carrying function and constitutes about half of the total urine protein excreted by mature male rats.³⁵ Bayard et al.²⁶ showed prealbumin, comprising about 10% of male urinary protein, to be an isoform of α_{2u} -globulin with no amino acid sequence homology to rat serum prealbumin (transthyretin).

Use of mAbs in the detection of airborne rat allergens has, to our knowledge, not been described previously. In the described two-mAb sandwich ELISA, prealbumin must be added at tenfold higher concentrations than α_{2u} -globulin to produce the same optical density values. Monoclonal antibody(6) detected both isoallergens equally, whereas mAb(1) had reduced affinity to prealbumin, especially aged prealbumin. The epitope on prealbumin to which mAb(1) can bind thus seems vulnerable to breakdown or conformational change (as also indicated in the Western blot, Fig. 2, A). Because α_{2u} -globulin is the main excreted protein in rat urine and because it is probable that the proteins in the air filter samples have undergone some breakdown, we conclude that detected protein consists principally of α_{2u} -globulin.

In the Western blot, in which electrophoretically separated and immobilized rat urinary proteins were probed with the mAbs, in addition to the strongly labeled isoallergens, very weak bands at larger protein sizes were visible (Fig. 2, A, lanes 2 to 4). However, because mAb(6) affinity purification of rat urine resulted in an allergen preparation of low molecular weight proteins and total absence of larger molecules (Fig. 3), we believe this to be nonspecific background staining, perhaps caused by the use of Tween during blocking.³⁶ The appearance of multiple bands during reducing conditions (Fig. 2, B) could be due to the oxidation products of the reducing agent.³⁷ The oxidative nature of polyacrylamide gel could also give rise to multiple bands caused by random oxidation of the sample when it enters the gel.

The mAb sandwich ELISA thus captures a marker for allergen load, the major allergen α_{2u} -globulin, in the same vein as using mAbs against Fel d 1 or Can f 1 to determine airborne cat and dog allergen levels.²⁴ Other allergenic proteins derived from, for instance, fur or saliva, may be present. However, α_{2u} -globulin was shown to be present and stable in both airborne and accumulated animal house dust.³⁸

To be used widely, the mAbs must detect α_{2u} -globulin from other strains of rats. The antibodies were prepared by immunization with urine proteins from Sprague-Dawley rats. Both a Wistar rat urine extract and samples from animal facilities containing only other rat strains (data not shown) were detectable in the assay. From this evidence and from reports of cross-reactivity between urinary proteins from different rat strains,³⁹ we deduce that the mAbs should detect α_{2u} -globulin from several, if not all, rat strains.

To protect personnel, employers now seek to reduce environmental allergen exposure by installation of efficient ventilation systems and ventilated laboratory benches and use of better cage cleaning practices and bedding material. The described sliding curtain system improved the environment in the aisle of the animal rooms, with statistically significant lower allergen levels. During cleaning procedures, however, the exposure to allergens was many times higher, because the curtains must be opened to perform the task and because the levels of airborne allergen in the breathing zone are influenced by the close contact with the animals. In high-allergen load situations, use of filtered-air helmets is recommended.

In conclusion, we have developed a method that can be standardized to measure low levels of airborne rat urinary allergen. What may be called a "safe" level of exposure for personnel new to laboratory animal work or for already sensitized individuals remains to be investigated.

We thank Conny Lindqvist, ENERGO AB, Stockholm, for help in the description of the sliding curtain system. We also wish to thank Mabtech AB, Stockholm, Sweden, for fruitful cooperation in the production of monoclonal antibodies against rat urinary allergens.

REFERENCES

- Eggleston PA, Wood RA. Management of allergies to animals. *Allergy Proc* 1992;13:289-92.
- Cockcroft A, Edwards J, McCarthy P, Andersson N. Allergy in laboratory animal workers. *Lancet* 1981;1:827-30.
- Andersson S, Banerdy K, Lindh G. Working with laboratory animals. Stockholm: Arbetarskyddsstyrelsen, Publikationsservice; 1990. Report no. AFS 1990:11 (in Swedish).
- Eggleston P, Ansari A, Ziemann B, Adkinson N, Corn M. Occupational challenge studies with laboratory workers allergic to rats. *J Allergy Clin Immunol* 1990;86:63-72.
- 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;49:548-52.
- Renström A, Malmberg P, Larsson K, Larsson PH, Sundblad B-M. Allergic sensitisation is associated with increased bronchial responsiveness. A prospective study of laboratory animal allergy. *Eur Respir J* 1995;8:1514-9.
- Aoyama K, Ueda A, Manda F, Matsushita T, Ueda T, Yamauchi C. Allergy to laboratory animals: an epidemiological study. *Br J Ind Med* 1992;49:41-7.
- Cullinan P, Lowson D, Nieuwenhuijsen MJ, Gordon S, Tee RD, Venables KM, et al. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to laboratory rats. *Occup Environ Med* 1994;51:589-92.
- Hunskar S, Fosse RT. Allergy to laboratory mice and rats: a review of the pathophysiology, epidemiology and clinical aspects. *Lab Anim* 1990;24:358-74.
- Hunskar S, Fosse RT. Allergy to laboratory mice and rats: a review of its prevention, management, and treatment. *Lab Anim* 1993;27:206-21.
- Slovak AJM, Orr RG, Teasdale EL. Efficacy of the helmet respirator in occupational asthma due to laboratory animal allergy (LAA). *Am Ind Hyg Assoc J* 1985;46:411-5.
- Gordon S, Tee R, Lowson D, Wallace J, Taylor A. Reduction of airborne allergenic urinary proteins from laboratory rats. *Br J Ind Med* 1992;49:416-22.
- Edwards RG, Beeson MF, Dewdney JM. Laboratory animal allergy: the measurement of airborne urinary allergens and the effects of different environmental conditions. *Lab Animals* 1983;17:235-9.
- Ziemann B, Corn M, Ansari AA, Eggleston P. The effectiveness of the Duo-Flo BioClean unit for controlling airborne antigen levels. *Am Ind Hyg Assoc J* 1992;53:138-45.
- Eggleston PA, Newill CA, Ansari AA, Pustelnik A, Lou SR, Even R III, et al. Task-related variation in airborne concentrations of laboratory animal allergens: studies with Rat n I. *J Allergy Clin Immunol* 1989;84:347-52.
- Swanson MC, Agarwal MK, Reed CH. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J Allergy Clin Immunol* 1985;76:724-9.
- Platts-Mills TAE, Heymann PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 1986;77:850-7.
- Price JA, Longbottom JL. ELISA method for measurement of airborne levels of major laboratory animal allergens. *Clin Allergy* 1988;18:95-107.
- Gordon S, Tee RD, Lowson D, Taylor AJN. Comparison and optimization of filter elution methods for the measurements of airborne allergen. *Ann Occup Hyg* 1992;36:575-87.
- Lindqvist C, Persson L, Iwarsson K, Lustig G, Renström A, Larsson PH. A sliding curtain system for improving air distribution of animal rooms in relation to working environment and cage climate. Conference Proceedings of the Joint International ICLAS/Scand-LAS/FinLAS Conference, July 2-6 1995, Helsinki, Finland. *Scand J Lab Anim Sci* 1996;23(suppl):135-43.
- Galfre G, Howe SC, Milstein C, Butcher GW, Howard JC. Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature* 1977;266:550-2.
- Jönsson U, Malmqvist M. Real time biospecific interaction analysis. The integration of surface plasmon resonance detection, general biospecific interface chemistry and microfluidics into one analytical system. *Adv Biosensors* 1992;2:291-336.
- Beatty JD, Beatty BG, Vlahos WG. Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay. *J Immunol Methods* 1987;100:173-9.
- King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TAE, Thomas W. Allergen nomenclature. *J Allergy Clin Immunol* 1995;96:5-14.
- Stewart GA, Thompson PJ. Letter to the Editors. Re: Nomenclature of rat allergens. Reply to Gordon S, Jones MG, Tee RD, Newman Taylor AJ. *Clin Exp Allergy*. 1997. In press.
- Bayard C, Holmquist L, Vesterberg O. Purification and identification of allergenic α_{2u} -globulin species of rat urine. *Biochim Biophys Acta* 1996;1290:129-34.
- Vesterberg O, Acevedo F, Bayard C. Sensitive quantification of proteins by electrophoresis in gels by use of chemiluminescence. *Electrophoresis* 1995;16:1390-3.
- Bobrow MN, Harris TD, Shaughnessy KJ, Litt GJ. Catalyzed reporter deposition, a novel method of signal amplification. *J Immunol Methods* 1989;125:279-85.
- Ekins R, Chu F, Micallef J. High specific activity chemiluminescent and fluorescent markers: their potential application to high sensitivity and "multi-analyte" immunoassays. *J Biolumin Chemilumin* 1989;4:59-78.
- Nieuwenhuijsen MJ, Gordon S, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Exposure to dust and rat urinary aeroallergens in research establishments. *Occup Environ Med* 1994;51:593-6.
- Ihre E, Zetterström O. Increase in nonspecific bronchial responsiveness after repeated inhalation of low doses of allergen. *Clin Exp Allergy* 1993;23:298-305.
- Larsson PH, Malmberg P, Wickman M. Assessment of airborne Fel d 1 concentrations using an amplified ELISA [abstract]. *J Allergy Clin Immunol* 1996;97:421.
- Walls AF, Longbottom JL. Comparison of rat fur, urine, saliva, and other rat allergen extracts by skin testing, RAST, and RAST inhibition. *J Allergy Clin Immunol* 1985;75:242-51.
- Gordon S, Tee RD, Taylor AJN. Analysis of rat urine proteins and allergens by sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotting. *J Allergy Clin Immunol* 1993;92:298-305.
- Mancini MA, Majumdar D, Chatterjee B, Roy A K. α_{2u} -Globulin in modified sebaceous glands with pheromonal functions: localization of the protein and its mRNA in preputial, meibomian, and perianal glands. *J Histochem Cytochem* 1989;37:149-57.
- Bird CR, Gearing AJH, Thorpe R. The use of Tween 20 alone as a blocking agent for immunoblotting can cause artefactual results. *J Immunol Methods* 1988;106:175-9.
- Suresh Kumar TK, Prasad VVH, Pandit MW. Origin of multiple bands of proteins on sodium dodecyl sulphate-polyacrylamide gel electrophoresis: intermolecular disulphide cross-linking due to the presence of oxidizing components in the reducing agent. *J Biochem Biophys Methods* 1994;28:243-7.
- Gordon S, Tee RD, Newman Taylor AJ. Analysis of the allergenic composition of rat dust. *Clin Exp Allergy* 1996;26:533-41.
- Lutsky I, Fink JN, Kidd J, Dahlberg MJ, Yunginger JW. Allergenic properties of rat urine and pelt extracts. *J Allergy Clin Immunol* 1985;75:279-84.