



The effect of relative humidity on mouse allergen levels in an environmentally controlled mouse room

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Abstract (riepilogo)

To determine the effect of humidity on the levels of the mouse allergen Mus m 1, an experimental animal room was constructed to control environmental variables. The sex, strain, age, and number of mice was constant in the room, so that the average daily production of Mus m 1 would not vary greatly. Six different levels of relative humidity from 15% to 65% were maintained for a minimum of a week each. Daily collections of airborne particulates were eluted from filters and Mus m 1 content measured by immunological assay. Increasing relative humidity caused a decrease in Mus m 1 levels from a high of 3 ng/m³ at 15% humidity to a low of 0.5 ng/m³ at 65% humidity. Thus, reduction of airborne allergen levels can be achieved by careful attention to humidity control, especially during the winter heating season when humidity levels may be low. This experimental room can be used to measure the effect of other variables such as ventilation rate, caging, bedding, and work practices on the levels of mouse allergen, in an animal facility.

Testo completo

Thirty-five thousand workers in the United States are exposed to rodent allergens in scientific laboratories or in the breeding and care of rodents,(1) and significant quantities of rodent allergen have been found in homes.(2) A review of 19 studies measuring the prevalence of laboratory animal allergy indicates that an average of 20% of exposed workers experience symptoms.(1) However, this prevalence is considered an underestimate of the true incidence of animal allergy because many workers who become allergic leave the work situation.

The mouse urinary protein, Mus m 1, is the major sensitizing antigen for individuals with mouse allergy.(3) This antigen is produced by the liver and salivary glands and excreted into urine and saliva. Some male mice excrete higher levels of Mus m 1 because it is induced by testosterone.(3,4) The quantity of Mus m 1 in urine also varies by strain.(5) Mus m 1 becomes associated with particulate matter which, when airborne, is subsequently inhaled resulting in allergic symptoms.

The authors studied airborne Mus m 1 in the Jackson Laboratory environment to elucidate the factors affecting allergen levels and to design ways to reduce exposure.(6) In previous studies of air quality the authors measured particulates and Mus m 1 levels in working mouse rooms. These survey data were useful, but interpretation was difficult due to differences among the rooms in strains of mice, density of mice, type of air filter, and ventilation rate. Even within the same animal room, changes in the number of workers or mice occurred over time. Consequently, a small experimental mouse room was constructed so that environmental variables could be controlled rigorously. For example, the number, sex, strain, and age of mice in the experimental room can be maintained constant, as can the humidity, air change rate, and working practices. In this report the authors describe the room and demonstrate that mouse allergen levels decrease as the humidity increases.

METHODS

Control of Air Flow and Humidity in the Experimental Room

To calculate the number of room air changes per hour, the authors measured air flow at the entrance of the supply duct to the room using a factory-calibrated thermoanemometer (Alnor Instrument Co., model 8565; Skokie, Ill.). Throughout this experiment the air change rate was maintained at 10 air changes/ hour and temperature at 22degC (72degF). Humidity was maintained at the determined levels with a duct-mounted steam humidifier (Armstrong EHU-600, Three Rivers, Mich.) controlled by a humidity sensor located in the experimental room (HygroDynamics, Jessup, M.D.). Two air conditioners were used for dehumidification (Heat Controller, Inc., model 3140-1-18A, Jackson, Mich.). The authors evaluated six relative humidity (Rh) levels (15, 25, 35, 45, 55, and 65% Rh) for a minimum of one week at each level; Rh levels of 35% and 65% were evaluated for two weeks. During each humidity setting, Rh was monitored daily; the humidistat controlled humidity within a 5% range. The humidity level in the experimental mouse room was changed on Monday morning and the room allowed to equilibrate for 24 hours. Sampling of air began on Tuesday; 3 samples of 12 hours each were obtained, and 1 set of paired samples of 6 hours each obtained on the fourth day. Experiments at lower humidity ranges (15-25 Rh) were conducted in February and March when ambient Rh is normally low, and the desired humidity could be achieved by humidification, because it is easier to increase humidity than to dehumidify the air. For the same reason experiments at higher humidity ranges (35-65 Rh) were conducted in October and November when ambient Rh is higher.

Mouse Colony

The room contained 1088 male C57BL/6J mice, a strain chosen for its high production of Mus m 1.(5) Since testosterone levels vary with mouse age, a constant average age of nine weeks was maintained. The colony initially was established with 10 age increments from 4 to 13 weeks old and then maintained by changing 10% of the mice weekly, adding mice 4 weeks old and removing the oldest mice. Mice were housed four per cage and provided with Old Guilford 911 grain (Emory Morse Co., Guilford, Conn.) and acidified water. The experimental protocols were approved by the Institutional Care and Use Committee. To replicate human activity and cage changing in a typical room, the caretaker changed one-third of the cages daily, Tuesday through Thursday. On a waist-high changing table, the caretaker transferred mice from soiled cages to clean ones with fresh white pine shavings (Crobb Box Co., Ellsworth, Maine). Cages with soiled bedding were stacked on pallets, which were transferred to a separate facility with an automatic cage washer, so removal of bedding did not occur within the room. General room cleaning occurred on Friday.

Measurement of Mouse Allergen

The allergen was collected as airborne particulate matter. A 20 L/min air sampling pump (Air Diagnostics, Naples, Maine) collected particulates for each of four working days (8 a.m. to 8 p.m. Tuesday through Friday). Monitoring equipment was placed at a standardized location midroom along one wall in front of an exhaust grill at a height of four and one-half feet. This placement permits a sampling of well-mixed room air with minimal influences from intermittent mouse handling activities. Mus m 1 was extracted from these filters with 2.5% Tween in phosphate buffered saline (PBS) pH 7.4 by shaking at 4degC for about 48 hours. The eluate was stored frozen at -80degC until a group of samples were lyophilized. The lyophilized powder was resuspended in 1 mL distilled water and

frozen at -20degC until assayed. A sandwich enzyme-linked absorbance assay (ELISA) was used to measure Mus m 1 based on a modification of the procedure described previously by Price and Longbottom.(7) The ELISA plates were coated (50 mL per well) with an affinity-purified monospecific anti-Mus m 1 sheep antibody (derived from its polydonal native serum by passing the serum through an affinity column with purified Mus m 1 linked to cyanogen bromide) in 0.05 M borate buffer, pH 8.6. Monospecificity of antibody was determined by testing against Mus m 1 and mouse albumin, which is the major contaminant of purified Mus m 1. The plates were incubated overnight at 4degC and washed four times with PBS pH 7.4 containing 0.05% Tween 20 (PBS-T). A standard curve of Mus m 1 quantitated by spectrophotometry at optical density of 280 nm was included in each assay procedure. The samples were diluted 1:1 with PBS pH 7.4 with 0.1% bovine serum albumin and 0.05% Tween 20, plated in triplicate, and incubated overnight at 4degC. The plates were washed four times with PBS-T and plated with a biotinylated anti-Mus m 1 antibody (used at a dilution of 1:2000) and incubated for one hour at room temperature. After four washes the plates were incubated with horse radish peroxidase conjugated streptavidin (TAGO Immunologicals, Burlingame, Calif.) and incubated 30 minutes at room temperature and 5 minutes at 37degC. The plates were washed three times with PBS-T and one time with PBS. They were then incubated with the color-developing reagent containing 0.5 mg/mL orthophenylenediamine and 0.006% hydrogen peroxide in 0.2 M potassium phosphate buffer pH 7.4 at room temperature for 20 minutes in the dark. The optical densities were read at 495 nm on a Dynatech MR 5000 microplate reader (Dynatech, Chantilly, Va.) equipped with a statistical data analysis package. Optical densities for the wells containing the standards were converted into a standard curve of concentrations using semi-logarithmic regression analysis. The levels of Mus m 1 are expressed given in units of ng/m sup 3 . The data were analyzed using the computer program Systat (Systat Inc, Evanston, Ill.).

RESULTS

The Experimental Room

The experimental room was constructed by dividing the room in two and placing the animals in one part and the equipment controlling the air supply in the other (Figure 1). (Figure 1 omitted) The animal portion of the room, which measures 3.35 m x 3.35 m x 2.44 m (11 ft x 11 ft x 8 ft), contains the cage-changing table, racks with mouse cages, and the air monitoring collection probes. The ventilation design replicates that found in a typical animal room and consists of two high-aspiration diffusers 15 cm (6 inches) in diameter, which are located side by side in the ceiling at the center of the room. Air leaves the room by two 30.5 cm x 10.2 cm (12 x 4 inch) return air grills located on the side wall.

The second room contains the mouse room supplies, the humidifier, air sampling pumps, and equipment necessary to control the air flow and humidity. Air entering the room passes through a prefilter and a high efficiency particulate air (HEPA) filter designed to capture 99.99% of particles larger than 0.3 mm in diameter. This air is already heated or cooled depending on the season to maintain a constant temperature. Air control gates in the side of the main air supply duct can be opened to reduce air flow, and a damper can be positioned to increase or decrease air flow.

Working practices in this room were controlled by having the same animal caretaker change a constant number of cages at the same time of day. The number of mice, age, sex, and strain were constant throughout the experiment. This level of reproducibility is difficult to achieve in a working mouse room.

Effect of Humidity on Mouse Allergen Levels

The levels of 15, 25, 35, 45, 55, and 65% Rh were maintained for at least one week. All Mus m 1 samples at each humidity level were averaged; the mean and SE as a function of Rh are depicted in Figure 2. (Figure 2 omitted) Increasing humidity is inversely proportional to mouse allergen levels; a change of humidity from 15% to 65% resulted in a decrease of Mus m 1 levels from 3 to 0.5 ng/m sup 3 , a six-fold drop.

DISCUSSION

Results demonstrate that increasing the Rh in an animal room decreased the quantity of mouse allergen found in the air. Increased Rh reduces static charges on particulates and also increases the weight of particulates. Either or both of these effects may contribute to reduced particle counts at higher humidities.

Although the decrease in mouse allergen was six-fold over the humidity range of 15-65% tested in this study, raising the humidity too high is not practical or comfortable for people. Experience at this facility indicates that animal care personnel report discomfort when humidity is above 60%. Furthermore, a damp environment is associated with increased respiratory illness primarily due to increased growth of molds and mites.(8-10) Most species of fungi require humidity above 60% for growth.(11) Respiratory infections are increased at humidities below 45% or above 70%.(11) Thus, a humidity level of 40-50% appears to be best for human occupant comfort, and maintaining this level is recommended. A decrease of Mus m 1 levels of about half (from 3-1.5 ng/m sup 3) can be achieved by careful attention to humidity, especially during the winter heating season.

The levels of Mus m 1 found at 45% Rh in this experimental mouse room are similar to those found in 13 different working mouse rooms at The Jackson Laboratory. Mus m 1 levels in the survey of working rooms ranged from 0.4-1.6 ng/m sup 3 ,(6) similar to the 1.5 ng/m sup 3 found in the experimental room.

A previous study reported dramatic decreases of up to 10-fold in allergen levels when humidity was increased from 54% to 77%.(12) The present study confirmed that increasing humidity results in decreased allergen levels but the authors did not find such a dramatic decline in allergen levels as reported by Edward and coworkers.(12) The two studies do not necessarily contradict each other, since there were several differences in methods. For example, Edward and coworkers tested a higher humidity level of 77% compared to the current authors' highest level of 65%; and they used sera from allergic individuals as the source of antibody to measure mouse allergen, while in the present study antisera was used to purify Mus m 1 prepared in a sheep. Furthermore, the experimental mouse room in this study permitted the careful control of mouse colony, working practices, and air flow. This would be expected to yield more reproducible measurements of mouse allergen.

The experimental mouse room described in this report offers several advantages for the study of environmental conditions affecting animal allergen levels. The rate of allergen production is controlled by keeping the age, sex, strain, and number of mice constant. Variables associated with air conditioning and work practices also can be controlled carefully. Future studies in this room will evaluate the effect of different types of caging, bedding, work practices, and the number of air changes/hour on airborne mouse allergen levels. Once the major variables affecting exposure are understood, effective reduction of allergen levels may be achieved with an improvement in employee health.

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