

Laboratory animal allergies

Use of the radioallergosorbent test inhibition assay to monitor airborne allergen levels

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Occupational exposure to laboratory animals frequently leads to the development of allergies in exposed workers. The incidence of laboratory animal allergies has been estimated to be between 11 and 30 % of the exposed workers in the United States (2, 3). Occupational allergies can have a significant effect in terms of lost time at work and can even cause people to change positions and/or vocations. Although numerous studies have documented the prevalence of occupational allergies in general, and laboratory animal allergies in particular, less is known about the environmental factors which influence the development and expression of these diseases. The current study was undertaken to develop methods of monitoring airborne levels of allergens so that environmental controls could be applied to prevent or lessen allergic reactions in workers exposed to laboratory animals. Previous studies have been reported in which airborne levels of mouse and guinea pig allergens were measured with a modification of the radioallergosorbent test (RAST), termed the RAST inhibition assay (4, 5). Although these studies demonstrated that the RAST inhibition assay could be used to quantify airborne allergen levels, they were performed using only area sampling techniques. We have investigated the utility of this assay to detect airborne rat allergen levels by both personal and area sampling, and we have attempted to make a better delineation of the rat allergens in the air.

Materials and methods

Extracts of rat epithelium and urine were prepared as previously described (5). Briefly, for epithelial extracts rat pelts were lyophilized, defatted by ether extraction in a Soxhlet apparatus, ground in a Wiley mill, and extracted as a 5 % suspension in 0.1 M phosphate buffer, pH 7.4, overnight at 4°C. The extract was dialyzed against 0.05 M ammonium bicarbonate, lyophilized, and stored at 4°C until used. The urine extracts were prepared by dialysis against 0.05 M ammonium bicar-

bonate buffer, filtration through a 0.45 µ porosity filter, and lyophilization. The extracts were coupled to cyanogen bromide activated Sepharose-4B beads according to the manufacturer's recommendations (Pharmacia, Piscataway, New Jersey). The extracts used in the inhibition assay were dissolved in saline at an epithelial protein concentration of 10 mg/ml and a urinary protein concentration of 20 mg/ml, as determined by the BioRad protein assay (BioRad Laboratories, Richmond, California).

Air samples were collected with DuPont personal sample pumps at a flow rate of 2 l/min and type FWS-D polyvinyl chloride membrane filters. The filters were extracted in 10 ml of 0.05 M ammonium bicarbonate, and the extract was lyophilized and redissolved in 1.0 ml of sterile saline.

The RAST procedure was performed as described (6) with the use of 50 µl of a 10 % suspension of the allergen-coupled Sepharose-4B beads per tube and iodine-125-labeled antihuman immunoglobulin E (IgE) obtained from Pharmacia Diagnostics. The RAST procedure was used to screen the sera from workers exposed to rats and identify those with high levels of IgE antibodies to rat allergens. Sera from five individuals were pooled and used as the reference serum for the RAST inhibition assay. The reference serum pool contained 160 IU/ml of IgE, and a 1:5 dilution of the pool would bind approximately 15 % of the radioactivity in the standard RAST assay.

The RAST inhibition assay (1) was performed using 200 µl of either the reference allergen or air sample extract mixed with 250 µl of diluted reference serum and 50 µl of allergen-coupled beads, the volume was brought up to 900 µl with sample diluent (0.1 M phosphate buffer, pH 7.4, containing 4 % fetal calf serum, 1 % Tween 20, and 0.01 % NaN₃), and the mixture was incubated overnight at room temperature on a tube rotator. The tubes were washed three times, radio-labeled anti-IgE was added, and the tube was incubated overnight at room temperature. After three additional washes, the radioactivity of the samples was determined in a gamma counter. The percentage of inhibition was calculated for each sample, and a standard curve was made by the plotting of the logit of the percentage of inhibition against the concentration of protein in the reference samples. By regression analysis the allergen content of each sample was calculated and

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expressed as micrograms of protein allergen per cubic meter of air.

Results and discussion

Initial studies were conducted to determine if the extracts of rat allergens coupled to the beads contained the appropriate allergens. For this purpose, sera from 36 individuals occupationally exposed to rats were assayed with the use of the reagents prepared in our laboratory and the commercially available RAST reagents. There was complete concordance between these assays in that all the subjects were either negative in both assays or positive in both assays. In addition, with IgE-positive subjects the relative reactivity (percentage of counts bound) was comparable in both assays (data not shown). The results from these assays were used to identify five individuals who had relatively high reactivity in the RAST, additional serum was obtained

from these subjects, and a reference serum pool was established.

A series of experiments was carried out to determine the optimal conditions for the RAST inhibition assay. The volume of each of the reactants and the incubation times and conditions were determined which gave the best results. As shown in figure 1, a linear relationship between the logit of the percentage of counts bound and the protein content of the extracts was obtained, correlation coefficients (r) of 0.97 or better typically being obtained. The lower limits of detection were estimated to be 0.6 $\mu\text{g}/\text{ml}$ for the urinary allergens, and 1.4 $\mu\text{g}/\text{ml}$ for the epithelial allergens.

Air samples were collected from a variety of sites and assayed for allergen content. In figure 2 the concentration of urinary and epithelial allergens have been plotted against each other. There were several samples in which only one of the allergens was detected. In addition the airborne levels of urinary and epithelial allergens did not correlate ($r = 0.36$). This finding would suggest that there are antigens that are unique to each extract. When these data were analyzed with respect to the site of sample collection, the results were generally as expected. In other words, areas with the highest allergen levels were areas with either many animals (eg, holding rooms) or a high dust exposure level (eg, cage dumping and cleaning areas). Of interest was the finding of detectable levels of the allergens in the locker room and work break room, ie, in the rooms where employees changed their clothes or took work breaks. These results suggest that the allergens may be carried on clothing and distributed throughout the worksite.

In order to define the allergens contained in the extracts better, a sample of the urine extract was chromatographed on a Sephacryl S-300 column, and the major protein fractions were assayed for allergen content. As shown in figure 3, the allergenic activity eluted in two peaks indicating that the urine contained at least two allergens. A sample of the epithelial extract was

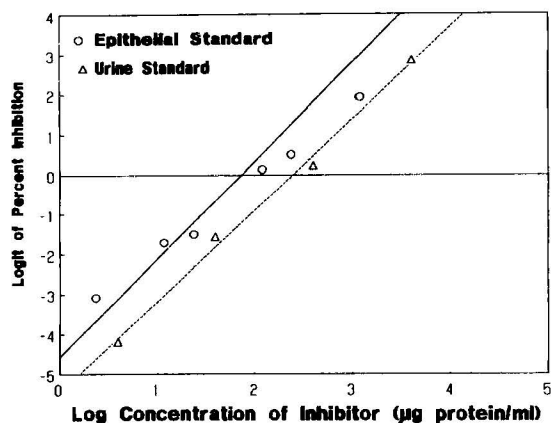


Figure 1. Linear relationship between the logit of the percentage of counts bound and the protein control of the extract.

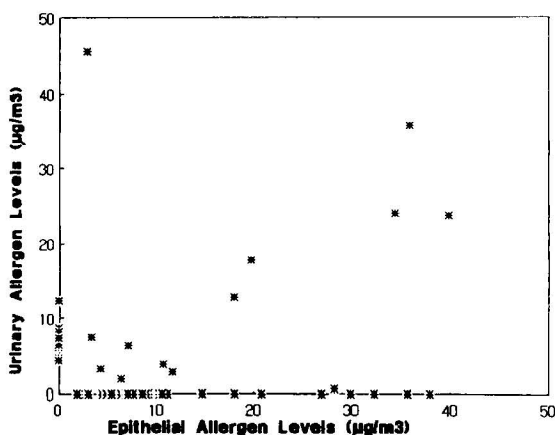


Figure 2. Concentration of urinary and epithelial allergens plotted against each other.

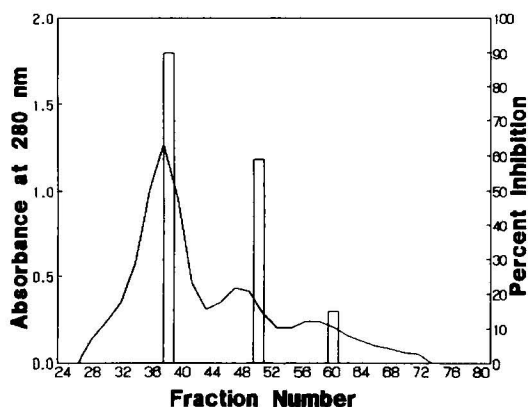


Figure 3. Chromatograph of the urine extract showing the major protein fractions.

chromatographed, and the allergenic activity was found to be widely distributed in the column eluate. This finding indicates that the epithelium is a much more complex mixture of allergens than the urine. It is hoped that, by using more purified allergens, we will be able to increase the sensitivity and specificity of the RAST inhibition assay.

In summary, the results obtained to date indicate that the RAST inhibition assay provides a very sensitive method to detect airborne allergens of laboratory animals. The use of serum from workers allergic to rats in this assay assures the specificity of the assay. We have found that, by concentrating filter extracts by lyophilization, we can detect as little as 1 µg of allergen on a filter and thus allow for the use of personal samplers to monitor the work environment. Even though we are continuing to attempt to improve the sensitivity of the assay, the assay has proved to be very reliable and consistent. Quantitative assessment of the airborne allergen levels in the workplace should allow for the development of control measures to

reduce allergen levels either by changes in work practices or by engineering controls.

References

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