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Diagnostic utility of concentrated Mus m 1 allergen extract in humans

Allison Norton, MD¹, Keegan Smith, MD², Kristina James, MD², Aimee Hoskins, RN, BSN², Theresa A Scott, MS³, Greg Plunkett, PHD⁴, John Fahrenholz, MD², and Ryszard Dworski, MD, PhD²

¹Division of Allergy, Immunology and Pulmonary Medicine, Department of Pediatrics, Vanderbilt Children's Hospital, Nashville, TN

²Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, TN

³Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN

⁴ALK-Abelló, Inc., Round Rock, TX

There is growing evidence that the mouse allergen is a major causative factor for allergic rhinitis, conjunctivitis, and asthma in children and adults of urban and rural populations.¹ Furthermore, exposure to mice by laboratory animal workers is associated with a high risk of developing occupational allergies.² Our Allergy practice at Vanderbilt University has recognized a number of patients with poorly controlled allergies to the mice that they work with. This clinical observation prompted an investigation for a better diagnostic and therapeutic option for our patients.

The major mouse allergens belong to the lipocalin family of proteins that are synthesized in the mouse liver and secreted in the urine. The major mouse allergen is known as Mus m 1 in the allergen nomenclature. Although detectable in serum and pelt extracts, Mus m 1 concentration is 10 times greater in mouse urine than serum.³ Yet, currently the only commercially available mouse extracts are mouse epithelial extracts which contain varying low concentrations of the major mouse allergen Mus m 1 (0.5–8 µg/mL).⁴

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Authors Contributions: Allison Norton, MD, designed the study, prepared IRB and IND applications, supervised or performed human experiments, collected and interpreted data, and greatly contributed to preparation of the manuscript. Keegan Smith, MD, contributed to designing the study, preparing IRB application, and writing the manuscript. Kristina James, MD, contributed to human experiments, collection of data, and preparation of the manuscript. Aimee Hoskins, RN, BSN, had a major contribution to recruitment of volunteers, performing human experiments, and collection of data. Theresa A Scott, MS, performed statistical analysis of data and contributed to preparation of the manuscript. Greg Plunkett, PHD, prepared the urine mouse extract and contributed to preparation of the manuscript. John Fahrenholz, MD, participated in designing the study and preparation of the manuscript. Ryszard Dworski, MD, PhD, coordinated all efforts associated with this investigation and had contributed to designing the study, interpretation of data, and preparing the manuscript.

We hypothesized that a highly concentrated major mouse allergen extract suitable for mouse allergy testing in humans could be isolated from mouse urine. The purpose of the study was to develop a new method for preparing mouse urine allergen extract and assess its diagnostic properties in humans. Volunteers underwent skin prick testing and intranasal challenge with the mouse urine extract to determine the diagnostic performance. To our knowledge, this is the first study to determine the diagnostic performance of mouse urine extract.

We asked ALK-Abelló Laboratory (Round Rock, TX) to prepare a new mouse urine extract to use in our investigation. Mouse urine collected from male laboratory mice was stored frozen until tested for Mus m 1 (Indoor Biotechnologies, Charlottesville, NC). Urine, containing 2,000 to 3,000 µg/mL of Mus m 1 was dialyzed using 0.4% phenol in normal saline, and then diluted to a concentration of 100 µg/mL. The final extract contained 100 µg/mL of Mus m 1 in 50% glycerin, 0.9% NaCl and 0.4% phenol. Qualitative analysis of protein content in the new mouse urine extract and the commercial mouse epithelial extract (ALK-Abelló) were analyzed by reversed-phase liquid chromatography-tandem mass spectrometry.⁵

Thirty nine healthy individuals (32 women, 7 men, age 18–60 years) with a history of mouse exposure were recruited from Vanderbilt University by means of mass e-mail and advertisement. Volunteers consented verbally and in writing to the protocol that was approved by the Vanderbilt University Committee for the Protection of Human Subjects. An Investigational New Drug agreement with the U.S. Food and Drug Administration for the use of mouse urine extract for skin testing and nasal provocations in humans was in place prior to initiation of the study. Subjects discontinued any medications that could interfere with testing at least 5 days prior to the study. Patients completed a questionnaire to assess mouse-related allergic symptoms and exposure.

Volunteers then underwent skin prick testing with common aeroallergens (cat and dog hair, mixed mites, German cockroach, alternaria, cladosporium, Bermuda grass, Johnson grass, pecan pollen, oak, cedar, ragweed, mixed lambs quarter) and the commercial mouse epithelial extract (ALK-Abelló). Subsequently, volunteers underwent titrated skin prick testing to the new mouse urine extract. Normal saline was added to the mouse urine extract containing 100 µg/mL of Mus m 1 to produce concentrations of extract ranging from 0.33 µg/mL to 100 µg/mL. Skin prick testing to the new mouse urine extract started at 0.33 µg/mL if positive to the commercial mouse epithelial extract or 1 µg/mL if the subject was negative to the commercial mouse epithelial extract in prior testing. Regardless of the starting point, the dose was then increased by ½ log increments until the subject was considered positive or reached the maximum concentration of 100 µg/mL. Allergy testing was performed using standard guidelines and was considered positive if the wheal was ≥ 3 mm than negative control at 15 min post-exposure.⁶ Intradermal testing was not done. In addition, all volunteers, regardless of symptoms, underwent nasal challenge to the new mouse urine extract using the procedure described by Bousquet and colleagues.⁷ The starting point for the nasal challenge depended on the titrated skin test results and clinical symptoms to mice, as evaluated by the mouse symptom and exposure survey. Each challenge was 0.1mL of full strength mouse extract diluted with 0.9% NaCl beginning with glycerin control followed by increases in concentration of mouse allergen extract until a

positive challenge or maximum dose (100 µg/mL) was reached. A positive challenge was determined by using a 13-point symptom score of sneezing, pruritis, rhinorrhea, nasal blockage and ocular symptoms.⁸ Each symptom was graded by the participant and accumulated to give a total score for each incremental challenge, including the glycerin control.⁹ The nasal challenge was considered positive if the subject had a non65 cumulative symptom score of 5. All participants tolerated the experiments well.

The primary statistical objective was to compare the results of the titrated skin prick testing and nasal challenge of the new mouse urine extract. The sensitivity, specificity, positive predictive value and negative predictive value of the titrated skin prick test were calculated using 95% confidence intervals for each of these values by employing the Wilson score-test-based method for calculating confidence intervals for binomial probabilities and R (www.r-project.org) software.

Qualitative reversed-phase liquid chromatography-tandem mass spectrometry analysis of the mouse urine extract revealed approximately 26 mouse urine proteins and a minute amount of albumin. In contrast, the commercial mouse epithelial extract contained more than 267 proteins including high amounts of albumin, diverse bioactive proteins including heat shock proteins and other poorly characterized proteins with only small quantities of mouse urine proteins.

The mouse exposure and symptom survey was completed by 39 volunteers. These volunteers were exposed to mice on average 14 hours per week. Ninety two percent of participants (36/39) handled mice as laboratory workers. Self diagnosis of allergy to mouse and other aeroallergens based on nasal, ocular, respiratory and skin symptoms was reported by 23/39 (59%) and 29/39 individuals (74%), respectively. Thirty seven had interpretable skin prick testing (2 individuals had dermatographism).

Among these 37 patients, 28 (76%) had a positive test with at least one aeroallergen, 13 (35%) with the mouse epithelial extract, and 9 (24%) with the mouse urine extract. Nasal challenge with the mouse urine extract was completed in 35 participants. Two volunteers were excluded from the study because they developed reaction to glycerin control either on skin testing or nasal challenge. Out of the 9 subjects with positive skin test to the mouse urine extract, 8 reported symptoms on the survey, and 7 were positive to nasal challenge using mouse urine extract. All subjects with negative mouse symptom questionnaires had negative nasal challenges. The sensitivity of the new mouse urine extract was 70% (7/10), specificity 92% (23/25), positive predictive value 78% (7/9), and negative predictive value 88% (23/26).

The most important characteristics of allergen extracts used for diagnostic challenges and immunotherapy include chemical purity and predictable concentration of the specific antigen. We describe a new mouse urine extract that has a greater concentration of the major mouse allergen Mus m 1 and chemical purity than the commercially available mouse epithelial extract. The urine extract contains mostly mouse urine proteins, whereas the epithelial extract is a mixture of albumin, nonspecific proteins including heat shock proteins with a small amount of Mus m 1. However, despite the improved purity, the mouse urine

extract did not demonstrate a clear improvement in diagnostic performance over the previously published data of the diagnostic performance of the mouse epithelial extract.⁴ Comparative performance is revealed in table 1.

These results may suggest that Mus m 1 is not the only allergen causing clinical symptoms to mice. There are studies suggesting that serum albumin may also play an etiologic role in mouse allergy in humans.¹⁰ Serum albumin is abundantly present in the mouse epithelial extract. Perhaps, the optimal diagnostic method for mouse allergy should include testing with both Mus m 1 and albumin proteins. Importantly, it is also possible, that the reaction to the highly impure epithelial extract is mediated by both IgE and non-IgE mediated inflammatory pathways, thus only in part assessing a true allergic response to mouse.

Thus, we describe a new mouse allergen extract containing pure and highly concentrated mouse urine protein that is suitable for diagnostic purposes in humans. Therefore, in our opinion, despite comparable diagnostic efficacy, the newly described urine extract could be superior to the epithelial extract as a diagnostic and research tool for mouse allergy in humans.

Furthermore, if we assume that human allergy to mice is similar to cat and dog in which there is a dose dependent response to the major allergen in immunotherapy, then in the future this new extract could be used to develop desensitization methods based on dose of major allergen.^{11,12} This could give laboratory workers as well as children and adults with severe allergy and asthma to mice an option for relief that was previously never available.

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Table 1

Comparative Performance of Mouse Urine Extract to Mouse Epithelia Extract

MOUSE EXTRACT	SENSITIVITY ¹	SPECIFICITY ²	POSITIVE PREDICTIVE VALUE ³	NEGATIVE PREDICTIVE VALUE ⁴
URINE	70% (95% CI=0.40, 0.89) ⁵	92% (95% CI=0.75, 0.98)	78% (95% CI=0.45, 0.94)	88% (95% CI=0.71, 0.96)
EPITHELIAL ⁶	67%	94%	83%	86%

¹ Proportion of true (new) mouse positives correctly identified by the titrated skin prick test (i.e., the probability that a patient's titrated skin prick test result was positive given their nasal challenge result was positive).

² Proportion of true negatives correctly identified by titrated skin prick test (i.e., the probability that a patient's titrated skin prick test result was negative given their nasal challenge result was negative).

³ Probability a patient's nasal challenge result was positive given their titrated skin prick result was positive.

⁴ Probability a patient's nasal challenge result was negative given their titrated skin prick result was negative.

⁵ 95% confidence intervals were calculated using the Wilson score-test-based method for calculating confidence intervals for binomial probabilities.

⁶ Sharma HP, Wood RA, Bravo AR, Matsui EC. A Comparison of Skin Prick Tests, Intradermal Skin Tests, and Specific IgE in the Diagnosis of Mouse Allergy. *J Allergy Clin Immunol* 2008; 121: 933–939.