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Letter

Diagnostic utility of concentrated Mus m 1 allergen extract in humans

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 with which they work. 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 that contain varying low concentrations of the major mouse allergen Mus m 1 (0.5–8 $\mu\text{g/mL}$).⁴

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 the ALK-Abelló Laboratory (Round Rock, Texas) 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, North Carolina). Urine that contained 2,000 to 3,000 $\mu\text{g/mL}$ of Mus m 1 was dialyzed using 0.4% phenol in normal saline and then diluted to a concentration of 100 $\mu\text{g/mL}$. The final extract contained 100 $\mu\text{g/mL}$ of Mus m 1 in 50% glycerin, 0.9% sodium chloride, and 0.4% phenol. The protein content in the new mouse urine extract and the commercial mouse epithelial extract (ALK-Abelló) was analyzed by reversed-phase liquid chromatography–tandem mass spectrometry.⁵

Thirty-nine healthy individuals (32 women and 7 men; age range, 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 US Food and Drug Administration for the use of mouse urine extract for skin testing and nasal provocations in humans was in place before initiation of the study. Participants discontinued use of any medications that could interfere with testing at least 5 days before 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, and 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 that contained 100 $\mu\text{g/mL}$ of Mus m 1 to produce concentrations of extract ranging from 0.33 to 100 $\mu\text{g/mL}$. Skin prick testing to the new mouse urine extract started at 0.33 $\mu\text{g/mL}$ if the participant was positive to the commercial mouse epithelial extract or 1 $\mu\text{g/mL}$ if the participant was negative to the commercial mouse epithelial extract in prior testing. Regardless of the starting point, the dose was then increased by half-log increments until the participant tested positive or reached the maximum concentration of 100 $\mu\text{g/mL}$. Allergy testing was performed using standard guidelines, and the result was considered positive if the wheal was 3 mm or larger than the negative control at 15 minutes after exposure.⁶ Intradermal testing was not performed. In addition, all volunteers, regardless of symptoms, underwent nasal challenge to the new mouse urine extract using the procedure described by Bousquet et al.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 0.1 mL of full-strength mouse extract diluted with 0.9% sodium chloride beginning with glycerin control followed by increases in concentration of mouse allergen extract until a positive challenge result or maximum dose (100 $\mu\text{g/mL}$) was reached. A positive challenge result was determined by using a 13-point symptom score of sneezing, pruritus, 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 result was considered positive if the individual had a noncumulative symptom score of 5 or higher. 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 using the Wilson score test–based method for calculating

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Table 1
Comparative performance of mouse urine extract to mouse epithelial extract

Mouse extract	Sensitivity, % (95% CI) ^a	Specificity, % (95% CI) ^b	Positive predictive value, % (95% CI) ^c	Negative predictive value, % (95% CI) ^d
Urine	70 (40–89) ^e	92 (75–98)	78 (45–94)	88 (71–96)
Epithelial ^f	67	94	83	86

Abbreviation: CI, confidence interval.

^aProportion of true (new) mouse positive results correctly identified by the titrated skin prick test (ie, the probability that a patient's titrated skin prick test result was positive given that their nasal challenge result was positive).

^bProportion of true-negative results correctly identified by titrated skin prick test (ie, the probability that a patient's titrated skin prick test result was negative given that their nasal challenge result was negative).

^cProbability a patient's nasal challenge result was positive given that their titrated skin prick result was positive.

^dProbability a patient's nasal challenge result was negative given that their titrated skin prick result was negative.

^eThe 95% CIs were calculated using the Wilson score test–based method for calculating CIs for binomial probabilities.

^fFrom Sharma et al.⁴

confidence intervals for binomial probabilities and R software (www.r-project.org).

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 an average of 14 hours per week. Thirty-six of 39 (92%) 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 of 39 (59%) and 29 of 39 individuals (74%), respectively. Thirty-seven had interpretable skin prick test results (2 individuals had dermatographism).

Among these 37 patients, 28 (76%) had a positive test result 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 a reaction to glycerin control on skin testing or nasal challenge. Of the 9 individuals with positive skin test results to the mouse urine extract, 8 reported symptoms on the survey, and 7 tested positive to nasal challenge using mouse urine extract. All study participants with negative mouse symptom questionnaire results had negative nasal challenge results. The sensitivity of the new mouse urine extract was 70% ($n = 7/10$), specificity was 92% ($n = 23/25$), positive predictive value was 78% ($n = 7/9$), and negative predictive value was 88% ($n = 23/26$).

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 and 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 given in Table 1.

These results may suggest that Mus m 1 is not the only allergen that causes clinical symptoms to mice. There are studies that suggest 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. 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.

We describe a new mouse allergen extract that contains 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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References

- [1] Matsui EC. Role of mouse allergens in allergic disease. *Curr Allergy Asthma Rep*. 2009;9:370–375.
- [2] Bush RK, Stave GM. Laboratory animal allergy: an update. *ILAR J*. 2003;44:28–51.

- [3] Lorusso JR, Moffat S, Ohman JL. Immunologic and biochemical properties of the major mouse urinary allergen (Mus m 1). *J Allergy Clin Immunol*. 1986;78: 928–937.
- [4] 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.
- [5] Kubota K, Kosaka T, Ichikawa K. Shotgun protein analysis by liquid chromatography-tandem mass spectrometry. *Methods Mol Biol*. 2009;519: 483–494.
- [6] Bernstein L, Li JT, Bernstein DI, et al. Allergy diagnostic testing: an updated parameter. *Ann Allergy Asthma Immunol*. 2008;100:S1–S148.
- [7] Bousquet J, Lebel B, Dhivert H, Bataille Y, Martinot B, Michel FB. Nasal challenge with pollen grains, skin-prick tests and specific IgE in patients with grass pollen allergy. *Clin Allergy*. 1987;17:529–536.
- [8] Rajakulasingam K. Nasal provocation testing. In: Adkinson NF, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER, eds. *Middleton's Allergy: Principles and Practice*. 6th ed. Philadelphia, PA: Mosby; 2003:644–655.
- [9] Wierzbicki DA, Majmundar AR, Schull DE, Khan DA. Multiallergen nasal challenges in nonallergic rhinitis. *Ann Allergy Asthma Immunol*. 2008;100:533–537.
- [10] Liccardi G, Asero R, D'Amato M, D'Amato G. Role of sensitization to mammalian serum albumin in allergic disease. *Allergy Asthma Rep*. 2011;11: 421–426.
- [11] Ewbank PA, Murray J, Sanders K, Curran-Everett D, Dreskin S, Nelson SN. A double blind, placebo-controlled immunotherapy dose response study with standardized cat extract. *J Allergy Clin Immunol*. 2003;111:155–161.
- [12] Lent AM, Harbeck R, Strand M, et al. Immunologic response to administration of standardized dog allergen extract at differing doses. *J Allergy Clin Immunol*. 2006;118:1249–1256.