

Characterization of occupational sensitization by multiallergen immunoblotting in workers exposed to laboratory animals

María Luisa Caballero, PhD ^{*}; Elena Ordaz, DVM [†]; Marta Bermejo, MD [‡]; Rosa Rodríguez-Perez, PhD ^{*}; Enrique Alday, MD [§]; Jerónimo Maqueda, MD [†]; and Ignacio Moneo, MD, PhD ^{*}

^{*} Department of Immunology, Hospital Carlos III, Madrid, Spain

[†] Escuela Nacional de Medicina del Trabajo, Facultad de Medicina, Ciudad Universitaria, Madrid, Spain

[‡] Unidad de Vigilancia de la Salud y Medicina del Trabajo, Consejo Superior de Investigaciones Científicas, Madrid, Spain

[§] Instituto Nacional de Seguridad e Higiene en el Trabajo, Unidad de Neumología y Alergia Laboral, Madrid, Spain

ARTICLE INFO

Article history:

Received for publication December 28, 2011.

Received in revised form January 10, 2012.

Accepted for publication January 16, 2012.

ABSTRACT

Background: Studies have estimated that 10% to 23% of workers exposed to laboratory animals report symptoms of laboratory animal allergy.

Objectives: To determine the level of occupational sensitization in workers exposed to laboratory animals and to develop a diagnosis system based on a multiallergen IgE immunoblot.

Methods: A total of 75 workers exposed to laboratory animals were initially studied with skin prick tests performed with animal epithelia extracts. The workers with suspected occupational disease and positive skin prick test results were further studied with the ImmunoCAP system to determine specific IgE levels to urine and epithelia allergens and with multiallergen IgE immunoblotting to detect specific IgE levels to epithelia allergens and bovine serum albumin.

Results: Twenty of the 75 workers were studied with ImmunoCAP and multiallergen IgE immunoblotting. Nine were polysensitized and 3 were sensitized to only one animal. The results obtained by ImmunoCAP and multiallergen IgE immunoblotting were concordant except for in 3 workers, who had low or negative values of specific IgE determined by ImmunoCAP but positive allergen detections by immunoblotting. On the basis of the results of the study and the clinical symptoms related by workers, 16% were diagnosed as having occupational allergy.

Conclusions: Multiallergen immunoblotting by means of a unique test offers a graphic representation of sensitization to the different animals to which workers are exposed, providing additional information on the clinical symptoms caused by the involved allergens. The results presented suggest that this system can improve the diagnosis of laboratory animal allergy by obtaining a sensitization profile for each exposed worker.

© 2012 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Laboratory animal allergy (LAA) is a frequent occupational condition in workers exposed to laboratory animals. Large studies have estimated that 10% to 23% of exposed workers report symptoms of allergy to these animals, and these estimates do not include the workers who may have left the workplace after developing symptoms.¹ The LAA syndrome has been defined as an allergic disorder characterized by conjunctivitis, rhinitis, urticaria, and asthma. The reaction is known to occur through either inhalation of or skin contact with animal allergens.² Most important animal-derived respiratory allergens belong to the lipocalin protein family. Aller-

gens Mus m 1, Rat n 1, Cav p 1, and Ory c 1, main allergens of mouse, rat, guinea pig, and rabbit, respectively, are all lipocalin proteins mainly found in urine and epithelium of animals.^{3–5} Lipocalins are produced in the liver or secretory glands and are present in body fluids and secretions. They act as carriers of retinol, odorants, steroids, lipids, and pheromones.⁶

Regarding the implication of specific animals, mice and rats are the most common causes of LAA. Moreover, sensitization to both animals can develop in laboratory animal workers exposed to only one species. This dual sensitization may reflect IgE cross-reactivity between mouse and rat major allergens because Mus m 1 and Rat n 1 share 66% homology, and these dual sensitized individuals are more likely to be sensitized to other animal allergens.⁷ In our study, we aimed to determine the level of occupational sensitization in workers exposed to laboratory animals and to develop a diagnosis system based on a multiallergen IgE immunoblot.

Reprints: María Luisa Caballero, PhD, Department of Immunology, Hospital Carlos III, C/Sinesio Delgado, 10 28029 Madrid, Spain; E-mail: mlcsoto@hotmail.com.

Disclosures: Authors have nothing to disclose.

Funding Sources: Financial support was provided by the Ministerio de Trabajo e Inmigración (grant code HCIII/SENPRO).

Table 1

Results of the 20 workers with suspected occupational disease studied by the ImmunoCAP system and multiallergen IgE immunoblotting

Worker No.	Allergen	IgE ImmunoCAP, kU/L ^a		Multiallergen IgE immunoblotting ^b	
		Urine	Epithelium	Epithelium	BSA
1	Mouse	21.1	7.66	3	
	Rat	6.58	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		0.4	4	
2	BSA				1
	Mouse	>100	>100	5	
	Rat	>100	36.6	3	
	Guinea pig		20.3	3	
	Rabbit	1.79	16.3	1	
3	Hamster		76.5	5	
	BSA				3
	Mouse	0.7	<0.35	1	
	Rat	5.97	<0.35	1	
	Guinea pig		0.42	1	
4	Rabbit	<0.35	0.43	4	
	Hamster		<0.35	1	
	BSA				1
	Mouse	0.68	1.12	3	
	Rat	8.6	0.71	3	
5	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster	0.81	7.71	5	
	BSA		<0.35	1	
	Mouse	<0.35	<0.35	1	
6	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	1.96	1.38	1	
	Hamster	1.44	<0.35	3	
	BSA	<0.35	<0.35	1	
7	Mouse	16	6.16	3	
	Rat	24.5	4.78	2	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
8	BSA				1
	Mouse	<0.35	<0.35	1	
	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	4.32	13	5	
9	Hamster		<0.35	1	
	BSA				1
	Mouse	1.99	0.92	3	
	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
10	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1
	Mouse	<0.35	<0.35	1	
	Rat	<0.35	<0.35	1	
11	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1
	Mouse	3.98	1.31	2	
12	Rat	3.67	<0.35	2	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1

Table 1 (Continued)

Results of the 20 workers with suspected occupational disease studied by the ImmunoCAP system and multiallergen IgE immunoblotting

Worker No.	Allergen	IgE ImmunoCAP, kU/L ^a		Multiallergen IgE immunoblotting ^b	
		Urine	Epithelium	Epithelium	BSA
13	Mouse	<0.35	<0.35	3	
	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
14	BSA				1
	Mouse	<0.35	<0.35	3	
	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
15	Hamster		<0.35	1	
	BSA				1
	Mouse	<0.35	1.87	3	
	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
16	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1
	Mouse	0.32	0.13	3	
	Rat	<0.35	<0.35	1	
17	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1
	Mouse	<0.35	<0.35	3	
18	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1
19	Mouse	3.39	2.41	3	
	Rat	1.03	<0.35	1	
	Guinea pig		0.83	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
20	BSA				1
	Mouse	<0.35	<0.35	1	
	Rat	<0.35	<0.35	1	
	Guinea pig		<0.35	1	
	Rabbit	<0.35	<0.35	1	
	Hamster		<0.35	1	
	BSA				1

Abbreviation: BSA, bovine serum albumin.

^aIgE ImmunoCAP results are reported as specific IgE levels (cutoff, 0.35 kU/L).

^bMultiallergen IgE immunoblotting results are reported as specific IgE detection signal scores: 1, negative; 2, doubtful; 3, weak detection; 4, positive; and 5, strongly positive.

Methods

Serum samples

Serum samples from 75 workers exposed to laboratory animals were included in this study. The Scientific Committee of the National Institute for Occupational Safety and Health at Work approved the study, and written informed consent was obtained from the study participants. Experiments were performed in adherence to the appropriate laws and institutional guidelines.

Workers were initially studied by skin prick tests (SPTs) performed with commercial extracts (ALK-Abelló, Madrid, Spain). Histamine dihydrochloride (1 mg/mL) and saline solution were used as positive and negative controls, respectively. A SPT response was considered positive if the largest wheal diameter was at least 3 mm greater than the produced by the negative control.

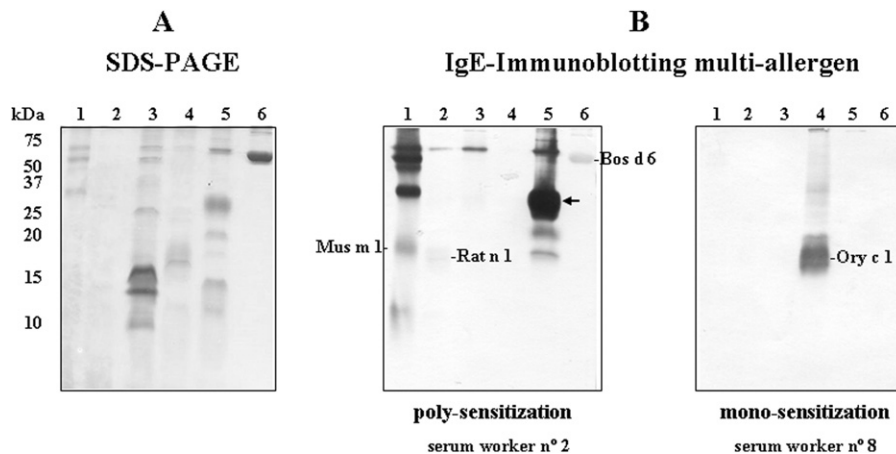


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of commercial extracts of epithelia (A) and multiallergen IgE immunoblotting (B) performed with serum samples from a polysensitized and a monosensitized worker. Lanes: 1, mouse; 2, rat; 3, guinea pig; 4, rabbit; 5, hamster; and 6, bovine serum albumin. The allergens most frequently detected by the workers are indicated in the figure and correspond to Mus m 1, Rat n 1, Bos d 6, and Ory c 1. The arrow indicates a 32-kDa hamster epithelium allergen, which is not included on the list of allergens of the International Union of Immunological Societies (www.allergen.org).

The workers with suspected occupational disease (symptoms of asthma, rhinitis, rhinoconjunctivitis, urticaria, or angioedema) and positive SPT results to animal epithelia were further studied by the ImmunoCAP system (Phadia, Uppsala, Sweden) to determine specific IgE levels to urine and epithelia allergens (cutoff, 0.35 kU/L) and by multiallergen IgE immunoblotting to detect epithelia allergens and bovine serum albumin (BSA).

Preparation of the extracts used for multiallergen IgE immunoblotting

Commercial extracts from mouse, rat, guinea pig, rabbit, and hamster epithelia (ALK-Abelló) were concentrated ($\times 16$) before use by centrifugation (14,000g for 30 minutes) in Microcon filters (YM10; Millipore Corporation, Billerica, Massachusetts).

SDS-PAGE Analysis and Multiallergen IgE Immunoblotting

Aliquots of 15 μ g of total protein from each epithelium extract or 2 μ g of BSA (Sigma-Aldrich, St Louis, Missouri) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on 16% acrylamide minigel, under standard conditions. After electrophoresis, proteins were stained with Coomassie Blue or were transferred onto nitrocellulose membranes (NitroPure supported, 0.45 μ m; Bio-Rad, Hercules, California) and incubated overnight with the workers' serum samples (diluted 1:20). Specific IgE determination was performed with a monoclonal anti-IgE antiserum (Ingenasa, Madrid, Spain) and an alkaline phosphatase-labeled goat anti-mouse antiserum (Biosource International, Camarillo, California). Finally, the signal was visualized with the alkaline phosphatase 5-bromo-4-chloro-3-indolyl phosphate/4-nitroblue tetrazolium system (Amresco, Solon, Ohio) for 30 minutes.⁸

Results

A total of 75 workers (33.3% male and 66.7% female; age range, 20–60 years) were studied. The mean time of exposure to laboratory animals was 9 years for males and 6 years for females. The main species handled by the workers were mouse (93.1%), rat (62.5%), rabbit (29.2%), guinea pig (15.3%), and hamster (12.5%).

The results of SPTs performed with animal epithelia extracts were positive to mouse in 13.6% of the workers, rat in 7.6%, rabbit and guinea pig in 6.1%, and hamster in 3%.

Twenty of the 75 workers (26%) were suspected of having occupational disease and were further studied by ImmunoCAP (urine and epithelium) and multiallergen IgE immunoblotting (epithe-

lium) (Table 1). The study revealed the existence of 2 profiles of sensitization among workers: polysensitized and monosensitized workers. Nine of the 20 studied workers were polysensitized, and 3 of the 20 workers were sensitized to only one animal (workers 5 and 8 were monosensitized to rabbit and worker 9 was monosensitized to mouse).

SDS-PAGE analysis and multiallergen IgE immunoblotting

The analyses by SDS-PAGE of the commercial epithelia extracts indicated an unequal content of proteins among the different animal extracts (Fig 1A, lanes 1–5). It is noteworthy, as shown in lanes 1 and 2, that the mouse and rat main allergens, Mus m 1 and Rat n 1, both with molecular weights of 17 kDa (www.allergen.org), are poorly represented in the total protein extracts in contrast to guinea pig, rabbit, and hamster extracts (lane 3–5).

Multiallergen IgE immunoblotting performed on the 20 workers with suspected occupational disease revealed the 2 mentioned profiles of sensitization (Fig 1B). Polysensitization to several animals' epithelia, as shown in worker 2, detected mouse (Mus m 1), rat (weakly Rat n 1), and hamster allergens (strongly 32 kDa) and BSA, and monosensitization, as shown in worker 8, detected only the rabbit allergen Ory c 1 (17–18 kDa). With reference to the 32-kDa hamster allergen, 3 of the 20 studied workers had positive detection results. BSA was detected only in worker 2.

The results obtained by both ImmunoCAP and multiallergen IgE immunoblotting in relation to the epithelia were concordant except in 3 of the 20 studied workers (workers 1, 3, and 6), who had low or negative values of specific IgE determined by ImmunoCAP but positive allergen detection results by immunoblotting (Fig 2). Finally, on the basis of the results of this study and the clinical symptoms related by workers, 16% were diagnosed as having occupational allergy.

Discussion

We studied 75 workers whose mean time of exposure to laboratory animals was 7.5 years, and after the study, 16% were diagnosed as having occupational allergy. This figure agrees with the estimations of LAA.¹

Polysensitization to several species of laboratory animals was found, but it cannot be attributed to cross-reacting proteins (as in BSA) because the frequency of detection of specific IgE to albumin in our study was 5%. However, polysensitization could reflect the cross-reactivity among lipocalin proteins as previously described.^{7,9} On the other hand, 2 of the 20 workers were sensitized

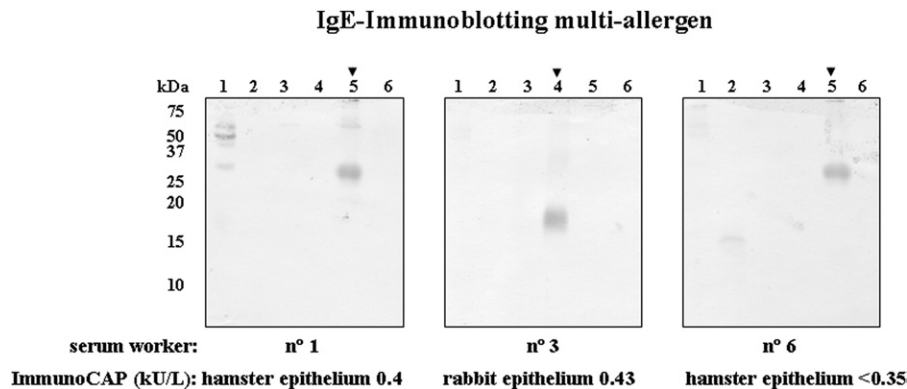


Figure 2. Multi-allergen IgE immunoblotting performed with commercial extracts of epithelia and serum samples from 3 workers who had low or negative IgE levels measured by the ImmunoCAP system. Lanes: 1, mouse; 2, rat; 3, guinea pig; 4, rabbit; 5, hamster; and 6, bovine serum albumin.

only to rabbit, and 1 worker was monosensitized to mouse. We do not know whether these workers handled only one species of animal.

The analysis by SDS-PAGE of commercial extracts of animal epithelia used for allergy diagnosis by SPTs revealed an unequal amount of proteins among the different animal extracts, with the presence of irrelevant proteins and, in most cases, low amounts of major allergens. These observations agree with those described by Fernandez-Caldas et al.¹⁰

Comparing our results obtained by ImmunoCAP with those obtained by a multi-allergen IgE immunoblot based on the SPT epithelia extracts revealed that in 3 patients specific IgE to hamster or rabbit would not have been detected (Fig 2). In such cases, the confirmation of the results by a second method was decisive for diagnosis.

In this sense, both SPTs and serologic tests are highly sensitive for detecting specific IgE and occupational asthma caused by most high-molecular-weight agents, but they are not specific for diagnosing occupational asthma.^{11–13}

These observations suggest that a correct diagnosis would require the purification and standardization of animal epithelia extracts or the use of recombinant allergens. In this sense, component-resolved diagnosis strategies used for the diagnosis of other allergies could help solve this problem.

Regarding the hamster allergens, the multi-allergen IgE immunoblot allowed us to identify a 32-kDa allergen, strongly detected by 3 of the workers with suspected occupational disease. Even though an allergen with the same molecular weight was previously described,¹⁴ there are no hamster allergens registered by the International Union of Immunological Societies Allergen Nomenclature Subcommittee.

In conclusion, multi-allergen immunoblotting offers, by means of a unique test, a graphic representation of sensitization to the different animals to which workers are exposed, providing additional information on the allergens involved in their clinical symptoms. The results presented in this pilot study suggest that this

system can improve the diagnosis of LAA by obtaining a sensitization profile for each exposed worker.

References

- [1] Elliot L, Heederik D, Marshall S, Peden D, Loomis D. Incidence of allergy and allergy symptoms among workers exposed to laboratory animals. *Occup Environ Med.* 2005;62:766–771.
- [2] Thulin H, Björkdahl M, Karlsson AS, Renström A. Reduction of exposure to laboratory animal allergens in a research laboratory. *Ann Occup Hyg.* 2002;46:61–68.
- [3] Virtanen T, Zeiler T, Mäntyjärvi R. Important animal allergens are lipocalin proteins: why are they allergenic? *Int Arch Allergy Immunol.* 1999;120:247–258.
- [4] Fahlbusch B, Rudeschko O, Szilagyi U, et al. Purification and partial characterization of the major allergen, Cav p 1, from guinea pig *Cavia porcellus*. *Allergy.* 2002;57:417–422.
- [5] Baker J, Berry A, Boscatto LM, Gordon S, Walsh BJ, Stuart MC. Identification of some rabbit allergens as lipocalins. *Clin Exp Allergy.* 2001;31:303–312.
- [6] Flower DR. The lipocalin protein family: structure and function. *Biochem J.* 1996;318:1–14.
- [7] Jeal H, Harris J, Draper A, Taylor AN, Cullinan P, Jones M. Dual sensitization to rat and mouse urinary allergens reflects cross-reactive molecules rather than atopy. *Allergy.* 2009;64:855–61.
- [8] Moneo I, Caballero ML, Gomez F, Ortega E, Alonso MJ. Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. *J Allergy Clin Immunol.* 2000;106:177–182.
- [9] Saarelainen S, Rytkönen-Nissinen M, Rouvinen J, et al. Animal-derived lipocalin allergens exhibit immunoglobulin E cross-reactivity. *Clin Exp Allergy.* 2008;38:374–381.
- [10] Fernández-Caldas E, Carnés J, Gallego M, Mari A, Pagán Alemán JA. Standardization of animal epithelia. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankfurt A M.* 2006;95:107–116.
- [11] Nicholson PJ, Cullinan P, Newman Taylor AJ, Burge PS, Boyle C. Evidence based guidelines for the prevention, identification, and management of occupational asthma. *Occup Environ Med.* 2005;62:290–299.
- [12] Sung SY, Lee WY, Yong SJ, et al. A case of occupational rhinitis induced by maize pollen exposure in a farmer: detection of IgE-binding components. *Allergy Asthma Immunol Res.* 2012;4:49–51.
- [13] Green BJ, Cummings KJ, Rittenour WR, et al. Occupational sensitization to soy allergens in workers at a processing facility. *Clin Exp Allergy.* 2011;41:1022–1030.
- [14] Bertó JM, Peláez A, Fernández E, Lombardero M, Ferrer M. Siberian hamster: a new indoor source of allergic sensitization and respiratory disease. *Allergy.* 2002;57:155–159.