Identification and isolation of a Fel d 1–like molecule as a major rabbit allergen

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Background: Rabbits are increasingly kept as domestic pets. Several rabbit allergens have been characterized. However, their sequences are still elusive, and none of these molecules are available for diagnosis.

Objective: We sought to isolate major allergens from the rabbit *Oryctolagus cuniculus* and to investigate their importance in sensitized patients.

Methods: Proteins were extracted from rabbit hair, and IgE-reactive proteins were purified by using sequential chromatography. Allergens were characterized by means of N-terminal sequencing and mass spectrometry. IgE reactivity to a new allergen was analyzed in sera of 35 patients sensitized to rabbits in a domestic setting. A model of the crystal structure of the isolated proteins was constructed.

Results: A new IgE-reactive allergen, Ory c 3, was identified as rabbit lipophilin. The molecule that belongs to the secretoglobin family is a heterodimer of 18 to 19 kDa composed of 2 polypeptide chains, CL2 and AL. CL2 has a predicted N-linked glycosylation site confirmed by using mass spectrometry. Of the 35 patients with rabbit allergy studied, 27 (77%) had IgE to both the glycosylated and deglycosylated Ory c 3 heterodimer. Allergenicity of Ory c 3 was confirmed by using skin prick tests and the basophil activation assay. Modeling of the structure revealed a marked homology to Fel d 1, the major cat allergen. However, no IgE cross-reactivity was detected between Fel d 1 and Ory c 3.

Conclusion: The rabbit lipophilin heterodimer AL-CL2 has been identified as a major rabbit allergen. After Fel d 1, Ory c 3 is the second mammalian secretoglobin shown to be a major allergen. (J Allergy Clin Immunol 2014;133:759-66.)

Key words: *Allergen, cross-reactivity, Fel d 1, IgE, inhibition, lipophilin, molecular modeling, Ory c 3, rabbit, secretoglobin*

Abbreviation used
MD: Molecular dynamics

Rabbits, which are usually raised for their fur and meat but also kept as laboratory animals, have become increasingly popular as pets over the last years.[1] According to pet industry statistics, 1 million rabbits are kept as pets in the United Kingdom; 24.6 million small animals, including rabbits, guinea pigs, and hamsters, are present in European Union households; and 16 million small animals are present in the United States.[2] Rabbits are considered the most popular mammalian pets after cats and dogs. About 1.5% to 3% of US and European households own a rabbit. The majority of these animals are kept indoors. In Italy, 7.6% (82/1085) of an atopic population with respiratory symptoms were sensitized to rabbits, whereas 20.9% were sensitized to dogs, and 20.7% were sensitized to cats.[3] In this study, as well as in occupational studies,[4-6] multiple sensitizations to several mammalian animals were found in a majority of patients. Animal extracts contain potentially cross-reactive molecules, such as serum albumins[7] and some lipocalins.[8] Skin prick testing and *in vitro* IgE diagnosis with animal extracts do not allow discrimination between primary sensitization and IgE cross-reactivity. Several single allergens are available for cats, dogs, horses, mice, and rats.[9] There are no purified natural or recombinant allergens for rabbits allowing determination of a specific sensitization. Neither are tests available to measure rabbit allergens at the workplace or at home. Asthma prevalence studies generally report sensitization to cats and dogs, as well as indoor exposure levels of cat and dog allergens.[10,11] Sensitization to small pets is generally neglected, possibly because the specific components for diagnosis are lacking.

Allergens from rabbit *(Oryctolagus cuniculus)* have been detected in fur, saliva, and urine.[12-14] N-terminal sequences were determined for 4 of them, which allowed identifying the 18- and 21-kDa proteins isolated from saliva as lipocalins, named Ory c 1 and Ory c 2.[12] In addition, an 8-kDa saliva protein was found to have some homology to Fel d 1.

In the present study we have isolated and identified a lipophilin heterodimer as a new major rabbit allergen. Specific IgE antibodies have been quantified in a cohort of 35 patients sensitized to rabbits in a domestic setting. Allergenicity was evaluated by using skin prick tests and the basophil activation assay. The new allergen has a high structural similarity to Fel d 1, but no IgE cross-reactivity could be shown in the sera tested. The detection of Ory c 3 in settled house dust from homes with a rabbit pet adds further relevance to the new allergen.

METHODS

Patients and sera

Patients with inhalant allergy or atopic dermatitis and sensitized to rabbits were selected from the outpatient population of the Luxembourg National Immunology-Allergology Unit. All had positive skin prick test responses to a

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TABLE I. Patients with rabbit allergy

Patient no.	Age (y)	Sex	Clinical symptoms	Rabbit epithelium e82 (kU_A/L)	Cat dander e1 (kU_A/L)	Dog dander e5 (kU_A/L)	Pets at home	nOry c 3 (kU_A/L)	rFel d 1 (kU_A/L)
1	30	Male	**A, R, C**	24	<0.35	0.49	Rabbits	13.0	0.45
2	15	Male	Rs ns	16	1.85	5.8	NI	19.6	3.19
3	46	Female	A	47	<0.35	1.44	Dog	33.7	0.13
4	23	Male	**R, C** A	56	38	11	Rabbit prev Dog	10.8	43.8
5	32	Female	**R**	30	NT	NT	Rabbit prev Cat	12.7	0.15
6	17	Female	**R, A**	>100	7.8	3.1	Rabbit prev	276.9	5.72
7	4	Female	Rs ns	82	NT	NT	NI	28.0	<0.1
8	32	Female	R, A	20	3.73	14	Dog	17.2	6.9
9	23	Male	R, A	3.26	28.3	6.85	Cat	<0.35	60.5
10	36	Male	A, **C**	22.4	<0.35	<0.35	Rabbit	9.3	0.38
11	40	Male	R	42.2	17.7	14.1	Rabbits Horse	35.6	24.9
12	5	Male	Rs ns	17	<0.35	27	NI	25.3	<0.1
13	47	Male	A, E	9.77	81	42	Dog, cat Ferret	9.6	37.5
14	29	Female	**R, A**	4.64	4.79	14	Rabbit, dog	<0.35	8.01
15	9	Male	**R, U**	4.24	<0.35	<0.35	Rabbit	2.2	<0.1
16	47	Female	**A**	78	1.76	0.34	Rabbit	74.8	0.19
17	17	Female	A	7.43	>100	18	Rabbit Cat, dog, horse	8.9	169.2
18	27	Female	Rs ns	25	<0.35	<0.35	NI	11.3	<0.1
19	35	Female	A, R	6.55	5.97	2.14	Cat Rabbit prev	<0.35	5.48
20	9	Male	**A**	9.61	<0.35	0.13	Rabbit	5.4	<0.1
21	58	Female	A	>100	>100	52	Rabbit Cat, guinea pig	96.0	>200
22	39	Male	E	27	NT	NT	Rabbit prev	15.5	0.29
23	11	Male	**C**, E	57	<0.35	<0.35	Rabbit	67.7	<0.1
24	35	Female	Rs ns	18	NT	NT	NI	<0.35	<0.1
25	34	Female	A, R	13	4.75	6.71	Rabbit prev Dog, horse	70.5	6.15
26	36	Female	**A**	>100	47	32	Rabbit, horse	59.2	33.2
27	28	Male	A, E	4.18	<0.35	<0.35	Rabbit	<0.35	<0.1
28	50	Male	Rs ns	45	NT	NT	NI	7.2	<0.1
29	31	Female	**A, R, C**	5.69	<0.35	0.68	Rabbit, guinea pig Hamster	<0.35	0.12
30	10	Female	**C, U**	3.26	1.87	0.478	Rabbit	<0.35	2.11
31	45	Female	R, A	3.84	<0.35	3.27	Rabbit, dog	<0.35	<0.1
32	12	Male	A, C	4.95	7.7	3.5	Rabbit	5.6	0.1
33	15	Female	Rs ns	53	NT	NT	NI	47.3	<0.1
34	29	Female	R, A	42	0.16	0.49	Rabbit prev	22.9	<0.1
35	54	Male	A	3.81	<0.35	1.42	Rabbit	4.7	<0.1

Symptoms are shown in boldface when caused by rabbit exposure.
A, Asthma; *C*, conjunctivitis; *E*, atopic eczema; *NI*, no information; *NT*, not tested; *R*, rhinitis; *Rabbit*, actual exposure to rabbit(s); *rabbit prev*, previous prolonged exposure to rabbit(s); *Rs ns*, respiratory symptoms not specified; *U*, urticaria after contact with rabbit.

commercial rabbit hair extract (Stallergenes, Antony, France) and specific IgE to rabbit epithelium (e82; range, 3.3 to >100 kU_A/L) quantified by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Age of the patients ranged from 4 to 58 years (mean age, 28.9 years; median, 30 years), and 19 of 35 patients were women. For 28 patients, complete data concerning clinical symptoms and rabbit exposure were available: 22 had confirmed exposure to rabbits at their homes, 15 had rabbits at the time of diagnosis, and 7 had rabbits previously. Thirteen patients had immediate-type allergic symptoms that were clearly related to rabbit exposure. Seven had asthma, 7 had rhinitis, 6 had conjunctivitis, and 2 had contact urticaria (Table I). The study was approved by the National Committee for Medical Research Ethics.

Sera of 18 nonallergic subjects with low total IgE levels (range, 2-7.8 kU/L) and sera of 22 patients with pollen allergy, mite allergy, or both (total IgE range, 16-370 kU/L) served as controls in the ELISA IgE quantification assays.

Protein extractions

Male adult rabbits (strain "Fauve de Bourgogne") were obtained from a local breeder, and hair was cut with scissors. Proteins were extracted overnight with PBS containing protease inhibitors (Protease Inhibitor Cocktail Tablets; Roche, Mannheim, Germany).

SDS-PAGE and immunoblotting

Proteins were separated by using 15% SDS-PAGE under reducing conditions. Immunoblotting was performed, as previously described.[15] Briefly,