

IgE-mediated anaphylaxis caused by bites of the pigeon tick *Argas reflexus*: Cloning and expression of the major allergen Arg r 1

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Background: Anaphylactic reactions caused by bites of the European pigeon tick *Argas reflexus* are repeatedly reported. This soft-backed tick is a parasite of wild pigeons colonizing urban buildings and houses. Occasionally the ticks can bite human beings, inducing anaphylactic reactions in sensitized patients.

Objective: Our aim was to characterize the major allergen implicated in a series of anaphylactic reactions caused by *Argas* bites and to produce the allergen as recombinant protein for diagnostic purposes.

Methods: Protein extracts were prepared from whole *A reflexus* bodies, and IgE immunoblots were performed with sera from 13 patients who had an anaphylactic reaction with pigeon tick bites. A cDNA expression library was constructed from whole ticks and screened with a polyclonal rabbit antiserum raised against the major allergen.

Results: The cDNA coding for the dominant allergen Arg r 1 could be isolated. It encodes a protein belonging to the lipocalin family. Allergenicity of the recombinant Arg r 1 was confirmed by immunoblot, ELISA, and intradermal skin tests.

Conclusion: The dominant allergen of *A reflexus* has been isolated and the corresponding cDNA cloned. The recombinant protein, a lipocalin, was expressed in *Escherichia coli* and was shown to be immunoreactive *in vitro* and *in vivo*.

Recombinant Arg r 1 was used as a diagnostic tool in a series of anaphylactic reactions caused by pigeon tick bites. (J Allergy Clin Immunol 2005;115:617-22.)

Key words: Anaphylaxis, pigeon tick, *Argas reflexus*, major allergen, cDNA cloning, lipocalin, Arg r 1

During the last several decades, severe anaphylactic reactions provoked by pigeon tick bites have been reported from France, Italy, Germany, and Poland.¹⁻⁸ In 1997, the authors⁹ published a series of 12 cases of

Abbreviations used

nArg r 1: Natural Arg r 1
PVDF: Polyvinylidene difluoride
rArg r 1: Recombinant Arg r 1
TSGP: Tick salivary gland protein

anaphylactic reactions caused by *Argas reflexus* bites. The overall clinical picture was characterized by sudden onset of severe mucocutaneous, respiratory, gastrointestinal, and cardiovascular allergic symptoms resulting in loss of consciousness and requiring intensive care in 8 cases. The clinical suspicion of pigeon tick anaphylaxis is based on a history of a bite, occurring often at night during the hot season. Further arguments are the presence of pigeons in the immediate environment and, eventually, the identification of the parasite. The diagnosis is confirmed when immediate-type sensitization is shown by positive skin prick tests performed with pigeon tick extracts^{2,7} or the demonstration of specific IgE *in vitro*.^{2,3,6-9}

The European pigeon tick *A reflexus* is a temporary parasite of wild and domesticated pigeons in southern and central Europe. *A reflexus* belongs to the Argasidae or soft tick family.¹⁰ It is a specific bloodsucking parasite of pigeons. It feeds briefly, generally for less than 15 minutes. These ticks live inside houses, especially in old urban housing and on higher floors in the proximity of pigeon colonies. Because of the growing populations of wild urban pigeons, the parasite is spreading. Pigeon ticks have been found in 45% of occupied pigeon nests and 14% of deserted nests.¹¹ When pigeons are not available or have been removed, ticks can leave their hiding places, invade nearby apartments, and bite human beings. Because of the remarkable fasting capabilities of the nymphs and adults, which easily survive 3 to 5 years without feeding,¹² pigeon tick bites can occur several years after complete removal of the pigeons. Tick bites generally induce harmless local manifestations, but in some people, they induce systemic anaphylactic reactions with increasing severity. Positive diagnosis of pigeon tick anaphylaxis is hampered by the fact that no commercial test is available. Besides specimen procurement problems, tick extracts harbor the potential risk of transferring infectious agents, at least when used for skin tests.^{13,14} The purpose of this study was to isolate the dominant

From ^athe Centre de Recherche Public-Santé/Centre Hospitalier de Luxembourg Laboratory of Immunogenetics and Allergology, Luxembourg; and ^bthe Hôpitaux Universitaires de Strasbourg, Service de Pneumologie. Supported by grant SAN/03/012 Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche (Centre de Recherche Public-Santé: C03/07). Received for publication September 2, 2004; revised November 23, 2004; accepted for publication November 29, 2004.

Available online February 2, 2005.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.11.052

TABLE I. Patient characteristics

Patient	Sex	Age	Symptoms	Episodes of anaphylactic shock	Total IgE kU/L	Specific IgE to rArg r 1 kU/L
1	F	36	U-Q, R, C-V	2	ND	5.9
2	F	23	U-Q, C-V	1	10.5	1.3
3	F	40	U-Q, C-V	1	698	115.3
4	M	24	U-Q, C-V, N	1	2650	2556.3
5	ND	ND	U-Q, C-V	1	ND	5.1
6	ND	ND	U-Q, C-V	1	1050	242.2
7	M	25	U-Q, R	2	816	423.5
8	ND	ND	U-Q, C-V	1	2534	0.5
9	F	47	U-Q, C-V, D	2	2100	2071.7
10	F	40	R, C-V, D, N	1	218	149.6
11	M	38	U-Q, C-V, D, N	1	1216	245.5
12	M	35	R, N	3	121.8	6.6
13	M	51	U-Q, R	2	203	6.5

C-V, Cardiovascular symptoms; D, digestive symptoms; N, neurological symptoms; ND, not defined; R, respiratory symptoms; U-Q, cutaneous or mucosal symptoms.

allergen of *A reflexus*, to clone the corresponding cDNA, to express the allergen as recombinant protein, and to test its usefulness as a diagnostic tool.

METHODS

Human sera

Sera of 13 patients who had an anaphylactic reaction with pigeon tick bites were used for allergen characterization (Table I). Five of the sera were frozen historic sera⁹ (patients 1, 4, 10, 11, and 12). Eight sera were from unpublished cases with a history of pigeon tick anaphylaxis.

Preparation of protein extracts

A reflexus ticks were obtained from H. Dautel (Insect Services, Berlin, Germany). Ticks were disrupted with pestle and mortar in liquid nitrogen and suspended in lysis buffer (50 mmol/L TRIS HCl, pH 7.5; 150 mmol/L NaCl; 0.1% SDS; 1% nonidet P40; 0.5% sodium deoxycholate) containing a mixture of protease inhibitors (Roche, Mannheim, Germany). The extract was centrifuged for 10 minutes at 12,000g at 4°C to remove insoluble particles. The protein extract was dialyzed against PBS and concentrated with an Ultrafree concentrator (Millipore, Bedford, Mass).

SDS-PAGE and immunoblot

Proteins were separated by 18% SDS-PAGE gels under denaturing conditions and visualized by Coomassie brilliant blue colloidal staining. For immunoblotting, proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The PVDF membrane was cut into strips, which were incubated overnight with patients' sera diluted 1:10 in blocking buffer (TRIS-buffered saline/Tween-20, 0.05%; 3% BSA). Control strips were incubated with the serum of a patient without allergy. Bound IgE was detected with antihuman IgE antibody labeled with alkaline phosphatase (Sigma, St Louis, Mo), and the blots were developed by addition of nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, Wis).

Protein sequencing

A reflexus whole-body extract was separated by preparative SDS-PAGE and blotted onto PVDF membrane. A piece of membrane

containing the band of interest was isolated. N-terminal sequencing was performed by Eurogentec (Berlin, Germany).

Production of polyclonal rabbit antisera

Argas reflexus extract was separated by preparative SDS-PAGE and stained with Coomassie brilliant blue. A protein band corresponding to the allergenic protein detected in immunoblots was cut out of the gel. The gel slice was sent to Eurogentec (Herstal, Belgium) for immunization of rabbits.

Extraction of RNA and construction of cDNA expression library

Whole bodies of *A reflexus* were ground with a mortar and pestle in liquid nitrogen, and RNA was extracted by using the RNeasy Fibrous Tissue kit (Qiagen, Hilden, Germany). A cDNA expression library was constructed in λ TriplEx2 by using the SMART cDNA library construction kit (BD Biosciences Clontech, Erembodegem, Belgium). Briefly, 1 μ g total RNA was reverse-transcribed with PowerScript reverse transcriptase. cDNA was amplified by PCR, digested with Sfi I, size-fractionated by using Chroma Spin-400 columns and ligated to λ TriplEx2. cDNA was packaged by using Gigapack III Gold packaging extract (Stratagene, La Jolla, Calif).

Screening of the tick cDNA library

The unamplified cDNA library was screened with rabbit polyclonal antiserum S2 raised against the purified tick allergen of 18 to 19 kd. Positive phage plaques were detected by using alkaline phosphatase-labeled goat antirabbit IgG (Southern Biotechnology, Birmingham, Ala) and were subcloned. cDNA inserts were amplified by PCR and sequenced. Sequences were compared with the N-terminal protein sequence obtained by microsequencing. λ TriplEx2 clones were converted to pTriplEx2 plasmids by *in vivo* excision and circularization.

Expression and purification of recombinant Arg r 1

The predicted mature protein coding sequence was cloned into the vector pQE60 (Qiagen). Arg r 1 was expressed in *Escherichia coli* M15 with a C-terminal 6-histidine tail. The recombinant protein was purified by nickel-nitrilotriacetic acid metal-affinity chromatography (Qiagen) under native conditions according to the instructions of the manufacturer. Recombinant protein was eluted in 250 mmol/L imidazol, 300 mmol/L NaCl, and 50 mmol/L NaH₂PO₄, pH 8.0.

Fractions were pooled, dialyzed against PBS, and concentrated by centrifugation with an Ultrafree concentrator (Millipore). Further purification was performed by cation exchange chromatography on an Econo-Pac CM column (Bio-Rad, Nazareth, Belgium) with 20 mmol/L sodium phosphate buffer, pH 7.2. The protein was eluted with 0.5 mol/L NaCl in 20 mmol/L phosphate buffer. The purity of the recombinant protein was analyzed by SDS-PAGE.

Quantification of specific IgE antibodies to Arg r 1 by ELISA

Purified recombinant *A reflexus* Arg r 1 (rArg r 1) was coated at a concentration of 5 µg/mL in PBS to microtiter plates. Patient sera were serially diluted in blocking buffer (1% BSA in PBS/0.1% Tween-20) and were added to the microtiter plate for 2 hours. Binding of IgE was measured by incubation for 1 hour with biotin-labeled antihuman IgE antibodies (1/2000; Southern Biotechnology), followed by an incubation for 30 minutes with alkaline phosphatase-labeled streptavidine (1/1000; BD Biosciences Pharmingen, Erembodegem, Belgium). p-Nitrophenyl phosphate was added as a substrate, and plates were left in the dark for approximately 30 minutes before OD was read at 405 nm. All determinations were performed in duplicate. A series of 21 patients allergic to pollen, 14 allergic to house dust mites, and 11 nonatopic patients were used as negative controls. The cutoff was determined as the mean of the controls plus 2 SD. The serum of a patient with a known titer of specific IgE to cat serum albumin determined by Pharmacia CAP-System (Pharmacia, Uppsala, Sweden) was serially diluted to generate a standard curve on which all determinations of specific IgE to rArg r 1 were calculated. Two columns of wells were coated with cat serum albumin (Sigma) at a concentration of 5 µg/mL. Cold water fish gelatine 2% (Sigma) was used instead of BSA in the blocking buffer.

ELISA inhibition experiments

In ELISA inhibition experiments, purified natural Arg r 1 (nArg r 1) was coated at a concentration of 5 µg/mL to microtiter plates as described. Patient sera were preincubated for 2 hours with 100 µg/mL rArg r 1 as inhibitor or BSA as a control before addition to the microtiter plate for 2 hours. The percentage of inhibition of IgE binding was calculated from the mean OD values measured with or without addition of inhibitor according to the following formula:

$$\% \text{inhibition} = \frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{inhibitor}})}{\text{OD}_{\text{control}}} \times 100$$

Intradermal testing

Informed consent was obtained from 1 patient allergic to *A reflexus* (no 13), 1 individual without allergy, and 2 atopic controls, and intradermal tests were performed on the forearms of the patients. Individuals were injected with increasing concentrations of rArg r 1 (0.01, 0.1 µg/mL) diluted in 0.03% human albumin, 0.9% NaCl, and 0.4% phenol (ALK-Abelló, Hørsholm, Denmark). Codein was used as a positive control and the diluent as negative control. Skin reactions were recorded 20 minutes after testing. rArg r 1 was produced in *E coli* M15 in growth media certified BSE-free and purified as described. Proteins were dialyzed against PBS and sterile-filtered.

Purification of nArg r 1

Argas reflexus protein extract was centrifuged in an Ultrafree concentrator with a cutoff of 30 kd (Millipore). nArg r 1 was recovered in the flow through fraction and was further purified and dialyzed against PBS by using Ultrafree concentrators with a cutoff of 5 kd.

Sequence analysis and database searches

Database homology searches, multiple sequence alignments with Clustal W, and sequence analysis were performed with HUSAR (Heidelberg Unix Sequence Analysis Resources; Deutsches Krebsforschungszentrum, Heidelberg, Germany).

RESULTS

Allergen characterization by immunoblot and N-terminal sequencing

The patient's IgE antibodies recognized predominantly a broad, intense band of about 18 to 19 kd (Fig 1). Several sera (5, 8, 11, 13) also recognized a strong band at 40 kd. Additional bands were detected by some sera at 30 kd as well as in the high molecular weight range.

The N-terminal sequence of the major band was D (V/L) E (S/A) G K T D A E T S I K. No significant results were obtained by searching the protein sequence databases with this peptide sequence. The allergenic protein was purified by SDS-PAGE and used for immunization of 2 rabbits. The sera obtained from those rabbits recognize a protein in the same molecular weight range as the IgE antibodies of the patients (Fig 1, lanes S1 and S2).

Cloning of the cDNA coding for Arg r 1

Sequencing of positive clones revealed an uninterrupted open reading frame of 480 nucleotides (EMBL Nucleotide Sequence Database accession number AJ697694). A hydropathy plot¹⁵ of the deduced amino acid sequence revealed a major hydrophobic region from amino acid 1 to 16. This region corresponds to a secretory signal peptide with a predicted cleavage site¹⁶ corresponding to the site determined by N-terminal sequencing. The determined peptide sequence starts at amino acid 17 and matches the sequence predicted by the cDNA clone except for the misidentification of D₁₈, C₁₉, and W₂₆. Cleavage of the signal peptide results in a 143 amino acid mature protein with a predicted molecular weight of 15.37 kd and an isoelectric point of 8.44. No asparagine-linked glycosylation sites were detected. This was confirmed by the absence of stained proteins in the tick extract by using a commercial glycoprotein staining kit (data not shown).

The deduced amino acid sequence reveals limited identity with the tick histamine-binding proteins (HBP) discovered in the saliva of *Rhipicephalus appendiculatus*¹⁷ (RaHBP1, RaHBP2, RaHBP3 sharing 25.3%, 26.3%, and 35.6% identity, respectively) and serotonin- and histamine-binding protein (SHBP) of *Dermacentor reticulatus*¹⁸ (30.2%), the tick salivary gland proteins (TSGPs) from *Ornithodoros savignyi*¹⁹ (TSGP1, TSGP2, TSGP3, and TSGP4 sharing 33.6%, 28.5%, 28.5%, and 29% identity, respectively) and moubatin, a platelet aggregation inhibitor from the soft tick *Ornithodoros moubata*²⁰ (25.6%). These tick salivary gland proteins are lipocalins. Arg r 1 shares with them the 4 conserved cysteine residues as well as several other conserved residues (Fig 2). The crystal structure of recombinant RaHBP2 has been determined.¹⁷ Disulfid bridges are

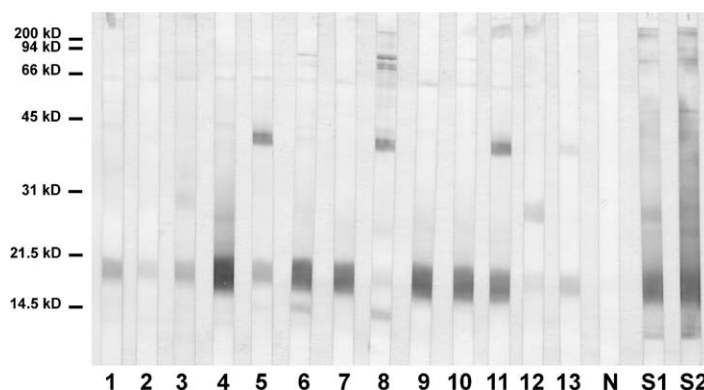


FIG 1. IgE immunoblotting of *Argas reflexus* protein extracts. Lanes 1-13, patient sera; lane N, nonallergic control; lanes S1-S2, rabbit antisera.

Arg r1 AJ697694	~~~~~MAII	ILLVACLS..		.VVSADDCSG	K..TDAWTSI	29
TSGP1 Q8I9U2	MQRLLLLLIA	LFSLSCAEAG	PDG	CVGSTEAKVA	VF.GEGGNAG	42
Moubatin Q04669	~~~MMLVLTT	LIFSFSAS..		IAYAQSGCSV	SDPLDALKAF	35
RaHBP3 O77422	~~~MKVLLLV	LGAALCON..	ADAN	PTWANEAKLG	SY.QDAWKS	38
SHBP Q8WSK7	MKMQVVLTLT	FVSAAALATQA	ETTSKAGEN	PLWAHEELLG	KY.QDAWKS	49
Arg r1 AJ697694	KGPKTGGYWL	KQTT.KT..G	ENECTY	VKGT.D.FKEN	TKTATYTYGY	71
TSGP1 Q8I9U2	.SPTIGYSYL	VKTTPD...	EHACVY	ILPPYGTADA	SGRYPYRMGY	84
Moubatin Q04669	KDG.AGTFL	QKSTDPQ...	ARDCLK	GTPNG..NRD	GNTLPVTMTY	75
RaHBP3 O77422	QQDQNKRYLL	AQATQTTD.G	VWGEETCVS	VTAEK.IGKK	KLNATILYKN	86
SHBP Q8WSK7	DQGVSVTVVL	AKTTYENDTG	SWGSQFKCLQ	VQEIE.RKEE	DYTVTSVTFE	98
Arg r1 AJ697694	KDAS.....G	KLTKTTGTAT	AKGSD.....	...IVVGS	SDT ST....VIYT	104
TSGP1 Q8I9U2	KDSN.....D	QWVKLDGKIK	TEGSKIIDND	...PEYCDTV	TT...VLYTH	123
Moubatin Q04669	KDDS.....	KWVSLNWMFT	LEGANIV..A	...TLECKRK	QR.GELVYDV	113
RaHBP3 O77422	KHLT...DLK	ESHETITVWK	AYDYT.TENG	IKYETQTRT	QTFEDVFVFS	132
SHBP Q8WSK7	RNASSPIKYY	NVTETVKAVF	QYGYKNIRNA	IEYQVGGGLN	IT...DTLIFT	146
Arg r1 AJ697694	DGKTCDVVKH	G.....	GH.TELWVHS	SKTSGGYNNC	CDKKFTETRG	144
TSGP1 Q8I9U2	LGGGCDVTLF	EGQKGQSKVQ	GPFLELWYHS	GASE.ESMRC	CEEEFRNLK	172
Moubatin Q04669	QSHDGHITKL	SS.....	G.VYQWQSN	GSADDKDIK	CDEKFKEKLS	154
RaHBP3 O77422	DYKNCDVIFV	PKERGSD..E	GD.YELWVSE	DKID.KIPDC	CKFTMAYFAQ	178
SHBP Q8WSK7	DGELCDVFYV	PN...AD..Q	G..CELWVKK	SHYK.HVPDY	CTFVFNVECA	188
Arg r1 AJ697694	..STPANEVY	KK..CPGMP~	~~	159	%I	%S
TSGP1 Q8I9U2	E.GTAVRKYN	KN..CDYGDV	A~	190	33.8	41.4
Moubatin Q04669	G.IDYTKPQE	KG..CETSAK	~~	171	25.6	31.4
RaHBP3 O77422	QKEKTVRNVY	TDSSCKPAPA	QN	200	35.6	40.9
SHBP Q8WSK7	K.DRKTIDIF	NEE CVYNGE	PWL	209	30.2	38.4

FIG 2. Amino acid sequence alignment of Arg r 1 and different proteins isolated from tick salivary glands. Percentage of identity (%) and similarity (%S) are referred to Arg r 1. Blue, All amino acids are identical; yellow, more than half are identical or highly similar.

formed between the first and the last of the conserved cysteins and between the second and the third cystein. Sequence-based secondary structure determinations on Arg r 1 predict an α -helix for the N-terminal secretory signal peptide followed by 8 β -sheets and a random coil at the C-terminus. The pattern G-X-W typical of the members of the lipocalin family is present on the N-terminus of Arg r 1.

Expression of recombinant Arg r 1 in *E coli*

The purified protein migrated as a single band with a molecular weight somewhat higher than the predicted 16.6 kd (including the His-tag; Fig 3). When run under

reducing conditions, purified nArg r 1 and rArg r 1 migrate at an apparently higher molecular weight than under nonreducing conditions. nArg r 1 migrates as 2 distinct bands in the extract and in the purified form. Both bands are recognized in the immunoblot by the polyclonal rabbit antiserum (data not shown). These bands possibly represent the nonprocessed and the mature protein forms, because the calculated difference in molecular weight between the 2 forms is 1.6 kd.

Allergenicity of the recombinant protein

The purified rArg r 1 was tested for its reactivity with IgE antibodies in immunoblot and ELISA. Rabbit poly-

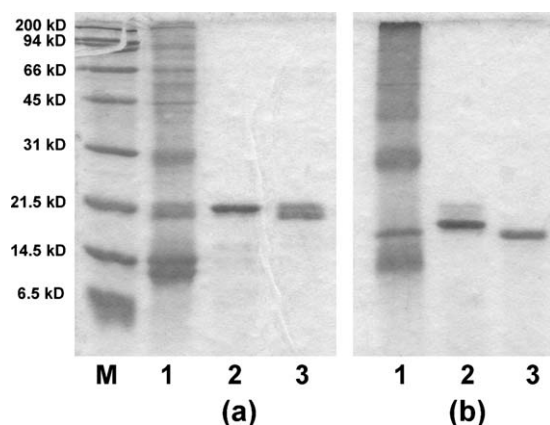


FIG 3. Electrophoretic analysis of purified Arg r 1. Coomassie stained SDS-PAGE. Lane M, molecular weight marker; lane 1, total *A reflexus* protein extract; lane 2, purified rArg r 1; lane 3, purified nArg r 1. Samples were run under reducing (a) and nonreducing conditions (b).

clonal antibodies as well as human IgE from sera of 5 patients allergic to *A reflexus* recognized the recombinant allergen in immunoblot (data not shown).

Specific IgEs to rArg r 1 were detected by ELISA in all 13 patients with IgE-mediated anaphylaxis to *A reflexus* (Fig 4; Table I). Two sera of patients allergic to mite had a titer slightly above the cutoff of 0.37 kU/L.

Inhibition experiments with rabbit polyclonal serum and 5 patient sera were performed by using nArg r 1 purified from *A reflexus* coated on microtiter plates and rArg r 1 as inhibitor. Preincubation with rArg r 1 abolishes IgE binding to nArg r 1 in 4 patient sera (inhibition 91.4% to 99.7%) as well as in the rabbit serum S2 (86.5%). In serum 7, IgE reactivity to nArg r 1 could only be inhibited to 29% by rArg r 1.

Patient 13 (6.5 kU/L specific IgE against rArg r 1), 2 atopic controls, and 1 nonatopic person were tested intradermally with rArg r 1. The intradermal injection of the purified rArg r 1 produced an erythema of 3 cm in diameter at the concentration of 0.01 µg/mL and produced a papule of 14 mm and an erythema at the concentration of 0.1 µg/mL. The 3 control persons were tested negative at the concentration of 0.1 µg/mL.

DISCUSSION

A major allergen of 18 to 19 kd was recognized in *A reflexus* extract by IgE antibodies of all 13 patients analyzed. Partial purification followed by N-terminal sequencing and production of antisera in rabbits allowed detection of specific clones in a cDNA expression library.

The potential diagnostic value of rArg r 1 was assessed by IgE immunoblot, ELISA, and intradermal testing. All 13 patients with a history of anaphylaxis caused by a tick bite were diagnosed positive by using an ELISA for detection of specific IgE to rArg r 1. Inhibition ELISA

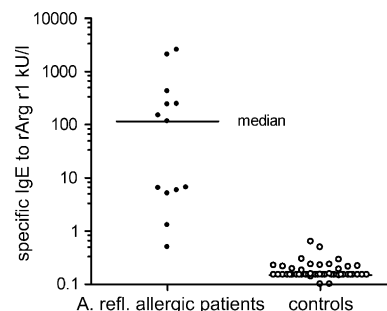


FIG 4. Detection of specific IgE to rArg r 1 in sera of patients allergic to Arg r 1 and controls.

performed with the sera of 6 patients showed that the recognition of the purified natural major allergen was largely inhibited by rArg r 1 in 5 of the 6 sera. This suggests that most of the IgE epitopes of the natural allergen are present on the recombinant molecule.

The specific IgE titer among the 13 patients varied greatly. Six patients had specific IgE between 0.5 and 10 kU/L; 7 patients had specific IgE above 100 kU/L. Because mites and ticks both belong to the subclass of the Acari, and to evaluate the occurrence of false positives caused by IgE cross-reactivity, 14 persons allergic to house dust mite (*Dermatophagoides pteronyssinus*) were included. Two of these patients allergic to mite had a weak titer of 0.49 and 0.64 kU/L, respectively, of specific IgE. This reactivity could not be confirmed by immunoblots.

The very high titers of *Argas* specific IgE of patients 4 and 9 at 2556 and 2071 kU/L seem quite exceptional. They could possibly be explained by a boosting effect caused by repeated *Argas* bites in already sensitized persons. A similar boosting of hen egg-specific IgE to as much as a titer of 2020 kU/L after repeated oral allergen intake has recently been described in an atopic child.²¹ High IgE titers above 600 kU/L have also been previously described by several authors in patients allergic to bee venom who failed to reach maintenance dosage during specific immunotherapy because of systemic reactions after injections.^{22,23}

Most major animal allergens described so far belong to the lipocalin family of proteins.²⁴ Lipocalins are a family of extracellular proteins with a molecular weight of about 20 kd that exhibit great structural and functional diversity. They include the rodent urinary allergens and allergens from dog, cow, and horse. Cockroach allergen Bla g 4 is also a lipocalin. Although lipocalins show only about 20% amino acid sequence homology, they have a conserved tertiary structure.²⁵ Lipocalins exhibit great functional diversity, with roles in retinol transport, cryptic coloration, olfaction and pheromone transport, and prostaglandin synthesis, and act in the general clearance of endogenous and exogenous compounds. Analysis of the protein sequence of Arg r 1, as deduced from the nucleic acid sequence, shows that the allergen harbors the sequence GYW (at positions 36-37-38), which permits us to classify it as a lipocalin.

A search for homologies with known tick lipocalin proteins shows that the pigeon tick allergen has a 35% identity with a histamine-binding protein isolated from the male *R appendiculatus* hard tick.¹⁷ The allergen has also 25% identity with moubatin, isolated from the salivary glands of the soft tick *O moubata*, a protein that inhibits platelet aggregation.²⁰ The biochemical properties of Arg r 1, which was shown by immunoblot to be localized in the tick salivary gland (data not shown), must be defined.

Soft back ticks parasitize birds worldwide. The species *A reflexus* parasitizing wild and domesticated pigeons (*Columba livia*) has been found in the Mediterranean region as well as in central and eastern Europe.^{13,14} Anaphylactic reactions have been described after bites by hard ticks.²⁶⁻³⁰ The feeding of hard ticks is different and lasts for several days compared with minutes for soft ticks. However, the implicated allergens have not yet been isolated.

In conclusion, this study identifies and characterizes Arg r 1 as the major allergen responsible for anaphylactic reactions caused by bites of the pigeon tick *A reflexus*. The recombinant allergen was able to bind specific IgE in all of the patients tested. The ELISA using the recombinant allergen was sensitive and specific. It remains to be seen whether rArg r 1 also has an indication in immunotherapy in persons allergic to *A reflexus* who cannot escape tick-infested housing and therefore stay at risk for anaphylactic reactions.

We gratefully acknowledge the assistance of Patrick Schmit with protein purification and the collaboration of Dr Hans Dautel, who supplied us with *A reflexus* ticks.

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