

Allergenic properties of rat urine and pelt extracts

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We studied the relative diagnostic efficacy of skin tests and RAST assays in laboratory animal allergy in 16 rat-sensitive animal workers with the use of epithelial extract and urinary antigens from three inbred rat strains. RAST inhibition was used to evaluate possible urinary antigen-strain specificity. The urinary antigens were more reliable skin test and RAST reagents than were epithelial extracts; data from urinary antigen testing correlated better with historical data. RAST inhibition did not detect strain specificity among the urinary antigens. Antigens in rat urine appear to be of better diagnostic value than do epithelial extracts. (J ALLERGY CLIN IMMUNOL 75:279-84, 1985.)

LAA has been described as a *bona fide* occupational disease.¹ In Britain it has been afforded the status of a prescribed compensable disease.² Common symptoms of LAA such as rhinitis and conjunctivitis frequently precede the development of asthma. Thus, early recognition and diagnosis of LAA are important in laboratory-worker health.

Information furnished by 155 animal-research facilities indicated that the rat was the laboratory-animal species most frequently evoking symptoms of LAA.³ Although laboratory studies have demonstrated that rat urine contains highly antigenic proteins,⁴ an investigation into the comparative allergenicity of urinary versus epithelial antigens has not been carried out. Information regarding possible strain specificity of urinary antigens is also not available.

In the present study we compared the relative diagnostic efficacy of skin tests and RAST assays with

Abbreviations used

LAA: Laboratory animal allergy

IgE: Immunoglobulin E

the use of rat urinary antigens as well as a rat epithelial extract. We also evaluated whether LAA in animal-research workers is related to the specific strain of rats from which the urinary antigens were derived.

METHODS

Subjects

The population evaluated included 16 symptomatic laboratory-animal workers with a history of rhinitis and/or asthma after occupational exposure to laboratory rats. The group consisted of nine male and seven female workers, ranging in age from 24 to 56 yr who had histories indicative of hypersensitivity to rats. Their clinical characteristics are illustrated in Table I. The mean length of time of their occupational exposure to laboratory animals was 9 yr; the average length of time of symptoms was eight years. Thirteen of the 16 workers reported both personal and family histories of atopy.

All subjects provided written informed consent for the study approved by the Human Research Review Committee in accordance with the principles of the Helsinki Declaration.

Skin test antigens

A 1:10 w/v commercial extract of rat pelts from random bred rats (Hollister-Stier, Spokane, Wash.) was used as the epithelial skin test antigen. Rat urinary-skin test antigens

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TABLE I. Clinical characteristics of workers symptomatic after rat exposure

Patient	Age (yr)	Sex	Occupational exposure (yr)	Type of symptoms	Skin reactivity to rats*			
					Epithelium	Urinary extracts		
						Fisher	Hooded	Lewis
1	54	M	20	R	3	4	3	3
2	34	M	15	A, R, U	1	3	3	3
3	41	F	9	R	0	3	3	4
4	41	M	16	R	2	4	3	3
5	24	F	2	R	0	1	1	2
6	34	M	12	R	2	4	4	4
7	32	F	6	A, R, U	0	4	4	4
8	29	F	1	A	2	4	4	4
9	28	F	2	A, R, U	4	3	4	4
10	26	M	7	A, R, U	2	4	4	4
11	49	F	3	R	0	2	1	2
12	31	M	5	R, U	2	4	4	3
13	47	M	24	R	0	4	2	3
14	56	M	20	R, U	1	3	4	4
15	50	F	<1	R	0	2	2	2
16	36	M	10	A, R	2	4	4	4
Control 1	41	M	None	None	0	0	0	0
Control 2	40	F	None	None	0	0	0	0
Control 3	30	M	None	None	0	0	0	0

A = asthma; R = rhinitis; U = urticaria.

*As determined by diameter of the wheal; number = positivity (see text).

were prepared in our laboratory from urine collected from 3- to 4-mo-old male rats from three inbred strains: Lewis, Fisher, and Hooded. The 90-day-old male rat was chosen since previous studies demonstrated peak levels of urinary protein excretion in such animals.⁵ Urine samples from each strain were collected daily by use of metabolic cages and were stored in aliquots at -20°C . The urine was concentrated by dialysis, after which the crude urinary protein was lyophilized. The lyophilized retentate was dissolved in phenolized phosphate-buffered saline to a final concentration of 10 mg/ml of urinary protein. The dialysand was not tested. All antigen preparations had Millipore (Millipore Corp., Bedford, Mass.) filtration (0.22 μm) and sterility testing before use in skin testing.

Skin tests

The study participants were skin tested on the forearm with rat epithelial antigen and urinary antigens by the prick method.⁶ The tests were graded according to the diameter of the wheal developing in 20 min as follows: negative, <3 mm; 1+, 3 mm to 5 mm; 2+, 5 mm to 7 mm; 3+, 7 mm to 10 mm; 4+, >10 mm and/or pseudopods. Diluent was used as a control. Nonallergic control subjects were also tested to determine possible nonspecific irritant effects of the antigens.

Immunologic studies

Preparation of RAST reagents. For RAST measurement of the IgE antibodies in sera from the 16 rat-exposed work-

ers, solid-phase antigens were prepared by reacting each dialyzed urine (10 mg protein per milliliter) or rat epithelium extract (1:10 w/v) with Whatman filter paper discs previously activated with BrCN.⁷ After incubation for 20 hr at 4°C , the allergen-coupled discs were washed extensively with NaHCO_3 (0.1M) and acetate buffer (0.1M, pH 4.0) before storage at 4°C in "incubation buffer" (NaCl 0.9%; phosphate buffer 0.05M; human serum albumin 0.3%; sodium azide 0.05%).

For subsequent investigation of cross-allergenicity among rat urines, particulate solid-phase antigens were prepared. Urines (5 ml from each of the three strains of rats) were dialyzed separately against three changes of 100mM NH_4HCO_3 by use of Spectrapor-3 casing (nominal molecular weight cutoff = 3600) at 4°C for 3 days and lyophilized; the yield of retentate ranged from 7 to 12 mg, and each unconcentrated rat urine contained 1.4 to 2.4 mg nondialyzable solids per milliliter. The entire quantity of each urine was dissolved in 5 ml of borate-saline buffer (200mM H_3BO_3 , 40mM NaOH, 160mM NaCl, pH 8.0) and mixed with 0.3 gm Matrex Pel-102 (Amicon Corp., Lexington, Mass.) for 36 hr at 4°C . The resulting polymer was washed 12 times with 30 ml aliquots of borate-saline until the 280nm spectrophotometric absorbance of the washes was <0.05, after which the Matrex was resuspended at 1 mg/ml in RAST diluent.⁸

Measurement of specific-IgE antibodies. Allergen-specific IgE antibodies were measured by the disc RAST procedure⁷ by use of sera drawn previously from both pa-

TABLE II. Total IgE levels and RAST results*

Patient	Total IgE levels (ng/ml)	Epithelial extract	Urinary extracts		
			Fisher	Hooded	Lewis
1	2372	116	846	776	588
2	647	89	395	350	237
3	86	82	118	119	118
4	177	96	188	205	86
5	374	166	121	138	107
6	95	167	380	438	319
7	1291	100	562	588	462
8	219	123	698	587	491
9	612	1636	720	624	530
10	881	331	560	535	454
11	70	139	115	104	101
12	833	253	798	836	564
13	161	98	171	184	145
14	995	94	179	230	118
15	199	90	285	281	199
16	171	233	251	307	242

*Expressed as percent of control; >200% considered significant.

tients and from normal control individuals. The percent total radioactive counts bound to the disc were calculated, and RAST results were expressed as a percent of the binding produced by a normal control serum. Values >200% of the negative control serum were considered positive.

RAST inhibition. To examine cross-allergenicity among rat urines, the mini-RAST⁹-inhibition procedure was used. Varying quantities of undialyzed, centrifuged rat urines were incubated with fixed quantities of patient sera (0.05 ml) and solid-phase rat urines (1 mg/ml, 0.05 ml). After overnight incubation at room temperature, the polymer was washed twice, after which ¹²⁵I-labeled anti-IgE (50 ng/ml, 0.1 ml) was added, and the tubes were incubated overnight at room temperature. After an identical washing procedure the tubes were counted by use of an automatic gamma spectrometer. Results were expressed as the percent inhibition of radioactive binding to the Matrex-urine polymer versus the quantity of fluid-phase inhibitor (rat urine) added.

Protein determination. The protein content of each undialyzed, centrifuged rat urine was measured by the Coomassie blue dye binding assay¹⁰ by use of human serum albumin as a standard.

Total serum IgE. Total serum IgE was measured by double antibody radioimmunoassay.¹¹ Values that were more than 780 ng/ml were considered elevated by use of this technique.

Statistics. Differences in the slopes of the various RAST-inhibition curves were tested by one-way analysis of covariance.¹²

RESULTS

All 16 subjects with histories compatible with hypersensitivity to rats demonstrated immediate wheal-and-flare skin reactions after prick testing with the

antigens. Skin tests on three individuals with no history of animal sensitivity were negative.

In 15 of the 16 sensitive individuals tested, skin reactivity was significantly higher with the urinary antigens than with the epithelial extract ($p < 0.001$) [Table I]. Furthermore, eight of the 16 subjects had skin reactions only to the urinary antigens ($p < 0.025$). In general the urinary antigens from the three inbred strains of rats had similar skin reactivities in the sensitive subjects.

Immunologic studies

The results of the immunologic studies are presented in Table II.

Total IgE levels

Total IgE levels were elevated in five of the 16 symptomatic workers. However, the total IgE levels could not be correlated with the degree of skin reactivity to rat antigens or with the symptom patterns of the studied workers.

RAST results

There was a considerable discrepancy evident between the RAST results when epithelial antigens were compared to urinary antigens. Significant RAST scores were detected in only four of the 16 subjects when epithelial antigens were used, but elevated scores were apparent in 12 of the 16 subjects when urinary antigens were used. Furthermore, these RAST scores correlated better with the histories and skin test reactions of the subjects than did the epithelial tests.

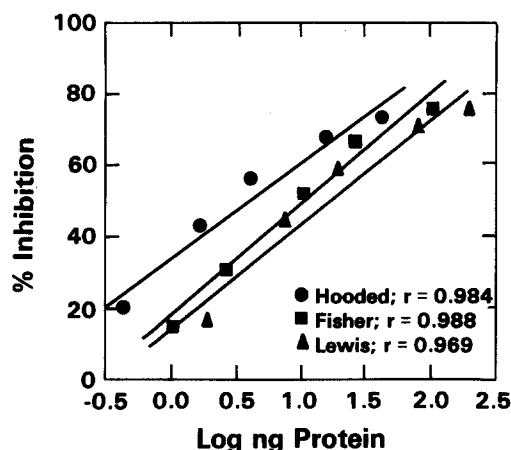


FIG. 1. Fisher rat-urine RAST inhibition with serum from patient 12. ($F [2,9] = 0.55$; $p = 0.6$)

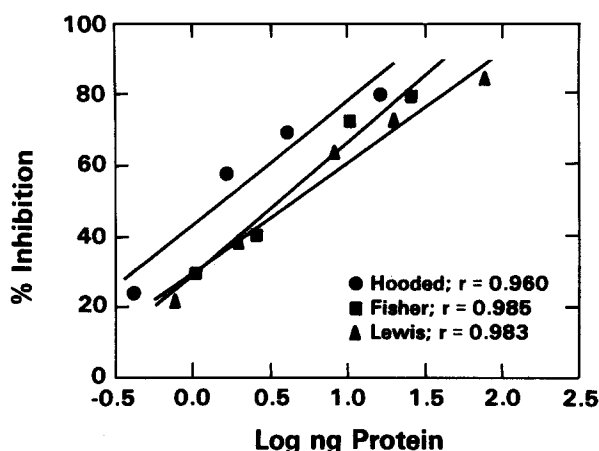


FIG. 2. Fisher rat-urine RAST inhibition with serum from patient 10. ($F [2,7] = 0.46$; $p = 0.65$)

As with the skin tests, no differential skin test reactivity was noted among the urinary antigens obtained from the three inbred strains of rats.

RAST inhibition

Each rat-strain urine was tested by RAST inhibition by use of separate solid-phase antigen preparations from each rat-strain urine; in each solid-phase antigen system individual sera from two of three patients with high urinary antigen RAST scores (patients 9, 10, and 12) were used as the IgE-antibody source. The resulting RAST-inhibition results (Figs. 1 to 6) indicate that in all cases there was a significant degree of cross-antigenicity of various rat urines.

With the use of Fisher rat urine as the solid-phase antigen and serum from either patient 12 (Fig. 1) or patient 10 (Fig. 2), all three urines produced significant dose-related RAST inhibition; there was no statistically significant difference in the slopes of the

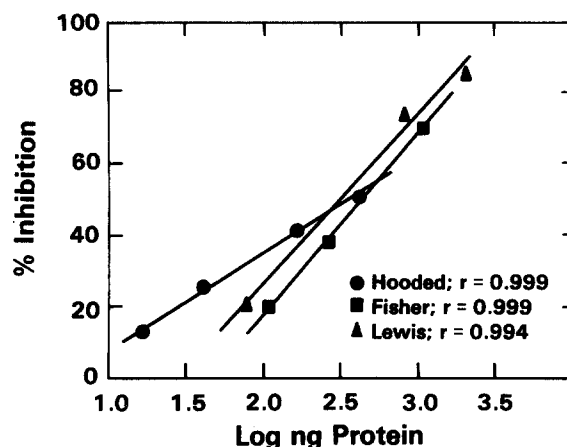


FIG. 3. Lewis rat-urine RAST inhibition with serum from patient 9. ($F [2,5] = 20.76$; $p = 0.0038$)

RAST-inhibition curves (F values are listed for each figure), suggesting that the three urines contained qualitatively similar antigens. However, with the use of Lewis rat urine as the solid-phase antigen, somewhat different results were obtained. There was a difference in antigenicity among the various rat-strain urines when serum from patient 9 was used as the IgE-antibody source (Fig. 3). In this case the IgE antibodies preferentially recognized antigenic determinants in Lewis and Fisher rat urines as compared to urine from Hooded rats. Conversely, IgE antibodies from patient 10 recognized antigenic determinants that were common to all three rat urines (Fig. 4). Finally, with the use of Hooded rat urine as the solid-phase antigen and IgE containing sera from either patient 10 (Fig. 5) or patient 12 (Fig. 6), the three inhibition curves produced appeared quite similar. However, because the curves with the use of serum from patient 12 were quite linear (all r values >0.99), the slopes of the three inhibition curves differed significantly by analysis of covariance testing. This represents more of a statistical quirk than a real difference in the antigenicity of the various urines, as all three urines were capable of inhibiting RAST binding by $>75\%$ in this case.

DISCUSSION

In a study characterizing the antigens of three inbred strains of mice (BALB/C, C57BL, and CBA), the purified proteins of the major urinary protein complex demonstrated considerable binding to IgE antibodies and cross-reacted extensively with one another.¹³ In the present study with three inbred rat strains (Fisher, Lewis, and Hooded), we also found considerable cross antigenicity among the urinary proteins as demonstrated by RAST-inhibition studies.

The possible existence of strain-specific antigens

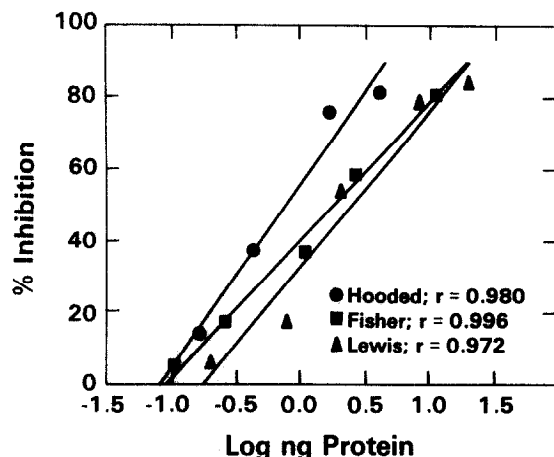


FIG. 4. Lewis rat-urine RAST inhibition with serum from patient 10. ($F [2,8] = 1.10$; $p = 0.38$)

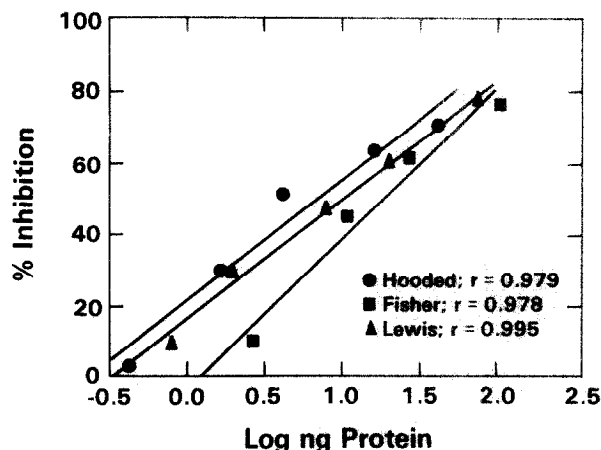


FIG. 5. Hooded rat-urine RAST inhibition with serum from patient 10. ($F [2,8] = 1.2$; $p = 0.35$)

has been suggested in another article¹⁴ in which some subjects reacted only to one or two urine preparations from three inbred strains of mice. However, results from our study by use of urinary antigens from three inbred rat strains, could not detect specific antigens, since the workers had skin reactivities to all three urinary antigenic mixtures. This lack of strain specificity is further supported by the RAST-inhibition studies, albeit only limited numbers of sera were tested.

The results of the present study confirm our previous findings with canine urine that urinary antigens are more reliable indicators of LAA than are epithelial antigens.¹⁵ This is supported by differential skin test reactivities observed in our study with the use of commercial rat epithelial antigens and urinary antigens from three inbred strains of rats. Although a comparison of the commercial pelt extracts from outbred rats with laboratory-prepared urine from inbred strains of rats is not strictly valid, it appears reasonable to assume that similar differences would be obtained by use of pelt extract and urine from the same strain. Similar differences were demonstrated in comparative studies of rat and mouse epithelial and urinary antigens by other investigators.^{4, 13, 14} Differences in protein-antigen concentrations between pelt extract and urine might explain some of the observed variations. However, adjusting the RAST-inhibition curves on the basis of milligrams of protein would shift only the position of the curve and not its slope. The present study thus indicates that commercially available rat epithelial extracts are in general less reactive than rat urines.

The reliability of the urinary antigens as skin test reagents is substantiated by the correlation with clinical symptoms as well as with RAST results. Skin reactions to urinary proteins were significantly more

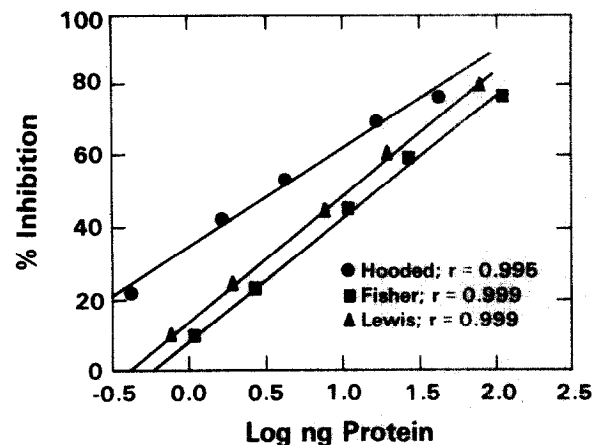


FIG. 6. Hooded rat-urine RAST inhibition with serum from patient 12. ($F [2,9] = 13.17$; $p = 0.002$)

frequently positive and correlated better with the history and RAST results than did the reactions to epithelial extracts. This emphasizes the importance of the use of relevant antigens in diagnostic evaluation for LAA.

Unfortunately, commercial rat-antigen extracts for diagnostic use are conventionally prepared from pelt and/or dander. On the basis of skin test findings in the present study, many rat-sensitive individuals might not be identified by use of only such epithelial extracts. Previous investigations^{1, 3} have demonstrated that LAA, particularly to rats, is a common and important occupational disease in laboratory workers. Accordingly, manufacturers of antigen extracts should be encouraged to market the most relevant test material (rat urine) so as to ensure the positive identification of such affected individuals.

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