

Analysis of rat serum allergens

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Previous immunoblotting studies of rat urinary proteins have identified the low molecular weight components (17, 21, and 23 kD) as being the major allergens involved in rat hypersensitivity.¹ In that study two "intermediate" (68 and 63 kD) urinary allergens and one "minor" (75 kD) urinary allergen were also described but remain unidentified. Circumstantial evidence suggests that these proteins are of serum origin because: (1) they have molecular weights similar to those of albumin (68 kD) and transferrin (75 kD), (2) these proteins are found in increased amounts in the urine of male rats who have age-related renal disease, and (3) the prevalence of specific IgE to the 68 kD allergen (28.9%¹) is similar to that described for albumin among other subjects with animal allergy.^{2,3} Although the prevalence of IgE to the 75 kD band was 15.7%, no independent report on the prevalence of IgE to transferrin is available for comparison. We now present further evidence, as determined by immunoblotting, that these high molecular weight urinary allergens are serum proteins and identify IgG as an additional rat serum protein with allergenic properties.

METHODS

Whole rat serum was obtained from male Wistar rats and purified rat transferrin; albumin and IgG were purchased (>99% pure) from Sigma-Aldrich Company Ltd. (Poole, Dorset, U.K.). The serum proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose as previously described¹ with the equivalent loading of 10 µg per track for the whole serum and approximately 1 to 3 µg per track for transferrin, albumin, and IgG. Serum samples from 15 subjects with rat hypersensitivity, who had a positive RAST response to rat serum, were used for the immunoblotting studies; all samples had additionally been used in the immunoblotting analysis of urine allergens¹ and had IgE to one or more of the 75, 68, and 63 kD allergens. The sera were diluted (2 ml; range, 1:3 to 1:40) and incubated with the nitrocellulose strips and anti-IgE label as previously described.¹ The immunoblots were exposed to x-ray film for 24 hours at -70° C before being classified as positive or negative.

RESULTS

The geometric mean of specific IgE to rat serum before dilution was 10.1% RAST binding (range percent binding, 1.5 to 52.8; geometric SD, 3.2), and after dilution was 3.9% RAST binding (range percent binding, 1.3 to 15.2; geometric SD, 2.5). Immunoblotting experiments identified five main allergens in whole rat serum with molecular weights of 68, 75, and greater than 94 kD ($n = 3$ allergens). All of the sera from subjects allergic to rats had some degree of IgE binding to the three allergens with molecular weights of greater than 94 kD and to the 68 kD allergen. Six sera contained specific IgE to the 75 kD band. No band was detected in rat serum with a molecular weight of 63 kD.

Table I summarizes the immunoblotting results obtained with the 15 serum samples from subjects allergic to rats with urine, whole rat serum, and with the purified serum proteins. The subjects with IgE to the 68 kD allergen in urine and serum all had a strong positive response to rat albumin. Similarly, there was agreement between those both with and without IgE to the 75 kD band in urine and/or serum and a positive (or negative) result for rat transferrin. Serum sample J had IgE to a band in serum with a molecular weight slightly less than 75 kD (also seen in serum samples F and I, ~70 kD Table I) but an equivocal response to transferrin (+(?), Table I), and whether this serum contains IgE specific for transferrin remains unclear. The sera with strong immunoblot reactivity to both the heavy and light chains when IgG was electrophoresed under reducing conditions also had strong radiostaining to the band greater than 94 kD, approximately 3 mm from the cathodic origin of the gel, but this was only observed for those with the strongest IgG reactions (serum samples I, K, M, and O).

DISCUSSION

Although rat albumin is known to be a minor allergen,^{3,4} the role of other serum proteins as allergens has not been determined. We have supplied further evidence that the 68 and 75 kD allergens first described in urine are likely to be albumin and transferrin, respectively, the latter of which is identified as an allergen for the first time. The subset of subjects allergic to rats who have IgE to serum proteins present in rat urine also commonly have specific IgE to a 63 kD component.¹ We have been unable to identify this allergen, but it seems improbable that it is of serum origin.

Preliminary data that rat IgG (and the heavy and light chains) are allergenic have also been presented. The prevalence of IgE to these proteins has not been deter-

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TABLE I. Summary of immunoblot analysis of 15 sera with IgE to rat serum proteins

Serum sample	Serum RAST (diluted) (%)	Molecular Weight		Albumin	Transferrin	IgG
		Urine (kd)	Serum (kd)			
A	3.7	68	75, 68	+	+	—
B	15.2	68, 63	68	+	—	—
C	1.4	68, 63	68	+	—	L
D	1.9	68, 63	68	+	—	—
E	1.4	68, 63	68	+	—	—
F	8.2	75, 68, 63	75, ~70, 68	+	+	—
G	1.9	68, 63	68	+	—	H
H	1.5	68, 63	68	+	—	—
I	6.3	75, 68, 63	>94, 75, ~70, 68	+	+	H & L
J	6.0	75, 68, 63	>94, ~70, 68	+	+(?)	—
K	13.7	75, 68, 63	>94, 75, 68	+	+	H & L
L	1.3	68, 63	68	+	—	H & L
M	9.4	68, 63	>94, 68	+	—	H & L
N	2.5	68, 63	68	+	—	—
O	10.8	75, 68, 63	>94, 75, 68	+	+	H & L

H, Heavy chain; L, light chain.

mined, but light chains found in rabbit urine and dust have been shown to be minor allergens for 30% of subjects allergic to rabbits.⁵ Immunoblotting experiments performed by Price and Longbottom⁵ suggested that the specific IgE-binding epitopes on the light chains were only revealed after the dissociation of IgG, and this may explain why no firm identification of the IgG band in whole serum was possible.

All of the 15 subjects were asked about their exposure to rat serum proteins. Of the seven who replied, all had been working with rats aged 6 months or older, and six had additionally handled rat tissue. It would seem that sensitization to rat serum proteins occurs in those exposed to rat tissue or urine from rats greater than 6 months of age.

REFERENCES

1. Gordon S, Tee RD, Newman Taylor AJ. Analysis of rat urine proteins and allergens by sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotting. *J Allergy Clin Immunol* 1993;92:298-305.
2. Spitzauer S, Pandjaitan B, Söregi G, Mühl S, Ebner C, Kraft D, et al. IgE cross-reactivities against albumins in patients allergic to animals. *J Allergy Clin Immunol* 1995;96:951-9.
3. Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin: II characterization of allergens extracted from rat, mouse, guinea pig and rabbit pelts. *J Allergy Clin Immunol* 1975;55:16-24.
4. Wahn U, Peters T, Siraganian RP. Studies on the allergenic significance and structure of rat serum albumin. *J Immunol* 1980;125:2544-9.
5. Price JA, Longbottom JL. Allergy to rabbits. I. Specificity and non-specificity of RAST and crossed-radioimmuno-electrophoresis due to the presence of rabbit light chains in rabbit allergenic extracts. *Allergy* 1986;41:603-12.