

Epitope mapping of Rhi o 1 and Generation of a Hypoallergenic Variant: a Candidate Molecule for Fungal Allergy Vaccines\*

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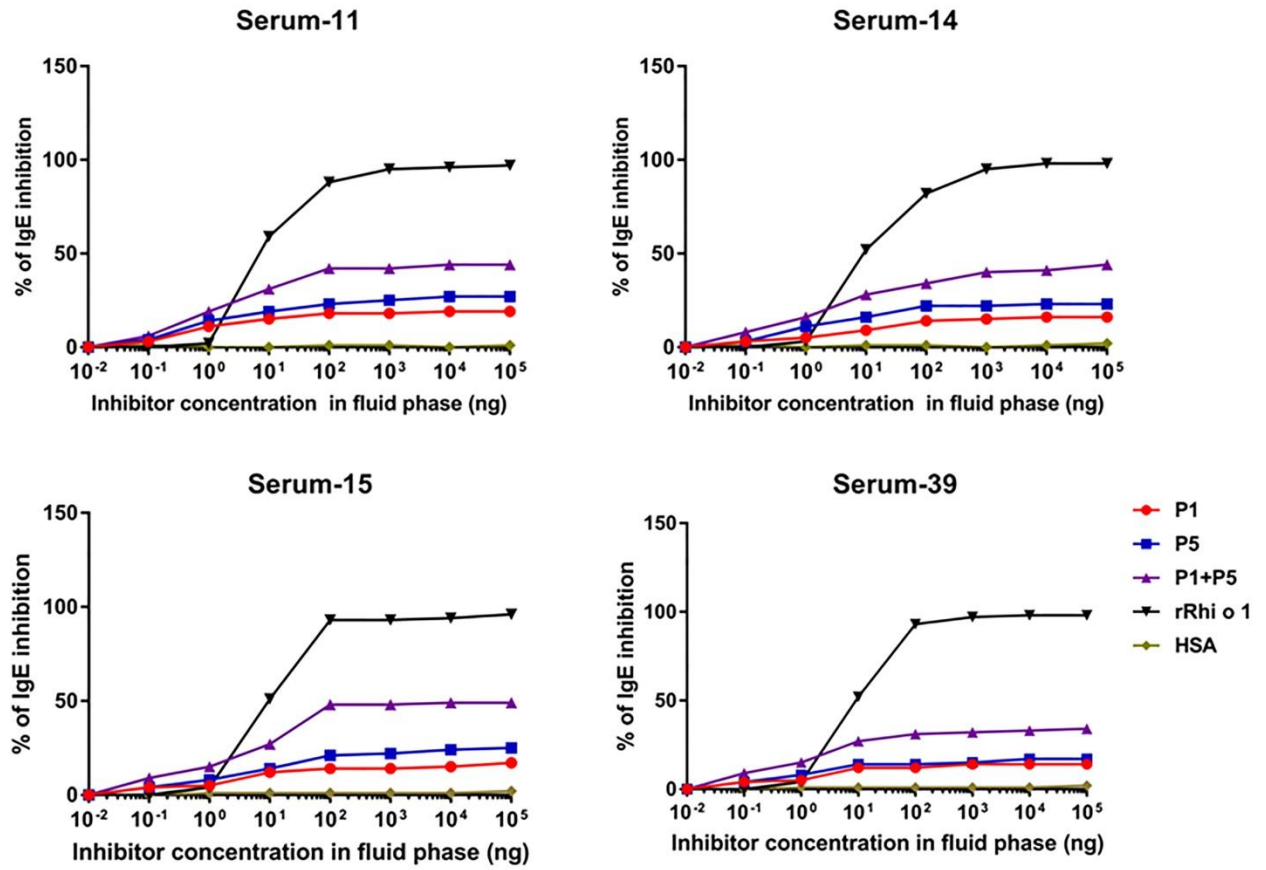
*\*Running title: IgE epitopes and Hypoallergenic Rhi o 1*

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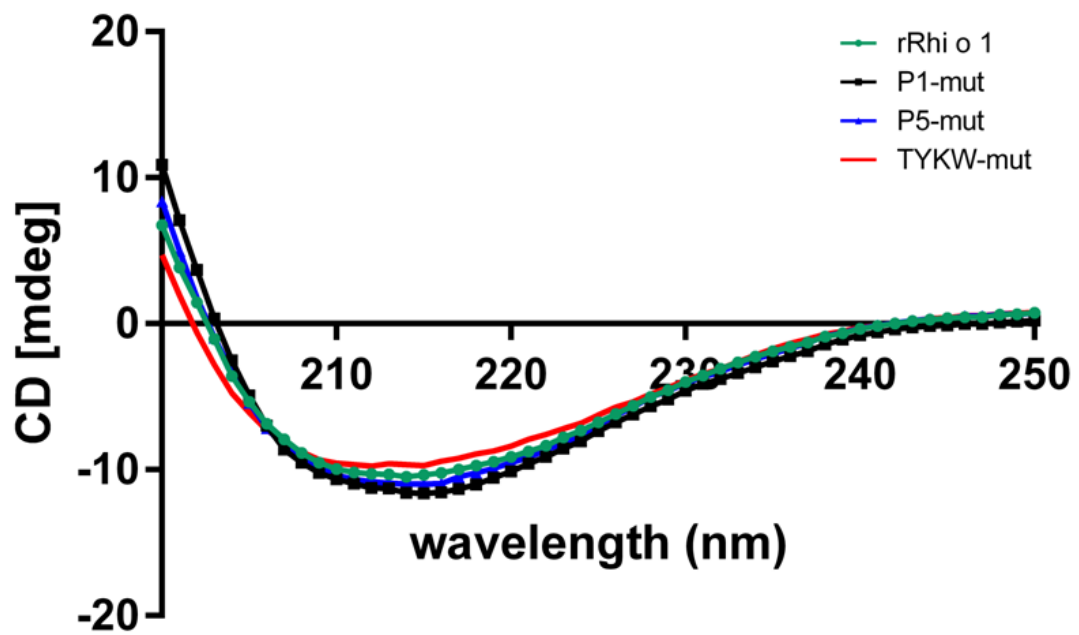
**Keywords:** Mold allergy, Asthma, Allergen, Epitope mapping, Immunoglobulin-E (IgE), Mutagenesis, Vaccine, Immunotherapy.

**Contents:** Supplementary figures S1-S2 and Supplementary table-S1

**Supplementary figure S1:** Competitive ELISA, in which immobilized rRhio 1 was exposed to four different patients' sera individually pre-incubated with increasing doses (x-axis) of peptides, rRhio 1, and HSA respectively. Consequently the IgE-binding to plate-bound rRhio 1 was inhibited (y-axis).



**Supplementary Figure S2:** CD spectra of 3  $\mu\text{M}$  each of three purified mutants were compared with that of equimolar rRhi o 1. Ellipticity signals (CD millidegrees; y-axis) of the proteins were plotted against different wavelengths (x-axis).



**Supplementary Table S1:** Demography and allergy-diagnostic data of human subjects (patients and controls) participated in the present study. Negative controls are in italics.

<b>Patient/ Subject No.</b>	<b>Age</b>	<b>Sex</b>	<b>tIgE IU/ml</b>	<b>sIgE-RCE (kU<sub>A</sub>/ml)</b>	<b>sIgE-CCE (kU<sub>A</sub>/ml)</b>	<b>Symptoms</b>
1	28	M	1525.28	7.80	0.14	BA
2	32	M	2228.32	10.20	4.80	BA+AD
3	31	M	4011.60	42.80	0.18	BA
4	48	M	3301.29	18.23	5.68	BA
5	51	F	4230.00	22.30	6.3	BA+AD+U
6	53	M	1689.20	9.36	0.07	BA+AR
7	47	F	1827.88	8.86	5.3	BA
8	46	F	1873.52	11.20	6.9	BA
9	31	F	2367.21	39.0	6.2	BA+AD
10	21	F	2169.24	18.00	4.8	BA
11	39	F	3512.33	26.30	0.10	BA+AD
12	54	F	4193.30	34.90	0.26	BA
13	25	F	3248.42	18.60	0.09	BA+AR
14	29	M	2365.26	8.36	0.64	BA+AD
15	28	M	3425.36	16.0	0.38	BA+AD
16	58	M	1569.36	25.32	8.01	BA
17	44	F	2458.33	28.8	15.23	BA
18	32	F	1897.34	31.8	0.41	BA
19	18	M	2201.65	17.35	8.5	BA+AD
20	21	M	1478.36	57.05	0.17	BA
21	26	M	4003.10	31.02	11.36	BA
22	27	M	1889.47	36.8	18.3	BA+AR
23	29	M	2954.44	18.0	0.35	BA+AD
24	31	M	2364.36	14.8	22.0	BA+AD
25	46	M	2564.14	16.3	0.08	BA+AD
26	42	M	3981.78	30.8	17.5	BA
27	50	M	3006.25	28.16	14.6	BA
28	40	M	3217.58	24.33	15.8	BA+AD
29	32	M	2698.02	18.63	0.06	BA+AR
30	36	M	1985.35	46.38	14.3	BA
31	48	F	14789.36	24.56	0.14	BA+AR
32	45	F	2374.05	28.36	16.3	BA+AR
33	31	M	3609.45	19.34	18.0	BA
34	26	F	2369.54	16.84	8.6	BA+AD
35	28	M	1569.08	8.86	6.3	BA+AD
36	31	F	1993.36	9.32	10.4	BA
37	20	M	2589.34	12.36	11.3	BA+AR
38	36	F	2489.34	14.55	14.5	AR+AD
39	46	F	1369.08	18.3	0.12	AR
40	48	F	23478.01	12.5	11.2	AR
41	51	F	3751.04	11.0	10.8	AR
42	23	M	16983.88	15.6	13.2	BA
43	61	F	4007.36	14.9	8.9	AR
44	45	F	3334.36	9.32	0.68	AR
45	44	M	3247.45	14.7	0.45	BA
<i>DMS</i>	28	<i>M</i>	<i>3008.36</i>	<i>0.13</i>	<i>0.68</i>	<i>BA+AD</i>
<i>N1</i>	29	<i>M</i>	<i>68.36</i>	<i>0.01</i>	<i>0.06</i>	<i>NS</i>
<i>N2</i>	50	<i>F</i>	<i>72.36</i>	<i>0.04</i>	<i>0.09</i>	<i>NS</i>
<i>N3</i>	19	<i>M</i>	<i>58.96</i>	<i>0.09</i>	<i>0.03</i>	<i>NS</i>

*Abbreviations* - tIgE: total IgE; sIgE: specific IgE; RCE: Rhizopus crude antigenic extract; CCE: Cockroach (*Periplaneta americana*) crude antigenic extract; BA: bronchial asthma; AD: atopic dermatitis; AR: atopic rhinitis; U: urticaria; NS: no symptom; DMS: dust mite sensitive serum; N1-N3: healthy subjects.

*Notes for Table S1:* All the human subjects were clinically tested for allergy/asthma at Mediland Diagnostics, Kolkata and B.R. Singh Hospital and Centre for Medical Education and Research, Kolkata. Total IgE was estimated by Human Total IgE ELISA kit (Omega diagnostics) and antigen specific IgE was estimated by ImmunoCAP (Phadia). The data of *in-vitro* serological tests were the part of clinical records collected from the pathological laboratory of the hospital.