

SUPPLEMENTARY MATERIAL

Table S1. Demographic and clinical features of the study population of 39 *Hymenoptera* venom-allergic patients and 22 controls.

Characteristics	Allergic (n=39)	Controls* (n=22)	P-value
Age in years (median, range)	46 (21-77)	35 (25-57)	0.0085
Female (%)	64.1	81.8	NA
Culprit (N°)			
HBV	22	NA	NA
YJV	17		
Reaction severity (Mueller grade; IV/III/II/I/LLR)	7/5/11/12/4	NA	NA
ID skin tests (HBV/YJV) (% of positive)	100	ND	NA
Specific IgE (HBV/ YJV) (kU _A /L)	4.11 (1.96-12.00)	<0.35	<0.0001
Total IgE (HBV/ YJV) (kU _A /L)	49.80 (19.80-115.0)	21.40 (10.24-75.80)	0.14
Basal tryptase (kU _A /L)	4.89 (3.63-6.33)	2.80 (2.08-3.49)	<0.0001
BAT CD63 HBV/ YJV 1 µg/ml (%)	78.0 (59.20-86.70)	3.11 (1.20-4.85)	<0.0001

HBV: honey bee venom; YJV: yellow jacket venom; BAT: basophil activation test; ND: not determined.

*The controls had negative venom sIgEs and no history of an allergic reaction after being stung.

Table S2. Diagnostic performance of LAD2 MAT for *Hymenoptera* venom allergy.

Protocol	AUC	Diagnostic cut-off* [% CD63+ LAD2 cells]
StemPro-34 CM	0.58 ($P=0.30$)	3.5
StemPro-34 CM +IL33+IL6	0.62 ($P=0.12$)	5.1
Tyrode buffer+SCF+IL33+IL6	0.67 ($P=0.03$)	5.5

CM: complete media; AUC: area under the curve

*Determined by the Youden Index

Table S3. Comparison of demographic and clinical features between patients with nonsevere anaphylactic allergic reactions and patients with severe anaphylactic allergic reactions.

Feature	Nonsevere allergic reaction*	Severe allergic reaction*	P value
N (%)	27 (69.0)	12 (30.1)	
Female (%)	16 (59.3)	9 (75.0)	0.34
Age (y)	54.0 (35.3-63.0)	46.0 (31.0-60.0)	0.47
BST ($\mu\text{g/ml}$)	5.2 (4.0-6.9)	4.1 (3.7-5.8)	0.32
tIgE (kU/l)	46.4 (19.6-92.9)	96.7 (24.4-212.8)	0.11
sIgE to HBV/YJV (kU/l)	3.6 (1.6-10.6)	9.3 (2.3-36.3)	0.13
% CD63+ BAT anti-Fc ϵ RI 0.55 $\mu\text{g/ml}$	90.7 (71.9-94.8)	89.3 (82.2-94.4)	0.96
% CD63+ BAT HBV/YJV 1 $\mu\text{g/ml}$	76.7 (53.5-90.8)	79.1 (75.3-85.7)	0.65
% CD63+ BAT HBV/YJV 0.1 $\mu\text{g/ml}$	55.2 (36.1-73.1)	40.7 (18.9-78.8)	0.67
% CD63+ LAD2 MAT anti-IgE 10 $\mu\text{g/ml}$	19.1 (15.6-27.7)	25.0 (21.0-37.5)	0.07

BST: Basal serum tryptase; tIgE: total IgE; sIgE: specific IgE; BAT: basophil activation test; LAD2 MAT: LAD2 mast cell activation test.

Data are expressed as numbers (percentages) or medians (interquartile ranges).

*The severity was evaluated according to the Mueller scale. Large local, grade I and II reactions were considered nonsevere, and grade III and IV reactions were considered severe.

Table S4. Clinical and laboratory characteristics of 37 *Hymenoptera* venom allergic patients with nonresponding basophils.

Characteristics	All (n=37)		
Age (median, range)	51 (24-80)		
Female (%)	22 (59%)		
Reaction severity (Mueller grade; IV/III/II/I/LLR)	18/8/3/6/2		
CD 63% basophils anti-FcεRI (median, IQR)	5.0 (3.5-6.9)		
CD63% LAD2 anti-IgE (median, IQR)	16.4 (12.9-21.1)		
Culprit	HBV (n=13)	YJV/ hornet (n=15)	NI (n=9)
Skin tests HBV (N of positive)	9*/13	3/15	1*/9
Skin tests YJV (N of positive)	2*/13	8/15	2*/9
sIgE HBV (median, IQR)	5.45 (1.10-21.40)	<0.10 (<0.10-1.0)	0.50 (0.0-4.99)
sIgE YJV (median, IQR)	0.99 (0.30-1.62)	2.73 (1.44-9.37)	0.51 (<0.10-1.46)
LAD2 MAT HBV (median, IQR)	8.29 (5.11-13.10)	4.63 (3.26-5.72)	4.97 (3.34-9.72)
LAD2 MAT YJV (median, IQR)	1.76 (1.29-13.10)	3.26 (2.37-6.81)	2.85 (1.48-4.02)

HBV: honey bee venom; YJV: yellow jacket venom; NI: not identified; LAD2 MAT: LAD2 mast cell activation test.

*2 patients ND

Table S5. Detailed clinical and laboratory data of 37 patients with uninterpretable BAT results.

Patient ID	Culprit	Reaction severity (Mueller stage)	Skin tests		tIgE (kU/l)	sIgE (kU/l)		BAT (% CD63+)	LAD2 MAT (% CD63+)		
			HBV	YJV		HBV	YJV		anti-FcεRI	HBV	YJV
1	unknown	2	neg	neg	110	0.56	0.72	3.5	7.38	4.41	17.9
2	unknown	4	ND	ND	27.9	<0.1	0.51	2.6	2.4	2.0	23.1
3	YJV	4	neg	pos	27.5	<0.1	0.39	7.7	3.0	2.9	11.2
4	HBV	LLR	ND	ND	129	81.6	<0.1	4.6	17.0	1.55	15.2
5	unknown	4	neg	neg	81.1	8.5	<0.1	5.7	7.44	2.99	32.0
6	unknown	3	pos	neg	60.9	61.6	0.16	3.8	12.5	1.46	21.2
7	YJV	1	neg	neg	92.3	<0.1	1.44	5.3	4.24	1.62	15.7
8	HBV	3	pos	neg	140	30.1	12.6	3.1	12.1	1.19	20.9
9	HBV	4	pos	neg	22.1	11.4	0.22	9.0	5.88	0.77	11.2
10	HBV	3	pos	pos	50.5	0.93	1.37	4.8	8.29	4.53	17.3
11	hornet	4	neg	pos	38.2	<0.1	2.73	5.4	2.19	7.27	27.3
12	HBV	1	ND	ND	54.8	6.32	0.38	2.6	1.9	3.7	22.3
13	HBV	2	pos	neg	236	5.45	0.73	5.9	12.2	1.76	18.3
14	YJV	4	neg	neg	308	<0.1	1.57	6.6	4.6	3.1	16.4
15	HBV	1	pos	neg	81.7	2.88	11.3	5.0	12.3	5.9	19.0
16	HBV	4	pos	neg	176	12.7	1.48	5.8	17.3	11.2	24.1
17	YJV	3	neg	neg	65	0.452	2.56	3.5	9.95	7.28	25.0
18	hornet	4	pos	pos	203	1.11	2.90	4.1	2.4	2.1	8.95
19	HBV	4	pos	neg	26.3	3.96	1.76	8.3	13.9	1.39	16.6
20	HBV	3	pos	neg	16	1.03	0.57	2.8	8.18	2.97	13.7
21	HBV	4	pos	neg	181	36.3	0.991	4.7	7.34	1.83	18.5
22	YJV	4	neg	pos	28.4	<0.1	38.6	3.3	5.2	6.81	14.5
23	unknown	2	neg	pos	192	1.48	1.78	5.5	2.9	3.7	20.1
24	YJV	4	neg	pos	20.2	<0.1	1.33	9.0	2.5	0.9	3.83
25	YJV	4	neg	neg	313	<0.1	1.55	3.3	4.3	1.0	12.8
26	YJV	3	neg	neg	29.3	<0.1	0.36	3.2	2.1	3.0	12.8
27	HBV	1	neg	neg	45.2	1.17	<0.1	9.5	5.2	3.6	32.4
28	hornet	4	neg	pos	15.8	0.26	1.12	1.81	5.72	4.62	16.5
29	YJV	4	pos	pos	129	ND	31.7	9.7	4.2	4.4	22.9
30	unknown	4	neg	neg	42.9	0.5	<0.1	7.1	12.0	5.81	11.4
31	unknown	3	neg	neg	27.4	<0.1	1.14	5.2	4.4	1.5	8.51
32	hornet	4	neg	neg	91.7	<0.1	3.97	4.5	5.4	3.3	15.7
33	YJV	1	pos	pos	33.7	5.56	4.70	7.3	4.9	2.4	13.5
34	unknown	3	neg	pos	79.1	<0.1	5.0	5.0	5.0	3.6	13.4
35	unknown	LLR	ND	ND	117	0.48	0.42	7.1	3.8	2.9	12.0
36	hornet	4	neg	neg	41.6	1	9.37	4.1	6.15	6.85	12.9
37	hornet	1	neg	pos	109	37.9	2.98	6.0	23.0	2.46	14.4

tIgE: total IgE; sIgE: specific IgE; BAT: basophil activation test; LAD2 MAT: LAD2 mast cell activation test; LLR: large local reaction; HBV: honey bee venom; YJV: yellow jacket venom; ND: not determined.

Positive LAD2 MAT results are bolded.