



Detection of Sensitization Profiles with Cellular In Vitro Tests in Wheat Allergy Dependent on Augmentation Factors (WALDA)

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Supplementary material

Table S1. Clinical characteristics of WALDA patients and healthy controls.

Clinical characteristics	WALDA patients, n=13	Controls, n=11	p-value
Age, median (range)	54 years (32-83)	32 years (25-67)	<0.05
Sex, male/female, n (%)	8/5 (61.5%/38.5%)	5/6 (54.5%/45.5%)	n.s.
Any self-reported atopic comorbidities, n (%)	3 (23%)	6 (54.5%)	n.s.
Total IgE, median (range)	234 kU/l (52.4-2848)	59.7 kU/l (3.69-488)	<0.05
sIgE to ω 5-gliadin, median (range)	5.40 kU/l (2.32-28.4)	<0.1 kU/l	<0.001
sIgE to wheat, median (range)	0.51 kU/l (<0.1-4.65)	0.12 kU/l (<0.1-1.19)	n.s.
sIgE to wheat gluten, median (range)	1.77 kU/l (0.13-9.95)	<0.1 kU/l (<0.1-0.3)	<0.001
sIgE to gliadins, median (range)	1.44 kU/l (<0.1-10.3)	<0.1 kU/l	<0.001
sIgE to rTri a 14 wheat, median (range)	<0.1 kU/l (<0.1-0.12)	<0.1 kU/l	n.s.
Positive SPT with wheat flour, n (%)	12 (92.3%)	0 (0%)	<0.001
Positive SPT with wheat gluten, n (%)	12 (92.3%)	1 (0.9%)	<0.001
Basal serum tryptase, median (range)	4.31 μ g/l (3.01-6.66)	2.75 μ g/l (0.4-11.7)	n.s.
Oral challenge test reaction threshold*, n (%)	1) 3 (23%) 2) 0 (0%) 3) 3 (23%) 4) 3 (23%) 5) 2 (15.4%) 6) 1 (7.7%) 7) 0 (0%) 8) 1 (7.7%) 9) 0 (0%) 10) 0 (0%)	-	-

* see Methods section for grading. Abbreviations: sIgE, specific IgE; SPT, skin prick test.

Table S2. Overview of the results of cellular in vitro tests in WALDA patients and in healthy controls.

In vitro test	Test substance	WALDA patients	Controls	p-value
BAT %CD63+max in any concentration, median (range)	Gluten	7.1 (1.2-64.8)	1.6 (0.4-3.7)	<0.001
	HMW-GS	10.7 (1.4-74.5)	1.0 (0.4-7.9)	<0.001
	ATI	10.7 (1.9-73.0)	1.6 (0.6-2.7)	<0.001
	Wheat beer	17.0 (1.0-87.4)	1.2 (0.2-22.5)	<0.001
	eHWP	6.9 (1.7-88.6)	2.0 (1.0-3.6)	<0.001
	sHWP	12.3 (2.8-77.2)	1.6 (0.2-7.4)	<0.001
	Rye gluten	10.8 (0.8-89.7)	1.2 (0.6-6.2)	0.002
	Rye secalins	10.5 (0.3-76.2)	1.2 (0.4-8.8)	0.004
aBHRA maximum histamine release (ng/ml) in any concentration, median (range)	Gluten	35.8 (11.5-73.5)	n.d.	
	HMW-GS	35.3 (24.0-207.0)	n.d.	
	ATI	24.5 (6.5-40.0)	n.d.	
	Wheat beer	38.3 (4.0-67.5)	n.d.	
	eHWP	25.0 (1.5-54.0)	n.d.	
	sHWP	44.0 (8.5-112.0)	n.d.	
	Rye gluten	23.0 (7.0-71.5)	n.d.	
	Rye secalins	31.8 (6.0-87.0)	n.d.	
pBHRA maximum histamine release (ng/ml exceeding the negative control) in any concentration, median (range)	Gluten	<10.0 (3.3-46.5)	5.7	
	HMW-GS	<10.0 (1.7-40.5)	5.0	
	ATI	<10.0 (2.0-14.3)	2.3	
	Wheat beer	<10.0 (7.0-34.0)	2.7	
	eHWP	<10.0 (2.7-32.0)	2.3	
	sHWP	<10.0 (5.3-33.3)	2.0	
	Rye secalins	<10.0 (1.0-18.3)	0.0	

Abbreviations: BAT, basophil activation test; aBHRA, active basophil histamine release assay; pBHRA, passive basophil histamine release assay; ATI, α -amylase/trypsin inhibitors; HMW-GS, high-molecular-weight glutenin subunits; eHWP, extensively hydrolyzed wheat proteins; sHWP, slightly hydrolyzed wheat proteins.

Control subject	slgE ω5- gliadin (kU/l)	BAT										pBHRA							
		Blanks	Anti- FcεRI	FMLP	Gluten	HMW- GS	ATI	Wheat beer	eHWP	sHWP	Rye gluten	Secalins	Gluten	HMW- GS	ATI	Wheat Beer	eHWP	sHWP	Secalins
c 1	<0.1	0.2	79.2	42.2	0.4	0.8	0.6	0.8	1.6	0.2	0.6	1.2							
c 2		0.9	84.4	38.0	0.6	0.8	1.4	1.4	2.2	2.9			5.7	5.0	2.3	2.7	2.3	2.0	0.0
c 3		0.4	19.4	16.2	0.6	0.4	1.6	0.2	1.0	0.4	0.6	2.4							
c 4		0.8	85.6	23.2	1.8	1.2	2.0	1.2	3.4	1.4	1.0	1.4							
c 5		1.4	62.0	17.2	3.4	2.2	1.2	2.4	3.6	7.4	6.2	1.7							
c 6		1.3	92.3	4.7	0.6	0.8	2.6	2.2	1.2	1.4	1.6	1.2							
c 7		0.8	53.2	13.6	2.4	1.0	2.1	2.5	2.1	0.6	1.2	0.6							
c 8		1.0	82.1	70.3	3.7	0.5	1.1	0.9	1.2	4.2	1.0	0.6							
c 9		0.4	86.8	10.8	1.7	7.9	2.7	22.5	1.4	1.7	3.4	0.6							
c 10		0.4	40.7	44.6	1.0	1.8	2.6	0.4	2.4	2.2	1.2	0.4							
c 11		1.5	52.0	13.5	1.6	4.0	1.4	1.2	2.0	1.6	1.6	8.8							
						Legend BAT								Legend pBHRA					
						<3 %CD36+max								<10 ng/ml					
						3-14 %CD36+max								10-24 ng/ml					
						15-49 %CD36+max								25-49 ng/ml					
						>50 %CD36+max								>50 ng/ml					
						not done								not done					

Figure S1. Overview of the *in vitro* basophil tests BAT and pBHRA in healthy controls. For the BAT, data are shown as maximum values of %CD63+ basophils in any concentration of the respective test substance (%CD63+max). Anti-Fc ϵ RI monoclonal antibodies

and N-formyl-methionine-leucyl-phenylalanine (fMLP) were used as positive controls, two blank determinations as negative controls (mean value shown in figure). For the pBHRA, the values are shown as histamine release in ng/ml exceeding the negative control. The colour scheme used is purely indicative.

Table S3. Concentrations of the allergen test solutions in µg/ml eliciting the highest histamine release in the aBHRA in WALDA patients.

Patient	sIgE against ω5-gliadin (kU/l)	Gluten	HMW-GS	ATI	Alcohol- free wheat beer	eHWP	sHWP	Rye gluten	Rye secalins
p 1	28.4	4000	4000	326.5	1:6430.7	1:12.2	1:12.2	4000	0.04
p 2	27.0	4000	4000	326.5	1:12.2	1:525.2	1:275487.5	1142.9	26.5
p 3	15.4	2.6	0.6	326.5	1:12.2	1:78710.9	1:275487.5	0.6	0.04
p 4	8.8	not done							
p 5	7.7	26.5	326.5	326.5	1:6430.7	1:78710.9	1:1838.8	1142.9	2.6
p 6	6.2	26.5	26.5	326.5	1:42.9	1:12.2	1:6430.7	93.3	26.5
p 7	5.4	26.5	1142.9	326.5	1:42.9	1:22517.4	1:12.2	1142.9	26.5
p 8	5.4	0.16	4000	326.5	1:12.2	1:6430.7	1:12.2	7.6	0.16
p 9	4.5	4000	4000	93.3	1:42.9	1:12.2	1:12.2	93.3	7.6
p 10	3.8	4000	4000	326.5	1:42.9	1:12.2	1:12.2	4000	0.16
p 11	3.6	4000	326.5	0.6	1:22517.4	1:42.9	1:42.9	2.6	0.04
p 12	2.4	4000	93.3	0.04	1:12.2	1:12.2	1:12.2	4000	0.6
p 13	2.3	93.3	93.3	93.3	1:12.2	1:1838.8	1:12.2	1142.9	26.5

All concentrations are given in µg/ml. Abbreviations: ATI, α-amylase/trypsin inhibitors; HMW-GS, high-molecular-weight glutenin subunits; eHWP, extensively hydrolyzed wheat proteins; sHWP, slightly hydrolyzed wheat proteins; sIgE, specific IgE.

Table S4. Concentrations of allergen test substances with highest median histamine release in patients with WALDA in the aBHRA.

Test substance in aBHRA	Concentration with highest median histamine release	Median histamine release (ng/ml)
Gluten	4000 µg/ml	25
HMW-GS	26.5 µg/ml	37.7
ATI	93.3 µg/ml	18.4
Alcohol-free wheat beer	1:12.2	31
eHWP	1:42.9	16.6
sHWP	1:42.9	29
Rye secalins	0.6 µg/ml	24.3
Rye gluten	1142.9 µg/ml	17.9

Table S5. Concentrations of allergen test substances with highest median histamine release in patients with WALDA in the pBHRA.

Test substance in pBHRA	Concentration with highest median histamine release	Median histamine release (ng/ml exceeding the negative control)
Gluten	3600 µg/ml	42.1
HMW-GS	3600 µg/ml	48.8
ATI	93.3 µg/ml	8.2
Alcohol-free wheat beer	1:12.2	36.1
eHWP	1:12.2	17.6
sHWP	1:12.2	18.3
Rye secalins	84 µg/ml	6.8

Table S6. Overview of the minimum concentration of test substances eliciting positive responses in WALDA patients.

In vitro test	Test substance	Minimum concentration
BAT %CD63+ >5%	Gluten	<800 µg/ml
	HMW-GS	<800 µg/ml
	ATI	80 µg/ml
	Wheat beer	<1:100
	eHWP	<1:50
	sHWP	<1:50
	Rye gluten	<800 µg/ml
	Rye secalins	<800 µg/ml
aBHRA histamine release >10 ng/ml	Gluten	<0.004 µg/ml
	HMW-GS	<0.004 µg/ml
	ATI	0.2 µg/ml
	Wheat beer	<1:964707
	eHWP	<1:964707
	sHWP	<1:964707
	Rye gluten	<0.004 µg/ml
	Rye secalins	<0.004 µg/ml
pBHRA histamine release >10 ng/ml exceeding the negative control	Gluten	293 µg/ml
	HMW-GS	293 µg/ml
	ATI	293 µg/ml
	Wheat beer	1:525.2
	eHWP	1:12.2
	sHWP	1:150.1
	Rye secalins	0.2 µg/ml

BAT, basophil activation test; aBHRA, active basophil histamine release assay; pBHRA, passive basophil histamine release assay; ATI, α -amylase/trypsin inhibitors; HMW-GS, high-molecular-weight glutenin subunits; eHWP, extensively hydrolyzed wheat proteins; sHWP, slightly hydrolyzed wheat proteins.