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Immunoprofile of α -Gal- and B-antigen-specific responses differentiate red meat allergic patients from healthy individuals

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Abstract

Background: The Galactose- α -1,3-galactose (α -Gal) epitope is involved in red meat allergy.

As α -Gal is structurally similar to the blood group B antigen, we explored the relationship between the immune responses to α -Gal- and the B-antigen in red meat allergic patients compared to healthy A/O or B blood donors.

Methods: Sera from 51 red meat allergic patients IgE positive to α -Gal and 102 healthy blood donors (51 blood group A/O; 51 blood group B), were included. α -Gal and B-antigen specific IgE (ImmunoCAP) and IgG/IgG₁₋₄ (ELISA) responses were determined. Basophil activation tests were performed.

Results: Fifteen healthy donors were IgE positive to α -Gal, of which 3 had blood group B. The allergic patients had significantly higher α -Gal IgE levels compared to the healthy donors. The majority of the allergic patients, but none of the healthy donors, had IgE against the B-antigen. Inhibition studies revealed cross-reactivity between α -Gal and the B-antigen. The biological activity of the B-antigen was confirmed by basophil activation tests. Anti- α -Gal IgG₁ and IgG₄ levels were significantly higher in the patients compared to the healthy donors. Moreover, the IgG response to the B-antigen was comparable between the allergic patients and healthy A/O donors.

Conclusion: Red meat allergic patients showed significantly higher α -Gal IgE, IgG₁ and IgG₄ levels, reflecting a Th2 response, compared to healthy blood donors. Blood group B donors had significantly reduced antibody responses to α -Gal, due to similarities with the B-antigen, resulting in a lower risk of sensitization to α -Gal and development of red meat allergy.

Key words: α -Gal, allergenicity, B-antigen, glycan, IgE, red meat allergy

Abbreviations: α -Gal: Galactose- α -1,3-galactose

Introduction

In the last decade, allergy to red meat has been identified worldwide as a novel type of severe food allergy (1-5). Patients report anaphylaxis, angioedema, urticaria or gastrointestinal symptoms 2 to 6 hours after red meat consumption. The allergic responses are due to IgE antibodies directed against the carbohydrate epitope galactose- α -1,3-galactose (α -Gal) which is abundantly expressed on glycolipids and glycoproteins from non-primate mammals and some lower primates, but not humans (6, 7). Several studies have provided evidence that the α -Gal IgE response in red meat allergic patients is induced by tick bites (5, 8-11). We have shown that α -Gal is present in the gastrointestinal tract of the European tick *Ixodes ricinus*, thus potentially explaining the relationship between tick bites and sensitization to α -Gal and the development of red meat allergy (8). Furthermore, red meat allergic patients have shown to have a selective IgE response to the pure α -Gal carbohydrate and not to common carbohydrates from plants and venoms (12).

Anti-Gal is a natural antibody produced in response to continuous antigenic stimulation by bacteria of the normal gastrointestinal flora, which have carbohydrate antigenic structures similar to the α -Gal epitope. These antibodies are produced in humans throughout life as ~1% of circulating immunoglobulins (13) of IgG, IgM, and IgA classes (6) and they are largely IgG₂ (14). These natural antibodies against α -Gal are thought to be involved in acute organ rejection in xenotransplantation (15).

The α -Gal epitope is structurally similar to the blood group B antigen which has an extra fucose residue on the glycan core in comparison to the α -Gal epitope. In subjects lacking the B-antigen, namely blood group A and O donors, α -Gal IgG antibodies react both to the α -Gal epitope and the B antigen. However, the α -Gal IgG antibodies in donors of blood group B

bind solely to the α -Gal epitope. The effect of blood type on anti- α -Gal immunity and incidence of infectious diseases was recently reported showing that susceptibility to malaria and tuberculosis correlates positively with the frequency of blood type B in endemic regions. These diseases are caused by pathogens with α -Gal on their surface. However, this positive correlation was not found in the case of dengue fever which is caused by a pathogen without α -Gal (16).

We were able to observe, for the first time, that more than 90% of our Swedish red meat allergic patients belonged to the B-negative blood groups (A or O). Only 2 out of 39 patients (5%) were blood group B-positive, which is significantly less compared to the expected frequency of blood group B in the Swedish population (18%) (5). These findings prompted us to further explore the relationship between α -Gal- and B-antigen-specific antibodies in a larger cohort of red meat allergic patients, as well as comparing the immune response to healthy donors with blood groups B or A/O. In addition, we explored whether the allergen-specific IgG and IgG subclass distribution could differentiate the meat allergic subjects from the blood group B or A/O donors. We furthermore elucidated the allergenicity of the B-antigen and its role in cross-reactivity with α -Gal.

Material and methods

Patients and sera

Sera from 51 patients reporting delayed allergic reactions following consumption of red meat attending the Allergy Unit at Södersjukhuset, Stockholm, were used in the study. The patients were examined by a physician experienced in allergic diseases. They were also subjected to a detailed questionnaire regarding clinical episodes and symptoms after consuming mammalian meat. All 51 patients had IgE antibodies to α -Gal (range 0.74–>100 kU_A/L) determined by ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden). The red meat allergic

patients were blood typed according to routine methods (Blood Center, Karolinska University Hospital) and three belonged to the B-positive blood groups (AB and B). Sera from 102 healthy blood donors from the greater Stockholm area (Blood Center, Karolinska University Hospital) were also included in the study. This group was further split into two subgroups consisting of 51 donors each: healthy B and healthy A/O blood groups, respectively. The A/O group was designed to include α -Gal positive (n=12) and negative (n=39) healthy donors, selected from a previously reported cohort (5). The study was approved by the local ethics committee.

ImmunoCAP IgE determination

Allergen-specific IgE antibody reactivity against α -Gal (ImmunoCAP o215, bovine thyroglobulin; Thermo Fisher Scientific Uppsala, Sweden) and the B-antigen (Glycotect, 01-092, Gaithersburg, MD, USA) coupled to Streptavidin ImmunoCAP (5 μ g of biotinylated antigen, as described by the manufacturer) were determined using the ImmunoCAP System according to the manufacturer's instructions (Thermo Fisher Scientific). Furthermore, the atopic status of all healthy individuals was determined using a mixture of the most common inhalant allergens (Phadiatop, Thermo Fisher Scientific). The results are presented as kU_A/L. The cutoff for allergen-specific IgE is ≥ 0.1 kU_A/L.

Indirect ELISA assay

Half-area microtiter plates (96 wells, Greiner bio-one, Frickenhausen, Germany) were coated for 6 h at RT with 0.1 μ g of α -Gal-HSA (Gal α 1-3Gal β 1-4GlcNAc-HSA) or B-antigen-HSA (NGP2334 and NGP9323, Dextra Laboratories, Reading, UK), or HSA as a control for unspecific binding (Sigma-Aldrich, Steinheim, Germany) followed by blocking (PBS containing 1% HSA and 0.5% Tween 20, pH 7.4) overnight at 4 °C. Sera were diluted in

PBS-t containing 0.1% HSA subsequently, (1:100 for IgG, 1:50 for IgG₁₋₃ and 1:20 for IgG₄) and incubated for 2 h at room temperature. After five washing steps, total IgG was analysed with rabbit anti-human IgG Fc γ fragment and goat anti-rabbit IgG HRP conjugated (Jackson, West Grove, PA, USA). IgG₁, IgG₂ and IgG₄ Ab were analysed with mouse anti-human IgG₁ (BD Pharmingen 555868, BD San Diego, CA, USA), IgG₂ (BD Pharmingen 555873), IgG₄ (BD Pharmingen 555881), IgG₃ (Sigma-Aldrich I7260), and visualized with HRP-conjugated anti-mouse IgG (Jackson, 515-035-071). The absorbance was read at 450 nm. The cut-off was determined as twice the mean OD value of serum-free controls and no unspecific binding to HSA was observed. Results from ELISA experiments are expressed as OD means of duplicate determinations with a deviation of <5%.

Inhibition experiments

Sera from 5 red meat allergic patients were pre-incubated with α -Gal trisaccharide (Gal α 1-3Gal β 1-4Glc, L330, Dextra Laboratories) at a concentration of 2-500 μ g/ml of inhibitor for 1 h at room temperature prior to indirect ELISA assay as described above. Detection was performed with mouse anti-human IgE antibody conjugated with HRP (1 h, 1:2500; Abcam, UK). The absorbance was measured at 450 nm. Inhibition of IgE binding was analyzed by ELISA and calculated as $[(OD_{no\ inhibitor} - OD_{inhibitor})/OD_{no\ inhibitor}] \times 100$.

Basophil activation assay

Allergen-specific basophil degranulation was analyzed by monitoring the basophil activation markers CD203c and CD63, as previously described (17). Heparinized venous blood samples from five red meat-allergic patients, one atopic birch-allergic patient as well as one non-allergic control were analyzed with HSA- α -Gal, HSA-B antigen (Dextra Lab) and HSA. Ten-

fold serial dilutions of antigen (from 100 - 0.1 $\mu\text{g/ml}$) were added to blood and incubated for 30 minutes at 37°C. Anti-Fc ϵ RI (Bühlmann Laboratories AG, Schönenbuch, Switzerland) was used as positive controls. The samples were further incubated with FITC conjugated anti-CD63 and PE conjugated anti-CD203c monoclonal antibodies (clones CLBGran/12 and 97A6, respectively, Immunotech, Marseille, France), lysed and analyzed by flow cytometry using a BD FACSCanto II (BD Biosciences, San Jose, CA) and data were analyzed using FlowJo (Treestar, Ashland, OR). Basophils were identified by gating at least 300 CD203c-positive cells and the magnitude of allergen-activation was calculated and expressed as the percentage of CD63-positive cells among the gated basophils.

Statistical analysis

Statistical analysis was performed with GraphPad Prism, version 6 (GraphPad software, La Jolla, CA). Quantitative measurements of IgE antibodies were compared using Spearman rank correlation and Mann-Whitney U tests. A *P* value of less than 0.05 was considered significant.

Results

The demographic data of the allergic patients and healthy A/O and B blood donors are shown in Table 1.

IgE reactivity to α -Gal and B-antigen

Twelve healthy A/O (n=51) and 3 healthy B donors (n=51) were IgE positive to α -Gal. The IgE levels against α -Gal were significantly higher in the meat allergic patients (median 11

kU_A/L; range 0.74->100 kU_A/L) compared to the α -Gal positive healthy A/O (n=12, median 0.15 kU_A/L; range 0.1-0.7 kU_A/L) and B donors (n=3; 0.36, 0.21 and 0.59 kU_A/L) (Fig. 1A).

The majority of the meat allergic patients had an IgE response against the B-antigen (39/51; median 1.03 kU_A/L; range 0.15->100 kU_A/l) whereas none of the healthy A/O or B donors exhibited a IgE response to B-antigen (Fig. 1B). The three meat allergic patients belonging to the blood groups AB/B were IgE negative to the B-antigen (Table S1). There was a moderate correlation between IgE levels to α -Gal and B-antigen among the red meat allergic patients ($r=0.51$; $P<0.001$) (Fig. 1C). The atopic status of all included healthy individuals was determined using Phadiatop to assess influence on IgE responses to α -Gal and B-antigen. In these groups 25-27% (A/O, 13/51; B, 14/51) were atopic and none of these had IgE to B-antigen. Furthermore, 3/11 atopic healthy A/O and 1/3 atopic healthy B had IgE to α -Gal which was not statistically different to the frequency of the groups as a whole.

Inhibition experiments were performed to assess whether α -Gal IgE antibodies in red meat allergic patients would also be directed against the B-antigen. Sera from 5 meat allergic patients were preincubated with stepwise dilutions of α -Gal trisaccharide and B-antigen and thereafter analysed for IgE binding against α -Gal (Figure S1). These patients exhibited a representative spread of IgE levels to α -Gal and the B-antigen (0.74-63 kU_A/L against α -Gal and 0.42-27 kU_A/L against B-antigen). A concentration of 31.25 μ g/ml of α -Gal induced a stronger drop in the IgE binding to the B-antigen compared to α -Gal itself in all sera tested. However, the inhibition was dependent on the IgE anti- α -Gal level. The serum with the highest IgE level to α -Gal (#18; 63 kU_A/L) had the least drop in IgE binding to the B-antigen (Figure S1 B).

Allergenic activity of B-antigen

The biological activity of the B-antigen in relation to α -Gal was evaluated by basophil activation test in five red meat-allergic patients. HSA- α -Gal induced degranulation in all patients at all concentrations tested (0.1-100 μ g/ml) and always to a higher degree than HSA-B antigen (Fig 2). The patient with the highest IgE level to B antigen (#48, 11 kU/L) was clearly positive at all concentrations of B antigen tested. Patient #49 was IgE negative to B antigen but granulation was observed at the highest tested concentration. For the remaining three patients, (#42, 46 and 47; 2.36, <0.1 and 6.62 kU/L to B antigen respectively), the B antigen induced degranulation to a degree just exceeding the HSA control. None of these antigens induced degranulation in the atopic (birch allergic, Fig. 2) or non-allergic (data not shown) controls, indicating that the observed reactions are IgE-dependent.

IgG and IgG subclass responses to α -Gal and B-antigen

We also investigated the IgG reactivity to α -Gal and B-antigen. We noted that red meat allergic patients had significantly higher total IgG levels against α -Gal ($p < 0.0001$; Figs. 3A and S2A) compared to the healthy A/O or B groups. Furthermore, the α -Gal-specific total IgG levels were also significantly higher in the healthy A/O donors compared to the B ($p < 0.0001$; Fig. S2A). With respect to IgG subclasses, α -Gal-specific responses of all subclasses were higher in meat allergic patients compared to both healthy groups ($p < 0.0001$ – 0.01 ; Figs. 3A and S2A). The largest differentiator was IgG₁, where the median level of meat allergic patients was more than 10-fold higher in comparison to healthy A/O and B donors ($p < 0.0001$). The IgG₂ subclass, on the other hand, induced a dominant response in all three groups. However, this response was significantly higher in the allergic patient and health A/O groups than the healthy B group (Fig. S2A). Regarding IgG₃, production was low in all groups, with a significantly lower level in the healthy B group. IgG₄ antibodies to α -Gal were

observed in the red meat allergic patients and the levels were significantly higher compared to the healthy donors. The healthy A/O donors were divided based on IgE reactivity to α -Gal (Fig. 4). The α -Gal IgE positive donors (n=12) showed significantly higher IgG1, IgG2 and IgG4 levels compared with the negative donors (n=39), whereas the total IgG levels were similar in the two groups. These subgroups were also compared to the red meat allergic and healthy B groups (Fig. S3), further illustrating that IgE to α -Gal is associated with higher IgG1, IgG2 and IgG4 levels in both red meat allergic patients and healthy controls.

In comparison to the α -Gal response, the IgG response to the B-antigen followed a similar pattern of a dominating IgG₂ response in the allergic and healthy A/O groups and in these groups the total IgG and IgG₂ levels were comparable (Figs. 3B and S2B). Significant but small increases in IgG₁, IgG₃ and IgG₄ levels to B-antigen were observed for the red meat allergic patients. As expected, very low IgG levels to B-antigen were observed among healthy B donors. The IgG levels against α -Gal and the B-antigen correlated moderately both in the meat allergic patients (r=0.49) and the healthy A/O group (r=0.65; Fig. 5), whereas there was a lack of correlation in the healthy B donors (r=0.08, data not shown).

Discussion

Here we report that the allergen-specific antibody responses differentiated red meat allergic patients from healthy A/O and B-donors. The allergic patients not only had significantly higher IgE levels to α -Gal but also higher total IgG and IgG₁₋₄ subclass levels towards α -Gal. The increase in IgG₁ subclass levels was the largest differentiator for meat allergic patients, in line with previous reports (18, 19). IgG₂, however, dominated the IgG response in all three groups, reflecting an antibody response to gut microbiota. Interestingly, we found that red

meat allergic patients induced significantly higher IgG₄ responses to α -Gal compared to the healthy donors.

Thus, the red meat allergic patients seem to have a Th2-driven immune response where α -Gal-specific IgG₁ and IgG₄ reactivity accompanies their IgE response.

It is known that the cat allergen cat IgA (Fel d 5) carries α -Gal (20) and an association between cat ownership and α -Gal sensitization has been reported (21). We noted that about one-fifth of the healthy A/O individuals had low IgE levels to α -Gal which most probably were induced by tick exposure, but cat exposure cannot be excluded. A similar IgG profile was seen among these 12 α -Gal IgE positive healthy A/O donors, but with lower antibody levels to α -Gal. This indicates that the route of α -Gal exposure is of importance for developing a strong IgE α -Gal response and symptoms, as in the case of tick bite exposure, which seems to be an important risk factor. This is in contrast to a weak IgE α -Gal response and lack of symptoms as seen in the few healthy A/O donors, which in turn may be related to cat exposure (21). When we compared the α -Gal IgE positive (n=12) and negative healthy A/O donors to the red meat allergic and healthy B groups we concluded that IgE to α -Gal is associated with higher IgG₁, IgG₂ and IgG₄ levels in both red meat allergic patients and healthy controls. We furthermore noted that the atopic status of healthy individuals did not influence IgE-binding to α -Gal or B-antigen.

As expected, the majority of the red meat allergic patients generated an IgE response to the B-antigen. However, the IgE levels were significantly lower compared to the anti- α -Gal levels and only a moderate correlation between the IgE anti- α -Gal and anti-B-antigen levels was noted. The cross-reactivity between α -Gal and the B-antigen was further elucidated using inhibition studies, where pre-incubation with α -Gal had a greater effect on the drop in IgE

binding to the B-antigen compared to homologous inhibition with α -Gal. This suggests that while α -Gal and the B-antigen have epitopes in common, there are unique IgE-binding structures or other factors affecting the affinity. These results on IgE and IgG subclass responses to α -Gal and B-antigen are in line with Galili *et al*, describing the “anti-Gal” IgG repertoire as comprising of both α -Gal and B-antigen binders depending on A/O or B/AB blood types (22). While AB/B individuals strictly had α -Gal specificities, the B-antigen response in A/O individuals was shown to mostly recognize both the α -Gal and B-antigens. We also find IgG responses to α -Gal irrespective of blood group where the IgG response to the B-antigen is seemingly dominated by cross-reacting α -Gal binders. As in our previous report (5), we observed a few B-positive subjects expressing IgE to α -Gal ranging from <1 kU_A/L in three healthy individuals to 5-51 kU_A/L in three red meat allergic patients. So while IgG responses to α -Gal are generated irrespective of blood group, IgE sensitization to α -Gal is associated with B-negative blood groups and thus natural tolerance mechanisms to B-antigen are reducing the IgE sensitization rate to the α -Gal epitope.

We also investigated the allergenic potential of the B-antigen in a basophil activation test using blood from five red meat allergic patients and noted that the B-antigen stimulated degranulation of basophils in all patients, demonstrating for the first time that the B-antigen is a biologically active allergen. However, compared to α -Gal the allergenic activity was lower. In contrast, none of the healthy A/O or B donors had an IgE response to the B-antigen. Thus, very low amounts of IgE to α -Gal, as in the healthy donor groups, seem not to be associated with a concurrent IgE response to the B-antigen.

With respect to the IgG and IgG subclass responses to the B-antigen, these were similar between the allergic and healthy A/O groups with IgG₂ being the dominating subclass but

only a small increase in IgG₁ levels for red meat allergic patients. This suggests that the IgG responses to the B-antigen are only affected to a small extent by the increased α -Gal responses in red meat allergic patients. This was further evidenced by studying the correlation between total IgG levels to α -Gal and B-antigen, where the correlation was found to be lower and skewed towards α -Gal among the allergic patients compared to the healthy A/O donors. As expected, the healthy B donors showed a contrasting response with almost nonexistent IgG levels towards B-antigen, a response in line with self-tolerance.

In conclusion, red meat allergic patients differ in their immune response to α -Gal and B-antigen compared to healthy donors. Their immunoprofile is characterized by increased IgE, IgG₁ and IgG₄ anti- α -Gal levels reflecting a Th2 response. However, red meat allergic patients' IgG reactivity to the B-antigen is similar to healthy A/O donors, suggesting a difference in IgG epitopes to α -Gal and B-antigen. Furthermore, for the first time we show that the B-antigen induces basophil degranulation and is thus a biological active antigen for red meat allergic patients. The healthy B-group on the other hand, is characterized by a nearly non-existing IgE and lower IgG responses to α -Gal and in addition, they almost completely lack IgG antibodies to the B-antigen. Thus self-tolerance to the B-antigen results in a reduced risk of sensitization to α -Gal and development of red meat allergy.

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Author contributions

The study was conceived by DA, CH and MvH. DA participated in all stages of the project and performed the experiments. DA, CH and MvH wrote the manuscript together with RDR and TH. MS and RDR provided the patient material. All authors contributed to interpretation of the data and provided critical review of the manuscript.

Conflict of interest

None of the authors has any conflict of interest to declare.

Supporting Information

Additional supporting information can be found in the online version of this article.

Table S1. Demographic and serological data of red meat allergic patients.

Figure S1. IgE cross-inhibition and homologous inhibition with α -Gal in five red meat allergic patients' sera.

Figure S2. Differences between IgG and IgG subclass (IgG₁₋₄) reactivity against α -Gal (A) and B-antigen (B) in sera from red meat allergic patients and healthy blood groups A/O and B using Mann-Whitney U-tests.

Figure S3. Differences between IgG and IgG subclass (IgG₁₋₄) reactivity against α -Gal (A) and B-antigen (B) in sera from red meat allergic patients, healthy blood group A/O divided

into IgE positive and negative to α -Gal as well as blood group B using Mann-Whitney U-tests.

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Table 1. Demographic data of patients

Group	Allergic	Healthy A/O	Healthy B
No	51	51	51
Age* (years)	47; 19-74	45; 23-70	43, 22-72
Gender*	27F, 24M	21F, 30M	24F, 27M
IgE to α -Gal** (kU _A /L)	51/51; 11; 0.74->100	12/51; 0.15; 0.1-0.7**	3/51; 0.36, 0.12-0.59***
IgE to B antigen** (kU _A /L)	39/51; 1.03; 0.15->100	0/51; <0.1	0/51; <0.1

* - median and range

** - frequency, median and range

F- female; M-male.

Figure legends

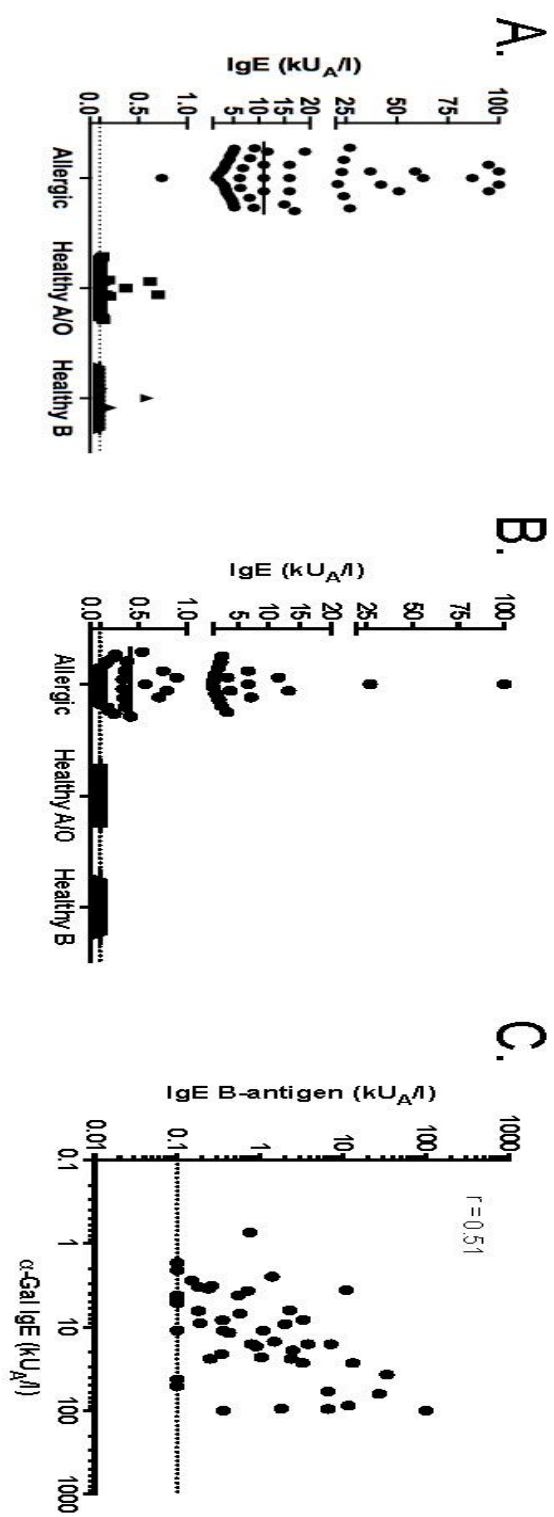
Figure 1. Analysis of IgE reactivity among red meat allergic patients and healthy blood A/O and B donors. IgE reactivity was measured using ImmunoCAP against α -Gal (A) and against the B antigen (B) among the three different groups. (C) Comparison of α -Gal and B antigen specific-IgE reactivity (kU_A/L) in the red meat allergic patient group.

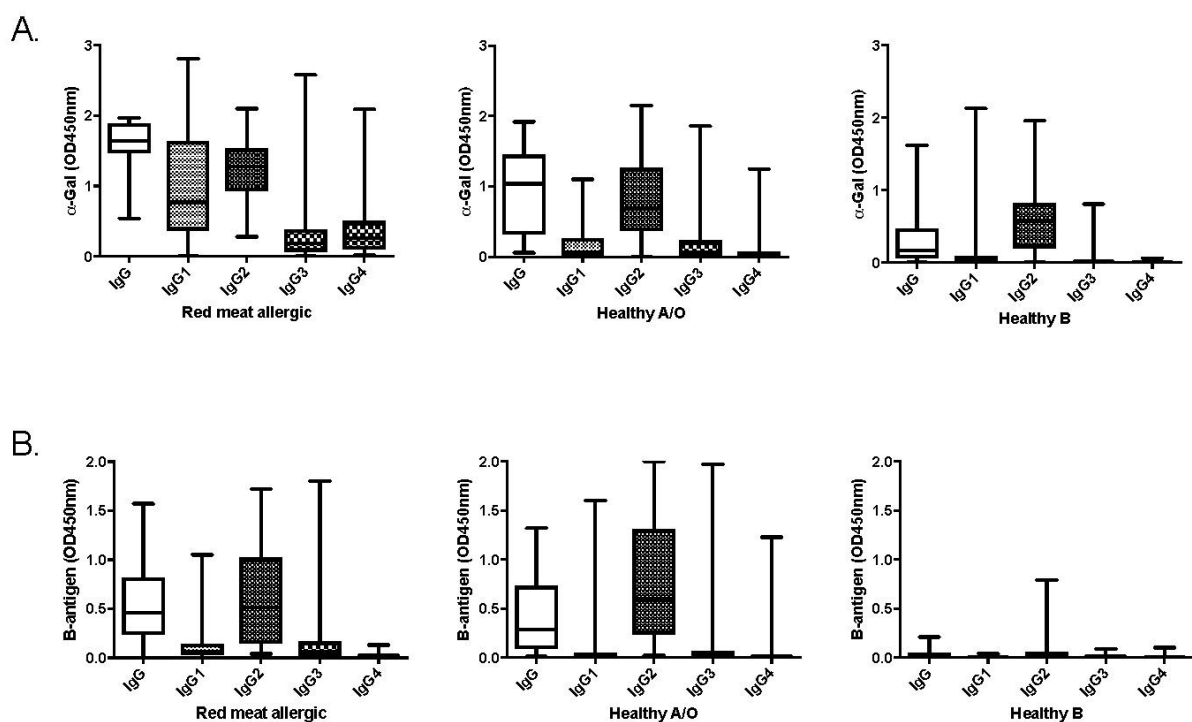
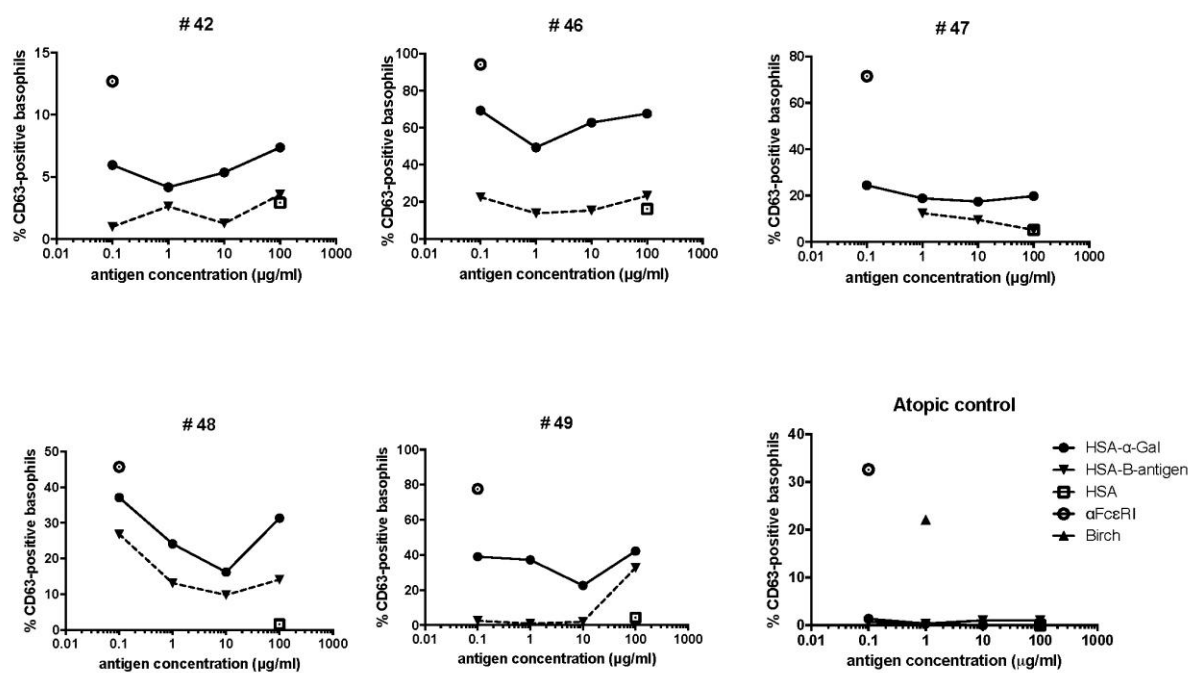
Figure 2. Allergenic activity of HSA- α -Gal, HSA-B-antigen and control molecules as determined by basophil activation in blood from five red meat allergic patients and one atopic control. Degranulation is presented as proportion (%) of CD63-positive out of CD203c-positive cells by flow cytometry (y-axes).

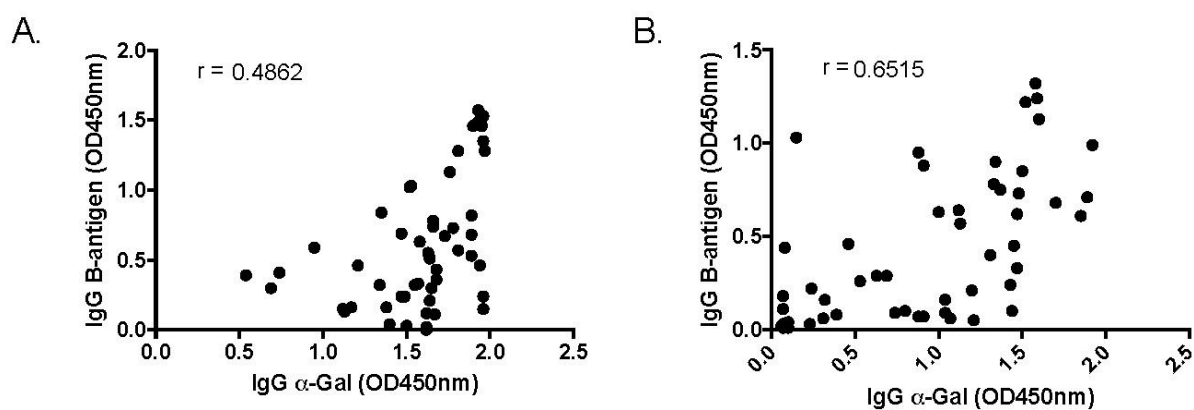
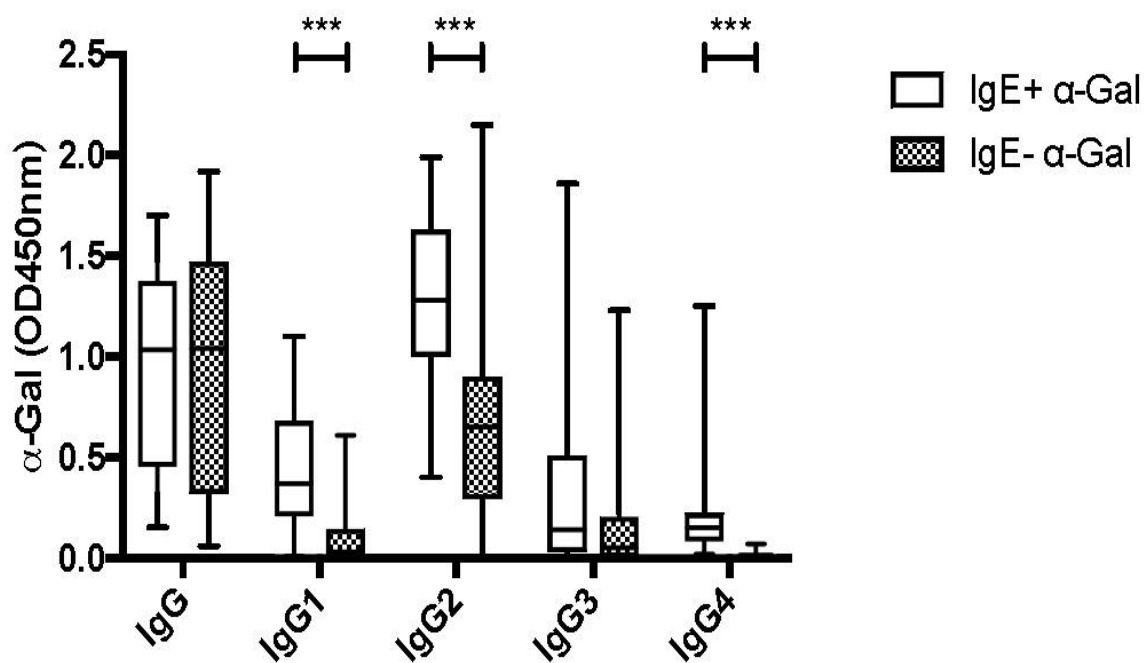
Figure 3. IgG, IgG subclass (IgG₁₋₄) reactivity against α -Gal (A) and B-antigen (B) in red meat allergic patients and healthy blood groups A/O and B donors. For a statistical analysis of differences per IgG subclass, please see Figure S2.

Figure 4. IgG and IgG subclass (IgG₁₋₄) reactivity against α -Gal in healthy blood group A/O donors IgE positive versus IgE negative against α -Gal. ** $p < 0.001$, *** $p < 0.0001$, **** $p < 0.00001$.

Figure 5. Correlation of α -Gal and B-antigen-specific IgG levels (OD) in sera from red meat allergic patients and healthy blood group A/O donors. No correlation was observed in healthy blood group B donors ($r=0.08$, data not shown).







Immunoprofile of α -Gal- and B-antigen-specific responses differentiate red meat allergic patients from healthy individuals

Supporting information

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Table S1. Demographic and serological data of red meat allergic patients

No	Blood group	Age/gender	α -Gal, IgE*	B antigen, IgE*
1	AB	50/F	5	<0.1
2	O	51/F	11.7	0.42
3	O	28/M	2.8	0.15
4	A	42/F	15	1.48
5	O	45/F	9	0.19
6	O	70/F	27	13.2
7	O	50/F	6.3	2.25
8	O	47/F	19	2.47
9	A	49/F	21	0.34
10	O	42/F	1.7	<0.1
11	O	50/M	17	0.89
12	A	40/F	100	0.36
13	A	75/F	2.1	<0.1
14	A	31/M	37	0,34
15	O	56/M	3.3	0.18
16	O	73/M	3.6	0,38
17	A	67/M	4.7	<0.1
18	O	74/M	63	27.3
19	A	44/M	24	0.25
20	A	48/M	16	3.77
21	A	49/M	6.4	0.18
22	A	42/F	11	1.09
23	O	60/M	2.5	1.4
24	O	65/M	8.3	0.35
25	A	50/F	4.2	0.54
26	B	19/M	51	<0.1
27	O	24/M	3.2	0.26
28	A	22/M	11	0.36
29	A	70/F	5.2	<0.1

30	A	39/F	6.9	0.57
31	A	56/M	16	7.12
32	O	21/M	8.2	3.33
33	A	56/M	16	0.79
34	A	50/F	4.9	<0.1
35	A	64/M	3.7	0.71
36	A	35/F	4.2	<0.1
37	O	19/M	>100	>100
38	O	62/F	59	6.66
39	O	63/F	0.74	0.75
40	O	30/M	9.2	2
41	A	56/F	3.46	0.24
42	O	45/M	24	2.36
43	A	25/M	23	1.03
44	A	46/F	4.4	<0.1
45	O	41/F	95	1.81
46	A	29/F	5.2	<0.1
47	O	33/F	95.2	6.62
48	O	46/M	87	11.6
49	B	50/F	42.2	<0.1
50	O	58/F	27	3.26
51	A	42/F	11	<0.1

F-female; M- male;

* - IgE level is expressed in kU_A/L

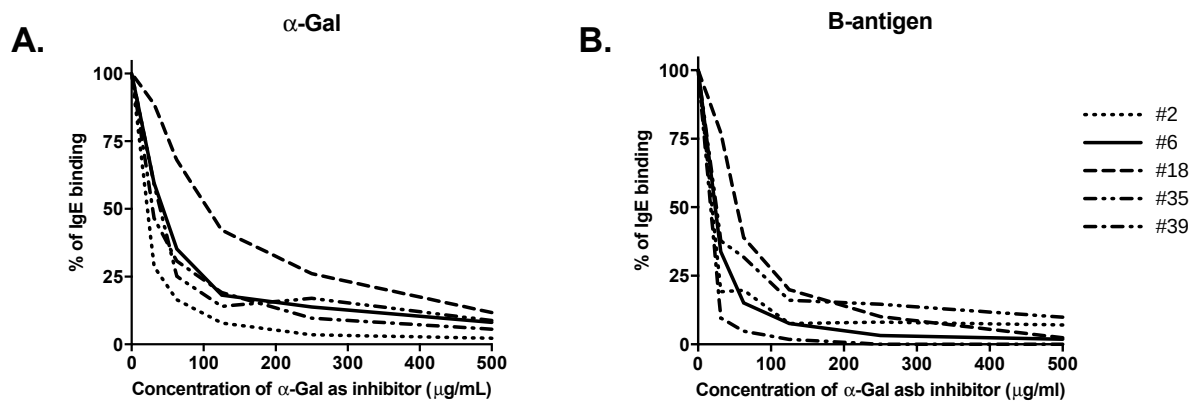
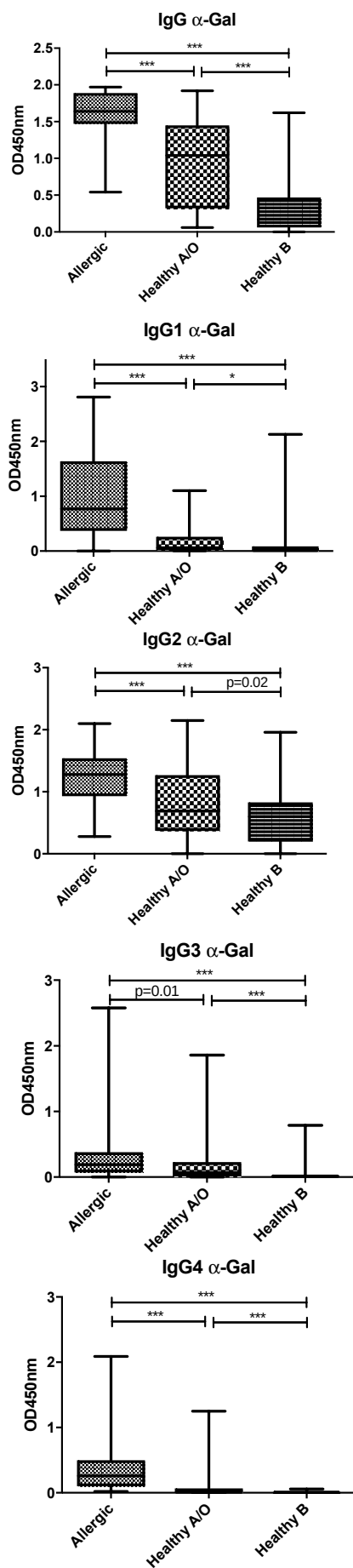


Figure S1. IgE cross-inhibition and homologous inhibition with α -Gal in five red meat allergic patients' sera. Sera were pre-incubated with α -Gal trisaccharide prior to ELISA measurement of IgE binding to α -Gal (A) and B-antigen (B). Percentages of inhibition (y-axes) are plotted against different concentrations of inhibitor allergens (x-axes).

A.



B.

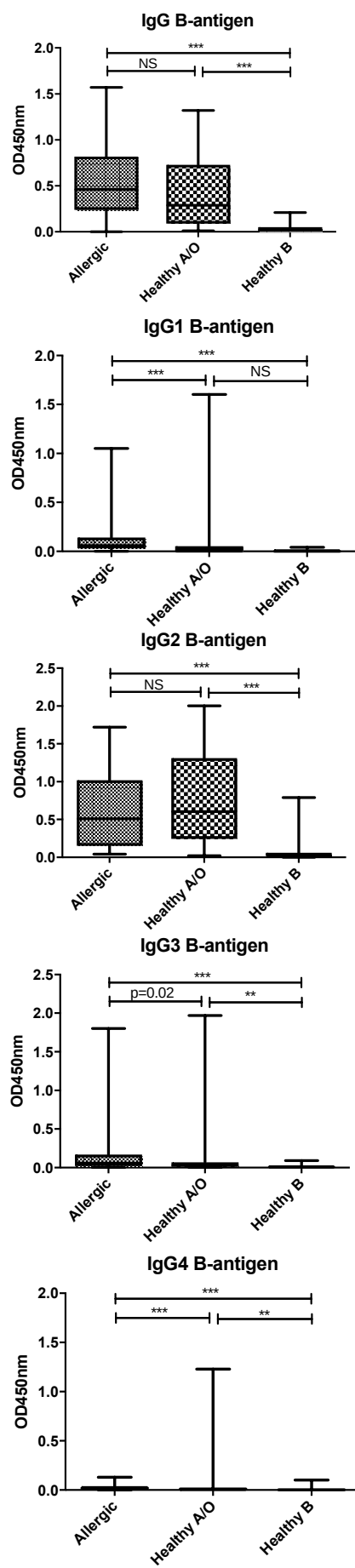
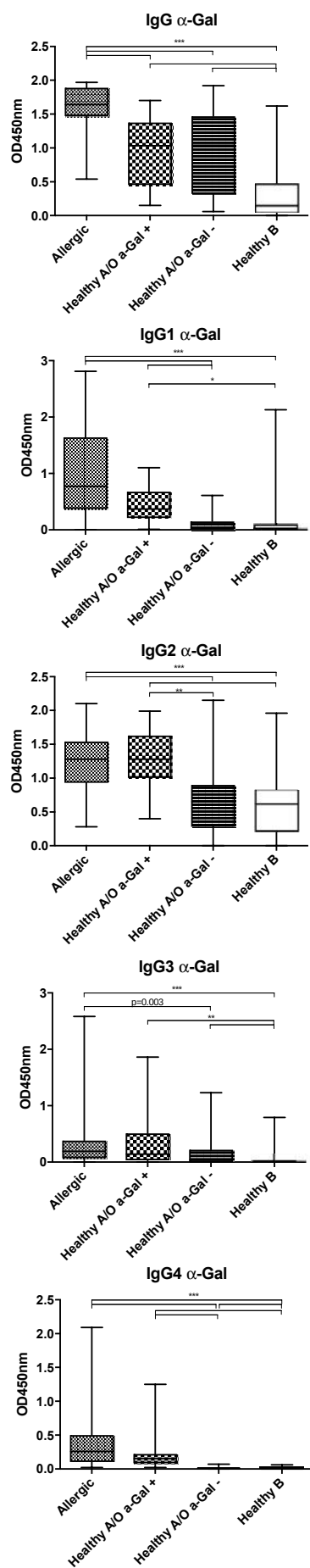


Figure S2. Differences between IgG and IgG subclass (IgG₁₋₄) reactivity against α -Gal (A) and B-antigen (B) in sera from red meat allergic patients and healthy blood groups A/O and B using Mann-Whitney U-tests. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

A.



B.

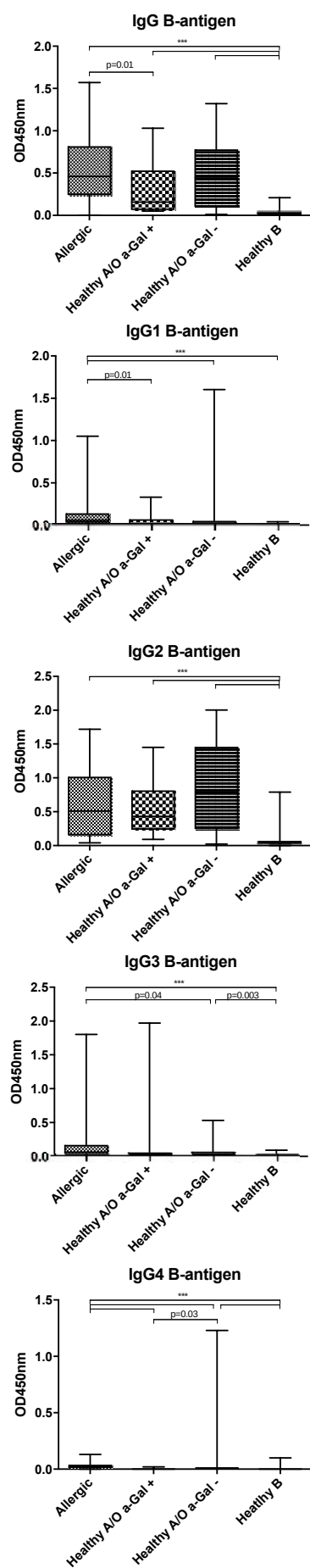


Figure S3. Differences between IgG and IgG subclass (IgG₁₋₄) reactivity against α -Gal (A) and B-antigen (B) in sera from red meat allergic patients, healthy blood group A/O divided into IgE positive and negative to α -Gal as well as blood group B using Mann-Whitney U-tests. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.