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Supporting Information

Red meat allergic patients have a selective IgE response to the α-Gal glycan

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Material and methods

Patient and control populations

Serum samples from 24 red meat allergic patients, reporting delayed allergic reactions following consumption of red meat and attending the Allergy Unit at Södersjukhuset, Stockholm, were used in the study. All patients were examined by a physician experienced in allergic diseases and responded to a detailed questionnaire regarding clinical episodes and symptoms of mammalian meat intake. Serum samples from 3 healthy blood donors (Blood Center, Karolinska University Hospital), IgE negative to thyroglobulin, were included in the study as controls. The study was approved by the local ethics committee.

ImmunoCAP IgE determination

Allergen-specific IgE reactivity to thyroglobulin (a protein that contains nine α-Gal epitopes on its surface and commercially used to test IgE against α-Gal), nCup a 1, nArt v 1 and MUXF^3 was determined (ImmunoCAP®, Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden). IgE antibodies against Neu5Gcα-sp-biotinylated and Gal-α1-3-Galβ1-4-GlcNAcβ-sp-biotinylated (the α-Gal glycan) (products #02-051 and #02-079, Glycotech Gaithersburg, MD, USA) were measured by coupling 5µg of biotinylated antigen to Streptavidin ImmunoCAP® as described by the manufacturer (Phadia AB/Thermo Fisher Scientific). All IgE determinations were analyzed using the ImmunoCAP System (Phadia AB/Thermo Fisher Scientific) according to the manufacturer's instructions. The results are presented as kU_A/l where the cut-off for allergen-specific IgE was ≤ 0.10 kU_A/l.

Deglycosylation assay

Enzymatic removal of glycans from thyroglobulin was performed with PNGase F kit (New England BioLabs, Ispwich, MA, USA) according to manufacturer's instructions. The enzyme cleaves all asparagine linked sugars, except for α1→3 fucose, under mild conditions and removes the intact oligosaccharide. The deglycosylated product was tested by SDS PAGE and immunoblot developed with an anti-α-Gal antibody (Enzo Life Science, Inc., Farmingdale, NY, USA) and patient serum.

CD spectroscopy

Far UV CD spectra were recorded on a JASCO J-815 spectropolarimeter (JASCO, Tokyo, Japan). Samples (1 mg/mL thyroglobulin and deglycosylated thyroglobulin in PBS pH 7.4) were analyzed at 25°C in a 0.1 mm path length quartz cell. The spectra were collected in 0.1 nm steps at a rate of 50 nm/min over the wavelength range 195-260 nm. Each spectrum was acquired five times and the results were averaged. Results were recorded in millidegrees and converted to molar Δε in M^{-1} cm^{-1} and mean residue ΔεMRV. To determine the percentage of secondary structure motifs, the CONTIN software and the SP37 protein set provided in CDPro software package were used.

SDS PAGE and immunoblot analysis

Thyroglobulin and deglycosylated thyroglobulin were separated by SDS PAGE carried out on Hoefer Scientific Instruments SE250 (Amersham Biosciences, Uppsala, Sweden), and Mini Protean Cell II (BioRad Laboratories, Hercules, CA, USA). The gel was stained with Coomassie Brilliant Blue or analyzed by immunoblotting under reducing conditions. Ten micrograms of proteins per lane were resolved by 6% PAGE and Any kD MiniProtean TGX precast Tris-glycine gels (BioRad), and electroblotted to Immobilon™ polyvinylidene difluoride transfer membranes (Millipore, Billerica, MA, USA) following manufacturer's instructions. Membranes were blocked for 1h at room temperature in blocking solution (1% HSA in PBS containing 0.05% of tween). IgE

binding was investigated by overnight incubation of membrane with human serum from one meat allergic patient (Patient #8, Table I), diluted 1:5 in 0.1% HSA in PBS-Tween. Detection was carried out by 1h incubation with mouse anti-human IgE conjugated with horse-radish peroxidase (HRP) (dilution 1:2000, Abcam, UK) and development with chemiluminescence substrate for HRP detection on ChemiDoc (BioRad). For α-Gal detection, mouse anti-α-Gal monoclonal antibody was incubated for 8h at room temperature (dilution 1:5, M86, Enzo Life Science, Inc., Farmingdale, NY, USA), following incubation with alkaline phosphatase conjugated goat anti-mouse IgM antibody (1:3000, SouthernBiotech, Birmingham, AL, USA) for 1h at room temperature.

Absorption experiment

The capacity of thyroglobulin (Sigma Aldrich, St. Louis, MA, USA) to inhibit IgE binding to solid phase-bound deglycosylated thyroglobulin was measured by the ImmunoCAP System. Serum from eight red meat allergic patients (# 3, 5, 6, 7, 13, 14, 19, and 24 from Table I) was pre-incubated with 500 µg/mL of Gal-α1-3-Galβ1-4-GlcNAcβ-sp-biotin (Glycotech).

Figure S1. Far UV CD spectra of thyroglobulin (solid line) and deglycosylated thyroglobulin (dash line).