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Insight into the effects of deglycosylation and glycation of shrimp tropomyosin on *in vivo* allergenicity and mast cell function

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Shrimp tropomyosin (TM) is a glycoprotein allergen with high lysine content, however, there is little data on how deglycosylation and glycation modify the allergenicity of TM. In this work, the effects of deglycosylation (enzymatic and chemical) and glycation of TM by glucose on *in vivo* allergenicity and mast cell function were investigated using BALB/c mouse and RBL-2H3 mast cells. Compared with TM, after deglycosylation, the deglycosylated TMs (DGTMs) exacerbated the *in vivo* allergenicity and mast cell allergy response. As for the TM glyated by glucose (TM-G), glycation led to weaker allergy responses on mouse and mast cells, which might be due to glucose destroying the epitopes with glycation sites. Moreover, the glucose residues on TM-G functioned as both a resistance to prevent the accessibility of TM-G to the IgE on mast cell and masking epitopes. Therefore, deglycosylation of TM was not suitable for desensitizing TM-induced food allergy, while glycation of TM might be applied to desensitize TM allergenicity, which could provide new insight into food allergy desensitization for larger cohorts of shrimp-allergic patients.

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1. Introduction

Shrimp food allergy is a severe adverse hypersensitivity reaction,^{1,2} the most common and potent allergen of shrimp is tropomyosin (TM). However, the current management of shrimp allergy is still the avoidance of shrimp diet, lacking efficient treatment and medical approaches.

Shellfish tropomyosin is a glycoprotein allergen.^{3–5} Glycans have vital physiological functions (e.g. in resistance to proteolytic degradation, intercellular transduction of information between cells and adhesion through ligand–receptor interactions),⁶ however, there is little data on how the glycans of TM affect their reactivity in triggering allergic reactions. In this study, the glycans of TM were investigated using enzymatic and chemical deglycosylation to elucidate the role of glycans in food allergy.

The destruction of epitopes of allergens could be a potent approach to reducing the allergenicity.⁷ Glycation is a common chemical reaction between proteins and saccharides during food processing and storage, which has been approved to be a promising approach to affect the allergenicity of aller-

gens by epitope modification.⁷ During glycation, the conformational epitopes could be altered by conformation changes, and the linear epitopes with glycation sites could be destroyed and masked by the saccharide residues on the glyated allergens.^{7,8} When allergy susceptible individuals are exposed to the glyated allergens, the allergens with less IgE-binding epitopes might sensitize the immune systems to a lesser extent, or not at all, to trigger a food allergy.

Our previous work has investigated the effects of deglycosylation (both enzymatic and chemical) and glucose glycation on *in vitro* allergenicity using western blot and ELISA,⁸ however, little is known about how deglycosylation and glucose glycation of TM affect the *in vivo* allergenicity and mast cell function. The purpose of this work is to evaluate the effects of deglycosylated TMs (DGTMs) and glyated TM by glucose (TM-G) on the *in vivo* allergenicity and mast cell function via BALB/c mouse and RBL-2H3 mast cell models. The results should enhance our understanding of how deglycosylation and glycation of allergens affected the allergenicity, which could provide pre-clinical data for food allergy desensitization for larger cohorts of shrimp-allergic patients.

2. Materials and methods

2.1 Materials

Shrimp tropomyosin was purified from *Exopalaemon modestus* according to a previous method.⁹ 2',7'-Dichlorodihydro-

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fluorescein diacetate ($H_2DCF\text{-}DA$, CAS: 4091-99-0), 4-nitrophenyl *N*-acetyl- β -D-glucosaminide (PNAG, CAS: 3459-18-5), β -mercaptoethanol (CAS: 60-24-2), cholera toxin (CT, CAS: 9012-63-9) and D-glucose (CAS: 50-99-7) were purchased from Sigma-Aldrich (St Louis, MO, USA). PNGase F and α 2-3,6,8 neuraminidase and *O*-glycosidase bundle were purchased from New England Biolabs Inc. (Ipswich, MA, USA). Anisole (CAS: 100-66-3), *n*-hexane (CAS: 110-54-3) and trifluoromethanesulfonic acid (TFMS, CAS: 1493-13-6) were obtained from Aladdin Industrial Corp. (Shanghai, China).

Female BALB/c mice were obtained from JOINN Laboratories (Suzhou, Jiangsu, China). The murine mast cell line (RBL-2H3) was obtained from the Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Mast cells were cultured in MEM culture medium and supplemented with 10% FBS, 100 $U\text{ mL}^{-1}$ penicillin and 100 $\mu\text{g mL}^{-1}$ streptomycin at 37 °C in a humidified incubator with a 5% CO_2 /95% air atmosphere (Forma 371, Thermo Fisher Scientific, Waltham, MA, USA).

2.2 Enzymatic and chemical deglycosylation

2.2.1 Enzymatic deglycosylation. TM was enzymatically deglycosylated according to our previous methods,⁸ using different enzymes (PNGase F for *N*-deglycosylation; α 2-3,6,8 neuraminidase and *O*-glycosidase for *O*-deglycosylation; PNGase F, α 2-3,6,8 neuraminidase and *O*-glycosidase for *N*-&*O*-deglycosylation). The deglycosylated TM sample was purified using centrifugal filter units (Amicon Ultra-4, 10 kDa, Merck Millipore Ltd, Tullagreen, Ireland), lyophilized and stored at $-20\text{ }^{\circ}\text{C}$.

2.2.2 Chemical deglycosylation. KOH treatment (β -elimination) and TFMS deglycosylation were applied to remove carbohydrates from TM. After deglycosylation, the protein was dialyzed in ultrapure water for 48 h, then lyophilized and stored at $-20\text{ }^{\circ}\text{C}$.

2.3 Glycation of TM by glucose

TM was dissolved in a buffer (1 mM $NaHCO_3$ and 5 mM β -mercaptoethanol) containing 0.6 M glucose, with TM concentration at 0.2 mg mL^{-1} , then lyophilized and stored at $-20\text{ }^{\circ}\text{C}$. The lyophilized mixtures were incubated in a desiccator containing saturated aqueous $Mg(NO_3)_2$ solution at 60 °C for 48 h. The glycosylated TM was dialyzed (10 kDa, Union Carbide Corp., Danbury, CT, USA) in ultrapure water, lyophilized and stored at $-20\text{ }^{\circ}\text{C}$. The carbohydrate content of deglycosylated TM and glycosylated TM was determined using the phenol-sulfuric acid method with glucose according to a previous reference, with slight modifications.⁴ The procedure involved adding a 500 μL sample, 0.3 mL 6% (w/v) phenol and 1.5 mL sulfuric acid together, then the mixture was incubated in a boiling water bath for 20 min and cooled to room temperature. The absorbance was measured at 490 nm.

2.4 Murine allergenicity model

2.4.1 Mice sensitization. Female BALB/c mice were housed in an SPF animal care facility in Jiangnan University. All proto-

cols were approved by the Ethics Committee of Jiangnan University for the care and use of experimental animals (JN. No. 20170930b1920120[38]). The experimental protocol for sensitizing and challenging mice was carried out as in previous methods (Fig. 2A).^{10,11} The mice were randomly divided into eight groups (eight mice per group): PBS negative control group (CT in PBS, served as adjuvant.), TM positive control group, *N*-deglycosylated TM (TM-DNG) group, *O*-deglycosylated TM (TM-DOG) group, β -eliminated TM (TM- β E) group, *N*-&*O*-deglycosylated TM (TM-DNOG) group, TFMS deglycosylated TM (TM-TFMS) and glucose glycosylated TM (TM-G) group. The mice in the PBS negative control group were treated by intragastric (i.g.) gavage of 0.2 mL PBS (containing 10 μg CT per mouse). In each immunized group, mice were sensitized by i.g. gavage of 0.2 mL PBS containing TM, DGTMs or TM-G (0.1 mg per mouse) and CT (10 μg per mouse) on day 0, 7, 14, 21 and 28. On day 35, after being challenged with i.g. administration of 0.5 mg TM, DGTMs or TM-G, the mice were fasted overnight and sacrificed on day 36.

2.4.2 Mouse anaphylactic reactions and serum analysis. Clinical anaphylactic reactions were evaluated using a previous scoring system.¹² The levels of TM-specific IgE, IgG1 and IgG2a in sera were measured using ELISA as previously described.¹³ Briefly, 96-well plates were coated with 100 μL TM solution (10 $\mu\text{g mL}^{-1}$) and incubated overnight at 4 °C. The wells were washed with 300 μL TBST buffer (0.1 M Tris, 0.15 M NaCl, 0.05% Tween-20, pH 7.5) and then blocked with 300 μL blocking solution (5% defatted milk in TBST) at 37 °C for 2 h. After washing, to each well was added 100 μL diluted sera (1:10 dilution) and incubated overnight at 4 °C. After washing, 100 μL of horseradish peroxidase (HRP) conjugated goat anti-mouse IgE secondary antibody (1:1000 dilution, Thermo Fisher Scientific, Rockford, IL, USA), HRP conjugated goat anti-mouse IgG1 heavy chain (1:5000 dilution, Abcam, Cambridge, UK) secondary antibody or HRP conjugated goat polyclonal secondary antibody to mouse IgG2a-heavy chain (1:5000 dilution, Abcam, Cambridge, UK) was added to each well and incubated at 37 °C for 2 h. After TBST washing, 100 μL of TMB substrate solution (Beyotime Biotech, Shanghai, China) was added to each well. The plates were incubated at 37 °C for 20 min. The color reactions were stopped with 100 μL stop solution (2 M H_2SO_4). The absorbance at 450 nm ($OD_{450\text{ nm}}$) was read with a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA).

The serum TM-specific IgE level was also detected by western blotting analysis according to the method of Huang *et al.*¹⁴ The proteins from electrophoretic gels were transferred to a PVDF membrane (0.45 μm , Merck Millipore Ltd, Darmstadt, Germany) by a Mini Trans-Blot cell (Bio-Rad, Philadelphia, PA, USA) using a Tris-glycine transfer buffer system. For the detection of IgE-binding activity of TM, DGTMs and TM-G, the PVDF membrane was incubated with human serum (1:10 dilution) at 4 °C for 16 h. HRP conjugated goat anti-human IgE antibody (1:2000 dilution) was then allowed to react with the membrane at room temperature for 1 h, followed by extensive washing and detection by ECL

(BeyECL Plus, Beyotime Biotech, Shanghai, China) and recorded using a ChemiDoc XRS⁺ imager (Bio-Rad, Hercules, CA, USA).

2.4.3 Fecal and serum histamine. The minced feces (1 g) were homogenized with 2 mL of 5% trichloroacetic acid (TCA) for 2 min. After centrifugation, the supernatant was collected. The residue was extracted again with 2 mL 5% TCA. Both supernatants were combined and adjusted to 4 mL for histamine determination. Mice venous blood was obtained when sacrificing on day 36, sera were collected for serum IgE analysis. The fecal and serum histamine were determined according to the manual of the histamine ELISA kit (IBL International, Hamburg, Germany).

2.4.4 Cytokines analysis. Individual spleens and mesenteric lymph nodes (MLN) were aseptically removed and minced to prepare cell suspensions. For the evaluation of cytokine production, spleen lymphocyte (SLP) and MLN cell suspensions were cultured in 24 well plates (1×10^6 cells per well) for 72 h with the presence of TM, DGTMs or TM-G ($10 \mu\text{g mL}^{-1}$). The IL-4, IL-13 and IFN- γ contents were determined using commercial ELISA kits (R&D systems, Minneapolis, MN, USA). The precipitates of SLP and MLN cells were collected for mRNA preparation and real-time quantitative PCR (RT-qPCR) for transcription factors (T-bet, GATA-3 and Foxp3).¹³

2.5 Murine mast cell model assay

2.5.1 Preparation of IgE-sensitized mast cell. RBL-2H3 mast cells (1×10^5 cells per well) were seeded on a 96-well plate cultured in MEM medium at 37 °C for 24 h. Cells were then washed with PBS and sensitized by adding the sera of TM sensitized mice (100 μL per well). After incubation (37 °C for 24 h), cells were washed three times with PBS. Allergy activation was induced by incubating with TM, DGTMs or TM-G in PBS (100 μL per well, 37 °C, 1 h).

2.5.2 Histamine and degranulation assay. The supernatant of IgE-sensitized RBL-2H3 mast cells (50 μL per well) was used to determine the histamine release according to the manual of the histamine ELISA kit (IBL International, Hamburg, Germany).

The degranulation of mast cells was measured by determining the enzymatic activity of β -hexosaminidase in the cell supernatant. The cell supernatant (30 μL per well) and PNAG (50 μL per well, 1 mM in 0.1 M citric acid buffer, pH 4.5) were added to a 96-well plate. After incubation at 37 °C for 1 h, 100 μL of 0.2 M glycine solution (pH 10.7) was added and the absorbance was measured at 450 nm. The results are presented as the percentage of β -hexosaminidase activity measured in supernatant samples compared to the total levels in corresponding cell lysates. For measurement of total activity, the cells were lysed with 0.1% Triton-X 100 prior to removing the supernatant. The β -hexosaminidase activity was quantified by measuring the absorbance of the hydrolyzed substrate using a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA).

2.5.3 Reactive oxygen species (ROS). The intracellular ROS was measured using H₂DCF-DA (2',7'-dichlorodihydrofluorescein diacetate), which was deacetylated by intracellular

esterases to non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH), which was in turn oxidized to the fluorescent compound 2',7'-dichlorodihydrofluorescein (DCF) by ROS. The IgE-sensitized mast cells (1×10^5 cells per well) were pre-incubated with H₂DCF-DA (5 μM) for 30 min at 37 °C, then washed to remove excess H₂DCF-DA, and treated with TM, DGTMs or TM-G for 30 min. The fluorescence intensity was measured, with 485 nm excitation and 530 nm emission, using a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA).

2.5.4 Cytokines (IL-4 and TNF- α). For cytokine immunoassays, the IgE-sensitized mast cells were activated with TM, DGTMs or TM-G. The secretions of IL-4 and TNF- α were quantified by mouse IL-4 and TNF- α ELISA kits (R&D systems, Minneapolis, MN, USA) using the supernatant of culture media cultured at 4 h and 6 h, respectively.

2.6 Statistical analysis

Data were presented as means $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS software (SPSS Inc., Ver. 19, Chicago, IL, USA). Statistical significance was assessed using two way ANOVA (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3. Results

3.1 Carbohydrate content

Shellfish TM is a glycoprotein allergen.^{3–5} The carbohydrate epitopes were highly stable and retained immune reactivity even after exposure to strong denaturing conditions.⁶ The data show (Fig. 1) that the carbohydrate content of TM was 3.031%; after deglycosylation, the carbohydrate content of DGTMs decreased to 1.408% (TM-DNG), 1.569% (TM-DOG), 1.611% (TM- βE), 0.059% ($\approx 0\%$, TM-DNOG) and 0.053% ($\approx 0\%$, TM-TFMS), which suggested that there might be both *N*-glycans and *O*-glycans in TM, and *N*-&*O*-deglycosylation and TFMS demonstrated similar high efficiency in removing TM

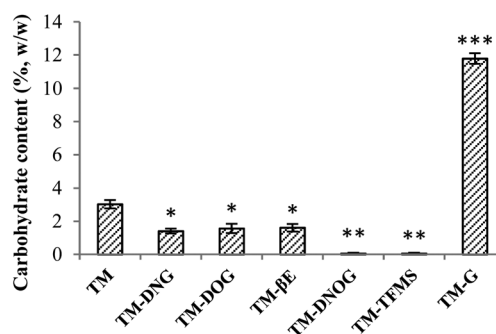


Fig. 1 The carbohydrate content of TM, DGTMs [*N*-deglycosylated TM (TM-DNG), *O*-deglycosylated TM (TM-DOG), β -eliminated TM (TM- βE), *N*-&*O*-deglycosylated TM (TM-DNOG), TFMS deglycosylated TM (TM-TFMS)] and glucose glycosylated TM (TM-G). Data are presented as the mean values $\pm$ standard deviation (SD) from triplicate determinations. * showed significant differences as compared with the TM group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

glycans. Our previous glycoprotein staining data in gel electrophoresis also showed that *N*-&*O*-deglycosylation and TFMS were highly effective in removing the glycans of shrimp tropomyosin allergens.⁸ Previous literature also reported the carbohydrate contents of other shellfish tropomyosins. Ruan *et al.*⁴ found that there was a 0.2% carbohydrate moiety that contained *O*-glycan in the TM of *Scylla paramamosain*. Wang *et al.*⁵ reported that the TM from *Penaeus chinensis* had carbohydrate content of 4.4%. The different carbohydrate contents of tropomyosins might be due to the fact that they came from different shellfish species. As for TM-G, after glycation, the carbohydrate content increased from 3.031% (TM) to 11.781% (TM-G), which suggested that TM could be highly glycosylated by glucose, and our previous SDS-PAGE data showed that after glycation, TM-G had an obvious increase in molecular weight as compared to TM,⁸ which further confirmed the glycation of TM.

3.2 Anaphylactic reactions and serum analysis

As shown in Fig. 2B and C, compared with the TM sensitized group, the mouse anaphylactic scores and diarrhea frequency of DGTMs (TM-DNG, TM-DOG, TM-βE, TM-DNOG and TM-TFMS) groups increased upon subsequent challenges, especially for the TM-DNOG and TM-TFMS group, while the scores and diarrhea frequency of TM-G group decreased significantly, which suggested that deglycosylation strengthened the allergenicity of DGTMs. However, in the TM-G group, mice had lower anaphylactic scores and diarrhea frequency, which suggested that glycation of TM contributed to lower allergenicity. Previous literature had similar findings. Li *et al.*¹⁵ reported that glycation of the tropomyosin from *Penaeus vannamei* reduced the allergenicity, and Gruber *et al.*¹⁶ found that glycation of Pru av 1 by glucose reduced its allergenicity. In addition, as shown in Fig. 2D and E, the fecal and serum histamine had similar tendencies of anaphylactic scores and diarrhea frequency, which suggested the histamine content might be correlated with allergy symptoms.

After i.g. treatment and digestion, the digests of TM, DGTMs and TM-G could be absorbed and transported to immune organs by blood circulation and trigger the secretion of a mediator (histamine) and TM-specific antibodies (IgE, IgG1 and IgG2a). In mice, the Th2 response leads to IgE and IgG1 generation, and Th1 response results in IgG2a production.^{13,17} As shown in Fig. 2F–H, compared with TM-sensitized mice, the serum histamine and Th2-dependent antibodies (IgE and IgG1) of DGTMs-sensitized mice significantly increased, the TM-specific IgE antibody was also confirmed with western blot (Fig. 2F), the Th1-dependent antibody (IgG2a) was insignificantly slight increased, while TM-G induced weaker anaphylactic symptoms, which indicated that the decreased accessible epitopes of TM-G led to the weaker allergy activation and lower secretion of TM-specific antibodies and cytokines.

3.3 Cytokines in mouse SLP and MLN cells

As shown in Fig. 3A1–3, compared with the TM group, the Th2 cytokines (IL-4 and IL-13) in SLP cells of DGTMs-sensitized

mice increased, and the Th1 cytokine (IFN-γ) had a slight increase. The Th1, Th2 and Treg cytokines of MLN cells (Fig. 3B1–3) demonstrated similar trends to SLP cells (Fig. 3A1–3).

To examine whether T-cell polarization was affected by deglycosylation or glycation, the expression of transcription factors (T-bet, GATA-3 and Foxp3) was evaluated as the reflection of Th1, Th2 and Treg cells, respectively. Compared with TM, the GATA-3 and Foxp3 of DGTMs-sensitized SLP cells increased, and the GATA-3 and Foxp3 of TM-G-sensitized mice tended to decrease, while the T-bet reduction of DGTMs and TM-G groups had no significant alterations. The transcription factor data (Fig. 3A4) were consistent with the cytokine results (Fig. 3A1–3), and the transcription factors of MLN cells (Fig. 3B4) had similar tendencies to those of SLP cells (Fig. 3A4).

3.4 RBL-2H3 mast cell assay

As shown in Fig. 4A, the DGTMs promoted the histamine release in IgE-sensitized RBL-2H3 cells, while TM-G weakened the histamine release. Compared with TM, DGTMs induced strong degranulation of IgE-sensitized RBL-2H3 mast cells, while TM-G significantly suppressed degranulation (Fig. 4B). Mast cells release ROS in response to various stimuli, and ROS from mast cells are involved in allergic inflammation.^{18,19} DGTMs upregulated the production of ROS (Fig. 4C), while TM-G downregulated ROS generation. DGTMs promoted the secretion of IL-4 and TNF-α in mast cells (Fig. 4D and E), while TM-G downregulated the secretion of IL-4 and TNF-α.

4. Discussion

Tropomyosin is the major allergen of shrimp that can cause severe allergic reactions.³ Generally, glycans play an important role during intercellular transduction of information between cells and adhesion through ligand–receptor interactions;⁶ however, little is known about the effects of tropomyosin glycans on the *in vivo* allergenicity. Moreover, tropomyosin is rich in lysine,⁸ which renders it amenable to glycation; the glycation might represent a potential approach to generating hypoallergenic variants of tropomyosin. Therefore, the purpose of this work is to investigate the role of glycans in allergenicity and how glycation of TM reduces the allergenicity in the mouse and mast cell models.

After deglycosylation, DGTMs aggravated anaphylactic symptoms, which might be due to the fact that after deglycosylation, the glycans that previously masked or blocked epitopes on TM got exposed, which contributed to more accessible epitopes.⁸ TM-G alleviated the allergic symptoms and histamine secretion in mice, which could result from the following: (1) glycation destroyed the conformational epitopes by conformation modification; (2) glycation destroyed linear epitopes with glycation sites; (3) the glucose residues occupied the surface space of TM-G to block and mask the interactions between TM epitopes and IgE; (4) glucose residues functioned as resistance to prevent the accessibility of TM epitopes to the

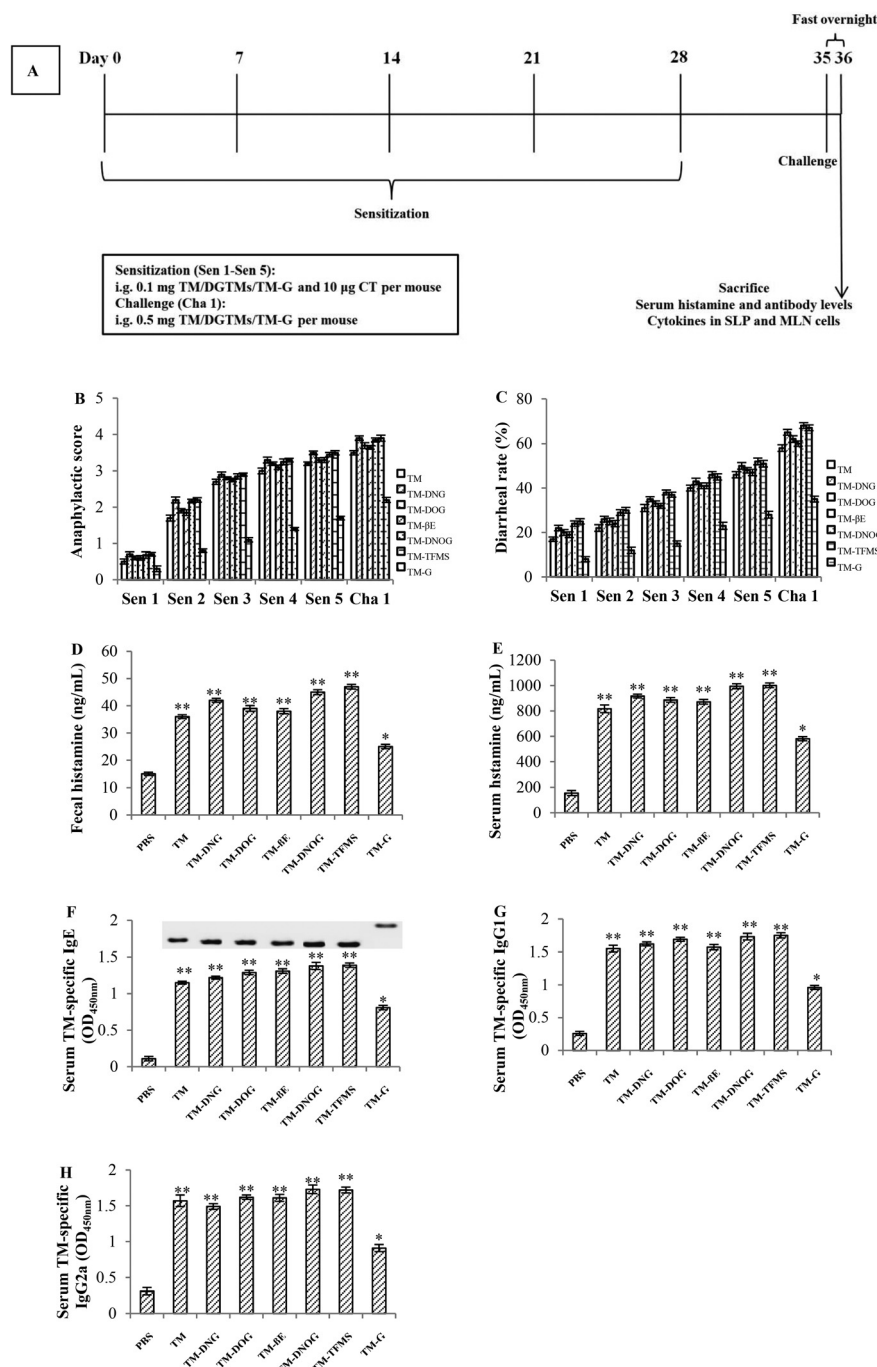


Fig. 2 The allergy sensitization and challenge protocol of the mouse model (A), anaphylactic score (B), diarrheal frequency (C), fecal histamine (D), serum histamine (E), serum TM-specific IgE (F), serum TM-specific IgG1 (G) and serum TM-specific IgG2a (H) of TM, DGTMs and TM-G. Data are presented as the mean values $\pm$ standard deviation (SD) from triplicate determinations. * showed significant differences as compared with the PBS group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

IgE on immune cells (e.g. mast cells); (5) glycans played an important role during intercellular transductions of information between cells and adhesion through ligand–receptor interactions,⁶ glucose residues might trouble the intercellular transductions, therefore reducing the allergenicity. Previous reports also found that glycation of tropomyosin weakened the allergenicity of shellfish tropomyosins.^{15,20,21}

IgE and IgG1 are Th2-dependent antibodies, and IgG2a belongs to Th1-dependent antibodies.^{13,17} After deglycosylation, the serum Th2-dependent antibodies (IgE and IgG1) of DGTMs-sensitized mice were significantly increased, Th1-dependent antibody (IgG2a) insignificantly changed, while TM-G had weaker secretion of TM-specific antibodies. Furthermore, IL-4 and IL-13 are important Th2 cytokines, and

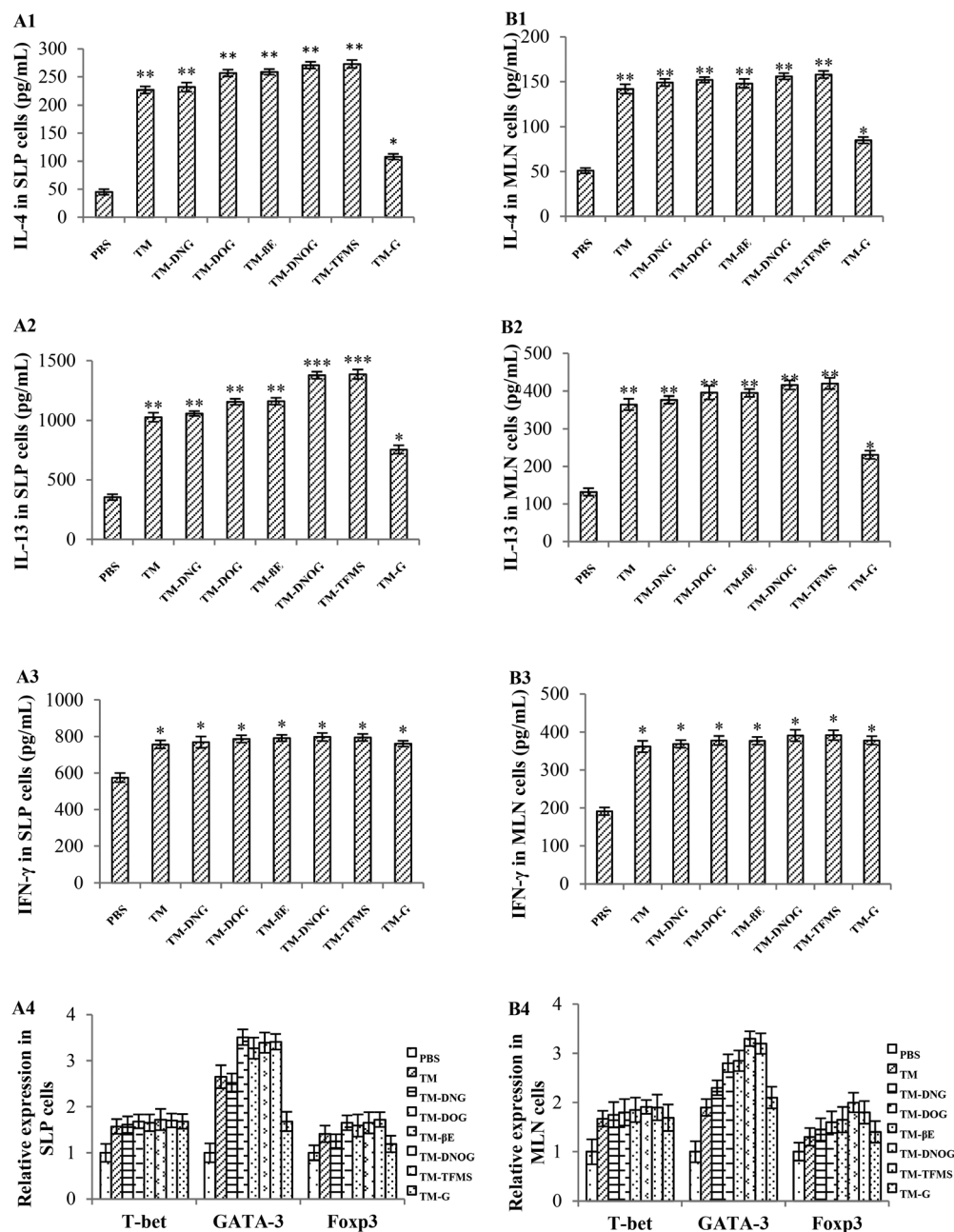


Fig. 3 The IL-4 (A1), IL-13 (A2), IFN- γ (A3) and T-cell transcription factors (A4) in the spleen lymphocytes (SLP) of mouse models sensitized by TM, DGTMs and TM-G. The IL-4 (B1), IL-13 (B2), IFN- γ (B3) and T-cell transcription factors (B4) in the mesenteric lymph nodes (MLN) of mouse models sensitized by TM, DGTMs and TM-G. Data are presented as the mean values $\pm$ standard deviation (SD) from triplicate determinations. * showed significant differences as compared with the PBS group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

IFN- γ belongs to Th1 cytokine.²² DGTMs upregulated Th2 cytokines in SLP and MLN cells, TM-G downregulated Th2 cytokines, while Th1 cytokine (IFN- γ) seemed to have no significant participation. Moreover, the expression of transcription factors (T-bet, GATA-3 and Foxp3) could reflect Th1, Th2 and Treg cells, respectively.¹¹ The GATA-3 and Foxp3 of DGTMs-sensitized SLP cells tended to decrease, while T-bet had insignificant changes. The SLP cells sensitized by TM-G had lower expressions of GATA-3 and Foxp3, and T-bet had no significant

change. The cytokines secreted from SLP cells were consistent with the MLN cell results. Considering the cytokine and transcription factor results, deglycosylation of TM strengthened the allergenicity through upregulating Th2 and Treg cytokines, while glycation of TM by glucose reduced the allergic reactions by downregulating Th2 and Treg cytokines, and Th1 cytokine (IFN- γ) had no significant participation in TM-sensitized mice. These results suggest that glycation of TM suppressed the allergic reaction through downregulation

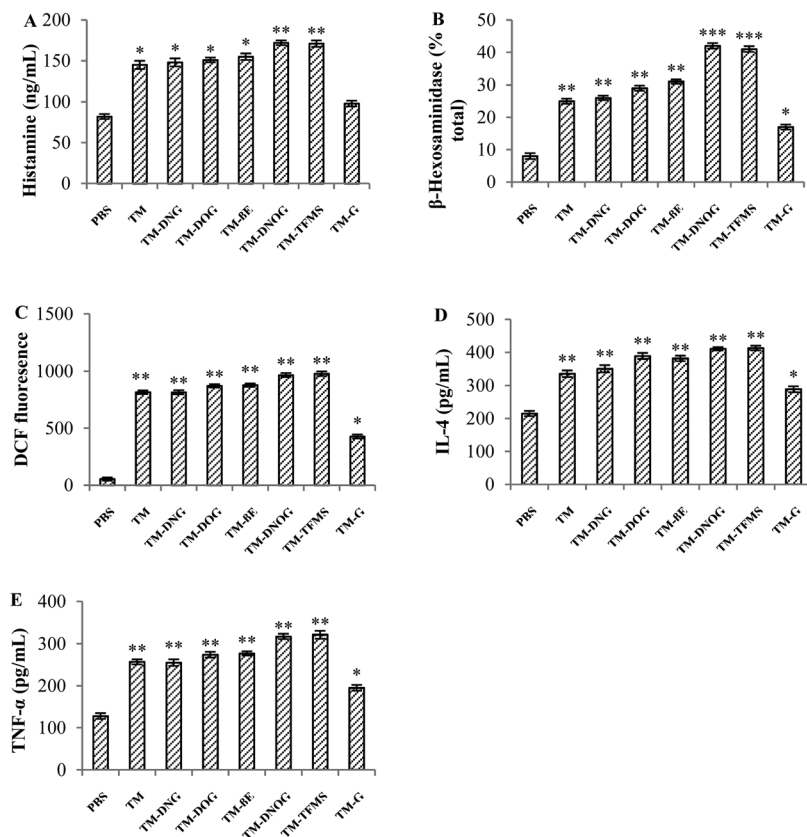


Fig. 4 The histamine release (A), degranulation (B), ROS generation (C), IL-4 content (D) and TNF- α content (E) of murine mast cells (RBL-2H3) sensitized by TM, DGTMs and TM-G. Data are presented as the mean values $\pm$ standard deviation (SD) from triplicate determinations. * showed significant differences as compared with the PBS group, * p < 0.05, ** p < 0.01, *** p < 0.001.

of the TM-induced Th2 cell. Lam *et al.*¹⁰ also found that after sensitization by Met e 1 (shrimp tropomyosin), mouse Th2 cytokines got promoted, while Th1 cytokine had no significant alteration.

Mast cells are well known as key effector cells of allergic disease.^{23,24} After deglycosylation, DGTMs aggravated the allergic reactivities of mast cells, while TM-G inhibited the allergic symptoms. This might be due to the fact that after deglycosylation, the more accessible epitopes might contribute to severe allergenicity. After glycation, TM epitopes with glycation sites could be destroyed and masked, resulting in lower allergenicity. Mast cells and basophils have similar characteristics in terms of their function in allergy diseases.²⁴ Our previous data also showed that DGTMs and TM-G led to similar exacerbated-allergy and weakened-allergy responses on basophil (KU812),⁸ like the mast cell in this work.

In summary, the exacerbated allergenicity of DGTMs on mouse and mast cells might be due to the more accessible epitopes, while the weaker allergenicity of TM-G may be due to glucose destroying and masking TM epitopes. Therefore, deglycosylation of TM was not suitable for desensitizing TM-induced food allergy through glycation, while glycation of TM by glucose could be applied to desensitizing the TM allergenicity.

5. Conclusions

Deglycosylation exacerbated the allergenicity of DGTMs on mouse and mast cells, possibly due to the more accessible epitopes. As for TM-G, glucose could destroy the epitopes with glycation sites; moreover, after glucose residues are added to TM molecules, the saccharide residues can function both as resistance to prevent the accessibility of TM to the IgE on immune cells (*e.g.* mast cell) and as masking epitopes. Deglycosylation of TM was not suitable for desensitizing TM-induced food allergy through glycation, while glycation of TM by glucose could be used to desensitize TM allergenicity, which could provide new insight into the prevention and desensitization of food allergy anaphylaxis for larger cohorts of shrimp-allergic patients.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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