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Reduction in Allergenicity and Induction of Oral Tolerance of Glycated Tropomyosin from Crab

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Abstract: Tropomyosin (TM) is an important crustacean (*Scylla paramamosain*) allergen. This study aimed to assess Maillard-reacted TM (TM-G) induction of allergenic responses with cell and mouse models. We analyzed the difference of sensitization and the ability to induce immune tolerance between TM and TM-G by in vitro and in vivo models, then we compared the relationship between glycation sites of TM-G and epitopes of TM. In the in vitro assay, we discovered that the sensitization of TM-G was lower than TM, and the ability to stimulate mast cell degranulation decreased from 55.07 ± 4.23% to 27.86 ± 3.21%. In the serum of sensitized Balb/c mice, the level of specific IgE produced by TM-G sensitized mice was significantly lower than TM, and the levels of interleukins 4 and interleukins 13 produced by Th2 cells in spleen lymphocytes decreased by 82.35 ± 5.88% and 83.64 ± 9.09%, respectively. In the oral tolerance model, the ratio of Th2/Th1 decreased from 4.05 ± 0.38 to 1.69 ± 0.19. Maillard reaction masked the B cell epitopes of TM and retained some T cell epitopes. Potentially, Maillard reaction products (MRPs) can be used as tolerance inducers for allergen-specific immunotherapy.

Keywords: allergenicity; Maillard reaction; mouse models; oral tolerance; *Scylla paramamosain*; tropomyosin

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1. Introduction

Food allergy, a conceptual term which covers many clinical implications, usually refers to an allergic reaction such as a type I hypersensitivity mediated by immunoglobulin E (IgE) [1]. About 8% of infants and 2–3% of adults have a food allergy with increasing incidence year after year [2,3]. Food allergens are the key of food allergy, usually some proteins or proteases with important structural or physiological functions in organisms.

Shellfish is also considered to be one of the most common aquatic products, which can induce severe food allergy diseases, tropomyosin (TM) is the pan-allergen [4]. TM with the 31 to 42 kDa molecular weight (MW), is a type of acidic glycoprotein with good stability to protease, acid, and thermal treatmed [5]. Nowadays, most food products are going through a thermal treatment before consumption, which causes changes in protein structure and physical and chemical properties. Glycation is known as a Maillard reaction (MR) or non-enzymatic browning, which in many studies has been demonstrated to change the allergenicity of food allergens, including Cor a 11, Pru av 1, Mal d 3, Pen a 1 and milk

allergen β-lactoglobulin [6–10]. MR of food allergens has been verified as affecting their IgE-binding ability by masking and destroying IgE-binding epitopes. Recently, more and more evidence has shown that Maillard reaction products (MRPs) may affect the interaction of immune cells in the immune system [10,11]. Glycated food allergens may influence the ability of the uptake and presentation by Dendritic cells (DCs), DCs affect the response of downstream immune cells. The food allergens are absorbed by DCs and degraded into peptides by lysosomal after entering the human body. The peptide is recognized by $CD4^+$ helper T cells (th) associated with allergy after binding to MHCII molecules [12,13]. In addition, activated Th2 cells secrete interleukins 4 (IL-4) to regulate B cells producing large amounts of IgE [14]. Basophil degranulation has been used to study the allergic reactions with a glycated food allergen, and basophil degranulation was induced by allergen cross-linking of IgE-bound to FcεR I receptors [15].

Oral tolerance is a state of systemic unresponsiveness, which is the default response to food antigens [16]. Failure of immune tolerance induction can cause food allergy [17]. Oral immunotherapy is training for the immune system to achieve a higher threshold, which there might be skewing towards a Th1 response, but mostly oral immunotherapy leads to a regulatory response, which suppresses the Th2 response to the allergen [18]. An ideal tolerance inducer could reduce the production of allergen-specific IgE, activate the tolerogenic DCs, and thus restore Th1/Th2 immune balance to induce regulatory T (Treg) cells responses. In the development of the study on the modulation of the allergic response, the treatment mode using allergen T cell epitopes peptides or processed products instead of allergens as a tolerance inducer was gradually evolved. Gouw et al. [19] demonstrated that the functional human leukocyte antigen DRB1-restricted T cell epitopes were found in the tested hydrolyzed infant formula, which can induce oral tolerance to whey potentially. Wai et al. [20] proved that the T cell epitopes peptides using allergens had the effect of inducing food tolerance after repeated oral administration of allergens. The use of processed food products as tolerance inducers have the characteristics of low production cost, large yield, and low risk [21]. In addition, Liu et al. [22] explored how the TM of crab treated by enzyme cross-linking reaction has the potential to induce oral tolerance in mice, without explaining modifications in the structure of allergenic proteins. Ren et al. [23] revealed the cross-linking reactions of Ara h 2 relevant reaction sites, unfortunately, they found its sensitization potential decreased in the mouse model, no experimental verification of mouse tolerance was carried out.

Antigenic epitopes are divided into two groups based on the cells which can be recognized: T cell epitopes and B cell epitopes [24]. Modifying B-cell epitope of allergens can reduce its allergic reaction, and retaining T-cell epitope of allergens can induce immune tolerance [25,26] Studies have shown that processed food could change allergen allergenicity by destroying antigenic epitopes or creating neoepitopes [27].

There is little data about the MRPs absorption and presentation of DCs, although it is important to explain the allergic responses induced by MRPs. Less information reported that MRPs could be as tolerance inducers. Whether the crab TM treated by MR has the potential to induce oral tolerance requires further exploration. Glycation sites of MRPs should be identified and the relation of epitopes and oral tolerance require further exploration.

In the present study, galactose could effectively decrease allergenicity in TM through MR in vitro assays. Besides, the mechanism of lower allergenicity and induced oral tolerance of Maillard-reacted TM (TM-G) was evaluated with cell and mouse models. Finally, the glycation sites of TM-G were identified to analyze the modification side on the T cell epitopes or B cell epitopes. Overall, these results would form the foundation to contribute to the future development of the Maillard reaction products as a functional food for tolerance induction.

2. Materials and Methods

2.1. Chemicals

Galactose was purchased from Macklin (Shanghai, China). Rabbit anti-crab TM IgG pAb was prepared in our laboratory previously and goat anti-rabbit IgG-HRP antibody was from Abmart (Berkeley Heights, NJ, USA). Granulocyte macrophage colony stimulating factor (GM-CSF), IL-4 were obtained from Sigma-Aldrich (St. Louis, MO, USA). RPMI 1640 and fetal bovine sera (FBS) were purchased from Invitrogen (New York, NY, USA). CD11c for flow cytometry analysis were purchased from Biolegend (San Diego, CA, USA). Imject Alum was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Goat anti-mouse IgE, IgG1, and IgG2a antibodies were purchased from Abcam (Cambridge, MA, USA). The ELISA kits of IL-4, IL-10, IL-13, interferon (IFN)-γ, mouse mast-cell protease-1 (mMCP-1) and transforming growth factor-β (TGF-β) were purchased from R&D Systems (Minneapoils, MN, USA). An ELISA kit of histamine was purchased from IBL (Hamburg, Germany).

2.2. Animals

Live crab (*S. paramamosain*) was purchased from a local aquatic products market in Xiamen. Specific pathogen-free female Balb/c mice weighing 14–16 g (4–6-weeks-old) were obtained from Shanghai Laboratory Animal Center of Chinese Academy of Sciences (Shanghai, China). The housing environment for mice under specific pathogen-free conditions was maintained at 20–25 °C and 60 ± 10% relative humidity with a 12 h light/dark cycle. Protocols were approved by the Institutional Animal Care and Use Committee of Animal Laboratory Center of Jimei University (Xiamen, China, No. SCXK 2016-0006), and all animals were used for academic research.

2.3. Human Sera

Sera were obtained by the Second Affiliated Hospital of Xiamen Medical College (Human ethical approval 2020018, Xiamen, Fujian, China), and signed informed consent was obtained from all individuals. Sera IgE antibodies (Table S1) to crab were measured by ImmunoCAP (Phadia AB, Uppsala, Sweden), when crab-specific IgE $\geq$ 0.35 kUA/L were defined as positive, the specific IgE levels with crab TM of positive sera were measured by ELISA previously, negative sera IgE < 0.35 kUA/L, and all sera were stored at −80 °C until further use.

2.4. Preparation and Immunobinding Capacity Analysis of TM-G

The purification of TM from *S. paramamosain* and preparing TM-G were accorded as described previously [28]. All samples were dialyzed by 10 mM PBS (pH 7.0) after reaction and then detected by 12% SDS-PAGE, western blot, and dot blot. Immune-binding capacity analysis was performed as described elsewhere with some modifications. Briefly, rabbit anti-crab TM IgG pAb with 1:1 $\times$ 10^4 dilution and HRP-labeled goat anti-rabbit IgG with 1:2 $\times$ 10^4 dilutions were used as the primary and secondary antibody, respectively, for immunoblot analysis. TM and TM-G were adjusted to 1 mg/mL to dot blot, the primary antibody was human sera (dilution 1:3), the secondary antibody was HRP-labeled goat anti-human IgE antibody (dilution 1:1 $\times$ 10^4).

2.5. Allergen Uptake and Presentation by Mouse Bone Marrow-Derived Dendritic Cells

Mouse bone marrow-derived dendritic cells (BMDCs) isolated from BALB/c mice were referred to the classical method by Perusko et al. [10]. After being cultured for 6 days, immature BMDCs were collected and used for the following experiments.

TM and TM-G were labeled with FITC according to the method of Liu et al. [23]. Briefly, FITC-proteins (TM, TM-G) were separated from unreacted FITC label using ultrafiltration centrifugal tube (MW cut off 3 kDa). Immature BMDCs were cultured at 1 $\times$ 10^5 cells per well in 24-well plates and incubated gradient time with 50 μg FITC-protein for 0, 10, 20, 30,

40, 50, 60, 90, 120, and 180 min at 37 °C. Then, the collected cells were stained with APC anti-mouse CD11c for 30 min at 4 °C for BMDCs uptake analysis by flow cytometry.

Similarly, immature BMDCs were cultured at 1×10^5 cells per well in 24-well plates and incubated 48 h with 50 μg proteins (TM, TM-G). The total RNA kit and TIANScript RT Kit (Tiangen, Beijing, China) was used to extract the cDNA from BMDCs for RT-PCR, according to the instructions with 20 μL final volume. The changes of CD86 and H2-Ob transcription levels in BMDCs stimulated by PBS, TM, and TM-G were detected by RT-PCR. The following primers were used for RT-PCR: CD86-F, 5′-ATG TCA CAA GAA GCC GAA TC-3′. CD86-R, 5′-TTC AGT GCT CTT GGC CTA TG-3′. H2-Ob-F, 5′-GTG ACC TGG GGA TGT TTG TTG-3′. H2-Ob-R, 5′-CAG GAG ATC CAG GCG TTT GTT-3′. β-actin-F, 5′-ACA GCA CCG TGT TGG CGT AGA G-3′. β-actin-R, 5′-TGC GGG ACA TCA AGG AGA AGC-3′. The result was chosen $2^{-\Delta\Delta Ct}$ method calculation the relative quantitative of CD86 and H2-Ob.

2.6. Degranulation Assay in RBL-2H3 Cells

RBL-2H3 was purchased from the American Type Culture Collection (Bethesda, MD, USA) and cultured as described previously [10]. MTT assay and assessment of the β-hexosaminidase release rate were conducted according to the method used by Han et al. [28]. RBL-2H3 cells were inoculated on 96-well plates and cultured at 37 °C for 12 h. The cells were washed three times with PBS, and then different concentrations of TM and TM-G were added to stimulate RBL-2H3 cells for 6 h, and then the MTT was added, after 4 h the value was measured at OD_{570} nm to assess cytotoxicity. Assessment of the β-hexosaminidase release rate, the serum of TM sensitized mice with RBL-2H3 cells was incubated for 16 h. Then allergic reactions were induced by co-incubation with PBS, TM-G, and TM [28].

2.7. Animal Models of Injection Sensitization and Oral Tolerance to TM-G

Injection sensitization and oral tolerance tests were based on Han et al. [28] with modifications. Mice were allocated randomly to three groups (n = 8). For the sensitization model, one week of adaptive culture female Balb/c mice were, respectively, exposed to TM (150 μg) or TM-G (150 μg) with alum adjuvant (6 mg) by injection on days 0 and 14. Mouse blood and spleen lymph cells were collected on day 15. The levels of TM-specific IgE, IgG1, and IgG2a in sera were measured using ELISA. After 3 days of culture, the cytokine assay of spleen lymph cells was measured by ELISA. For oral tolerance, exposed to TM (150 μg) with alum adjuvant (6 mg) by injection on days 0 and 14; next, on days 21 and 28, mice received 2 mg TM or TM-G via intragastric gavage. On day 33, 10 mg TM was given to the mouse. The diarrhea rate within one hour after three times (days 21, 28, and 33) of gavage was recorded, and the anaphylactic score and rectal temperature of mice were measured one hour after gavage on day 33. The method used for the anaphylactic score was according to Liu et al. [29]. Furthermore, blood, spleen, and mesentery lymph cells were collected on day 34 [22,28,29] The mMCP-1 and TGF-β were determined by ELISA kit using mouse sera. The IL-4, IL-10, IL-13, and IFN-γ were determined by ELISA kit using cellular supernatant of the cultured spleen, and mesentery lymph cells.

2.8. Identification of Glycation Sites and Modification Analysis of the Epitopes

The amino acid sequence of crab of TM was obtained from NCBI (GenBank number: ABS12233.1). The predicted T cell epitopes' results from the Prediction System (http://tools.iedb.org/mhcii/ (accessed on 12 January 2022)) were analyzed based on Gouw et al. [19,30,31]. B cell epitopes of TM were referred to Liu et al. [32]. Glycation sites testing was according to Han et al. [28] with modifications. The band of TM-G was hydrolyzed using trypsin (Promega, Madison, WI, USA) for 20 h, and then separated by capillary high-performance liquid chromatography (Orbitrap-ELite, Thermo, Finnigan, San Jose, CA, USA). The separated products were analyzed by Q-Exactive mass spectrometer. The result was named as "RAW file" and analyzed using the Maxquant software to find

the modified amino acids. Finally, the sites that were modified by MR were compared with TM epitope.

2.9. Statistical Analysis

Data were presented as means ± standard deviation (SD). Statistical significance was assessed using a two-way ANOVA. # $p < 0.05$ and ## $p < 0.01$ was used to compare with PBS group. * $p < 0.05$ and ** $p < 0.01$ was used to compare with TM group.

3. Results

3.1. IgG/IgE-Binding Capacity Analysis of TM-G

The bands of TM and TM-G are represented in Figure 1A. The MW of TM-G was higher than that of TM, and the band of TM-G performed diffusion. Moreover, the IgG/IgE-binding capacity of TM-G was lower than that of TM (Figure 1B,C) using western blot and dot blot. The band of TM-G was not bound with the specific IgG antibody of TM. Compared with TM, TM-G exhibited weaker binding capacity with patients' sera.

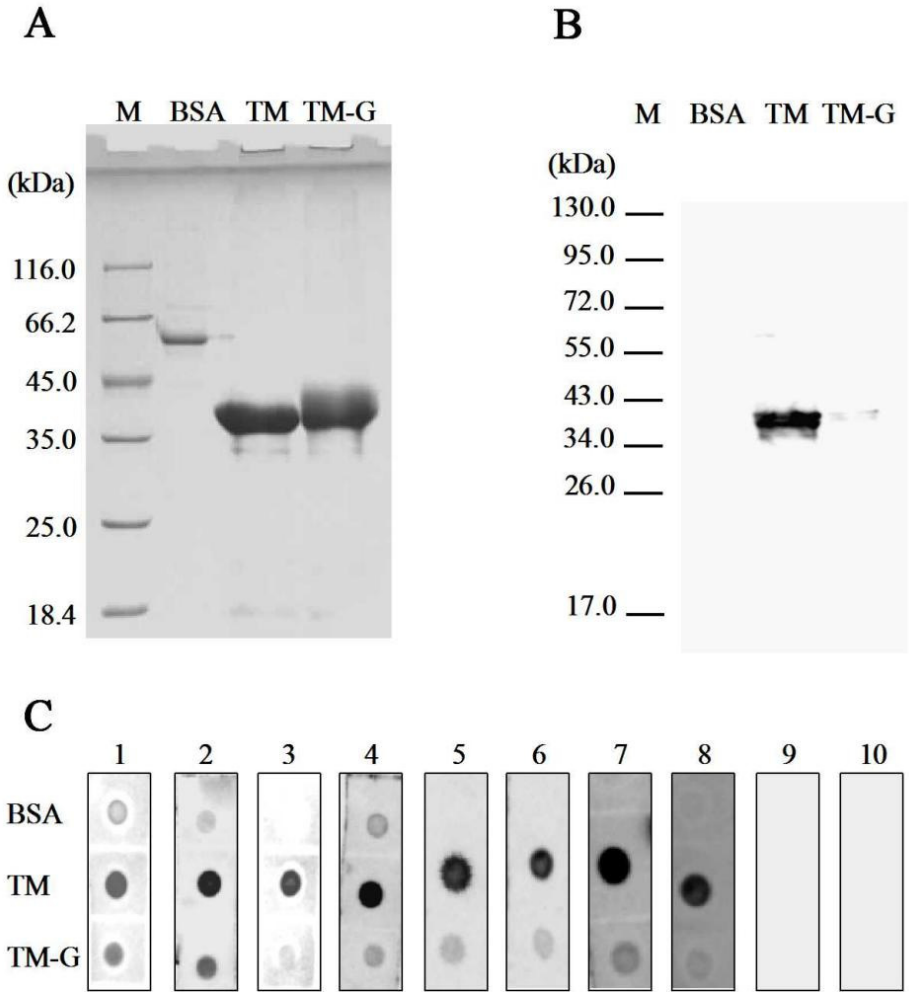

Figure 1. Immunoactivity analysis of TM-G: (**A**) SDS-PAGE analysis of TM-G. Lane M: protein marker. (**B**) IgG-binding capacity analysis of TM-G. Primary antibody: crab-TM-polyclonal antibodies, dilution 1:1 × 10^4. Lane M: protein marker. (**C**) IgE-binding capacity analysis of TM-G. BSA was used as the negative control, TM was used as the positive control, and TM-G was used to compare with BSA and TM. Primary antibody: crab-sensitized patients' sera (No. 1–8), negative sera (No. 9–10), dilution 1:5.

3.2. TM-G Uptake by BMDCs

Furthermore, to investigate whether glycation affects interactions of TM with BMDCs, TM and TM-G with FITC-label were incubated with BMDCs, and then the uptake ability of TM and TM-G was detected in time by flow cytometry. Uptake of TM and TM-G measured as the percent of $CD11c^+$ and FITC-positive cells were time-dependent, while the mean fluorescence intensity continuously increased up to 180 min (Figure 2A), the gradient time were 0, 10, 20, 30, 40, 50, 60, 90, 120, and 180 min. TM-G had lower efficiently, reaching 72.53% of FITC-positive BMDCs, whereas BMDCs were incubated with FITC-labeled TM, the uptake ability was up to 94.23% (Figure S1).

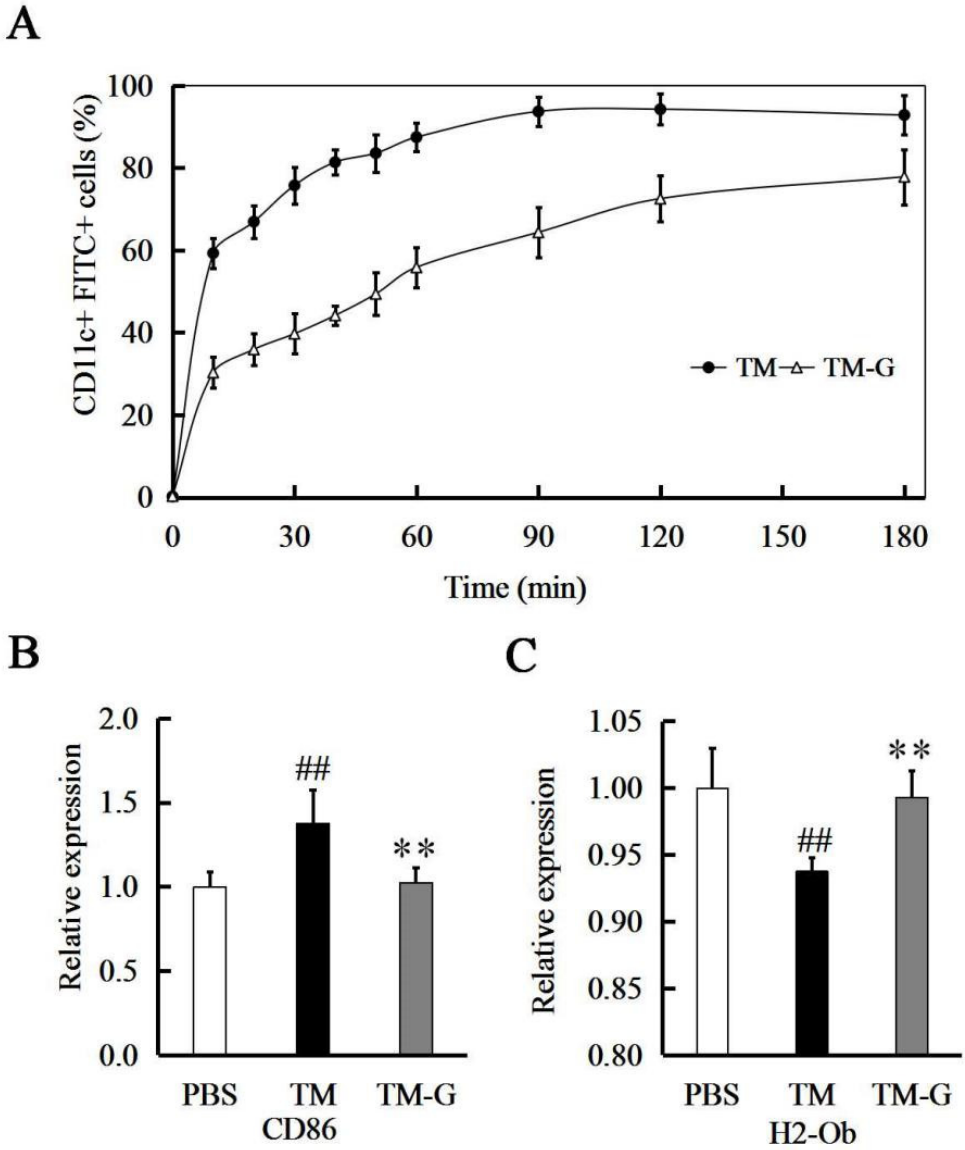

Figure 2. Allergen uptake and presentation by BMDCs: (**A**) TM-G uptake by BMDCs with different times (the gradient times were 0, 10, 20, 30, 40, 50, 60, 90, 120, and 180 min). (**B**,**C**) TM and TM-G presentation by BMDCs. All data are presented as the mean ± SD (n = 3). $^{\#\#}$ $p < 0.01$ was used to compare with PBS group. ** $p < 0.01$ was used to compare with TM group.

3.3. TM-G Presentation by BMDCs

The changes of CD86 and H2-Ob transcription levels in BMDCs cells stimulated by TM and TM-G were detected by RT-PCR. Compared with the PBS group, the expression level of CD86 on BMDCs increased by 37.60 ± 20.12% after stimulation in the TM group.

However, the expression level of CD86 on BMDCs stimulated by the TM-G group was significantly lower than that of the TM group ($p < 0.01$), without significant difference to the PBS group (Figure 2B). Furthermore, the expression level of H2-Ob was significantly up-regulated in the TM-G group in comparison to the TM group ($p < 0.01$), compared with the PBS group, the expression level of H2-Ob decreased by 6.24 ± 1.02% after stimulation in the TM group (Figure 2C).

3.4. Effect of TM-G on Cell Activity and Degranulation in RBL-2H3

MTT assay was used to assess the cytotoxic effects of TM-G by RBL-2H3 cells. It was found that there was no effect on cell viability after TM and PBS stimulation. The cell viability rate of TM-G was more than 90% at different concentrations without significant difference to TM group (Figure 3A). The sera of TM sensitized mice, using the 15-days injection model, was collected to sensitize RBL-2H3 cells. The effect of TM and TM-G on degranulation of RBL-2H3 cells was shown in Figure 3B, compared with the PBS group, the degranulation rate of the TM group was significantly decreased ($p < 0.01$), and the degranulation rate of the TM-G group was significantly decreased compared with the TM group ($p < 0.01$); meanwhile, the degranulation rate of the TM-G group was significantly increased compared with the PBS group ($p < 0.05$).

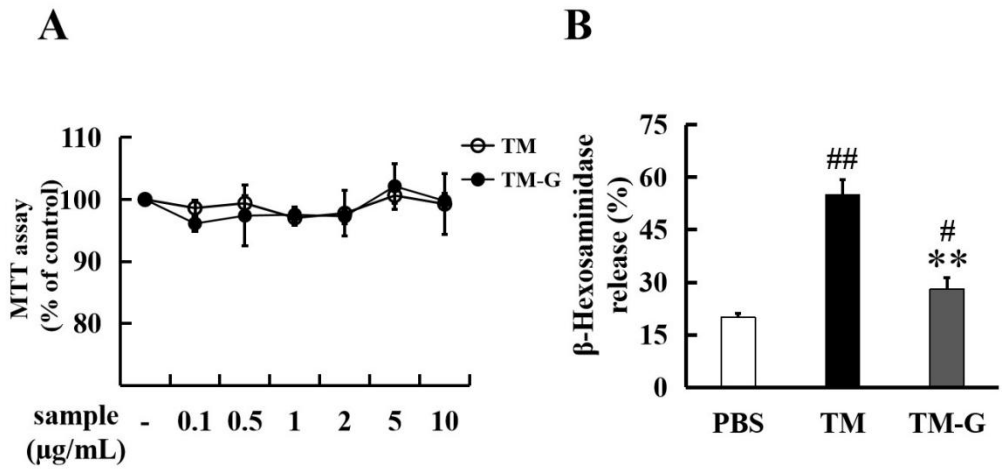

Figure 3. Bioavailability analysis of TM-G using RBL-2H3: (**A**) Cell viability analysis of TM and TM-G with different concentrations (0, 0.1, 0.5, 1, 2, 5, 10 μg/mL). (**B**) Effects of TM and TM-G on the release of β-hexosaminidase. All data are presented as the mean ± SD ($n = 3$). # $p < 0.05$ and ## $p < 0.01$ was used to compare with PBS group. ** $p < 0.01$ was used to compare with TM group.

3.5. Injection Sensitization to TM-G

In vivo experiments further proved our hypothesis. The effect of TM and TM-G on mice can be most intuitively analyzed by using the detection of sera antibodies. A 15-day mouse model was used to test the allergenicity of TM-G in vivo (Figure 4A). On the 15th day, the level of serum IgE in the TM group was significantly higher than the PBS group, which proved that the TM sensitized-mouse model was successfully constructed. In Figure 4B, the specific IgE level in the TM group performed the significant difference in the PBS group ($p < 0.01$), and there was no difference in the PBS group and the TM-G group, whereas the specific IgE level in the TM-G group was only one-tenth of that in the TM group ($p < 0.01$). Additionally, the level of IgG1 and IgG2a in the TM and TM-G group was higher than the PBS group, but the level of IgG1 and IgG2a in the TM-G group was significantly lower than the TM group ($p < 0.01$). Meanwhile, the released level of the Th1-type cytokines IFN-γ in spleen cells was similar to both the PBS group and the TM-G group; however, the IFN-γ value of the TM group was significantly lower than the TM-G group ($p < 0.01$) (Figure 4C). The level of released IL-4 and IL-13, cytokine from the Th2-type cell, is shown in Figure 4D,E, the TM group was significantly increased in comparison to

the PBS group ($p < 0.01$), whereas the TM-G group was significantly decreased compared with the TM group ($p < 0.01$). Overall, it is further demonstrated that MR could reduce the immunogenicity of allergens.

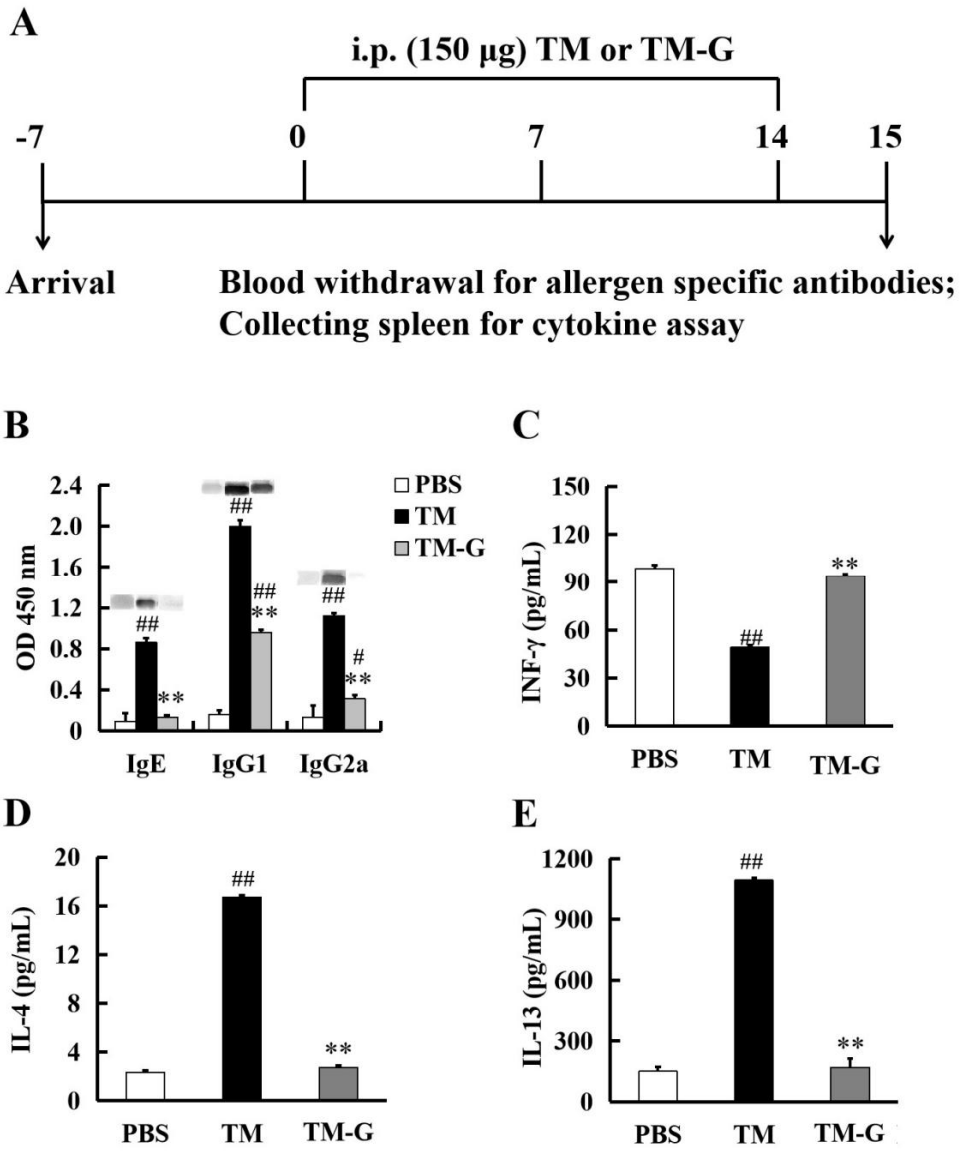

Figure 4. Sensitizing potential of TM-G: (**A**) Experimental design of in vivo sensitization. (**B**) Serum levels of IgE, IgG1, and IgG2a. (**C**–**E**) IFN-γ, IL-4, and IL-13 production by individual spleen lymph cells, was evaluated by ELISA in culture supernatants. All data are presented as the mean ± SD ($n = 3$). # $p < 0.05$ and ## $p < 0.01$ was used to compare with PBS group. ** $p < 0.01$ was used to compare with the TM group.

3.6. Oral Tolerance to TM-G

Furthermore, the potential of TM-G supporting the development of oral tolerance in mice was explored with a mouse model (Figure 5A). Anaphylaxis in mice was assessed by anaphylactic score, rectal temperature, and diarrhea rate (Figure 5B–D). All the figures showed the same result, the TM group can induce a significantly anaphylactic reaction in mice through the higher anaphylactic score, lower rectal temperature, and higher diarrhea rate in comparison to the PBS group, while the group of TM-G can relieve these symptoms.

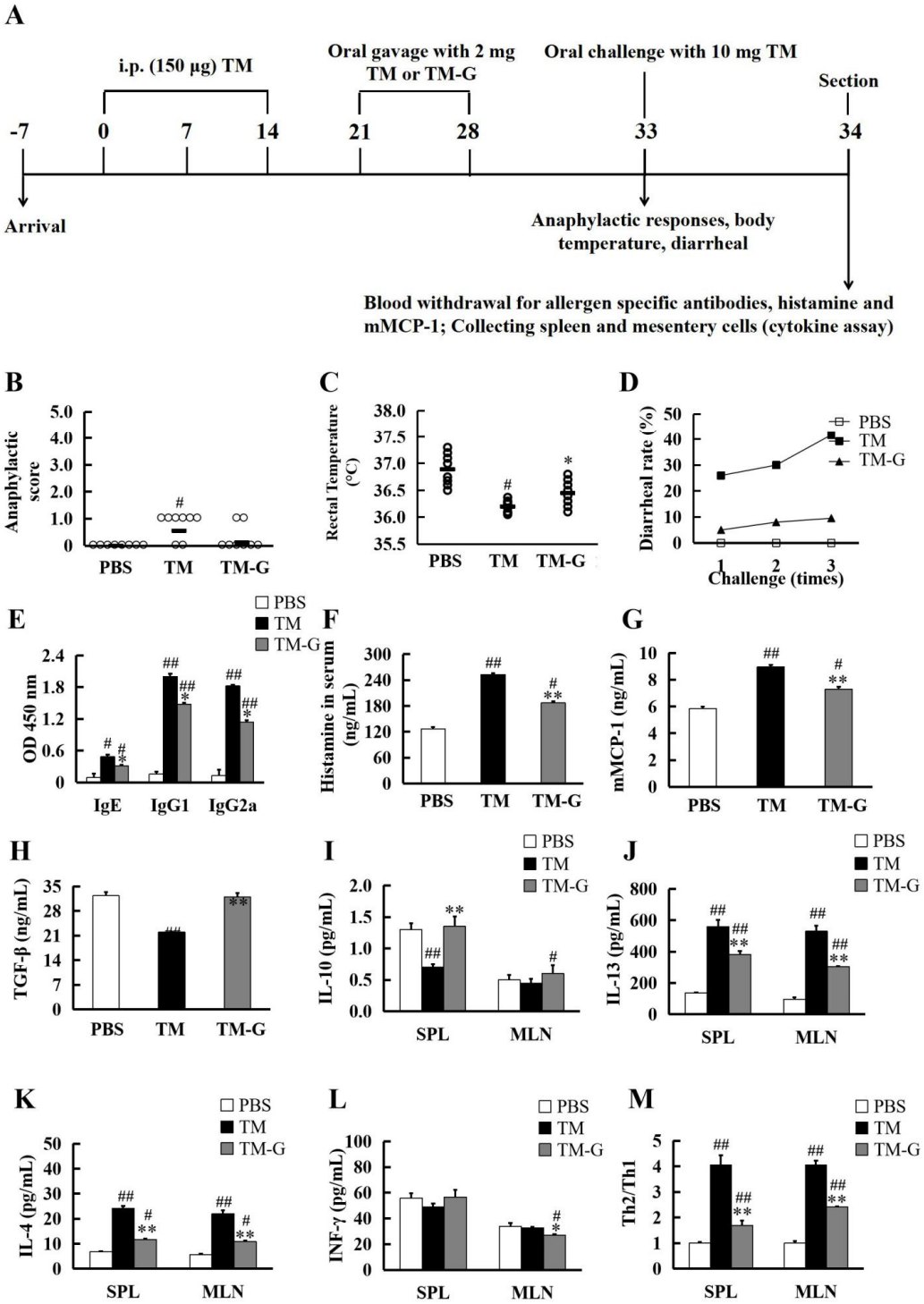

Figure 5. Oral tolerance induced by TM-G: (**A**) Experimental design of oral tolerance. (**B**) Anaphylactic scores. (**C**) Rectal temperature was measured 1 h after intragastric TM delivery. (**D**) Rates of diarrhea for 1 h after every challenge. (**E**) The levels of IgE, IgG1, and IgG2a in the serum of mice on day 34 were measured. (**F**–**H**) The levels of histamine, mMCP-1, TGF-β measured in sera (dilution 1:5, 1% skimmed milk). (**I**–**L**) The production of IL-10, IL-13, IL-4, and IFN-γ was measured in cultured spleen lymph cells and mesentery lymph cells, respectively. (**M**) The ratio of Th2/Th1. All data are presented as the mean ± SD (n = 3). $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ was used to compare with PBS group. * $p < 0.05$ and ** $p < 0.01$ was used to compare with TM group.

Further, assessing the tolerance potency of TM-G at sera level, normally the activation of mast cells was evaluated by specific antibody, histamine, and mMCP-1 (Figure 5E–G). Compared to the PBS group, the TM group had a higher level in those indexes, whereas the TM-G group alleviated these symptoms. The T-cell level (Figure 5H–L) showed the same results. As the response of Treg cells, TGF-β and IL-10 in the TM group significantly decreased than in the PBS group, while the TM-G group performed a similar degree with the PBS group (Figure 5H,I). Both IL-13 and IL-4 are generally involved in the response of Th2 cells, the TM group had a higher level than the PBS group, and the TM-G group mitigated this phenomenon, while the IFN-γ response of Th1 cells in the TM-G group increased slightly. The index of Th2/Th1 was regarded by IL-4 to IFN-γ ratio. As shown in Figure 5M, TM-G, but not TM, can regulate the balance of Th2/Th1 cells in mice. The up-regulation of IL-10 and TGF-β suggested that Treg cells inhibit allergic reactions by regulating the balance of Th2/Th1, thus relieving allergic symptoms. These results indicated the oral tolerance capacity of TM-G. The MRPs could promote the balance of Th2 cells and Th1 cells in mouse spleen lymphocytes, making them tend to the cell level of normal mice.

3.7. Modification of TM Epitopes via the MR

The results of the prediction of T cell epitopes can support information (Tables S2 and S3). The main T cell epitopes are distributed in the M1-D20, S102-M126, E145-R182, E219-Q247. The glycation site of TM-G was identified using LC-MS/MS (Figure S2). Glycated peptides were identified by molecular mass compared with the TM peptide masses, which showed in supporting information (Table S4).

To compare the epitopes and modification sites, the glycated residues of TM-G were marked by a solid triangle, the predicted T cell epitopes of TM are marked by single-underline, and reported B cell epitopes of TM are marked by double-underline (Figure 6). It was found that many glycated residues were located at the T cell epitopes or reported B cell epitopes. The modified specific amino acids on the B cell epitopes of the TM were R21, R101, R105, R160, K161, R178, R182, K213, R217, and this observation provides the mechanistic explanation of the decreased IgE capability of TM-G.

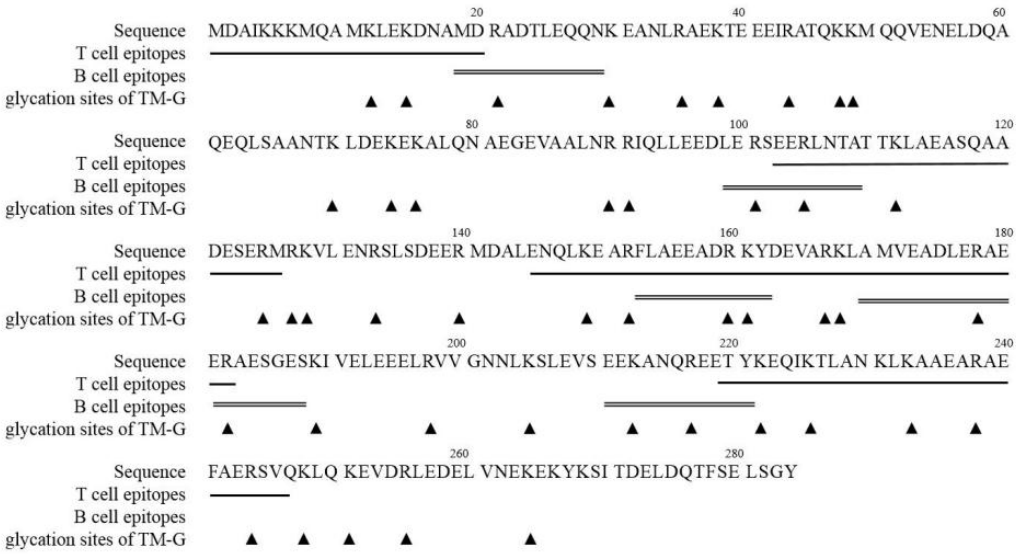

Figure 6. The modification analysis of the epitopes of TM-G. B cell epitopes of TM that have been identified, T cell epitopes of TM were predicted by prediction system. The T cell (underlined) and B cell (double underlined) reactive regions was compared with glycation sites of TM-G (marked by triangle).

4. Discussion

MR usually occurrs in processed food to improve food quality and taste, and now MR is gradually used to reduce the sensitivity of food allergens. In the previous study,

we found that both galactose and arabinose could reduce the allergenicity of TM, while galactose worked better than arabinose [28]. In this study, the mechanism has been further clarified. We found that the IgG/E-binding activity of TM-G was reduced, Nakamura et al. [9] found that the allergenicity of squid TM after MR was reduced, which is similar to this study, and the changes of the immune binding capacity of MRPs to mouse-specific IgE antibody could be detected indirectly by the RBL-2H3 cell model. TM-G could significantly inhibit the β-hexosaminidase release rate, a similar study found that after the Maillard reaction, the degranulation of KU812 cells also decreased [33], which indicated that the immune binding activity of MRPs to IgE antibody from mouse and human serum were also significantly decreased.

To analyze the immunogenic properties of TM-G, the influences on the uptake activity of DCs by TM-G were investigated. Perusko et al. [10] found that the phagocytic function of DCs to milk allergen after MR was slightly enhanced, which is similar to this study. It had a strong phagocytosis ability toward glycosylation products, due to DCs having a variety of cell surface receptors. CD86 is a co-stimulatory factor on DCs, which can activate T cells, H2-Ob has the function of inhibiting and regulating DCs' presentation [34]. Rupa et al. [35] found that ovalbumin after MR could inhibit the activation of T cells by DCs. The co-stimulatory factor CD86 on DCs' surface which could activate T cell surface receptor was decreased [17]. In this study, T cell epitopes were not identified, and they will be explored and elaborated on at a later stage. This result may be due to reduced immunogenicity. The in vivo experiments further supported our hypothesis. The TM-specific IgE antibody in mouse serum was decreased in the mouse sensitized model, which further demonstrated the effect of MR on allergen immunogenicity reduction. In this study, the MRPs did not affect the uptake function of DCs; however MRPs could reduce the presentation level of DCs and the signal transduction effect on downstream $CD4^+$ T cells.

Furthermore, the potential of TM-G to support the development of oral tolerance in mice was explored. It has been previously shown that MRPs could promote the balance of Th2 cells and Th1 cells in mouse spleen lymphocytes, making them reach the cell level of normal mice [28]. Treg cells mediate specific suppression by depleting peptide-MHC-II from DCs [36]. The activation of Treg cells is helpful to induce oral tolerance [37]. Multiple tolerance induction makes the body increase the potential of producing immune tolerance. Furthermore, $IgG1^+$ B cells do not compete with IgE^+ B cells as IgE^+ B cells cannot switch towards IgG1; moreover, allergen-specific IgG1 might block IgE binding to the allergen [14].

Meanwhile, we explained the molecular mechanism of inducing tolerance of TM-G. Though the molecular weight of amino acids on the peptide can be analyzed by mass spectrometry [38], the amino acid changes in TM-G were obtained. We found that TM-G has more glycosylated residues than TM with Maillard reaction with arabinose [28] and many glycosylated residues are located at T cell epitope or reported B cell epitope. Thus, the reaction of T cells could be activated without reacting with IgE on activating effector cells, so as to reduce allergic symptoms and improve the tolerance of the body.

In this study, the glycosylation for TM could reduce its sensitivity and preserve immunogenicity, aimed at improving the efficacy of TM tolerance induction and reduce TM occurrence of serious side effects. In theory, immunogenicity and a low sensitivity allergen vaccine can be used in high doses regardless of the rapid response mediated by IgE, for the significant decrease in the effect of TM-G on the degranulation of mast cells, but still retains the ability to induce Th1 cells to produce IFN-γ [39]. T cell epitopes of TM-G may be modified by MR, MRPs will affect the presentation function of DCs and then affect the immunogenicity of allergen. For allergen tolerance induction with modified low allergenicity protein vaccine, the dosage of tolerance inducer is critical [39]. Individualized treatment should be made according to the specific allergic protein of the patient [40]. Allergen-specific immunotherapy refers to the stimulation of a certain small dose of allergen in allergic patients after the clinical diagnosis of an allergen, and then gradually increase the dose to achieve the purpose of desensitization [41]. Therefore, the MRPs, which can be used as a vaccine or as inducing food, have a great application prospect,

but the safety and effectiveness of the MRPs as therapeutic vaccines and food need to be evaluated in clinical trials.

5. Conclusions

In summary, due to specific amino acid residues on the T cell epitopes and the B cell epitopes of TM being glycated, the TM-G could reduce the degranulation release rate, and inhibit the uptake and presentation function of BMDCs. Additionally, the IgE levels in mice sera of the TM-G group were significantly decreased in the mouse sensitized model. The TM-G could activate Treg to produce TGF-β and IL-10 without causing allergic symptoms. TM-G positively modulates the Th1/Th2 immune balance, which can decrease the specific IgE antibody and histamine in sera. Potentially, the MRPs as tolerance inducers are used for allergen-specific immunotherapy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27062027/s1, Table S1: Specific-IgE Levels and Symptoms of the Crab-Sensitive Patients. Table S2: Prediction results of MHC-II binding area for H-2 T cell epitopes from TM. Table S3: Prediction results of MHC-II binding for human HLA T cells epitopes from TM. Table S4: Identification of glycation sites of TM. Figure S1: BMDCs uptake of FITC-labeled TM and TM-G followed in time. Figure S2: Identification of glycation sites of TM-G.

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Abbreviations

BMDCs: bone marrow-derived dendritic cells; FBS: fetal bovine sera; GM-CSF: granulocyte macrophage colony stimulating factor; Ig: immunoglobulin; IL: interleukin; IFN: interferon; MR: Maillard reaction; MRPs: Maillard reaction products; MHC: major histocompatibility complex; TM: tropomyosin; TM-G: Maillard reacted TM with galactose; Th: helper T cells; Treg: regulatory T; TGF: transforming growth factor.

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Table S1 Specific IgE Levels and Symptoms of the Crab-Sensitive Patients

No.	Serum No.	Age	Gender	Crab specific IgE (kUA/L)	Specific IgE of TM from crab (OD 450 nm)	Symptoms
1	B119847	17	F	3.69	0.12	Cough
2	A281913	55	F	15.50	0.16	Eczema
3	B159030	8	M	24	0.14	Allergic purpura
4	B134851	1	F	6.47	0.15	Systemic lupus erthematosus
5	A819807	31	M	5.36	0.14	Eczema
6	B156635	12	M	2.69	0.12	Eczema
7	B134016	29	M	11.20	0.17	Cough
8	B450961	48	F	9.81	0.13	Lungs infected
9	B127123	67	M	0.15	0.07	-
10	A820983	23	F	0.09	0.06	-

Table S2 Prediction results of MHC-II binding area for mouse H-2 T cell epitopes from TM

No.	Allele	Start	End	Peptide	Adjusted rank
1	H2-IAd	1	13	MDAIKKKMQAMKL	22.38
2	H2-IAd	4	20	IKKKMQAMKLEKDNAMD	24.19
3	H2-IAd	55	69	NELDQAQEQLSAANT	22.2
4	H2-IAd	60	74	AQEQLSAANTKLDEK	27
5	H2-IAd	100	114	ERSEERLNTATTKLA	30
6	H2-IAd	111	127	TKLAEASQAADESERMR	28.87
7	H2-IAd	124	138	ERMRKVLENRSLSDE	25.5
8	H2-IAd	145	159	ENQLKEARFLAEEAD	29.5
9	H2-IAd	160	173	KYDEVARKLAMVE	24.95
10	H2-IAd	168	182	KLAMVEADLERAEER	26.5
11	H2-IAd	217	231	REETYKEQIKTLANK	25.95
12	H2-IAb	231	247	KLKAAEARAEFAERSVQ	28.96
13	H2-IEd	236	250	EARAEFAERSVQKLQ	14.5

Table S3 Prediction results of MHC-II binding for human HLA T cells epitopes from TM

No.	Allele	Start	End	Peptide	Adjusted rank
1	HLA-DRB4*01:01	1	16	MDAIKKKMQAMKLEKD	2.89
2	HLA-DRB4*01:01	2	16	DAIKKKMQAMKLEKD	2.4
3	HLA-DRB4*01:01	2	17	DAIKKKMQAMKLEKDN	2.42
4	HLA-DRB4*01:01	3	17	AIKKKMQAMKLEKDN	1.8
5	HLA-DRB4*01:01	3	18	AIKKKMQAMKLEKDNA	1.85
6	HLA-DRB4*01:01	3	16	AIKKKMQAMKLEKD	2.15
7	HLA-DRB4*01:01	3	19	AIKKKMQAMKLEKDNAM	2.9
8	HLA-DRB4*01:01	4	18	IKKKMQAMKLEKDNA	1.7
9	HLA-DRB4*01:01	4	17	IKKKMQAMKLEKDN	1.94
10	HLA-DRB4*01:01	4	19	IKKKMQAMKLEKDNAM	1.96
11	HLA-DRB4*01:01	4	16	IKKKMQAMKLEKD	2.81
12	HLA-DRB4*01:01	5	19	KKKMQAMKLEKDNAM	2.1
13	HLA-DRB4*01:01	5	18	KKKMQAMKLEKDNA	2.15
14	HLA-DRB4*01:01	5	20	KKKMQAMKLEKDNAMD	2.54
15	HLA-DRB1*03:01	8	22	MQAMKLEKDNAMDRA	2.6
16	HLA-DRB1*03:01	9	22	QAMKLEKDNAMDRA	1.83
17	HLA-DRB1*03:01	9	23	QAMKLEKDNAMDRAD	2.4
18	HLA-DRB1*03:01	10	23	AMKLEKDNAMDRAD	1.94
19	HLA-DRB1*03:01	10	22	AMKLEKDNAMDRA	2.03
20	HLA-DRB1*03:01	10	24	AMKLEKDNAMDRADT	2.6
21	HLA-DRB1*11:01	81	95	AEGEVAALNRRIQLL	2.7
22	HLA-DRB1*11:01	82	95	EGEVAALNRRIQLL	2.05
23	HLA-DRB1*11:01	82	96	EGEVAALNRRIQLLE	2.3
24	HLA-DRB1*11:01	82	94	EGEVAALNRRIQL	2.81
25	HLA-DRB1*11:01	83	96	GEVAALNRRIQLLE	1.83

26	HLA-DRB1*11:01	83	95	GEVAALNRRIQLL	2.03
27	HLA-DRB1*11:01	83	97	GEVAALNRRIQLLEE	2.1
28	HLA-DRB1*11:01	83	98	GEVAALNRRIQLLEED	2.89
29	HLA-DQA1*05:01/DQB1*02:01	86	100	AALNRRIQLLEEDLE	2.5
30	HLA-DQA1*05:01/DQB1*02:01	87	100	ALNRRIQLLEEDLE	2.15
31	HLA-DQA1*05:01/DQB1*02:01	88	100	LNRRIQLLEEDLE	2.49
32	HLA-DQA1*01:02/DQB1*06:02	102	117	SEERLNTATTKLAEAS	2.08
33	HLA-DQA1*01:02/DQB1*06:02	102	118	SEERLNTATTKLAEASQ	2.73
34	HLA-DQA1*01:02/DQB1*06:02	103	117	EERLNTATTKLAEAS	1.6
35	HLA-DQA1*01:02/DQB1*06:02	103	118	EERLNTATTKLAEASQ	1.62
36	HLA-DQA1*01:02/DQB1*06:02	103	119	EERLNTATTKLAEASQA	2.55
37	HLA-DQA1*01:02/DQB1*06:02	104	118	ERLNTATTKLAEASQ	1.3
38	HLA-DQA1*01:02/DQB1*06:02	104	117	ERLNTATTKLAEAS	1.4
39	HLA-DQA1*01:02/DQB1*06:02	104	119	ERLNTATTKLAEASQA	1.62
40	HLA-DQA1*01:02/DQB1*06:02	104	120	ERLNTATTKLAEASQAA	2.73
41	HLA-DQA1*01:02/DQB1*06:02	105	118	RLNTATTKLAEASQ	1.4
42	HLA-DQA1*01:02/DQB1*06:02	105	119	RLNTATTKLAEASQA	1.4

Continuation Table S3

43	HLA-DQA1*01:02/DQB1*06:02	105	117	RLNTATTKLAEAS	1.72
44	HLA-DQA1*01:02/DQB1*06:02	105	120	RLNTATTKLAEASQAA	1.85
45	HLA-DQA1*01:02/DQB1*06:02	105	121	RLNTATTKLAEASQAAD	2.73
46	HLA-DQA1*01:02/DQB1*06:02	106	121	LNTATTKLAEASQAAD	2.65
47	HLA-DQA1*01:02/DQB1*06:02	106	119	LNTATTKLAEASQA	2.69
48	HLA-DQA1*01:02/DQB1*06:02	106	118	LNTATTKLAEASQ	2.81
49	HLA-DQA1*03:01/DQB1*03:02	109	123	ATTKLAEASQAADES	2.1
50	HLA-DQA1*03:01/DQB1*03:02	109	124	ATTKLAEASQAADESE	2.54
51	HLA-DQA1*03:01/DQB1*03:02	110	124	TTKLAEASQAADESE	1.6
52	HLA-DQA1*03:01/DQB1*03:02	110	123	TTKLAEASQAADES	2.26

53	HLA-DQA1*03:01/DQB1*03:02	110	125	TTKLAEASQAADESER	2.65
54	HLA-DQA1*03:01/DQB1*03:02	111	124	TKLAEASQAADESE	1.72
55	HLA-DQA1*03:01/DQB1*03:02	111	125	TKLAEASQAADESER	2.4
56	HLA-DQA1*03:01/DQB1*03:02	111	126	TKLAEASQAADESERM	2.77
57	HLA-DQA1*03:01/DQB1*03:02	112	125	KLAEASQAADESER	2.8
58	HLA-DQA1*03:01/DQB1*03:02	112	126	KLAEASQAADESERM	2.8
59	HLA-DQA1*03:01/DQB1*03:02	112	124	KLAEASQAADESE	2.81
60	HLA-DQA1*04:01/DQB1*04:02	145	159	ENQLKEARFLAEEAD	2.9
61	HLA-DQA1*04:01/DQB1*04:02	146	159	NQLKEARFLAEEAD	2.37
62	HLA-DRB5*01:01	148	162	LKEARFLAEEADRKY	3
63	HLA-DRB5*01:01	149	162	KEARFLAEEADRKY	2.8
64	HLA-DRB5*01:01	149	163	KEARFLAEEADRKYD	2.9
65	HLA-DRB5*01:01	150	163	EARFLAEEADRKYD	2.58
66	HLA-DRB5*01:01	150	164	EARFLAEEADRKYDE	2.8
67	HLA-DRB5*01:01	151	164	ARFLAEEADRKYDE	2.69
68	HLA-DRB5*01:01	151	165	ARFLAEEADRKYDEV	2.8
69	HLA-DQA1*03:01/DQB1*03:02	163	177	DEVARKLAMVEADLE	2.9
70	HLA-DQA1*03:01/DQB1*03:02	164	177	EVARKLAMVEADLE	2.8
71	HLA-DQA1*03:01/DQB1*03:02	165	181	VARKLAMVEADLERAEE	1.6
72	HLA-DQA1*05:01/DQB1*02:01	165	179	VARKLAMVEADLERA	2.6
73	HLA-DQA1*03:01/DQB1*03:02	165	180	VARKLAMVEADLERAE	3
74	HLA-DQA1*03:01/DQB1*03:02	166	181	ARKLAMVEADLERAEE	0.99
75	HLA-DQA1*03:01/DQB1*03:02	166	182	ARKLAMVEADLERAEER	1.67
76	HLA-DQA1*05:01/DQB1*02:01	166	179	ARKLAMVEADLERA	2.15
77	HLA-DQA1*04:01/DQB1*04:02	166	181	ARKLAMVEADLERAEE	2.31
78	HLA-DQA1*05:01/DQB1*02:01	166	180	ARKLAMVEADLERAE	2.7
79	HLA-DQA1*05:01/DQB1*02:01	166	178	ARKLAMVEADLER	2.96
80	HLA-DQA1*03:01/DQB1*03:02	167	181	RKLAMVEADLERAEE	0.92

81	HLA-DQA1*03:01/DQB1*03:02	167	182	RKLAMVEADLERAEER	0.99
82	HLA-DQA1*03:01/DQB1*03:02	167	183	RKLAMVEADLERAEERA	1.55
83	HLA-DQA1*04:01/DQB1*04:02	167	181	RKLAMVEADLERAEE	1.6
84	HLA-DQA1*04:01/DQB1*04:02	167	182	RKLAMVEADLERAEER	2.31
85	HLA-DQA1*05:01/DQB1*02:01	167	181	RKLAMVEADLERAEE	2.6

Continuation Table S3

86	HLA-DQA1*03:01/DQB1*03:02	168	181	KLAMVEADLERAEE	1.29
87	HLA-DQA1*03:01/DQB1*03:02	168	182	KLAMVEADLERAEER	1.4
88	HLA-DQA1*04:01/DQB1*04:02	168	181	KLAMVEADLERAEE	1.72
89	HLA-DQA1*03:01/DQB1*03:02	168	183	KLAMVEADLERAEERA	1.73
90	HLA-DQA1*04:01/DQB1*04:02	168	182	KLAMVEADLERAEER	2.1
91	HLA-DQA1*03:01/DQB1*03:02	168	184	KLAMVEADLERAEERAE	2.73
92	HLA-DQA1*03:01/DQB1*03:02	169	182	LAMVEADLERAEER	1.18
93	HLA-DQA1*03:01/DQB1*03:02	169	181	LAMVEADLERAEE	1.29
94	HLA-DQA1*03:01/DQB1*03:02	169	183	LAMVEADLERAEERA	1.3
95	HLA-DQA1*03:01/DQB1*03:02	169	184	LAMVEADLERAEERAE	1.62
96	HLA-DQA1*04:01/DQB1*04:02	169	182	LAMVEADLERAEER	1.83
97	HLA-DQA1*04:01/DQB1*04:02	169	181	LAMVEADLERAEE	1.87
98	HLA-DQA1*04:01/DQB1*04:02	169	183	LAMVEADLERAEERA	2.2
99	HLA-DQA1*04:01/DQB1*04:02	169	184	LAMVEADLERAEERAE	2.65
100	HLA-DQA1*03:01/DQB1*03:02	169	185	LAMVEADLERAEERAES	2.73
101	HLA-DQA1*03:01/DQB1*03:02	170	183	AMVEADLERAEERA	1.4
102	HLA-DQA1*03:01/DQB1*03:02	170	184	AMVEADLERAEERAE	1.5
103	HLA-DQA1*03:01/DQB1*03:02	170	182	AMVEADLERAEER	1.72
104	HLA-DQA1*03:01/DQB1*03:02	170	181	AMVEADLERAEE	1.78
105	HLA-DQA1*03:01/DQB1*03:02	170	185	AMVEADLERAEERAES	2.42
106	HLA-DQA1*03:01/DQB1*03:02	171	184	MVEADLERAEERAE	1.29
107	HLA-DQA1*03:01/DQB1*03:02	171	183	MVEADLERAEERA	1.47

108	HLA-DQA1*03:01/DQB1*03:02	171	185	MVEADLERAEERAES	1.5
109	HLA-DQA1*03:01/DQB1*03:02	171	182	MVEADLERAEER	1.78
110	HLA-DQA1*03:01/DQB1*03:02	171	186	MVEADLERAEERAESG	2.42
111	HLA-DQA1*03:01/DQB1*03:02	172	187	VEADLERAEERAESGE	2.65
112	HLA-DQA1*03:01/DQB1*03:02	182	196	RAESGESKIVELEEE	2.4
113	HLA-DQA1*03:01/DQB1*03:02	182	197	RAESGESKIVELEEEL	3
114	HLA-DQA1*05:01/DQB1*02:01	183	197	AESGESKIVELEEEL	2
115	HLA-DQA1*03:01/DQB1*03:02	183	196	AESGESKIVELEEE	2.05
116	HLA-DQA1*03:01/DQB1*03:02	183	197	AESGESKIVELEEEL	2.1
117	HLA-DQA1*05:01/DQB1*02:01	184	197	ESGESKIVELEEEL	1.62
118	HLA-DQA1*03:01/DQB1*03:02	184	197	ESGESKIVELEEEL	1.72
119	HLA-DQA1*05:01/DQB1*02:01	184	198	ESGESKIVELEEELR	2.2
120	HLA-DQA1*03:01/DQB1*03:02	184	196	ESGESKIVELEEE	2.49
121	HLA-DQA1*05:01/DQB1*02:01	184	199	ESGESKIVELEEELRV	2.89
122	HLA-DQA1*05:01/DQB1*02:01	185	198	SGESKIVELEEELR	1.83
123	HLA-DQA1*05:01/DQB1*02:01	185	199	SGESKIVELEEELRV	1.9
124	HLA-DQA1*05:01/DQB1*02:01	185	197	SGESKIVELEEEL	2.03
125	HLA-DQA1*03:01/DQB1*03:02	185	197	SGESKIVELEEEL	2.96
126	HLA-DQA1*05:01/DQB1*02:01	186	199	GESKIVELEEELRV	1.62
127	HLA-DQA1*05:01/DQB1*02:01	186	198	GESKIVELEEELR	2.34
128	HLA-DQA1*05:01/DQB1*02:01	186	200	GESKIVELEEELRVV	2.4

Continuation Table S3

129	HLA-DQA1*05:01/DQB1*02:01	186	197	GESKIVELEEEL	2.85
130	HLA-DQA1*05:01/DQB1*02:01	187	200	ESKIVELEEELRVV	2.26
131	HLA-DQA1*05:01/DQB1*02:01	187	199	ESKIVELEEELRV	2.34
132	HLA-DRB5*01:01	194	207	EEELRVVGNNLKSL	2.8
133	HLA-DRB5*01:01	195	208	EELRVVGNNLKSLE	2.8
134	HLA-DRB1*08:02	219	234	ETYKEQIKTLANKLKA	1.96

135	HLA-DRB5*01:01	219	234	ETYKEQIKTLANKLKA	1.96
136	HLA-DRB1*08:02	219	233	ETYKEQIKTLANKLK	2.6
137	HLA-DRB5*01:01	219	235	ETYKEQIKTLANKLKAA	2.73
138	HLA-DRB5*01:01	220	234	TYKEQIKTLANKLKA	1.1
139	HLA-DRB1*08:02	220	234	TYKEQIKTLANKLKA	1.2
140	HLA-DRB1*08:02	220	235	TYKEQIKTLANKLKAA	1.73
141	HLA-DRB5*01:01	220	235	TYKEQIKTLANKLKAA	1.73
142	HLA-DRB1*08:02	220	233	TYKEQIKTLANKLK	2.26
143	HLA-DRB5*01:01	220	233	TYKEQIKTLANKLK	2.58
144	HLA-DRB5*01:01	220	236	TYKEQIKTLANKLKAAE	2.73
145	HLA-DRB1*08:02	220	236	TYKEQIKTLANKLKAAE	2.9
146	HLA-DRB5*01:01	221	234	YKEQIKTLANKLKA	0.84
147	HLA-DRB1*08:02	221	235	YKEQIKTLANKLKAA	0.86
148	HLA-DRB5*01:01	221	235	YKEQIKTLANKLKAA	0.88
149	HLA-DRB1*08:02	221	234	YKEQIKTLANKLKA	1.18
150	HLA-DRB1*08:02	221	236	YKEQIKTLANKLKAAE	1.73
151	HLA-DRB5*01:01	221	236	YKEQIKTLANKLKAAE	1.73
152	HLA-DRB5*01:01	221	237	YKEQIKTLANKLKAAEA	2.73
153	HLA-DRB1*11:01	221	235	YKEQIKTLANKLKAA	2.8
154	HLA-DRB5*01:01	221	233	YKEQIKTLANKLK	2.81
155	HLA-DRB1*08:02	221	237	YKEQIKTLANKLKAAEA	2.9
156	HLA-DRB1*08:02	222	235	KEQIKTLANKLKAA	0.66
157	HLA-DRB5*01:01	222	235	KEQIKTLANKLKAA	0.78
158	HLA-DRB5*01:01	222	234	KEQIKTLANKLKA	0.84
159	HLA-DRB1*08:02	222	236	KEQIKTLANKLKAAE	0.84
160	HLA-DRB5*01:01	222	236	KEQIKTLANKLKAAE	0.88
161	HLA-DRB1*08:02	222	237	KEQIKTLANKLKAAEA	1.73
162	HLA-DRB5*01:01	222	237	KEQIKTLANKLKAAEA	1.73

163	HLA-DRB1*08:02	222	234	KEQIKTLANKLKA	1.87
164	HLA-DRB5*01:01	222	238	KEQIKTLANKLKAAEAR	2.73
165	HLA-DRB1*08:02	223	236	EQIKTLANKLKAAE	0.72
166	HLA-DRB1*08:02	223	235	EQIKTLANKLKAA	0.78
167	HLA-DRB5*01:01	223	236	EQIKTLANKLKAAE	0.85
168	HLA-DRB5*01:01	223	235	EQIKTLANKLKAA	0.87
169	HLA-DRB1*08:02	223	237	EQIKTLANKLKAAEA	0.9
170	HLA-DRB5*01:01	223	237	EQIKTLANKLKAAEA	1.1
171	HLA-DRB5*01:01	223	234	EQIKTLANKLKA	1.45

Continuation Table S3

172	HLA-DRB1*08:02	223	238	EQIKTLANKLKAAEAR	1.85
173	HLA-DRB5*01:01	223	238	EQIKTLANKLKAAEAR	1.85
174	HLA-DRB1*08:02	223	234	EQIKTLANKLKA	2.28
175	HLA-DRB1*11:01	223	237	EQIKTLANKLKAAEA	3
176	HLA-DRB1*08:02	224	239	QIKTLANKLKAAEARA	1.85
177	HLA-DRB5*01:01	224	239	QIKTLANKLKAAEARA	2.42
178	HLA-DQA1*03:01/DQB1*03:02	229	243	ANKLKAAEARAEFAE	2.7
179	HLA-DQA1*03:01/DQB1*03:02	229	244	ANKLKAAEARAEFAER	2.89
180	HLA-DQA1*03:01/DQB1*03:02	230	244	NKLKAAEARAEFAER	1.6
181	HLA-DQA1*03:01/DQB1*03:02	230	243	NKLKAAEARAEFAE	2.26
182	HLA-DQA1*03:01/DQB1*03:02	230	245	NKLKAAEARAEFAERS	2.89
183	HLA-DQA1*03:01/DQB1*03:02	231	244	KLKAAEARAEFAER	1.94
184	HLA-DQA1*03:01/DQB1*03:02	231	245	KLKAAEARAEFAERS	2.3
185	HLA-DQA1*03:01/DQB1*03:02	231	246	KLKAAEARAEFAERSV	2.89
186	HLA-DQA1*03:01/DQB1*03:02	232	245	LKAAEARAEFAERS	1.94
187	HLA-DQA1*03:01/DQB1*03:02	232	244	LKAAEARAEFAER	2.18
188	HLA-DQA1*03:01/DQB1*03:02	232	246	LKAAEARAEFAERSV	2.3

189	HLA-DQA1*03:01/DQB1*03:02	232	247	LKAAEARAEFAERSVQ	2.89

Table S4 Identification of glycation sites of TM

Modified amino acid	Peptide sequence	Δ Mass	Molecular formula	MV
K12	MQAMK(1)LEK	52	C2H4O	54
K15	LEK(1)DNAMDR	52	C2H4O	54
R21	DNAMDR(1)ADTLEQQNK	52	C2H4O	54
K30	ADTLEQQNK(1)EANLR	52	C2H4O	54
R35	EANLR(1)AEK(1)TEEEIR	110	C6H8O2	112
R44	TEEEIR(1)ATQK	52	C2H4O	54
K49	K(1)MQQVENELDQAQEQLSAANTK	148	C5H10O5	150
K70	MQQVENELDQAQEQLSAANTK(1)	14	CH3	15
K74	LDEK(1)EK(1)ALQNAEGEVAALNR(1)R(1)	144	C5H10N2O3	146
K76	EK(1)ALQNAEGEVAALNR	52	C2H4O	54
R90	ALQNAEGEVAALNR(1)R	68	C4H6O	70
R91	R(1)IQLLEEDLER	72	CH2CH2COOH	73
R101	RIQLLEEDLER(1)	14	CH3	15
R105	IQLLEEDLER(1)SEER(1)	52	C2H4O	54
K112	LNTATTK(1)LAEASQAADESER	79	C5H4O	80
R125	LAEASQAADESER(1)	14	CH3	15
R127	LAEASQAADESER(1)MR(1)	52	C2H4O	54
K128	K(1)VLENR(1)SLSDEER	52	C2H4O	54
R133	VLENR(1)SLSDEER(1)	52	C2H4O	54
R140	SLSDEER(1)	14	CH3	15
K149	MDALENQLK(1)	14	CH3	15
R152	EAR(1)FLAEEADR	58	CH2COOH	59
R160	FLAEEADR(1)	14	CH3	15
K161	FLAEEADR(1)K(1)	52	C2H4O	54
R167	KYDEVAR(1)K	52	C2H4O	54

K168	K(1)LAMVEADLER	14	CH3	15
R178	LAMVEADLER(1)	14	CH3	15
R182	AEER(1)AESGESKIVELEEELR	148	C5H10O5	150
K189	AESGESK(1)IVELEEELR	132	C5H10O5	150
R198	IVELEEELR(1)	14	CH3	15
K205	IVELEEELR(1)VVGNNLK(1)	52	C2H4O	54
K213	SLEVSEEK(1)	14	CH3	15
R217	ANQR(1)EETYK(1)EQIK	52	C2H4O	54
K222	ANQR(1)EETYK(1)EQIK	52	C2H4O	54
K226	EQIK(1)TLANK	52	C2H4O	54
K231	TLANK(0.995)LK	52	C2H4O	54
K233	LK(1)AAEAR	52	C2H4O	54
R238	AAEAR(1)AEFAER	52	C2H4O	54
R244	AEFAER(1)SVQK	52	C2H4O	54
K248	AEFAERSVQK(1)	52	C2H4O	54
K251	LQK(1)EVDRLEDELVNEK	52	C2H4O	54
Continuation Table S4				
R255	EVDR(1)LEDELVNEKEK	68	C4H6O	70
K264	EVDRLEDELVNEK(1)EK	79	C5H4O	80

Figuer S1 BMDCs uptake of FITC-labeled TM and TM-G followed in time

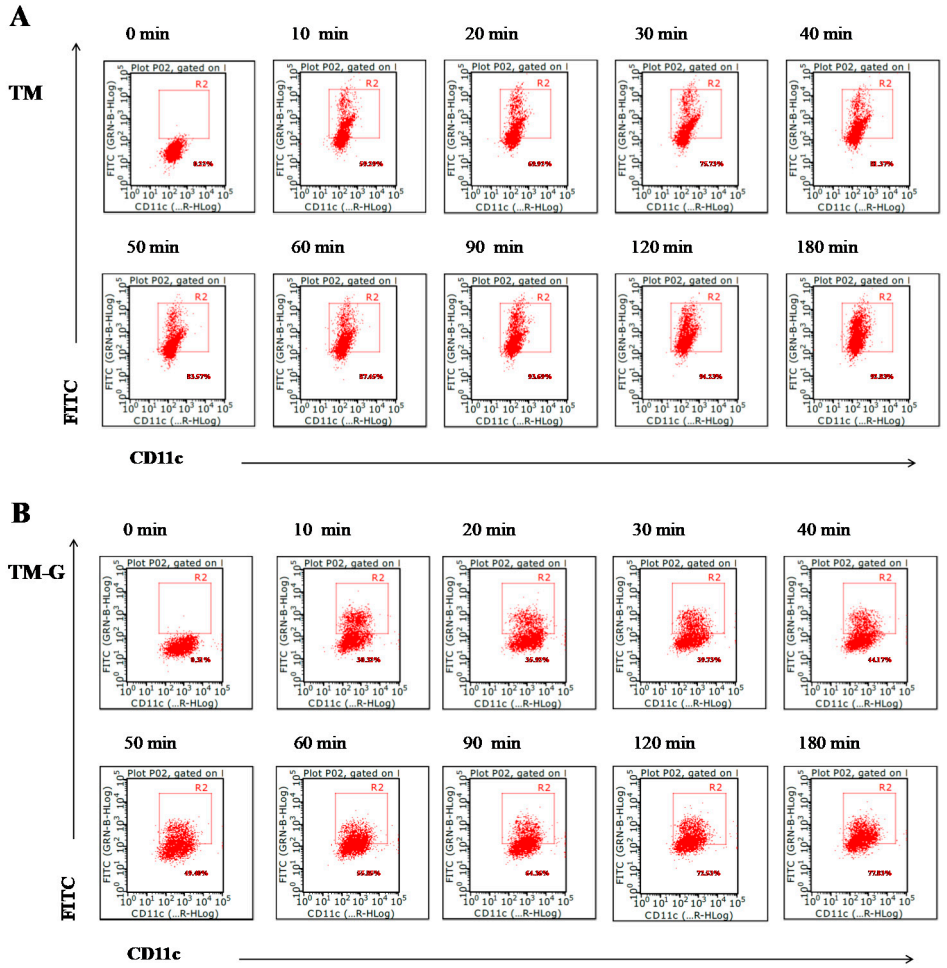
(A) BMDCs uptake of FITC-labeled TM followed in time.

(B) BMDCs uptake of FITC-labeled TM-G followed in time.

Figuer S2. Identification of glycation sites of TM-G

(A-D) identification of the specific amino acids of TM modified by the Maillard reaction using LC-MS/MS. The fragmention spectrums were shown in the graph with the amino acid in the figure corresponding to A_{22}-R_{35}, K_{49}-K_{70}, R_{91}-R_{101} and E_{150}-R_{160} of TM-G.

Figuer S1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120 min
180 min
B
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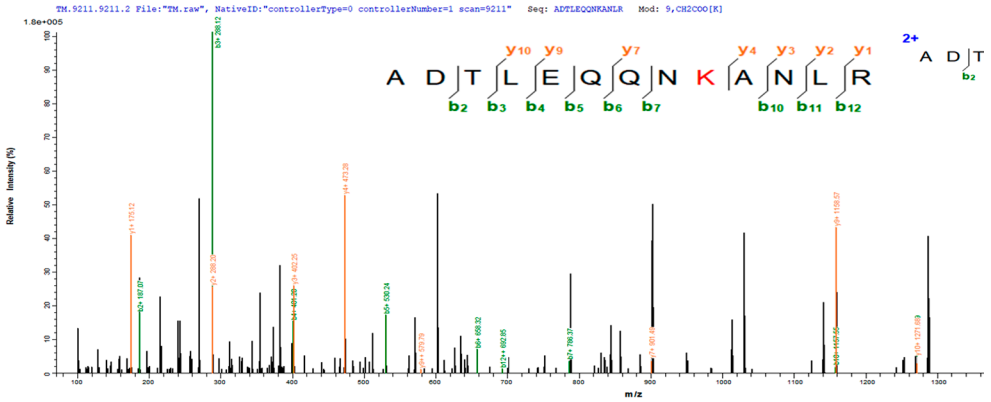
20 min
30 min
40 min
50 min
60 min
90 min
120 min
180 min

Figuer S2

A



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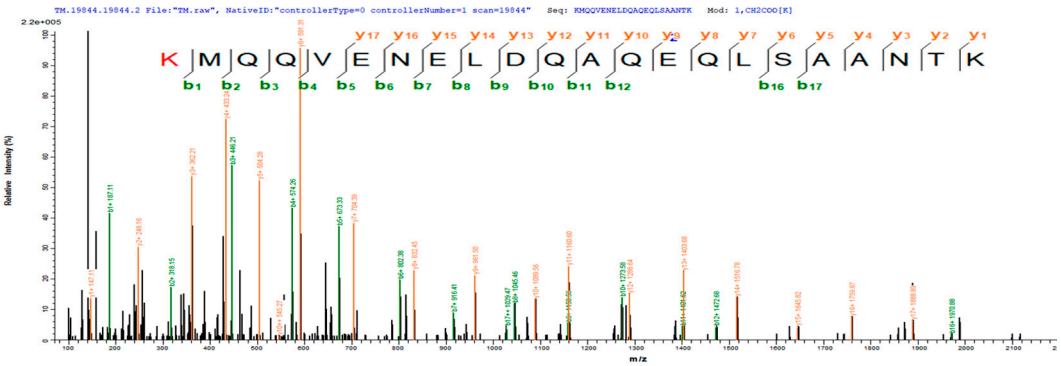

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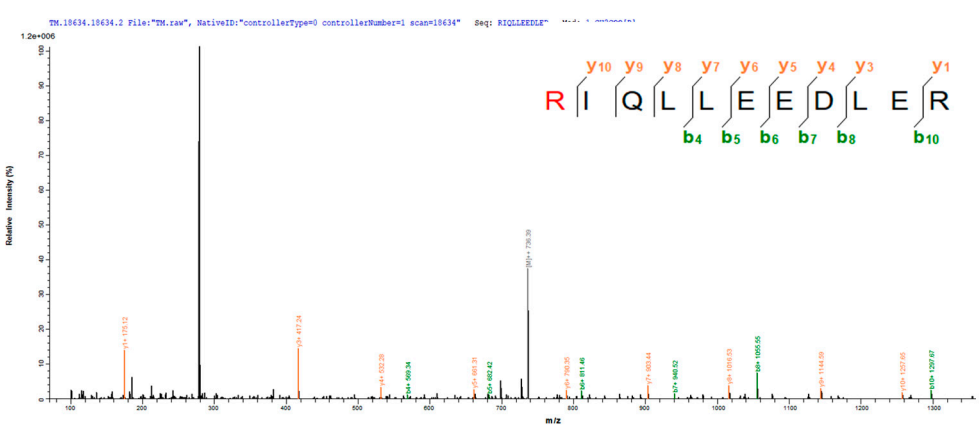

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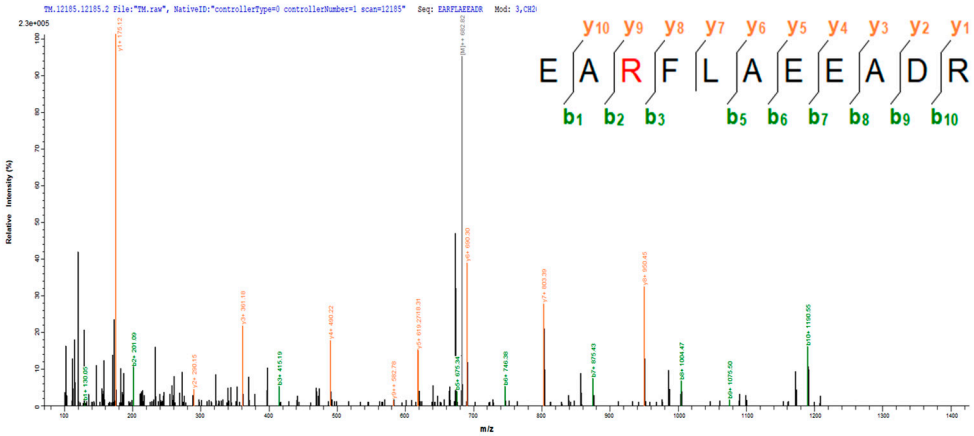
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b1 b2 b3 b5 b6 b7 b8 b9 b10