

Modulation of Allergen-Specific Immune Responses to the Major Shrimp Allergen, Tropomyosin, by Specific Targeting to Scavenger Receptors on Macrophages

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Key Words

Allergen · Th1/Th2 · Scavenger receptor · IgE regulation

Abstract

Background: Tropomyosin from shrimp is the major cross-reacting crustacean food allergen. Earlier studies have led to the purification and immunochemical characterization of the major IgE binding epitopes of the allergen. Maleylated proteins are known to be specifically targeted to scavenger receptors on macrophage. Since antigens processed and presented by macrophages are known to elicit Th1 type of responses and allergic responses are characterized by polarization towards Th2 phenotype, the possibility of modulation of allergen-specific immune responses by targeting of tropomyosin to macrophage via scavenger receptor was explored.

Methods: The IgG and IgE binding potential of the native maleylated form of tropomyosin was carried out by ELISA and immunoblot. The ability of the native and maleylated form of allergen to induce in vitro proliferation of splenocytes from BALB/C mice immunized with both forms of allergen was tested. The in vitro production of IL-4 and IFN- γ by splenocytes from mice immunized with the two forms of allergen was determined

from culture supernatants. The in vivo production of serum IgG1 and IgG2a antibodies following immunization with native and modified allergens was monitored by ELISA. **Results:** The maleylated form of tropomyosin was found to have reduced antigenicity and allergenicity as compared to its native counterpart. The modified allergen was, however, found to elicit a cellular response similar to native tropomyosin in vitro. Analysis of the cytokine profiles showed a modulation from an IL-4-dominant, proallergic, Th2 phenotype to an IFN- γ -dominant, antiallergic, Th1 phenotype that could also be correlated to a modulation in the in vivo antibody isotype. **Conclusion:** The results suggest the possible potential for modulating allergic responses in vivo by selective targeting to macrophages.

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Introduction

Initiation of an immune response occurs by the primary encounter of the T cells with the antigen-presenting cells (APC). The specific CD4⁺ T cells or T helper cells are activated by peptides presented to them by the MHC class II-expressing APC after intracellular processing of the

antigen [1, 2]. The functional outcome of selective T cell activation and subsequent effector responses are to a great extent dependent on the phenotype of the individual T cell and the cytokines that dominate the response to activation [3]. The classification of T helper subtypes into Th1 and Th2 is also primarily functional and based on the cytokine profiles of the T cells [4–6]. Functionally, Th1 cells secrete IL-2, IFN- γ and TNF- β , mediate macrophage activation and delayed-type hypersensitivity, while Th2 cells secrete IL-4, IL-5 and IL-10, provide help to the B cells and stimulate eosinophils [7–9]. The central Th2 cytokine is IL-4 [10, 11], the production of which is under the stringent control of other cells of allergic inflammation. Studies on allergen-specific T cell clones both in mice and humans demonstrate that atopic individuals respond to allergen molecules with a Th2 pattern while nonatopic individuals exhibit a Th1 kind of response [12]. It is widely assumed that APC and inflammatory cells have a decisive role to play in the polarization of the T helper cell response [13]. Although not clear-cut, there seems to be an association between Th1 polarization and a nonallergic response to allergens [14, 15]. One of the most prominent features of the Th1/Th2 reciprocal regulation is the synthesis of the IgE isotype [16, 17]. Hence, modulation of an allergen-specific CD4⁺ T cell-mediated response away from a pathological pathway towards the tolerant and even protective pathway observed in nonallergic individuals may serve as a potentially effective strategy for immunomodulation-based therapy for allergic diseases. Some of the microenvironmental factors which influence the differentiation of a naïve T cell to either a Th1 or a Th2 phenotype include the type of APC [18, 19], the profile of cytokines in the local milieu [20] and the quantitative nature of the MHC-peptide interactions [21]. The topology of the immune system and the route of entry of the antigen or in other words the way in which the immune system first ‘sees’ the antigen determine the effector functions implicated to influence the functional dichotomy of the APC.

Based on experiments using T cell clones and TCR transgenics, it has been proposed that the development of a Th1 phenotype is macrophage-dependent [22]. Scavenger receptors constitutively expressed on macrophages [23] have been shown to deliver protein antigens intracellularly following maleylation [24]. It has been shown that such targeting of protein antigens via scavenger receptor results in enhanced immunogenicity [25] and development of a Th1-dominating T cell response [26]. There has been increasing evidence that the pathogenesis of an allergic response is a consequence of activation of T cells hav-

ing a Th2 phenotype [27]. The possibility of deviating an allergic response from a proallergic Th2 phenotype to an antiallergic Th1 phenotype by specific targeting to macrophage scavenger receptors was therefore investigated. The model system used for the current study was the major cross-reacting crustacean food allergen, tropomyosin, which is a 34-kD heat-stable muscle protein known to be the causative allergen for IgE-mediated type I hypersensitive reactions to sea foods, leading to gastrointestinal cramps [28].

Materials and Methods

Purification of Shrimp Tropomyosin

Tropomyosin was isolated according to the procedure of Greaser and Gergely [29] with modifications. Briefly, 500 g of fresh shrimp (*Penaeus indicus*) was minced and the soluble sarcoplasmic proteins removed by extraction in a low salt buffer containing 20 mM KCl, 1 mM KHCO₃ and 0.1 mM CaCl₂. The suspension was filtered through a fine nylon mesh, the residue washed with 2 vol of ethanol, subjected to ether extraction and allowed to dry at room temperature for 2 h. The dried powder was then extracted overnight at 25 °C in a high salt buffer containing 25 mM Tris (pH 7.5), 1 M KCl and 0.1 mM CaCl₂, filtered through a nylon sieve and subjected to 33–60% ammonium sulfate fractionation. The precipitate was dissolved in 10 mM Tris·Cl (pH 7.5) containing 1 M KCl, and tropomyosin selectively separated by precipitation at its isoelectric pH of 4.5–4.7, leaving the contaminant troponin complex in the supernatant. The precipitate was dissolved in 10 mM Tris·Cl buffer (pH 7.5) and subjected to gel filtration chromatography on a Sephacryl S-200 column equilibrated in the same buffer.

Maleylation of Tropomyosin

Maleylation was carried out according to the procedure of Haberland and Fogelman [30] with modifications. Briefly, 50 mg of finely powdered maleic anhydride (Sigma Chemical Co., St. Louis, Mo., USA) was added to tropomyosin (10 mg) in 5 ml of 0.1 M Na₂CO₃ buffer (pH 9.6). The reaction was carried out with constant mixing and pH maintained in the range of 9–9.6 using 10 N NaOH. Excess unreacted maleic anhydride was removed by extensive dialysis against 10 mM Na₂CO₃ buffer (pH 9.6). The extent of maleylation assessed by TNBS assay [31] for the loss of free amino groups revealed that the modification was >98%.

Synthetic Peptides

Five peptides corresponding to various stretches of primary amino acid sequence of tropomyosin as putative T cell epitopes based on our previous study [32] were synthesized and purified by HPLC. The peptides were numbered serially, the sequences of which are as follows: peptide 1 (NKEANNRAEKSEEEVHNQKRMNQQLNDLDQVQESLLKANN), peptide 2 (NRRIQLLEEDLERSEERLNTATTKL), peptide 3 (TATTKLAEASQAADSESRMRKVLN-RSL), peptide 4 (LAEEADRKYDEVARKLA-MVEA), and peptide 5 (KEYKYSITDELDTFSELSGY), spanning regions 29–69, 89–113, 108–135, 154–174, and 264–284, respectively, of the primary amino acid sequence of tropomyosin.

Analytical Methods

Protein estimation was done by Bradford's dye binding assay using crystalline BSA as a standard [33]. SDS-PAGE analysis was performed using Laemmli's discontinuous buffer system [34]. Electrophoresis was carried out at room temperature for 2 h at 120 V and 30 mA. Native PAGE analysis was performed under similar conditions but in the absence of SDS. The gels were stained with Coomassie brilliant blue R-250. Immunoblotting was carried out according to the procedure of Towbin et al. [35]. The immunoblot was probed with either a 1:10 dilution of serum from a shrimp-sensitive patient (for IgE) or a 1:1,000 dilution of serum from mice immunized with native tropomyosin (for IgG). Normal human serum or normal mouse serum (as the case may be) was used as a negative control.

Immunization of Mice

Female BALB/c mice (4–6 weeks old) were immunized with 200 µg of either maleyl or native tropomyosin adsorbed on alum (intraperitoneally). Mice were bled on day 10 after the intraperitoneal administration and serum IgG or IgE titers determined by AB-microELISA. Eight mice per group were taken for analysis of the antibody titers and experiments were repeated thrice.

Quantitation of Allergen-Specific IgG and IgG1/IgG2a Isotype of Antibodies

Shrimp allergen (tropomyosin)-specific IgG antibodies were determined by AB-microELISA [28]. Briefly, the allergen (native or modified) was immobilized on polystyrene microtiter wells at a concentration of 0.5 µg/well and incubated for 1 h at 37°C. The plates were washed and blocked with 2% goat serum in rinse buffer (PBS, pH 7.4 containing 0.05% Tween 20). Serial double dilutions of the serum were done and incubation carried out for 1 h at 37°C. Biotin-conjugated anti-mouse IgG (Sigma Chemical Co.) was added at a dilution of 1:3,000 in blocking buffer and incubated for 1 h. Horseradish peroxidase-avidin D conjugate (Vector Laboratories, Burlingame, Calif., USA) was subsequently added and incubated for 15–20 min. Color was developed using *o*-phenylenediamine (OPD) and hydrogen peroxide (H₂O₂) as the substrate and the reaction was arrested by adding 2 *N* hydrochloric acid. Absorbance was measured at 490 nm. For quantitation of IgG1 and IgG2a isotype of antibodies, the assay was similar to that used for total IgG except that biotin-conjugated γ -chain-specific anti-mouse IgG1 or IgG2a (Sigma Chemical Co.) was used in place of anti-mouse IgG. The G1/G2a ratios were calculated based on a linear regression analysis of the serum dilution curves.

Quantitation of Allergen-Specific IgE Antibodies

Tropomyosin-specific IgE antibodies were determined by AB-microELISA [28]. The antiserum against mouse IgE was a kind gift from Dr. Henry Metzger, NIAMS, NIH. The IgG fraction was purified by a 40% ammonium sulfate fractionation followed by dialysis against 0.1 *M* sodium acetate buffer (pH 4.1) to precipitate albumin. The clear supernatant was dialyzed against borate-buffered saline, pH 8.2, and chromatographed on a column of mouse IgE coupled to cyanogen-bromide (CNBr)-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The bound fraction was eluted using 4 *M* MgCl₂, dialyzed against borate-buffered saline, and passed over a column of mouse IgG coupled to CNBr-activated Sepharose to remove antibodies cross-reacting with mouse IgG. The affinity-purified Fc-specific anti-mouse IgE antibodies collected in the flow-through were conjugated to biotin using *N*-hydroxysuccinimide ester [36]. The reactivity of the biotin conjugate was assessed against both mouse

IgG as well as mouse IgE. A dilution of 1:600 where there was no cross-reactivity with mouse IgG was used in all assays.

Lymphocyte Proliferation Assays

For proliferation assays, mice were immunized with 200 µg of native or maleyl tropomyosin adsorbed on alum (intraperitoneally). Seven days after the immunization, mice were killed by cervical dislocation and the spleen excised aseptically. The splenocytes were isolated, red blood cells lysed and the resulting cell suspension cultured in Iscove's modified Dulbecco's medium (GIBCO-BRL, Grand Island, N.Y., USA) containing penicillin 100 U/ml, streptomycin 100 µg/ml, sodium bicarbonate 3 g/l, *L*-glutamine 2 mmol/l, 2-mercaptoethanol 5×10^{-5} mol/l, and 10% heat-inactivated fetal calf serum (GIBCO-BRL). The cells were plated at a density of 5×10^5 cells per well in 96-well, flat-bottom plates (Costar, Cambridge, Mass., USA) in the presence of dilutions of the antigen, and cultured for 96 h at 37°C in a 5% CO₂ atmosphere. The cultures were pulsed with 1 µCi/well ³H-methyl thymidine (Bhabha Atomic Research Center, Bombay, India) for the last 15 h of culture and harvested on glass fiber filters. The amount of radioactivity incorporated was measured by liquid scintillation counting. The results are expressed as mean $\pm$ SEM for each triplicate set.

Cultures for Cytokine Assays

In order to assay for cytokines, cultures were set up using splenocytes isolated 7–10 days after the primary immunization. The cells were plated at a density of 5×10^5 cells/well in the presence of various dilutions of the antigen or cultured with 30 µg/ml of synthetic peptides corresponding to various regions of native tropomyosin under similar culture conditions. The culture supernatants were harvested 60–72 h after initiation of the cultures and stored at –70°C until the cytokine assays were performed.

Assay for IL-4

The levels of IL-4 were assayed using CTLL-2 cell line. The starved cells were washed and plated at a density of 1×10^4 cells/well in the presence of various dilutions of the culture supernatants or a known reference standard for IL-4 (Genzyme, Cambridge, Mass., USA). The proliferation specific to IL-4 was assessed by the inclusion of 11B11 (HB188, ATCC), an anti-IL-4 monoclonal antibody, in the assay [37]. The cells were cultured for 24 h at 37°C in a humidified CO₂ incubator and pulsed with 0.5 µCi of tritiated thymidine per well for an additional 12 h. The 11B11 inhibitable counts were extrapolated to the concentration of IL-4 based on a standard curve drawn with known concentrations of IL-4.

Assay for IFN- γ

The IFN- γ levels in the culture supernatant were assayed by a monoclonal antibody-based sandwich ELISA (Genzyme). Briefly, plates were coated overnight with 1 µg/ml of the capture antibody in coating buffer (pH 9.6) at 4°C. Blocking was done for 2 h at 37°C with 1% fatty acid-free BSA (Sigma Chemical Co.). The culture supernatants were added to a volume of 100 µl and incubated overnight at 4°C. The biotin-conjugated secondary antibody was added at a concentration of 1 µg/ml and incubated for 2 h at 37°C. Horseradish peroxidase-avidin D was added at a dilution of 1:2,000 in dilution buffer, and color developed using *o*-phenylenediamine/H₂O₂ as the substrate. The reaction was arrested using 50 µl of 2 *N* HCl and absorbance measured at 490 nm. The levels of IFN- γ were quantitated based on a standard curve drawn with known concentrations of standard IFN- γ (Genzyme) under similar assay conditions.

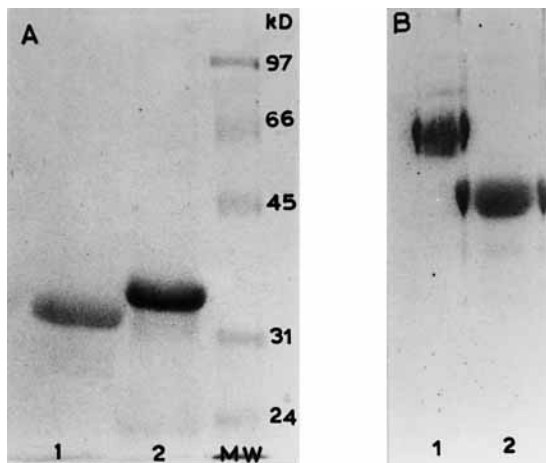


Fig. 1. Comparison of electrophoretic mobility of native and maleyl tropomyosin. Native or maleyl tropomyosin were electrophoresed on 12.5% polyacrylamide gels under denaturing conditions (SDS-PAGE) (**A**) or native, nondenaturing conditions (native PAGE) (**B**). **A, B.** Lane 1 = Native tropomyosin; lane 2 = maleyl tropomyosin; MW = standard protein molecular weight markers. Electrophoresis was performed as described in Materials and Methods.

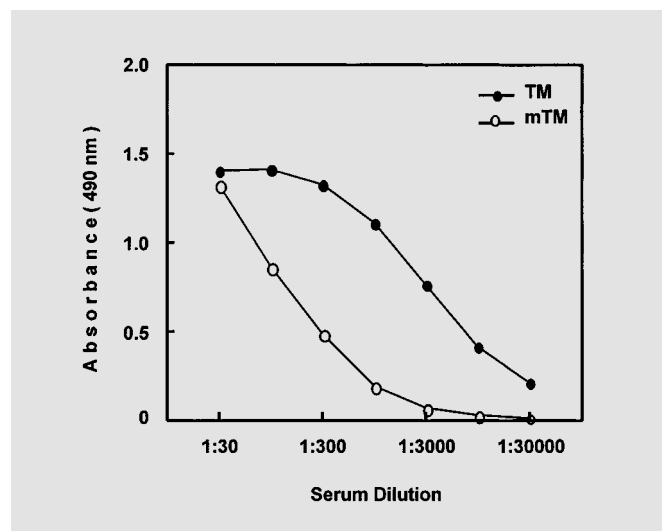


Fig. 2. Decrease in cross-reactivity with the native allergen following maleylation. Native (TM) or maleyl tropomyosin (mTM) was coated at a concentration of 5 µg/ml on 96-well microtiter plates and probed with various dilutions of mouse serum raised against native tropomyosin. The specific IgE binding to microtiter plate-coated TM and mTM was determined by AB-microELISA as described in Materials and Methods. The assay was performed in triplicates and the mean absorbance values are shown. Serum from normal unimmunized mice was used as a negative control.

Results

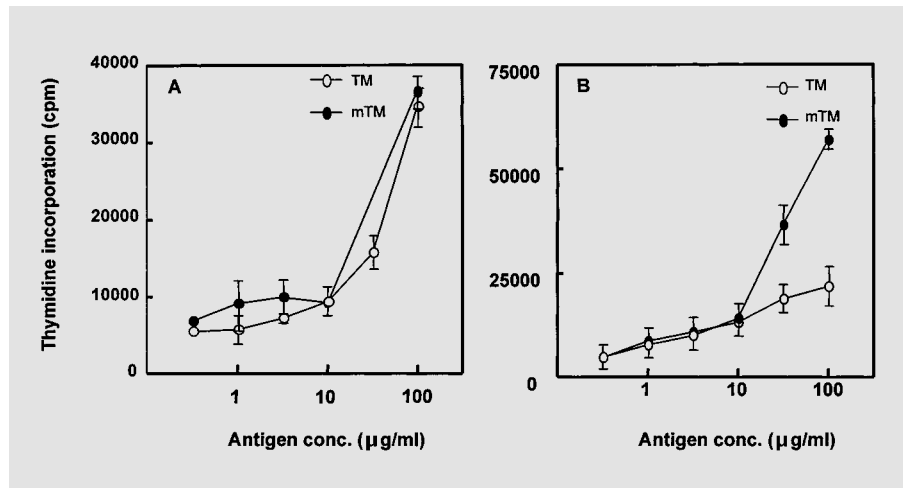
Immunochemical Characterization of Maleyl Tropomyosin

SDS-PAGE analysis of maleyl tropomyosin revealed a shift in mobility as compared to native tropomyosin showing a 2- to 3-kD increase in molecular weight due to the addition of maleyl groups to the protein (fig. 1A). Native PAGE analysis, however, showed that the maleylated form of tropomyosin migrated faster as compared to its native counterpart (fig. 1B). The enhanced anodic mobility of maleyl tropomyosin is due to the addition of negatively charged maleyl groups to the modified allergen. A comparison of the antigenicity of native and maleylated tropomyosin by ELISA revealed that the binding of mouse IgE antibodies raised against native tropomyosin to the latter was drastically reduced (fig. 2). A similar result was also observed with mouse tropomyosin-specific IgG (data not shown). A Western blot analysis using serum from a shrimp-sensitive patient also showed that there was a complete loss of reactivity to allergen-specific IgE antibodies (fig. 3). The above results suggest that there



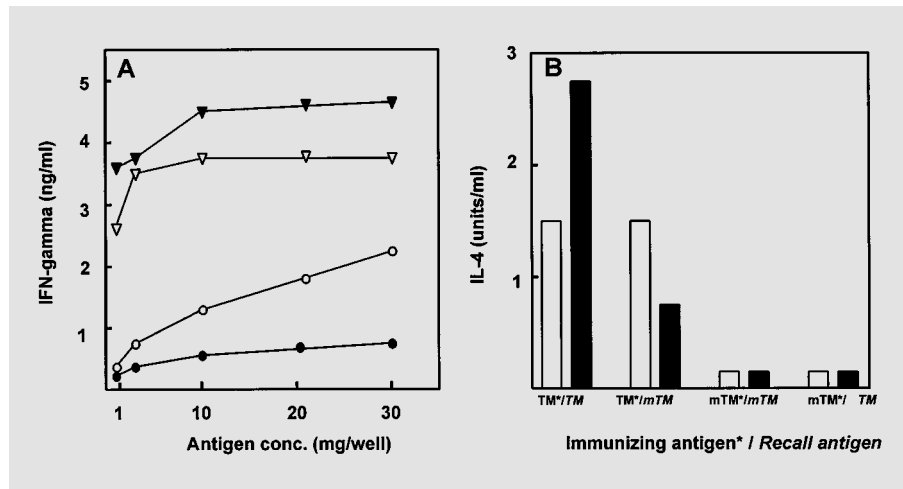
Fig. 3. Immunoblot analysis showing the loss of the ability of maleyl tropomyosin for binding to antitropomyosin IgE antibodies from the serum of a shrimp-sensitive patient. Maleyl and native tropomyosin were electrophoresed on 12.5% polyacrylamide gel in the presence of SDS and subsequently electroblotted to nitrocellulose. The protein blots were probed with a 1:10 dilution of serum from a shrimp-sensitive patient or a normal subject. The subsequent steps in the processing of the blot are described in Materials and Methods. Lane 1 = native tropomyosin; lane 2 = maleyl tropomyosin. The blots probed with normal human serum did not show any bands and are not shown.

Fig. 4. Proliferative response of splenocytes from mice immunized with native (TM) or maleyl tropomyosin (mTM) in a homologous or heterologous recall assay in vitro. Splenocytes from mice immunized with native (A) or maleyl tropomyosin (B) were cultured in vitro in the presence of titrating amounts of native or maleyl tropomyosin for 96 h and the proliferative response was monitored by ³H thymidine incorporation. Average background counts were 4,400 ± 200 cpm for mice immunized with maleyl tropomyosin and 3,900 ± 400 cpm for tropomyosin immune mice. The data are represented as a mean ± SEM of triplicate cultures.



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Fig. 5. Cytokine secretion profiles of splenocytes from mice immunized with native (TM) and maleyl tropomyosin (mTM) in homologous and heterologous assays in vitro. **A** IFN-γ levels from culture supernatants of splenocytes from mice immunized with maleyl (▽, ▼) or native tropomyosin (○, ●). Homologous recall is shown as white symbols and heterologous recall as black symbols. Titration doses of the respective antigens were used as indicated. **B.** Analysis of the same culture supernatants as in A for levels of IL-4 at antigen concentration of 10 μg/ml (□) and 30 μg/ml (■). The source of splenocytes is marked with an asterisk while the antigen used for the in vitro recall is italicized. Levels of cytokine in culture supernatants were determined by ELISA, as described in Materials and Methods.



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was an alteration of IgG as well as the IgE binding properties of the allergen following maleylation.

Characterization of T Cell Responses to Native and Maleyl Tropomyosin

Following immunochemical characterization of the maleyl tropomyosin the cellular responses were assessed by in vitro proliferation assays. Splenocytes from mice immunized with either the native or maleyl tropomyosin were cultured with homologous or heterologous antigen in recall assays in vitro. While mice immunized with maleyl tropomyosin show a better proliferative response to homologous antigen compared to the recall response to native tropomyosin (fig. 4A), splenocytes from mice immunized with native tropomyosin proliferate similarly to both forms of allergen (fig. 4B). These results suggest that the maleylated form of the allergen was recognized equal-

ly well by the splenocytes of mice immunized with native tropomyosin, unlike weak cross-reactivity observed at the B cell level.

Cytokine Profiles in vitro

The modulation of the immune response can be assessed in terms of the pattern of the cytokines' secreted sequel to the activation and proliferation of T cells. The polarization of the response in terms of patterns of secretion of the two predominant Th1 and Th2 cytokines, IFN-γ and IL-4, was therefore investigated. The levels of IFN-γ in culture supernatants of splenocytes isolated from mice immunized with maleyl tropomyosin were much higher than that observed in splenocyte cultures from immunized mice with native tropomyosin, regardless of the nature of the recall antigen – native or maleylated (fig. 5A). In contrast, the levels of IL-4 in culture

Fig. 6. Comparison of IFN- γ secretion of splenocytes from maleyl- and native tropomyosin-immunized mice in response to synthetic peptides of native tropomyosin. Splenocytes from mice immunized with native (TM) (A) or maleyl tropomyosin (B) were cultured in the presence of 30 μ g/ml of synthetic peptides corresponding to various stretches of native tropomyosin or with the corresponding homologous antigen (native or maleyl tropomyosin). The levels of IFN- γ were measured in the culture supernatants as described in Materials and Methods.

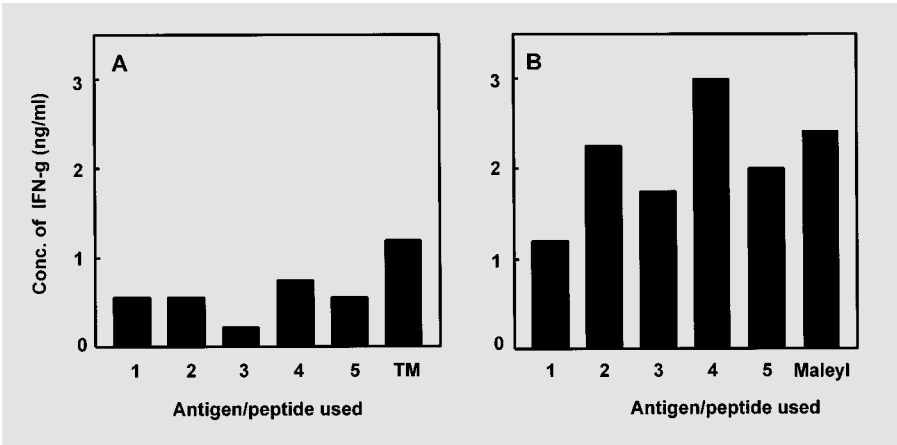


Table 1. Comparison of IgG1/G2a ratios in mice immunized with native and maleyl tropomyosin

	Maleyl tropomyosin			Native tropomyosin		
	IgG1	IgG2a	ratio	IgG1	IgG2a	ratio
1	503,931	5,886	40	2,067,898	20,325	101
2	153,084	947	155	548,319	1,235.5	443
3	2,586	100	25.86	62,636	754	83

Mice were immunized i.p. with 200 μ g of alum-adsorbed maleyl or native tropomyosin and serum IgG1 and IgG2a titers determined by AB-microELISA using isotype-specific secondary antibody. Mice were grouped based on their response as high, moderate and low responders. The G1/G2a ratios were calculated based on a linear regression analysis and values corresponding to absorbance values of 0.75 were chosen for calculation of the ratios. The ratios indicated are those obtained from a serum pool of 4 mice from each group classified on the basis of their serum IgG responses.

supernatants from the mice immunized with maleyl tropomyosin (<0.3 U/ml) were minimal as compared to those observed in mice immunized with native tropomyosin (>0.75 U/ml) (fig. 5B). Thus, immunization with maleyl tropomyosin resulted in an IFN- γ -dominant, Th1-like response instead of an IL-4-dominant Th2 response generated by native tropomyosin.

Synthetic peptides from the primary amino acid sequence of tropomyosin as putative T cell epitopes [32] selected based on a computer algorithm [38, 39] were used as recall antigens for analyzing cytokine responses from mice immunized with native or maleyl tropomyosin. IFN- γ secreted in response to peptide antigens was uniformly higher from mice immunized with maleyl tropomyosin as compared to the mice immunized with native tropomyosin (fig. 6A,B).

Antibody Isotype in vivo

The pattern of cytokine secretion is known to modulate the synthesis of a particular antibody isotype. Having observed an IFN- γ dominance in the cytokine profiles following immunization with maleyl tropomyosin, it was of interest to see whether the pattern of cytokines observed in vitro could be correlated in vivo with respect to the modulation of specific antibody isotypes. The results in table 1 are a representative of the titer values for IgG1 and IgG2a in mice grouped as high, moderate and low based on their antibody responses to the allergen. The results clearly show that in all the cases maleyl tropomyosin-immunized mice show a reduction in the G1/G2a ratio as compared to those immunized with tropomyosin, a characteristic outcome of an IFN- γ -dominant Th1-type response. The antibody isotype of consequence in allergic reactions is the IgE isotype. Results indicated that there

was a reduction in the production of specific IgE on immunization with maleyl tropomyosin, further emphasizing that the profiles of cytokines observed *in vitro* correlate to the isotype of antibody produced *in vivo* (fig. 7).

Discussion

Though exposure to allergens is a more or less uniform phenomenon, the induction of IgE responses following allergen exposure is determined by the qualitative nature of the T cell response. Advances in our understanding of the molecular and immunological basis of allergic diseases suggest a critical role for T-cells in the induction of IgE synthesis [40]. Naïve CD4⁺ T cells differentiate along a Th1 or a Th2 pathway depending on the mode and route of the antigen [41, 42], activation state of the APC and the presence of cytokines during T cell activation [43]. The Th2-derived cytokine, IL-4, is known to play a fundamental role in inducing and increasing the production of specific IgE antibodies [44, 45]. The profile of cytokines produced by antigen-specific CD4⁺ T cells has been proposed to determine the type or form of immune response that occurs *in vivo* [46, 47]. The concept that allergic disorders are caused by inappropriate cytokine profiles was suggestive of a possible strategy for specific immunotherapy based on the functional inactivation or deviation of the cytokine patterns of the specific T cells *in vivo*.

The present study, therefore, investigates the outcome of specific targeting of the major shrimp allergen, tropomyosin, to macrophage scavenger receptors. Cytokine profiles of the homologous recall assays indicated a modulation of the responses towards an IFN- γ -dominated, Th1-like response following immunization with maleyl tropomyosin (fig. 5A,B) while levels of IL-4 showed a reciprocal correlation. In addition, the *in vivo* antibody isotypes showed a low IgE and IgG1/G2a ratio in maleyl tropomyosin-immunized mice, further establishing that the cytokine profiles observed *in vitro* correlate well with the antibody isotype produced *in vivo*. Results with synthetic peptides corresponding to predicted T cell epitopes on native tropomyosin [32] indicated an IFN- γ -dominated cytokine profile following immunization with maleyl tropomyosin (fig. 6A,B). Regulatory cytokines such as IL-10 for Th2 responses and IL-2 and TNF- β for Th1 responses have an important role to play in the polarization of the helper T cell responses. Though the results on recall assays with peptides conform to an IFN- γ -dominated Th1-like response following maleyl tropomyosin immunization, the response pattern of other cytokines

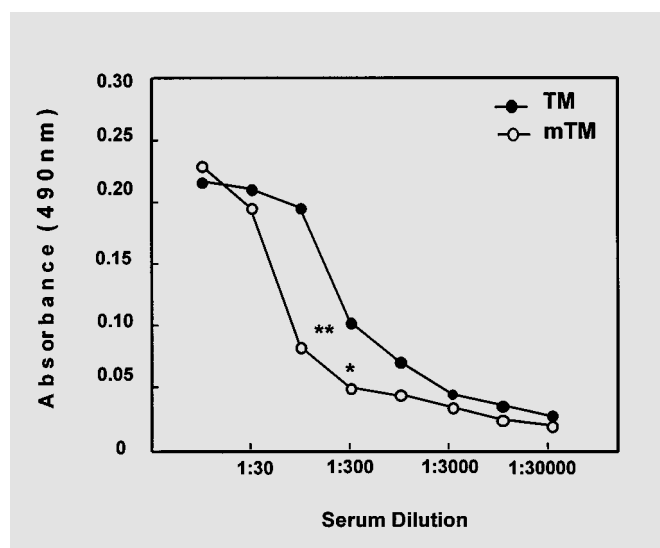


Fig. 7. Representative serum dilution curve showing a reduction in the *in vivo* serum IgE titers in mice immunized with native and maleyl tropomyosin. Female BALB/c mice were immunized i.p. with 200 μ g of alum-adsorbed native (TM) or maleyl tropomyosin (mTM). Mice were bled on day 10 after administration of the antigen and serum IgE titers assessed by AB-microELISA. The curves depicted represent the profile from a serum pool of 4 mice. Serum from normal nonimmunized mice used as a negative control showed almost negligible absorbance values (<0.006) and hence is not shown. * $p < 0.05$, ** $p < 0.001$.

needs to be assessed. Results of the cytokine profiles observed in heterologous recall assays, however, suggest that although administration of maleyl tropomyosin does lead to a skewing of the cytokine profiles to an antiallergic IFN- γ -dominant phenotype, the possible potential ability of maleyl tropomyosin to divert an already established allergic phenotype would have to be based on a detailed study of the cytokine profiles of other regulatory cytokines as well.

The current mode of therapy for allergic inflammatory responses is 'hyposensitization' by the administration of increasing doses of native or crude allergenic extracts [48, 49]. A major point of concern in such a therapeutic strategy is the possible induction of local or systemic anaphylaxis due to the binding of the native allergen to the circulating IgE antibody molecules [50, 51]. Our studies indicate that the maleylated allergen is unable to bind IgE antibodies directed against the native allergen (fig. 2, 3) but is potent in terms of its cellular functions (fig. 4A,B) in being able to bring about a modulation to an antiallergic phenotype, or an IFN- γ -dominant response to the allergen (fig. 5). Further, immunization with maleyl tro-

pomyosin resulted in considerably low IgG1/G2a ratios (table 1) as well as reduced titers of IgE (fig. 7) in vivo as compared to the native allergen. The normal course of an allergic reaction is accompanied with an observed 4- to 5-fold increase in the serum IgE titer that manifests in an allergic inflammatory reaction. Consequently, a 5-fold reduction in the in vivo serum IgE observed in the mice immunized with maleyl tropomyosin is of significance. Preliminary experiments also demonstrate that preadministration of maleyl tropomyosin resulted in a marked decrease in the induction of tropomyosin-specific IgG as well as IgE in a primary response in vivo. Although a direct extrapolation from these data to a clinical situation may not be possible, the results nevertheless suggest the

possible potential of targeting allergens to macrophages as a means of a modulation of allergic responses in vivo in addition to reemphasizing the regulatory role of APC in directing T cell effector responses in general.

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