



Identification of the amino acid residues in trichosanthin crucial for IgE response[☆]

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Abstract

Trichosanthin (TCS) is the major effective component from Chinese herb *Trichosanthes kirilowii*. TCS has been approved to be effective in clinical treatment of HIV infection and leukemia, but its allergenicity has limited its clinical usage. To identify amino acid residues in TCS with an important role in IgE induction, TCS-specific IgE mAb (TE1) was used to serve as a probe and TE1 epitope was determined by a random phage-peptide library. Based on phage peptide sequences, TE1 epitope was predicted at amino acid residues 169–174 (**QQIGKR**) of TCS protein. Based on modeling data, two amino acids (Lys173 and Arg174) on TCS were considered to have a crucial role in binding to TE1. After lysine 173 and arginine 174 were mutated to glycine, the mutant TCS protein specifically lost the binding activity to TE1 mAb and exhibited reduced IgE induction in the immunized mice. The data showed that the IgE epitope of TCS was determined and shown to play a critical role in induction of IgE, and the modification of IgE-epitope may be a useful strategy to reduce the allergenicity of an allergen. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Trichosanthin; Allergen; IgE-epitope; Phage-peptide library; Mutation

Immunoglobulin E (IgE) is believed to be one of the major mediators of immediate hypersensitivity reactions that underlie atopic conditions such as urticaria, seasonal allergy, asthma, and anaphylaxis, which represents a health problem for more than 20% of the population in industrialized countries [1]. Factors that control IgE production are therefore essential to the pathogenesis of these important afflictions [2]. The nature of allergens plays a determining role in induction of IgE. A complete understanding of the structure of allergen and IgE induction is very important to prevent IgE-mediated reactions.

Trichosanthin (TCS), an active plant protein extracted from Chinese herb of *Trichosanthes kirilowii*, has

been used as a traditional Chinese medicine for inducing abortion for a long time and has a variety of medical functions in clinical practice. It has been showed that TCS is able to inhibit tumor growth and human immunodeficiency virus (HIV) replication [3–7].

However, TCS is a strong allergen and can cause anaphylaxis in the patients, and the TCS-specific IgE-secreting cells have been detected even three years after the stop of TCS treatment [8]. To investigate the mechanisms of IgE production induced by TCS in human, we have developed an animal model in which TCS alone can induce TCS-specific IgE response in C57BL/6 mice without using any adjuvant, which is more closer to the human allergy reaction [9]. Our animal model provides an invaluable tool to study general IgE induction mechanisms in vivo.

Our previous work has demonstrated that IL-4, IL-13, and CD40L expression has been increased in draining lymph node cells of TCS-immunized mice, which are involved in IgE induction [10]. Furthermore, TCS was able to inhibit IL-12 and TNF- α and promote

[☆] Abbreviations: TCS, Trichosanthin; mAb, monoclonal antibody; 9aa, nine amino acids; wTCS, wild-type TCS protein; mTCS, mutant TCS protein; nTCS, native TCS protein.

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IL-10 and MCP-1 expression in TCS-treated murine macrophages [11]. All these data suggested that IgE-induction in TCS-immunized mice is related to the properties of TCS structure. Since TCS crystal structure is available, we have succeeded in identifying the Ag epitopes by structural prediction as well as by using random phage-peptide library, TCS protein expression, and site-directed mutagenesis [12,13].

In this study, we focus on analysis of the IgE-epitopes of TCS and intend to address the correlation between IgE induction and the structure of TCS IgE-epitopes. TCS-specific IgE mAb (TE1) was selected as a probe to screen a random phage-peptide library for the clones that mimic the IgE-epitopes of TCS. After comparing the sequences of phage peptides with TCS, the sequence of IgE-epitope that corresponds to TE1 was predicted on the three-dimensional structure of TCS. The predicted TE1-TCS binding sites were confirmed by site-directed mutagenesis. Our data demonstrated that the IgE-epitope of TCS has been identified and the mutated TCS protein lost the binding activity to TE1 in ELISA assay and reduced IgE induction in immunized mice. The results clearly show that IgE-epitopes of allergen play a crucial role in induction of IgE and that few amino acids that were mutated in IgE-epitopes will eliminate the IgE production.

Methods

MAb and reagents. TCS-specific monoclonal antibody (TE1) was made in our laboratory [14]. It was purified through affinity chromatography using TCS-Sepharose 4B conjugate column (constructed according to the instructions of Pharmacia, USA) from the cultured supernatant of hybridoma. Crystallized TCS in Trichosanthin injection solution (1.2 mg/ml) was produced by Jin-Shan pharmaceutical Factory (Shanghai, China). Random phage-peptide libraries [13,15] expressing linear (pVIII 9 aa) nine peptides fused to pVIII of filamentous bacteriophage fd, vector pC89, wild-type phage f1, and bacterial strain DH5 α F' were all provided by Dr. Paolo Monaci of IRBM (Istituto di Ricerche di Biologia Molecolare P. Angeletti SPA, Rome, Italy).

Mice and IgE induction. C57BL/6J mice, female, 9–12 weeks old, were obtained from the Animal Center of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The animals were breed according to the institutional guidelines. Experiments were carried out as previously described [9]. Briefly, the mice were immunized by i.p. injection with 0.5 ml TCS solution (native TCS protein (nTCS), wild-type TCS protein (wTCS), and mutant TCS protein (mTCS) 5 μ g/per mice, respectively) for three times on days 0, 14, and 28. The mice were bled on 7 days after last immunization. The immunized serum was used to test specific binding activity with TCS protein in ELISA. The sera were diluted at 1:100. Each control or experimental group consisted of five mice. Each experiment was repeated at least twice.

Affinity selection. Four rounds of affinity selection were performed as follows: 200 μ l TE1 mAb in coating buffer (50 mM NaHCO₃, pH 9.6) at 2 μ g/ml was incubated in 96-well plate (Immuno plate Maxisorp, Nunc) at 4°C overnight and then blocked with blocking buffer (1 $\times$ PBS, 3% BSA, and 0.05% Tween 20) at 37°C for 1.5 h. Wild-type phage f1 (10^{11}) in 200 μ l blocking buffer was added to the well and incubated at room temperature for 1 h. The plate was washed exten-

sively with PBST (1 $\times$ PBS, 0.1% Tween 20). Then, about 1×10^{10} phages from 9 aa peptide library in 200 μ l blocking buffer were added to the well and incubated at room temperature for 2 h. After washing, the absorbed phages were eluted and neutralized with Tris-HCl as described [13]. The eluted phages were amplified by infecting DH5 α F' and purified with 20% PEG/2.5 M NaCl precipitation, which could be stored for latter immuno-screening. The purified phages were used for next round of affinity selection.

Immuno-screening. After four rounds of affinity selection, DH5 α F' cells were infected with eluted phages at a multiplicity of infection (m.o.i.) of 10^{-3} . The mixture was incubated at 37°C for 30 min. Then, helper phage M13 K07 was added at an m.o.i. of 20–50 and incubated at 37°C for another 15 min. The infected bacteria were centrifuged for 5 min at 3000g. The supernatant was discarded and bacteria pellet was washed three times in 1 ml LB medium to eliminate the non-absorbed phages. One hundred μ l resuspended pellet with serial of dilutions (10^{-3} , 10^{-4} , and 10^{-5}) was plated on LB plates containing ampicillin (100 μ g/ml), kanamycin (50 μ g/ml), and IPTG (30 μ g/ml). After incubation at 37°C overnight, the plates containing 200–400 separated clones (10-cm plate) were layered with nitrocellulose filters and marked with needle. The filter was taken out immediately and blocked with blocking buffer (5% non-fat milk powder, 1 $\times$ PBS, 0.05% Tween 20, and 0.05% NaN₃) at room temperature for 2 h. The mAb (10 μ g) was preincubated with 25 μ l bacterial extract and 25 μ l f1 Phage (2.3×10^{13} /ml) in 5 ml blocking buffer at room temperature for 1 h and then was added to the blocked filters. Filter with mAb mixture was then incubated at room temperature for 1.5 h and then extensively washed with washing buffer (1 $\times$ PBS, 0.05% Tween 20). Then, secondary antibody (alkaline phosphates-conjugated goat-anti-mouse Abs, Sigma) diluted to 1/5000 in blocking buffer was incubated with the filter for 1.5 h at room temperature. The filters were then washed as above and developed by incubation with developing solution (330 μ g/ml nitroblue tetrazolium, 165 μ g/ml 5-bromo-4-chloro-3-indolylphosphate in 100 mM Tris-HCl, 100 mM NaCl, and 5 mM MgCl₂, pH 9.6) at room temperature for 10 min. Reaction was stopped by washing with water.

Positive phage clones were screened by ELISA. In brief, multi-well plates (Immuno plate Maxisorp, Nunc) were coated overnight at 4°C with TE1 at a concentration of 2 μ g/ml in 50 mM NaHCO₃, pH 9.6. After washing several times with PBS/0.05% Tween 20 (PBST), plates were incubated at 37°C for 60 min with ELISA blocking buffer (5% non-fat dry milk in PBST). Sample phages (4×10^9) were diluted in 100 μ l blocking buffer, then added to each well, and allowed to bind for 1 h at 37°C. The equal amount of wild-type phages was used as a negative control. Plates were then washed with PBST and 100 μ l/well rabbit anti-phage IgG HRP-conjugated antibodies (Sigma, 1/5000 dilution in ELISA blocking buffer) were added. After incubation for 1 h at room temperature, plates were then washed, developed by adding 100 μ l substrate TMB-H₂O₂, and incubated at 37°C in dark for 15 min. Optical density was measured in an ELISA reader (Bio-Trk, USA) at 450 nm.

DNA sequencing. Single-stranded DNA of the positive clones was extracted from the amplified phage using DNA Purification Kit according to the instructions (Promega). Sequencing was performed by the Sanger dideoxy method in Genecore, Shanghai.

Site-directed mutagenesis. Site-directed mutagenesis was performed according to our previous work [13]. Briefly, mutated primers were designed according to the experiments. For TE1 epitope mutation, the primers MF and MR were used. A pair of primers F and R were used to amplify the whole-length TCS gene. MF: 5' ATTGGGGGGGGT GTTGACAAAACCTTC 3'; MR: 5' GTCAACACCCCCCCCCAA TTTGTTGCTC 3'; F: 5' GGATCCATGGATGTTAGCTCCG 3'; R: 5' GAATTCATGCCATATTGTTTC 3'.

Protein expression and purification. By site-directed mutagenesis, the TCS gene and mutant TCS gene for expression were digested with *Eco*RI and *Bam*HI, cloned into pP_{RO}EX-HTb (Gibco), and then transformed to *Escherichia coli* SG13009. The transformed *E. coli*

strain was cultured at 30 °C in 250 ml LB medium with ampicillin and kanamycin until the cell density reached OD₆₀₀ = 0.6. Then, the IPTG was added to a final concentration of 0.1 mM and the cells were cultured at 30 °C for 2 h and collect the bacteria. Resuspend all pellets in 8 ml lysis buffer for native purification and sonicate in 10 min and 30 s with 30 s pause at 120 W. Centrifuge lysate at 6500 rpm at 4 °C for 10 min. Collect the supernatant and save on ice. Resuspend the pellet in 5 ml lysis buffer for assay. Add 1 ml of 50% Ni-NTA agarose to the supernatant and mix gently by shaking at 4 °C for 60 min. Wash the resin in column with 3 ml wash buffer and elute the protein for three times with 1 ml elution buffer.

Western blotting. Run 15% SDS-PAGE, load 10 µl sample each well, then run under 50 V until the marker enters the separation gel, adjust the run voltage to 130 V, and run for another 2 h. Transfer the protein onto the PVDF membrane with Bio-Rad equipments, under 90 V for about 1 h in ice. Dry the membrane at room temperature for about 15 min. Wash the membrane twice with TBS, 10 min each time. Block the membrane with 3% BSA for 1 h. Wash the membrane with TTBS (0.1% Tween 20 in TBS) for about twice each 10 min. Wash the membrane with TBS for another 10 min. Block the membrane with mouse anti-His-tag antibody for 1 h. Repeat wash procedure. Block the membrane with goat-anti-mouse IgG-HRP-conjugated antibody (Sigma) for 1 h. Repeat wash step four times with TTBS each 10 min and the membrane was developed by developing solution.

Analysis of RNA N-glycosidase activity. Ribosomes were isolated from rat liver as previously described [16]. One point five A₂₆₀ units of ribosomes (27 pmol) were incubated with TCS in 50 ml buffer (Tris-HCl, 25 mM, pH 7.6, 25 mM KCl, and 5 mM MgCl₂) at 37 °C for 15 min. The reaction was stopped by adding 5 ml of 10% SDS solution and then kept in ice-bath. Ribosomal RNAs were extracted with phenol-chloroform and precipitated with ethanol. After treatment with acidic aniline at 60 °C for 15 min, the ribosomal RNAs were separated by 8 M urea-denatured PAGE (3.5%). The RNA bands were stained by methylene blue.

Serum IgG and IgE were determined by ELISA. The plates with 96 wells were coated with 20 µg/ml antigen overnight at 4 °C with coating buffer. After washing several times with PBST, plates were incubated at 37 °C for 60 min with ELISA blocking buffer (3% BSA in PBST). Then add goat-anti-mouse IgE-Biotin, streptavidin-HRP, or goat-anti-mouse IgG HRP-conjugated antibodies, add 50 µl substrate TMB-H₂O₂ and incubated at 37 °C in dark for 15 min. Optical density was measured by ELISA reader (Bio-Tek, USA) at 450 nm.

Results

The phage clones that carried peptide-specific binding to TE1 were selected from phage-peptide library

After four rounds of affinity selection and immunoscreening, mAb TE1 binding phage clones were picked out and amplified. The binding activity of selected clones to TE1 mAb was assayed by ELISA and the sequences of the clones were analyzed by sequencing in Table 1. The results showed that 19 positive clones had relatively higher specific binding activity to TE1 mAb. According to their sequence analysis, all 19 clones were divided into three groups. It was interesting to observe that the 16/19 clones showed an identical peptide sequence (**DLHQQIHGR**) and other 3/19 clones had two different sequences. Although three groups had the different sequences, but all of them were able to bind to TE1 as determined by ELISA. Based on the peptide

Table 1

IgE-binding peptides selected from the phage-displayed random-peptide library

Sequence	No. of selected clones	ELISA (OD units)
DLHQQIHGR^a	16	0.66 ± 0.03
RELRYLRRA	2	0.53 ± 0.02
RRPMHDYPA	1	0.49 ± 0.01

Nineteen phage clones that had relative high binding activity to TE1 mAb were selected. Their sequences were analyzed and their specific binding activities to TE1 were determined by ELISA.

^a The selected clone appeared with high frequency.

sequences among three groups, it failed to find a conserved sequence or motif. The results suggested that the available information of Ag structure and sequence are required to further identify the Ag epitope.

The IgE-epitope that confers to binding TE1 was predicted on the three-dimensional structure of TCS

Since the crystal structure of TCS is available [17], the peptide sequences were compared with the TCS sequence by using computer software produced by R. Sayle, which can be freely downloaded from Internet. The data revealed that a peptide sequence (**DLHQQIHGR**), which represents most clones (16/19 clones), has a sequence (**QQIXXR**) which is similar to the sequence (**QQIGKR**) that was located at amino acid residues 169–174 of TCS protein (Fig. 1). The results suggested that the IgE-epitope of TCS that corresponds to TE1 can be predicted in the TCS protein.

The predicted IgE-epitope of TCS was confirmed by site-directed mutagenesis analysis

To confirm whether the predicted IgE-epitope of TCS is correct, the IgE epitope that corresponds to TE1 was

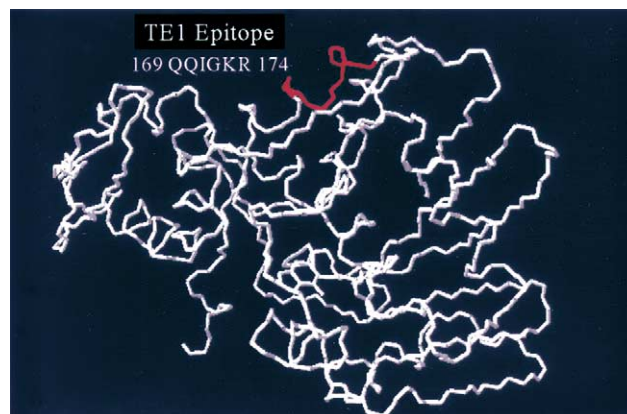


Fig. 1. The three-dimensional structure of predicted IgE-epitope recognized by mAb TE1 in TCS protein. The structure is generated by using software R.Sayle. The sequence of TE1 epitope (QQIGKR, red line) was predicted and it was located at positions 169–174 amino acid residues of TCS protein.

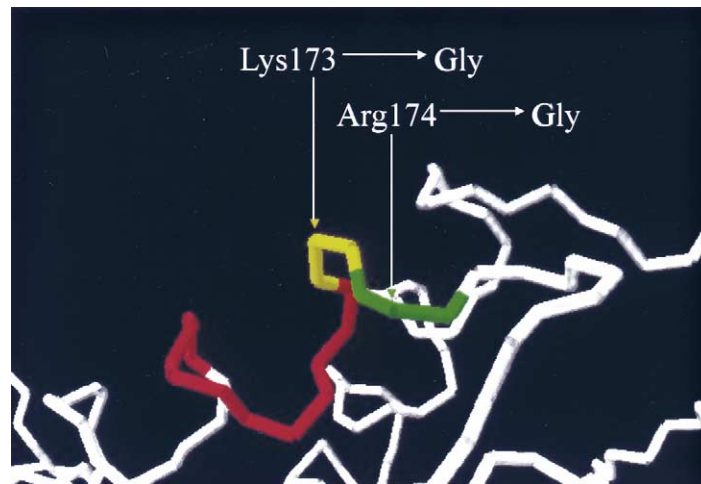


Fig. 2. Lys173 and Arg174 were mutated to Gly in the predicted TE1 epitope of TCS protein.

mutated by site-directed mutagenesis. Our previous work has demonstrated that TE1 epitopes have been predicated on two sites in TCS protein, one of them is located at amino acid residues 172–178 (GKRVDKT) of TCS by molecular modeling analysis, which was determined by analysis of the preferable interaction between TCS and the complementarity-determining region (CDR) loops of TE1 [18,19]. When we compared TE1 epitopes deduced by phage-peptide library (169 QQIGKR 174) and modeling analysis (172 GKRVDKT 178), the residues Lys173 (K) and Arg174 (R) had long and positively charged side-chains, which can form a hydrophilic surface with higher polarity than other residues. Those two amino acids are likely to play a crucial role in determining the binding activity to TE1. To confirm this prediction, the TE1 corresponding epitope on TCS was mutated as follows: Lys173 → Gly, Arg174 → Gly (Fig. 2). The DNA sequence of the mutant was verified by DNA sequencing.

To express the TCS protein, the expressing vector containing the TCS gene and mutant TCS gene was transformed into *E. coli*. After expression and purification, Western blotting was used to detect the recombinant wTCS and mTCS (Fig. 3). Since the RNA N-glycosidase activity is very important for the function of TCS protein, initially the RNA N-glycosidase activities of wTCS and mTCS were determined by using an in vitro rat liver ribosome system. As shown in Fig. 4, nTCS, wTCS, and mTCS were incubated with rat 80S ribosome and treated with acidic aniline, while the specific R-fragment was detected by urea-denatured PAGE. The data showed that all of them exhibited the RNA N-glycosidase activity, suggesting that the expressed

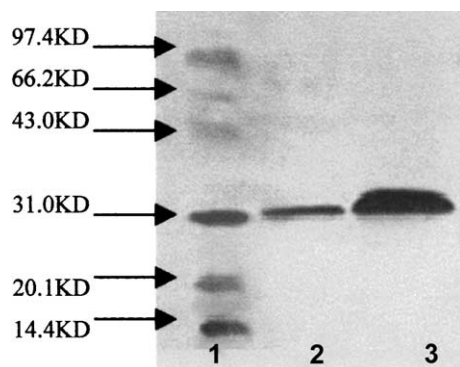


Fig. 3. The expressed wTCS and mTCS proteins were confirmed by Western blotting analysis. The recombinant wild-type TCS protein (wTCS) and mutant TCS protein (mTCS) were analyzed by SDS-PAGE gel electrophoresis and transferred onto membrane. Then, they were detected with mouse anti-His-tag Ab and anti-mouse IgG-HRP-conjugated Ab. Lane 1: protein marker. Lane 2: wTCS. Lane 3: mTCS.

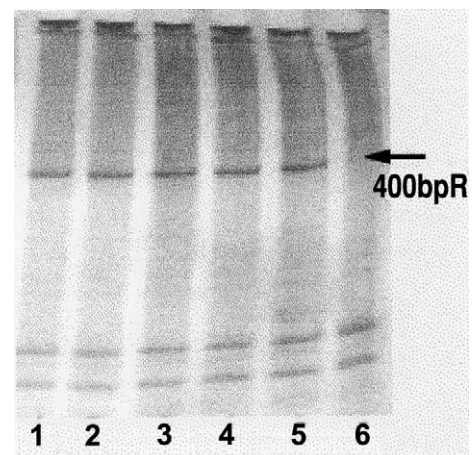


Fig. 4. The expressed wTCS and mTCS proteins had the TCS's functional characteristics of RNA N-glycosidase activity. The in vitro rat liver ribosome system was used to determine the RNA N-glycosidase activity of TCS. nTCS, wTCS, and mTCS all exhibit RNA N-glycosidase activity, only the specific R-fragment is detected by urea-denatured PAGE. Lanes 1–2: wTCS; lanes 3–4: mTCS; lane 5: nTCS to serve as positive control; and lane 6: negative control.

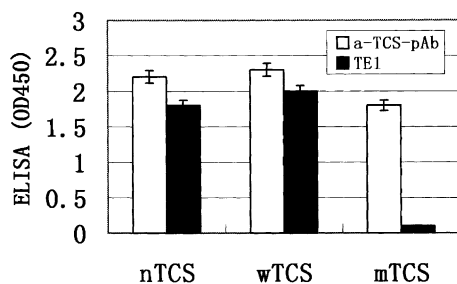


Fig. 5. The mutated TCS protein retained the binding activity to TCS-specific polyclonal Abs, but lost the specific binding activity to TE1. TCS-specific polyclonal Abs were collected from TCS-immunized mice and the wTCS and mTCS-specific reactivities to polyclonal Abs (IgG) and TE1 mAb (IgE) were measured by ELISA.

wTCS and mTCS proteins had a similar functional activity to nTCS.

Second, the expressed protein's (wTCS and mTCS) specific-binding activities to Abs were determined by ELISA. The TCS proteins (nTCS, wTCS, and mTCS) were coated on the plate and their specific binding activities to IgG polyclonal Abs of anti-TCS from nTCS immunized mice were determined by ELISA. The data showed that wTCS and mTCS had a similar binding activity to the IgG. In contrast, when mAb TE1 was selected to determine its binding activity to nTCS, wTCS, and mTCS proteins by ELISA, only mTCS significantly lost its binding activity to mAb TE1, but the nTCS, wTCS proteins retained binding activity to mAb TE1 (Fig. 5). The data suggest that the predicted TE1 epitope (169 QQIGKR 174) identified by phage-peptide library is correct.

The mutant TCS protein exhibited reduced allergenicity in TCS-immunized mice

Since TCS immunized mice were able to induce TCS-specific IgE Ab. This system allows us to understand the antigenicity of TCS in vivo with whole protein and especially TCS-specific IgE-epitopes. To evaluate the allergenicity of mTCS in vivo, mice were immunized with

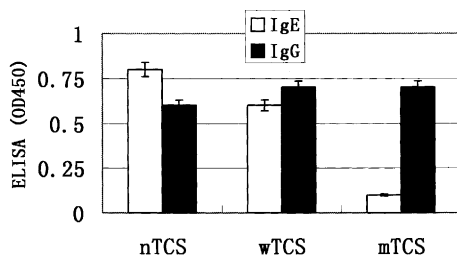


Fig. 6. The TE1-epitope mutated TCS protein was capable of inducing IgG production, but eliminated the ability to induce IgE production. nTCS, wTCS, and mTCS were used to immunized mice and serum was collected on day 7 after third immunization. The TCS-specific IgG and IgE levels in the serum were determined by ELISA.

mTCS and mTCS-induced IgE and IgG Abs in the serum were determined by ELISA. It was obviously observed that mTCS lost the ability to induce IgE production in immunized mice, but maintained the ability to induce IgG production in immunized mice (Fig. 6). In the control groups, both Ag-specific IgG and IgE Abs were detectable in nTCS and wTCS immunized mice. The data indicated that IgE-epitope plays a crucial role in IgE induction and that few amino acids that are mutated in the IgE-epitope were able to reduce allergenicity of the allergen.

Discussion

IgE response is frequently induced by allergens. The IgE-mediated allergic reactions are multiple steps. The IgE secreted by B cells binds to the cell surface of IgE receptors on mast cells and basophils. After re-exposure to the specific multi-valent allergen, the fixed IgEs are cross-linked by the allergen, leading to the release of inflammatory mediators. The regions of an allergenic molecule capable of cross-linking IgE are defined as epitopes, most of them are discontinuous and consist of amino acid residues not contiguous in the primary structure but are brought together by the folding of the polypeptide chain [20]. Therefore, primary allergen sequences can provide only information regarding the molecular native allergen and three-dimensional structure of allergen is essential to identify the potential epitopes in an allergen [21]. Since IgE-epitopes play a determining role in triggering the inflammatory response, the allergenic epitopes are potential candidates for immunotherapy and diagnosis [22].

Our previous work has demonstrated that TCS is a strong allergen and TCS immunization alone can induce TCS-specific IgE response in mice without using any adjuvant [9]. Despite the extensive researches concerning the regulatory mechanism involved in specific IgE induction, only a few studies have dealt with the identification of TCS-specific IgE-epitopes.

The monoclonal Abs have been widely used in structural and functional analysis. Analysis of Ab–Ag complexes by X-ray crystallography [23,24], chemical modification [22], or deuterium exchange analysis by nuclear magnetic resonance [26] has provided a detailed picture of the amino acids that comprise a given epitope. Few Ab–Ag complexes have been described in such detail because these methods require substantial effort and are technically difficult. Recent alternative techniques for epitope identification have been established, such as large libraries of random peptides displayed on the surface of filamentous bacteriophage [27,28] and chemically synthesized [29,30]. However, most studies demonstrated only the sequence of epitopes in given Ag, but rare study provided structure information of Ag due

to the lack of a three-dimensional structure of Ag protein.

In this study, initially one TCS-specific IgE mAb (TE1) was chosen due to its high affinity to TCS protein. After the screening by phage-peptide library, many phage clones binding to TE1 mAb were selected by the random-peptide library technique. To evaluate whether the sequences of peptide from the phage clones mimic TCS, ELISA was performed in individual clone with mAb (TE1). The results suggest that the selected clones were able to bind to mAb (TE1).

Second, to determine whether the peptide sequence of phage clones is similar to epitopes of TCS, all the sequences of selected clones were deduced by DNA sequence analysis. It was interesting to find that a peptide sequence of DLHQQIHGR was generated in a higher frequency in the selected phage clones in which the sequence of QQIXXR was predicted to locate at amino acid residues 169–174 (QQIGKR) of TCS protein. Based on our previous modeling work, the epitope of mAb TE1 has been predicted at amino acid residues 172–178 (GKRVDKT) of TCS protein [15,16]. Based on physical–chemical properties of amino acids, lysine (K) 173 and arginine (R) 174 carry a polar group and locate on the surface of TCS protein. They may contribute to binding activity between mAb TE1 and its corresponding IgE-epitope on TCS protein.

To further prove that our speculation is correct, site-directed mutagenesis analysis is performed in the TE1 epitope that was predicted on the surface of TCS protein. The amino acid residues of lysine and arginine in the positions 173 and 174 of TCS were mutated to glycine. When expressed wTCS and mTCS proteins were used to test the binding activity to TCS-specific polyclonal Abs (IgG) and mAb TE1 (IgE), expressed wTCS protein showed a relatively high binding activities to both of them. Conversely, expressed mTCS lost the binding activity to TE1 mAb, but the binding activity to TCS-IgG Abs was retained. The data suggest that the mTCS protein in TE1 epitope eliminated only its specific binding reactivity to TE1, but maintained the binding activity to IgG-Abs of TCS. The results confirmed that the predicted TE1 binding sites (Lys173 and Arg174) are correct in the three-dimensional structure of TCS protein and indicated that the portion of TCS protein from 169 to 174 amino acid residues is recognized by mAb TE1 (IgE). This region in TCS structure may be very important for forming the epitope required for IgE-binding. It may also be the crucial structure to induce allergenicity in TCS protein.

The relationships between allergenic structure and IgE induction have been studied in past decades. It is suggested that the allergens cannot be classified as the same subpopulation, because they have no characteristic structural features [20,31]. To date, the most research works were only limited to epitope-binding

activity with Abs by using site-directed mutagenesis [32,33].

To address whether IgE-epitopes play a determining role in IgE induction in vivo, the allergenicity of mTCS was determined in mice. When Ig isotypes were measured by ELISA, nTCS, wTCS, and mTCS-immunized mice produced comparable anti-TCS IgG levels. In contrast, mTCS-immunized mice significantly reduced IgE production, but nTCS- and wTCS-immunized mice had relatively higher levels of anti-TCS IgE Ab. Our data demonstrate that IgE-epitope is very important to induce IgE production.

In summary, TCS is a potent allergen. Its IgE-epitope has been successfully identified by using phage-peptide library and the available three-dimensional structure of TCS. TCS can be converted into hypo-allergic immunogens by the amino acid alteration in the IgE epitope. The mutated TCS protein has a low allergenicity, but maintains other functions such as ribosome-inactivating activity, which is an essential for its therapeutic effects on AIDS and tumor treatment. So the mutant protein can be used in clinical treatment by reducing the risk of anaphylaxis. Our work highlights the possibility that site-directed mutagenesis in IgE-epitopes may represent a useful strategy to modify the structure of allergic drug and food and to reduce their side effects in induction of allergic reaction during treatment or eating.

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