

A peptide binding to dimerized translationally controlled tumor protein modulates allergic reactions

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Abstract Translationally controlled tumor protein (TCTP) is believed to be involved in a variety of inflammatory processes: secretion of histamine and cytokines such as IL-4, IL-8, IL-13, and granulocyte/macrophage colony-stimulating factor; chemoattraction for eosinophils; augmentation of B cell proliferation; and immunoglobulin production, thereby potentially regulating allergic phenomena. In a previous study, we showed that the cytokine-releasing activity of extracellular TCTP is generated only when TCTP dimerizes via the intermolecular disulfide bond of NH₂-terminal truncated TCTP implying that the dimerized TCTP (dTCTP) promotes the inflammatory phenomena. Modulation of dTCTP, thus, may offer a strategy for the treatment of chronic allergic diseases. In this study, we searched for dTCTP-binding peptides (dTBP) by screening a phage-displayed 7-mer peptide library. We identified one peptide in the library, designated as dTBP2, which showed higher affinity to dTCTP than to full-length, monomeric TCTP. dTBP2 inhibited the induction of IL-8 by dTCTP from BEAS-2B

cells. dTBP2 also reduced symptom score and eosinophil infiltration in a mouse rhinitis model. This study suggests that the dTBP2 binding to dTCTP modulates the release of inflammatory mediators of dTCTP. This result may provide a rational strategy for the treatment of allergic diseases.

Keywords Anti-allergic peptide · dTCTP · Eosinophil infiltration · Phage-displayed 7-mer peptide · Rhinitis model · Peptide libraries · Asthma · Drug development · Biochemistry

Introduction

Upon exposure to allergens, various cells associated with inflammation converge. After several hours, the late-phase reaction (LPR) occurs by histamine and other cytokines secreted from basophils, eosinophils, and lymphocytes [1–3]. Endothelial cells express several intercellular adhesion molecules 1 (ICAM-1), ICAM-2, vascular cell adhesion molecule-1, and selectin, which enhance leukocyte infiltration of airway tissue [4]. As the cellular and molecular mechanisms of allergic inflammation became better understood, many new therapies were developed for downregulation of T_H2 cytokines such as IL-4 [5] and IL-5 [6], augmentation of T_H1 cytokines such as IFN- γ [7] and IL-12 [8], reduction of immunoglobulin (Ig)E responses by humanized anti-IgE antibodies [9, 10], and modulation of ICAMs [11].

Translationally controlled tumor protein (TCTP) was identified in the culture supernatant of U937 cell line as an IgE-dependent histamine releasing factor (HRF) [12]. Human recombinant HRF (HrHRF) directly stimulates the secretion of histamine, IL-4, and IL-13 from a subpopulation of basophils; enhances their secretion from all IgE-bearing basophils activated by anti-IgE antibody [13]; causes

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chemotaxis of eosinophils; and increases IL-8 secretion from eosinophils [14]. HrHRF stimulates the secretion of IL-8 and granulocyte/macrophage colony-stimulating factor in both primary cultures of human bronchial epithelial cells and human bronchial epithelial cell line, BEAS-2B [15]. HrHRF directly enhances B cell proliferation, activates B cell growth, and increases Ig production from murine splenic B cells [16]. Also, extracellular TCTP appears during the LPR of allergic disease [15], which suggests that TCTP might play a role in LPR. We showed in a previous report that only NH₂-terminal truncated TCTP, but not full-length TCTP or COOH-terminal truncated TCTP, could form homodimers through intermolecular disulfide bond and that dimerization of TCTP was essential for its cytokine-like activity [17]. Thus, modulation of the release of inflammatory or allergic mediators exerted by the dimerized TCTP (dTCTP) may provide a way to develop therapeutic strategies for chronic allergic diseases.

In the present study, we tried to identify dTBPs that inhibit the cytokine-like effect of dTCTP. We screened phage-displayed 7-mer peptide library and evaluated the activity of the selected peptides by measuring IL-8 secretion in BEAS-2B cells as well as by assessing inflammatory reaction in a rhinitis mouse model.

Methods

Construction of rat recombinant TCTP and truncated TCTPs

The sequences coding rat TCTP [17] and truncated (residue-deleted) rat TCTPs (RrTCTP; residue 1–172; Del-N11TCTP, residue 11–172; 1–38TCTP, residues 1–38; 39–110TCTP, residues 39–110; 111–172TCTP, residues 111–172; 84–108TCTP, residues 84–108) were amplified using the oligonucleotide primers for cloning into the bacterial expression vector, pRSET A (Invitrogen). The residue 1–38 was amplified using forward—G GAA TTC (*EcoRI*) TCT ACT GAC CAT CTT GC—and reverse primer—CG GGA TCC (*BamHI*) ATG ATT ATC TAC CGG GAC, the residue 111–172 was amplified using forward—CG GGA TCC (*BamHI*) GTA AAG CCT TTT ATG ACT—and reverse primer—CCC AAGCTT (*HindIII*) ACA TTT TTC CAT CTC TAA, and the residue 84–108 was amplified using forward—CG GGATCC (*BamHI*) ACA AAA GAG GCC TAC AAA—and reverse primer—CG GGATCC (*BamHI*) TGG TTT CTG TTC TTC AAG.

Preparation of recombinant proteins

We prepared the recombinant proteins as previously described [17]. In brief, *Escherichia coli* BL21(DE3)pLysS

cells were transformed with the pRSET A/TCTPs, and the overexpressed TCTP was purified using a Ni²⁺-charged His-Bind column according to manufacturer's protocol (Novagen). The NH₂-terminal fusion proteins of TCTP were separated by fast protein liquid chromatography on a Mono Q HR 5/5 column (Amersham Pharmacia Biotech) using a NaCl gradient. Limulus amoebocyte lysate assays (Cambrex Bio Science Walkersville, Inc.) performed to detect LPS contamination in the protein preparations showed that LPS contaminations ranged from 1 to 100 pg/g protein.

Phage selection

A phage-displayed peptide library (New England Biolabs), expressing 7-mer random peptides, was screened for peptides that specifically bound to Del-N11TCTP. Since the activity of Del-N11TCTP was slightly better than that of Del-N35TCTP, we used Del-N11TCTP for screening. The M13 phage displays randomized peptide 7-mers on the pIII minor coat protein, and the complexity of library is about 2.8×10^9 . Panning was repeated five times, and individual plaques were randomly isolated from either the fourth or fifth round of amplification. Single-stranded DNA preparations from 35 selected phages were sequenced. For further experiments, representative phage clones were precipitated with polyethylene glycol (PEG)/NaCl (4% (w/v) polyethylene glycol-8000, 3% (w/v) NaCl). To evaluate the relative binding strengths and specificity of isolated phage clones, PEG-precipitated phage clones were applied to wells containing immobilized dTCTPs or a control protein (bovine serum albumin, BSA). After 2 h incubation at room temperature, the wells were washed with phosphate buffered saline—Tween 20 (PBST; 137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, and 1.5 mM KH₂PO₄, pH 7.4, 0.05% Tween 20) five times. Each well was incubated with 1/5,000 diluted horseradish peroxidase (HRP)-conjugated anti-M13 monoclonal antibody (Amersham Pharmacia Biotech) to detect the phages bound to the immobilized protein. The wells were washed five times with PBST and once with PBS, and HRP substrate solution was added to each well. The optical density of the constituent of each well was measured at 405 nm by microplate reader (BioRad).

Synthetic peptides

Peptides, corresponding to sequences displayed by dTCTP-selected phage, were synthesized by Fmoc solid-phase method (Pepton Inc., Daejeon, South Korea). For biotinylated peptides, linker sequences (GGGK) were added, and the COOH-terminus of the peptide was biotinylated through the primary amine of lysine. Synthetic peptides

were purified to >90% homogeneity and analyzed by high-performance liquid chromatography to ensure purity and by mass spectroscopy to confirm identity.

Measurement of IL-8

Human bronchial epithelial cells, BEAS-2B (ATCC), were maintained in bronchial epithelial growth medium (BEGM, Lonza). When BEAS-2B cells became 80% confluent, they were passaged into 48-well culture plates, incubated for 20–24 h in BEGM, and washed twice with 1% penicillin–streptomycin/bronchial epithelial basal medium (BEBM). The indicated amounts (0, 0.75, 7.5, and 75 nM) of dTBP2 and 75 nM of Del-N11TCTP were pre-incubated for 10 min, added to cells in 1% penicillin–streptomycin/BEBM, and incubated for 20 h. IL-8 secreted into medium was measured by ELISA using a commercial kit (PIERCE).

Western blot using biotinylated dTBP2

Two hundred or 400 ng of RrTCTP and Del-N11TCTP was fractionated by non-reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose. The membrane was incubated in 3% BSA/TBS containing 5 μ M biotinylated dTBP2 (dTBP2-B, dTBP2-GGGK-biotin, Peptron), washed, and then incubated with HRP-conjugated avidin (BioRad). After treatment with ECL plus Western blot detection reagents (Amersham Pharmacia Biotech), the membrane was exposed in LAS-3000 (Fujifilm). It was stripped and reprobed with a rabbit anti-TCTP antibody [17]. We also analyzed bronchoalveolar lavage (BAL) fluids of OVA-sensitized OVA-challenged mice which had been used in our previous work [17]. BALB/c mice were sensitized at days 0 and 14 (i.p.) with OVA. Two weeks after the last injection, the sensitized mice were challenged with OVA by intranasal administration for 12 days on alternate days. At day 40, the mice were sacrificed and BAL fluids were collected with 1 ml of PBS. The supernatants of BAL fluids were used for Western blotting using dTBP2-B.

Flow cytometry

Jurkat T cells (ATCC) were pre-incubated in dilution buffer (3% BSA/PBS with 0.04% NaN_3) at room temperature for 10 min. Cells were treated with dilution buffer (negative control) or 5 μ M of Del-N11TCTP. For inhibition assay, cells were treated with 5 μ M of Del-N11TCTP and 0.5 μ M of dTBP2 in dilution buffer on ice for 1 h. To detect cell-surface binding of Del-N11TCTP (histidine-tagged protein), cells were incubated with phycoerythrin (PE)-conjugated anti-poly-histidine antibody (R&D system) on ice for

30 min. After washing, cells were analyzed by FACS Calibur and CellQuest program (BD Biosciences).

OVA-induced allergic rhinitis model

Male BALB/c mice, 8 to 9 weeks of age, were injected intraperitoneally, with 25 μ g of OVA (Sigma) conjugated with 1 mg of alum, on days 1, 5, 14, and 21. One week after the last injection, 30 μ l of saline containing 0 (negative control) or 100 μ g of OVA was introduced into the airway via the nasal cavity for seven consecutive days. Thirty minutes before nasal challenge, saline alone (positive control) or saline containing dissolved dTBP2 (2.5 and 5 mg/kg) was injected intraperitoneally into each of six mice. The frequency of sneezing and nasal rubbing events was recorded for 15 min after the last intranasal challenge, using a video recorder. The mucosa was fixed with formaldehyde, sectioned (thickness of 5 μ m), and stained with hematoxylin and eosin. The number of eosinophils in the inferior turbinate was counted under microscope at high power ($\times 400$). All experiments were performed in accordance with the National Research Council (USA) guidelines for the care and use of animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Clinical Research Institute in Seoul National University Hospital.

Statistical analysis

Results are expressed as means $\pm$ SD or means $\pm$ SE. We evaluated the results with a one-way ANOVA followed by multiple-comparison tests. Differences were considered significant if p values were <0.05 . Tukey's honestly significant difference test was applied to all pairwise differences between means, and Dunnett's test was performed to compare each group with the control.

Results

Three kinds of phage-displayed peptide clones bind to Del-N11TCTP

In the previous study, we found that only NH_2 -terminal truncated TCTP, in contrast to COOH -terminal truncated TCTP or full-length TCTP, formed homodimers through intermolecular disulfide bond and that the dimerized TCTP increased IL-8 secretion from BEAS-2B cells and enhanced allergic response in T cells [17]. This suggests that the dimerized NH_2 -terminal truncated TCTP, not the intact monomer, is the active form in vivo. We then explored whether the biological activity of the TCTP dimer can be modulated by a dimer-binding peptide. We screened a

Table 1 Phage-displayed peptide clones which preferentially bind to Del-N11TCTP

Peptide	Sequences	Phage clone #
dTBP1	LVTYPLP	4
dTBP2	WYVYPSM	2, 5, 6, 8, 9, 18–25, 28, 30, 31, 34, 35
dTBP4	WEFPGWM	10–17, 26, 27, 29, 32, 33

phage-displayed 7-mer peptide library for peptides that block IL-8 release by Del-N11TCTP. After the fourth and fifth round of panning, 35 Del-N11TCTP-binding phage clones were isolated and screened for specific Del-

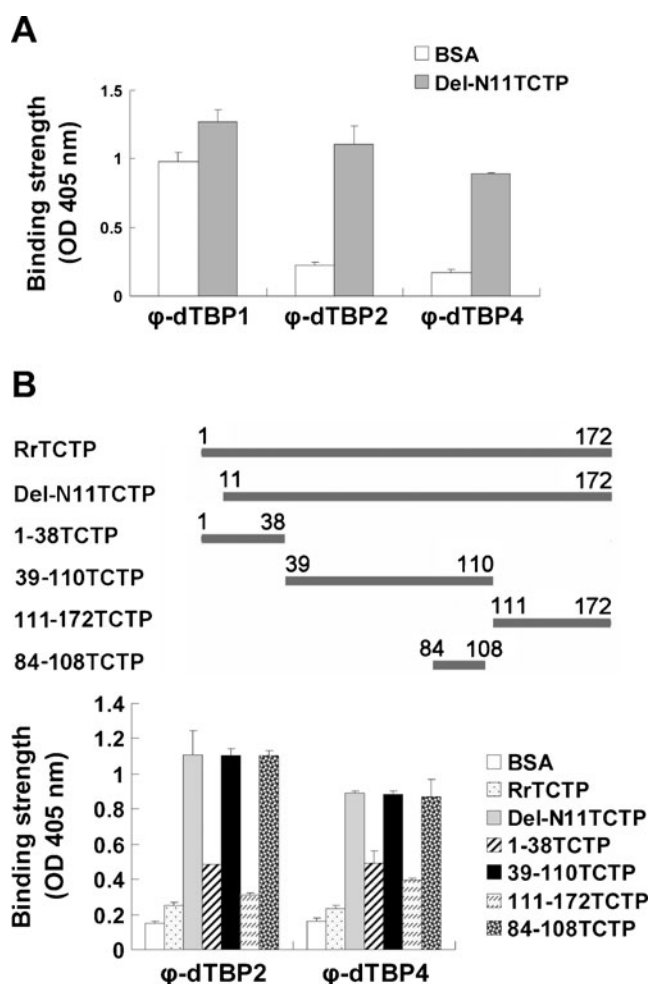


Fig. 1 Binding of phage clones to TCTP. **A** Three representative phage clones were precipitated with PEG, and the binding strengths to Del-N11TCTP were compared with that to BSA. Del-N11TCTP or BSA was immobilized to polystyrene plates, and one of the three phage clones was added. After wash, the bound phages were detected by a HRP-conjugated anti-M13 antibody. The relative binding strength was determined as optical density at 405 nm. **B** PEG-precipitated phage clones were allowed to bind to plates with immobilized BSA, RrTCTP, or deletion constructs of RrTCTP (Del-N11TCTP, 1–38TCTP, 39–110TCTP, 111–172TCTP, and 84–108TCTP), and the bound phages were detected by HRP-conjugated anti-M13 antibody

N11TCTP-binding activity. Thirty-two of these clones showed a specific Del-N11TCTP-binding activity in ELISA (supplementary Fig. 1). DNA sequencing led us to identify three distinct sequences: LVTYPLP (dTBP1), WYVYPSM (dTBP2), and WEFPGWM (dTBP4) with specific Del-N11TCTP-binding activity (Table 1). While dTBP1 identified from fourth round of panning appeared from only one clone, dTBP2 identified from fourth and fifth round of panning and dTBP4 identified from fifth round of panning appeared in several clones. Output from fourth round of panning consisted of less enriched population of library than output from fifth round. We assumed that the enriched population might represent the peptide having high binding affinity. We observed a similar pattern in affinity assay. dTBP2-displayed phage clone (φ-dTBP2) and dTBP4-displayed phage clone (φ-dTBP4) exclusively bound to Del-N11TCTP, while TBP1-displayed phage clone (φ-dTBP1) bound not only to Del-N11TCTP but also to BSA (Fig. 1A).

To map the region of Del-N11TCTP that interacts with the selected phage-displayed peptide clones, four deletion constructs including 1–38TCTP, 39–110TCTP, 111–172TCTP, and 84–108TCTP, each representing distinct subdomains, were prepared and used in ELISA binding assays (Fig. 1B). We could not evaluate the φ-dTBP1 binding region of TCTP because it exhibits non-specific affinity to other proteins including BSA (data not shown). The binding affinities of φ-dTBP2 and φ-dTBP4 to 39–110TCTP and 84–108TCTP were similar to that to Del-N11TCTP, suggesting that the major binding region of phage-displayed peptide is the residue 84–108 of rat TCTP.

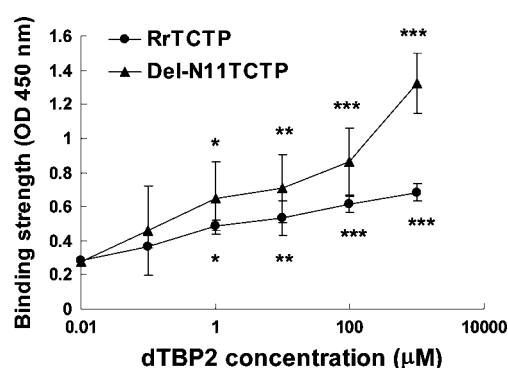


Fig. 2 Differential binding of biotinylated dTBP2 to RrTCTP and Del-N11TCTP. The indicated concentrations of biotinylated dTBP2 were immobilized to streptavidin coated polystyrene plates (PIERCE); 0.1 μg/ml of RrTCTP and Del-N11TCTP was added into the plates and incubated. TCTP bound to the biotinylated dTBP2 was detected with anti-TCTP polyclonal antibody and HRP-conjugated anti-rabbit IgG. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, significantly different from the value of 0.01 μM peptide. These values are expressed as the mean $\pm$ SE

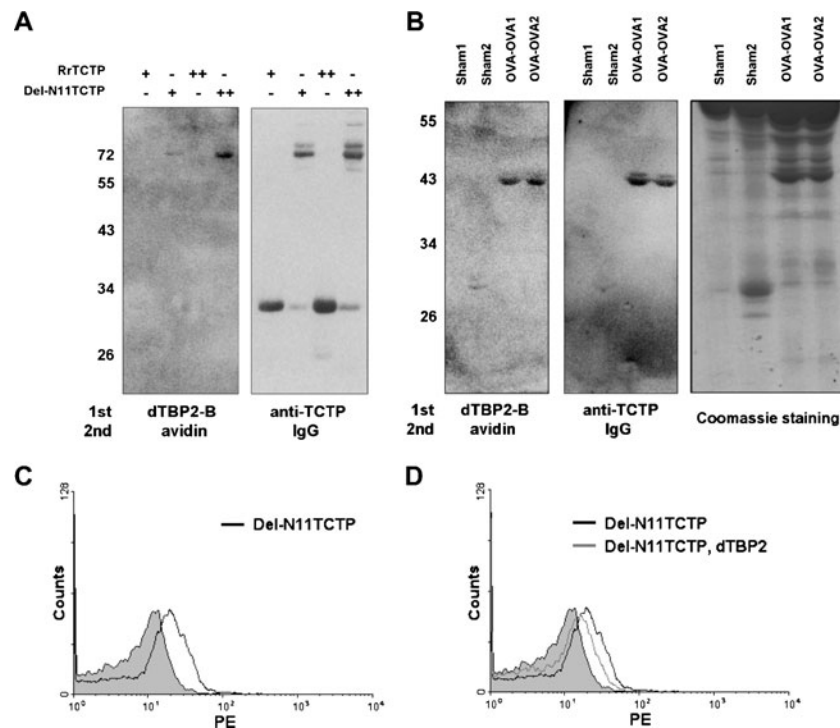


Fig. 3 **A** dTBP2 binds to dimeric Del-N11TCTP, but not to monomeric RrTCTP. 200 ng (+) or 400 ng (++) of recombinant proteins was analyzed with non-reducing SDS-PAGE and Western blotting. The blot was probed with 5 μ M of dTBP2-B (biotinylated dTBP2, dTBP2-GGGK-biotin) and HRP-conjugated avidin and then reprobed with a rabbit anti-TCTP antibody and HRP-conjugated rabbit IgG. **B** dTBP2 binds to dimeric TCTP in BAL fluids from OVA-sensitized and OVA-challenged mice. Thirty microliters of BAL fluids from Sham (OVA-sensitized and PBS-challenged, lanes 1 and 2) and OVA-OVA (OVA-sensitized and OVA-challenged, lanes 3 and 4) mice were analyzed by non-reducing SDS-PAGE and Western blotting. The blot was probed with 5 μ M of dTBP2-B and HRP-conjugated avidin

and then reprobed with a rabbit anti-TCTP antibody and HRP-conjugated rabbit IgG. Equal amounts of BAL fluids were analyzed by non-reducing SDS-PAGE and Coomassie brilliant blue staining. **C**, **D** Interaction between dTBP2 and Del-N11TCTP reduces the binding of Del-N11TCTP to the cell surface. Jurkat T cells were incubated with buffer (negative control, gray histogram), 5 μ M of Del-N11TCTP, or 5 μ M of Del-N11TCTP and 0.5 μ M of dTBP2 for 1 h. Cells were labeled with phycoerythrin-conjugated anti-polyhistidine antibody and analyzed the fluorescence by PE using FACS Calibur. The number of cells was expressed as counts in y-axis, and 10,000 cells per experiments were counted. The fluorescence intensity by PE was given in x-axis

dTBP2 has higher affinity to Del-N11TCTP than to RrTCTP

We compared the binding affinities of ϕ -dTBP2 and ϕ -dTBP4 to RrTCTP and Del-N11TCTP (Fig. 1B). Interestingly, these selected phage clones showed high affinity to Del-N11TCTP, but not to RrTCTP. Since it was possible that phage-bound peptides and synthetic peptides showed different binding affinities to the target, we tested the binding affinities of synthetic peptides to RrTCTP and Del-N11TCTP. Because dTBP2 was the most frequently appearing sequence in the phage clones (Table 1), we compared the binding affinities of biotinylated dTBP2 to RrTCTP and Del-N11TCTP (Fig. 2). Again, biotinylated dTBP2 showed stronger affinity to Del-N11TCTP than to RrTCTP. We also tried to screen the phage-displayed 7-mer peptide library using full-length RrTCTP but failed to detect any RrTCTP-binding peptides (data not shown), probably due to the conformational differences between RrTCTP and NH₂-terminal truncated RrTCTP.

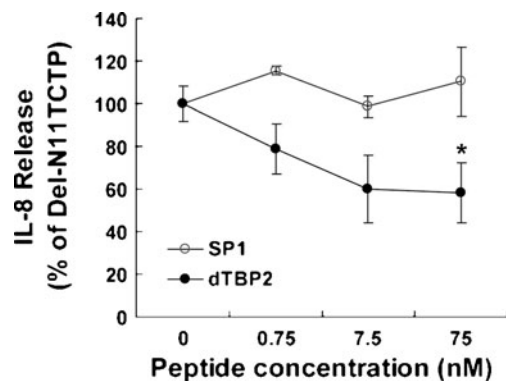


Fig. 4 dTBP2 reduces IL-8 release by Del-N11TCTP in BEAS-2B cells. The indicated nanomolars of dTBP2 (closed circle) or SP1 (a scrambled version of dTBP2, YSYMVWP; open circle) was premixed with 75 nM of Del-N11TCTP for 10 min and added to BEAS-2B cells. After 20 h, the supernatants were harvested and their IL-8 levels were measured. These values are expressed as the mean $\pm$ SD. $n=3$, * $p<0.05$, significantly different from the Del-N11TCTP value

dTBP2 binds to dimeric Del-N11TCTP and inhibits its binding to Jurkat cells

To reconfirm the specific interaction between dTBP2 and dTCTP, we performed non-reducing SDS-PAGE and Western blot using two recombinant proteins, RrTCTP and Del-N11TCTP, BAL fluid, and biotinylated dTBP2 (dTBP2-B). In these blots, dTBP2-B preferentially bound to dTCTP (Del-N11TCTP), but not to monomer TCTP (RrTCTP) (Fig. 3A). Furthermore, dTBP2-B could specifically detect dTCTP in BAL fluid which contains a variety of proteins in addition to TCTP (Fig. 3B). These results suggest that dTBP2 might bind to the discontinuous epitope of dimerized TCTP.

When using native gel electrophoresis, dTBP2 did not prevent dTCTP dimerization (data not shown). It is, therefore, possible that dTBP2 might bind to a receptor binding site of dTCTP. We performed FACS analysis with Del-N11TCTP and dTBP2 to determine whether dTBP2 inhibits the binding of Del-N11TCTP to the cell surface. We measured binding of Del-N11TCTP, a NH₂-terminal poly-histidine fusion protein, to Jurkat cells using PE-conjugated anti-poly-histidine antibody (Fig. 3C). In this FACS analysis, we found that fluorescence by the binding of Del-N11TCTP was reduced by dTBP2 to about 50% (Fig. 3D).

dTBP2 inhibits IL-8 release induced by Del-N11TCTP

Next, we examined whether synthetic dTBP2 modulates the cytokine-like activity of dTCTP. When BEAS-2B cells were incubated with 75 nM of Del-N11TCTP plus various amounts (0, 0.75, 7.5, and 75 nM) of dTBP2, for 20 h in 1% penicillin–streptomycin/BEEM, the release of IL-8 decreased in a dTBP2 dose-dependent fashion (Fig. 4). We could not detect any significant effect of a scrambled version of the dTBP2 (SP1, YSYMVWP) on the activity of Del-N11TCTP. On the other hand, dTBP2 inhibited IL-8 release by Del-N11TCTP, to approximately 61±13%, while IL-8 release from untreated BEAS-2B cells was about 4±3% of IL-8 released by Del-N11TCTP. It seems that inhibition by dTBP2 was not potent. However, these observations suggest that dTBP2 may interact with dTCTP at a binding site to the cell surface, thereby inhibiting the cytokine-like effect of dTCTP.

dTBP2 inhibits OVA-induced rhinitis

We tested the effect of dTBP2 in a mouse model of OVA-induced rhinitis. OVA challenge is known to stimulate the secretion of TCTP and infiltration of eosinophils in OVA-immunized mice [17, 18]. We measured the severity of rhinitis in the mice by determining the frequency of their

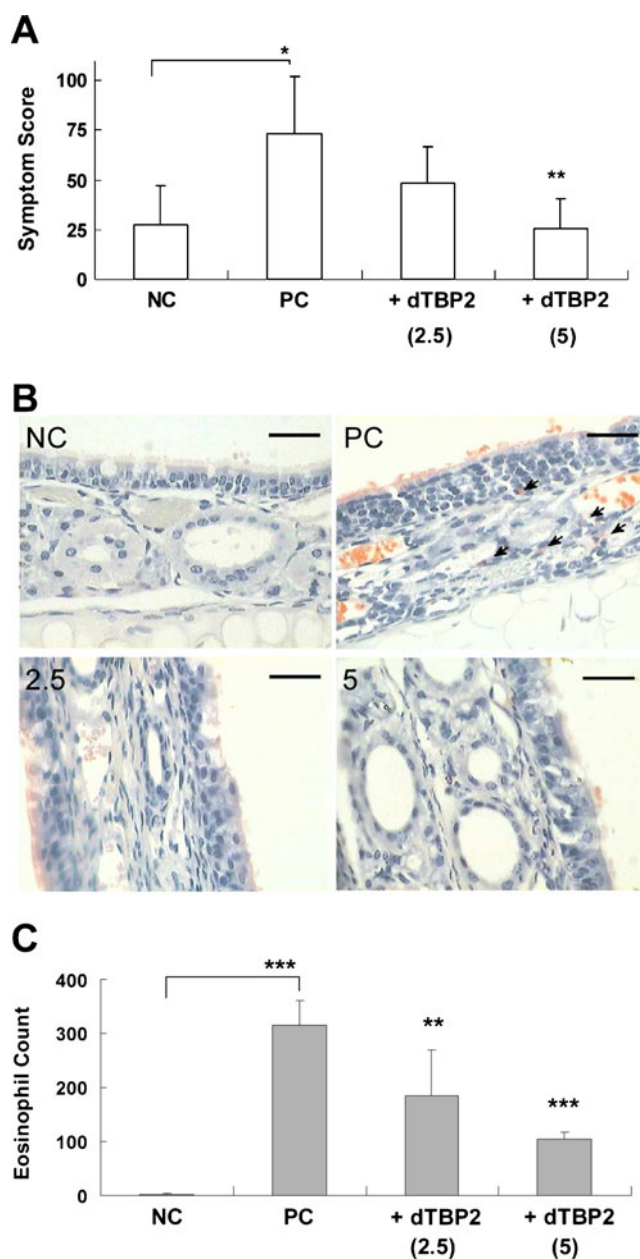


Fig. 5 dTBP2 reduces allergic symptoms in a mouse rhinitis model. Male BALB/c mice were OVA-sensitized and OVA-challenged. Each group of mice (six mice/group) was treated with 2.5 or 5 mg/kg of dTBP2 before OVA-challenge. **A** Allergic symptom scores are expressed as the mean ± SD. * $p < 0.05$; ** $p < 0.01$, significantly different from the negative control (NC) or the positive control (PC) value. **B, C** The nasal mucosa of dTBP2-treated or dTBP2-untreated mice was sectioned (thickness of 5 μ m), hydrated with 100–70% ethanol, and stained with hematoxylin. Decolorized sections were counter-stained with eosin. The infiltration of eosinophils in the inferior turbinate was counted under microscope at high power ($\times 400$). **B** 2.5, 2.5 mg/kg dTBP2-pretreated; 5, 5 mg/kg dTBP2-pretreated. Scale bars indicate 25 μ m. The cytosols of eosinophils were stained pink by eosin due to their eosinophilic granules. They could be differentiated from cytosols of erythrocytes that were intensely red without blue-colored nuclei. Eosinophils were indicated by arrows. **C** Eosinophils count is expressed as the mean ± SD. ** $p < 0.01$; *** $p < 0.001$, significantly different from the NC or the PC value

sneezing and nasal rubbing events (symptom score) and the degree of eosinophil infiltration in their nasal mucosa. The frequency of sneeze and nasal rub was significantly inhibited in dTBP2-treated mice compared to the positive control (Fig. 5A). Also, the infiltration of eosinophils and possibly other cells significantly decreased in the dTBP2-pretreated mice in a concentration-dependent manner (Fig. 5B, C). In contrast, the eosinophils were massively recruited in the positive control.

Discussion

In an earlier study, we found that the dimeric dTCTP, found in sera from atopic/asthmatic patients and in BAL fluids from airway inflamed mice, exhibited more potent cytokine-releasing activity than monomeric full-length RrTCTP [17]. In the present study, we identified a dTCTP-binding peptide named dTBP2, which had stronger binding affinity to dTCTP (Del-N11TCTP) than to RrTCTP. The anti-parallel helical hairpin structure generated by α -helices H2 and H3 of TCTP may promote coiled-coil structure in the TCTP dimer [19]. We tested whether dTBP-binding regions could be dimerized using size exclusion column chromatography (data not shown). 39–110TCTP showed just one peak corresponding to the dimeric size. However, 39–110TCTP did not have any disulfide bond in its dimer and was not biologically active in IL-8 assay [17]. 84–108TCTP showed one major peak corresponding to the monomeric size. However, when it was incubated at 4°C longer, the peak shifted to the left corresponding to the dimeric size. These results suggest that α -helix H2 domain contributes to coiled-coil structure, but the structure is not sufficient to generate the cytokine-like activity of TCTP. Therefore, dTBP2 possibly binds to intermolecular region between TCTPs, which is absent in monomeric RrTCTP but is present in the dimer, and the intermolecular region may be a cell-surface binding site of dTCTP.

We showed here that dTBP2 inhibited IL-8 release by Del-N11TCTP, which existed as a dimer, but the inhibitory activity was not strong. We speculated that this low inhibitory activity might be due to incomplete blocking which might be caused by competition of dTBP2 with other factors for the binding site at dTCTP. The major ϕ -dTBP2-binding region of TCTP, residue 84–108, belongs to the epitope region [20], which reportedly plays roles in tubulin binding (residue 79–123) [21] and Ca^{2+} binding (residue 80–116) [22, 23]. It is, therefore, possible that the binding between NH_2 -terminal truncated TCTP and dTBP2 can be influenced by other factors, such as extracellular Ca^{2+} .

dTBP2 could reduce IL-8 release by Del-N11TCTP and inhibit several allergic symptoms in the animal model of

rhinitis. IL-8 is an important product of bronchial epithelia, and IL-8 levels are several fold higher in BAL fluids from patients with asthma than in those from healthy individuals [24]. The accumulation of eosinophils in airway tissue contributes to the airway congestion, increase mucus production, and bronchial hyper-responsiveness seen in LPR [25]. Also, cytokines produced by eosinophils are chemotactic to inflammatory cells and prolong the survival of eosinophils [26]. Thus, dTBP2 may offer an important new approach to treatment in allergic inflammation.

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Disclosures M. Kim and K. Lee have submitted an application for a patent on the use of the peptides to treat allergic diseases.

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