

Expression of C–C chemokine TARC in human nasal mucosa and its regulation by cytokines

N. TERADA, T. NOMURA, W. J. KIM, Y. OTSUKA, R. TAKAHASHI, H. KISHI, T. YAMASHITA*, N. SUGAWARA*, S. FUKUDA*, T. IKEDA-ITO† and A. KONNO

Department of Otorhinolaryngology, School of Medicine, Chiba University, Chiba, *R & D Mitsubishi Kagaku Bioclinical Laboratories Inc., Tokyo, and †Research Institute, Nissui Pharmaceutical Co. Ltd, Hokunanmoro, Yuki, Japan

Summary

Background Although interleukin (IL)-4 and IL-5 have been demonstrated to play a critical role in the pathophysiology of allergic diseases such as allergic rhinitis, the mechanism that causes the predominance of Th2 lymphocytes has yet to be clarified. Thymus and activation-regulated chemokine (TARC) has been known to facilitate the recruitment, activation and development of Th2 polarized cells, leading investigators to suggest a role for TARC in the development of Th2 responses.

Objective To gain a better understanding of the role of TARC in the pathogenesis of allergic rhinitis we investigated the cellular sources of this chemokine in nasal mucosa. In addition, the effect of cytokines on TARC production has been investigated.

Methods The expression of TARC in human nasal mucosa was assessed by immunohistochemistry. To study the effect of cytokines on TARC production, epithelial cells, endothelial cells and fibroblasts, isolated from inferior nasal mucosa samples, were stimulated by a variety of cytokines including IL-4, IL-13, tumour necrosis factor (TNF)- α and interferon (IFN)- γ .

Results Epithelial cells in nasal mucosa in subjects with allergic rhinitis expressed higher signal level than those in non-allergy patients. Combined stimulation with IL-4 and TNF- α , as well as IL-13 and TNF- α , synergistically induced TARC expression in epithelial cells. Furthermore, the amount of TARC induced by these cytokines was higher in epithelial cells obtained from patients with allergic rhinitis than in those from non-allergic patients.

Conclusion These results demonstrate a crucial role of nasal epithelial cells in the expression of TARC, and that Th2 cytokine IL-4 and IL-13 may promote Th2 responses by inducing TARC production from epithelial cells.

Keywords: TARC, chemokines, IL-4, IL-13, epithelial cells, allergic rhinitis, nasal mucosa

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Introduction

Clinical symptoms of sneezing, rhinorrhea and congestion are primarily a consequence of mast cell and basophil release of chemical mediators such as histamine, platelet

activating factor (PAF) and leucotrienes released from other inflammatory cells. A predominance of CD4⁺ T cells and eosinophils, synthesis and release of T helper type 2 (Th2) cytokines, and the coordinate expression of chemokines and adhesion molecules, are all characteristic of the allergic response observed in allergic rhinitis [1,2]. Using *in situ* hybridization an increased expression of interleukin (IL)-3, IL-4, IL-5 and GM-CSF has been observed in

Correspondence: Nobuhisa Terada, Department of Otorhinolaryngology, School of Medicine, Chiba University, 1–8-1 Inohana, Chuo-ku, Chiba City, Chiba 260–0856, Japan. E-mail: terada@med.m.chiba-u.ac.jp

allergic nasal mucosa after local provocation [3–5]. Furthermore, an increased expression of IL-4R and IL-5R has recently been demonstrated in allergic nasal mucosa after allergen challenge [6]. Although IL-4 and IL-5 have been demonstrated to play a critical role in the pathophysiology of allergic diseases, including allergic rhinitis [1,2,7,8], the mechanisms underlying the predominance of Th2 lymphocytes have yet to be clarified.

More recently, chemokines, a superfamily of small proteins, have been known to be key molecules involved both in routine leucocyte trafficking and in the activation and recruitment of specific cell populations to sites of inflammation and infection. The complexity and functions of the chemokine family have become increasingly diverse as more members have been identified and characterized. In various *in vitro* assays, chemokines have been shown to facilitate the chemotaxis or transendothelial migration of leucocytes [9], while *in vivo* injection [10] or overexpression of chemokines [11] results in leucocyte accumulation at the site of chemokine expression.

To date, it has been reported that *in vitro*-derived Th1 lymphocyte lines selectively express CXCR3 and CCR5, whereas Th2 lymphocyte lines selectively express CCR3, CCR4 and CCR8 [12–16].

Thymus and activation-regulated chemokine (TARC) was recently found to be a highly specific ligand for CCR4 [17]. TARC is likely to be produced by monocytes and dendritic cells and may facilitate the recruitment, activation and development of Th2 polarized cells that express CCR4, leading investigators to suggest a role for TARC in Th2 responses [18].

To gain a better understanding of participation of TARC in the pathogenesis of allergic rhinitis, we have examined the cellular sources of this chemokine in nasal mucosa. In this study, we have also investigated the effect of cytokines on TARC production in nasal epithelial cells, endothelial cells and fibroblasts. In addition, we report that the amount of TARC induced by cytokines was higher in epithelial cells obtained from patients with allergic rhinitis than in those from non-allergic patients.

Methods

MAbs and cytokines

Recombinant human IL-4, IL-13, interferon (IFN)- γ and tumour necrosis factor (TNF)- α were purchased from R & D systems (Minneapolis, MN, USA). Rabbit polyclonal Ab recognizing TARC was purchased from PeproTech EC (London, UK). Mouse anti-human monoclonal TARC Ab recognizing TARC and biotin-streptavidin system (LSAB-AP Kit) were purchased from DAKO (Glostrup, Denmark).

Anti-human cytokeratin antibody and antifactor VIII antibody were purchased from DAKO.

Collection of mucosa from the inferior turbinate

Inferior turbinate mucosa samples were collected from patients with allergic rhinitis sensitized to house dust and/or mites ($n = 6$, average age 18.9 ± 8.6 years) at the time of surgery for submucosal resection. Non-allergic patients were selected from among those who met the following conditions: absence of eosinophilia in nasal smears; CAP RAST negative to house dust mites, Japanese cedar pollen, ragweed pollen and mugwort pollen; IgE not higher than 300 U/mL; and absence of symptoms of nasal hyper-reactivity, such as sneezing and watery nasal discharge ($n = 6$, average age 24.7 ± 7.7 years).

Immunohistochemistry

Specimens of nasal mucosa were fixed in 4% paraformaldehyde for 4 h, frozen in liquid nitrogen and stored at -70°C until processing for immunohistochemistry. Cryostat sections ($6\ \mu\text{m}$) were obtained and air dried for 1 h. The sections were then blocked by incubation with Tris-buffered saline, pH 7.6, containing 2% BSA and 5% normal goat serum for 2 h at room temperature (25°C). To detect TARC, the sections were incubated with rabbit anti-human TARC overnight and stained with a biotin-streptavidin system.

Immunohistochemically specific controls were obtained by replacing the primary antibody to the rabbit immunoglobulin fraction (DAKO, Kyoto, Japan), and omitting the first antibody. Cells were also counterstained with Myer's haematoxylin. The specificity of this antibody was confirmed by direct-binding ELISA: it did not react with either macrophage-derived chemokine, pulmonary and activation-regulated chemokine, liver and activation-regulated chemokine, lymphotactin, fractalkine, eotaxin, eotaxin-2, eotaxin-3, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 α , IL-8 or RANTES (data not shown).

Isolation and propagation of epithelial cells, endothelial cells and fibroblasts from human nasal mucosa

Human nasal epithelial cells (HNECs) and human mucosal microvascular endothelial cells (HMMECs) were prepared as described previously, with some modifications [19]. Briefly, surgically removed mucosa specimens were cut into 4-mm square sections and then incubated separately for 1 h at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 0.25% collagenase (Wako Pure Chemicals, Osaka, Japan). Following incubation in collagenase, these sections were transferred to 10 mL of

DMEM containing 50 µg/mL gentamicin. HNECs and HMMECs were released into medium by applying pressure on the tissue across the surface with a pair of curved forceps 10 times. To separate HNECs from HMMECs, the cell pellets were resuspended in DMEM supplemented with 20% fetal calf serum (FCS, Costa Mesa, CA, USA) and placed onto a plastic culture dish, 60-mm in diameter, coated with type I-rich collagen (Sigma, St Louis, MO, USA). The dish was incubated for 1 h at 37 °C under a humidified atmosphere of 5% CO₂ to allow HMMECs to bind to the collagen matrix. The culture was then washed twice with plating medium to remove unattached cells and tissue fragments, and resuspended with 15 mL of molecular cellular developmental biology (MCDB) 104 (Nissui, Tokyo, Japan) containing 10% FCS, 75 mg/mL endothelial cell growth supplement (Collaborative Research Inc., Bedford, MA, USA) and antibiotics. The floating HNECs were collected, centrifuged and plated in MCDB153 (Nissui) medium containing 10 ng/mL epidermal growth factor and 1% bovine pituitary extract.

Morphological observations using a phase-contrast microscope showed that the HNECs consisted primarily of epithelial cells. More than 95% of the HNECs showed positive reactions with anti-human cytokeratin antibody. Based on the morphology and positive reactions with antifactor VIII antibody, it was confirmed that more than 95% of the HMMECs were endothelial cells.

HNMFs were isolated from the specimens by explant cultures following the removal of endothelial cells and epithelial cells. The specimens were cut into 1–2-mm square sections. Each of the explants was transferred to a well of a 12-well plate (Iwaki Glass Co. Ltd, Chiba, Japan) and centrifuged for 5 min at 110 g using a swinging plastic plate holder. The attached explants were cultured with DMEM containing 2% FCS, 10 ng/mL recombinant basic fibroblast growth factor and antibiotics. Based on the morphology and positive reactions with monoclonal anti-human fibroblasts (Dakopatts), it was confirmed that more than 97% of the HNMFs were fibroblasts.

Cell culture

HNECs, HNMFs and HMMECs were cultured directly onto collagen-type-I coated, 24-well culture plates (Sumitomo Bakelite Co. Ltd, Tokyo, Japan). They were checked by phase-contrast microscopy. At confluence, they were washed with phosphate buffered saline (PBS) containing 10 mg/mL gentamicin (Sigma) three times and were stimulated with various kinds of cytokines for 1–36 h at 37 °C in 5% CO₂. The supernatants were collected, centrifuged and stored frozen at –80 °C until use. Cells were harvested by trypsinization and immediately frozen in liquid nitrogen until use.

Extraction of total RNA and RT-PCR

RNA extraction, cDNA synthesis and PCR reaction were carried out essentially according to the methods described elsewhere [19].

Total RNA was extracted using the acid guanidinium-phenol-chloroform method, and the complementary DNA was synthesized using a cDNA synthesis kit (Boehringer Mannheim, Mannheim, Germany). PCR was performed in a DNA thermal cycler (Quick Thermo Systems, Nippon Genetics, Tokyo, Japan). The PCR was performed for 30 cycles under the following conditions: (1) pre-denaturation at 94 °C for 5 min; (2) denaturation at 94 °C for 2 min; (3) annealing at 55 °C for 2 min; (4) extension at 72 °C for 2 min. After repeating steps (2) to (4) for 30 cycles, re-extension was performed at 72 °C for 5 min.

Real-time PCR

The analysis was carried out on a Light Cycler (Roche Molecular Biochemicals, Mannheim, Germany) [20]. PCR was performed by rapid cycling in a reaction volume of 20 µL with 0.5 µmol/L of each primer, 0.2 µmol/L of the anchor probe and 0.4 µmol/L of the detection probes, and 50 ng of genomic DNA.

As the reaction buffer, the Light Cycler FastStart DNA Master Hybridization Probes buffer from Roche Molecular Biochemicals was used. This buffer is provided as a 10-fold stock solution containing nucleotides, *Thermus aquaticus* DNA polymerase, and 10 mmol/L Mg²⁺. The final Mg²⁺ concentration in the reaction mixture was adjusted to 2 mmol/L for TARC and 3 mmol/L for β-actin. The samples were loaded into glass capillary cuvettes (Roche Molecular Biochemicals) and centrifuged to place the sample at the capillary tip before capping. After an initial denaturation step at 95 °C for 10 min, amplification was performed using 40 cycles of denaturation (95 °C for 15 s), annealing (60 °C for 15 s) and extension (72 °C for 10 s). The temperature transition rates were programmed at 20 °C from denaturation to annealing, 20 °C from annealing to extension, and 20 °C from extension to denaturation. Fluorescence was measured at the end of the annealing period of each cycle to monitor amplification.

To detect TARC mRNA, four oligonucleotides were used in the assay, of them two were the labelled anchor and detection probes and two served as amplification primers.

All of the hybridization probe PCR assays were performed in quadruplicate. The intra-assay variation was 5.4% and the interassay variation was 10.3%, respectively. The ratio of TARC mRNA to β-actin mRNA was determined.

The probes used in this study are shown in Table 1. A schematic representation of the location and orientation of the oligonucleotides is shown in Fig. 1.

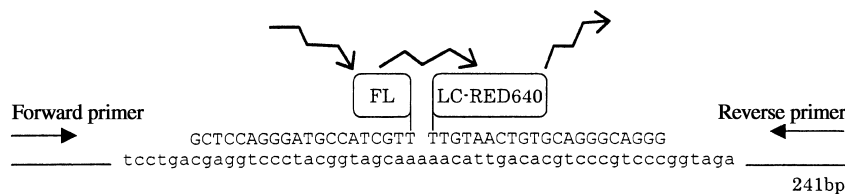


Fig. 1. Schemes of the primer and probe designs for TARC. The spacing between the hybridization probes is 1 base for TARC and β -actin (data not shown). When both probes are hybridized, FRET occurs over the 1-bp gap.

ELISA for TARC

Flat-bottomed 96-well microtitre plates (Maxisorp; Nunc A/S, Roskilde, Denmark) were coated with 5 μ g/mL of mouse anti-human monoclonal TARC antibody (100 μ L/well) in PBS, and incubated overnight at 4 $^{\circ}$ C and blocked with 100 μ L/well of blocking solution (1% BSA, 1% skim milk in PBS) for 1 h at room temperature; 100 μ L of the standard or sample was added to each well and incubated for 2 h at room temperature. Next, 100 μ L of the biotin-labelled goat anti-human polyclonal TARC antibody (DAKO) in assay buffer (1% BSA, 0.01% Tween 20 in PBS) was added and incubated for 1 h at room temperature. Thereafter, 100 μ L of the avidin-horseradish peroxidase conjugate (Vector Laboratories, Burlingame, CA, USA) (diluted 2000-fold in assay buffer) was added to each well and incubated for 30 min at room temperature. Finally, 100 μ L per well of tetra metyl benzidine (DAKO) was added as substrate, and the mixture was incubated for 10 min at room temperature. The enzyme reaction was stopped with 100 μ L of 2 mol/L H_2SO_4 and the absorbance at 450 nm was measured in a microtitre plate reader. The sensitivity of this assay was 3.90 pg/mL.

Statistical analysis

The data are expressed as the mean value $\pm$ SEM.

Table 1. Oligonucleotides used in this study. FL, 5,6-carboxyfluorescein attached to 3'-O-ribose; LC, light cycler RED 640 attached to 5' terminus.

	Sequence 5'-3'
β -actin	
Forward primer	CCAACCGCGAGCAGCTG
Reverse primer	GGAAGGAAGGCTGGAAG
Anchor probe	CCTCCCCATGCCATCCTGCGTC-FL
Detection probe	LC-GGACCTGGCTGGCCGGGACCTGA
TARC	
Forward primer	TCTC TGCAGCACATCCACGC
Reverse primer	GGCTTCAAGACCTCTCAAGGCT
Anchor probe	GCTCCAGGGATGCCATCGTT-FL
Detection probe	LC-TTGTAAGTGTGCAGGGCAGGG

Statistical significance was determined by using paired Wilcoxon's test, defining $P < 0.05$ as statistically significant.

Results

There was strong expression of TARC in the nasal mucosal epithelial cells both from subjects with allergic rhinitis and those without (Figs 2a and b, respectively). Epithelial cells from the nasal mucosa of subjects with allergic rhinitis expressed higher signal levels than those from non-allergic patients. No reaction was observed with the isotype-matched control antibody (Fig. 2c). In addition, various leucocyte types also stained positively, including eosinophils, lymphocytes and macrophages (Fig. 2d). Somewhat weaker expression of TARC was observed on vascular endothelium in subjects with allergic rhinitis.

We next studied the effect of cytokines on TARC production in cultured epithelial cells isolated from inferior nasal mucosa samples. In addition, we compared the amount of TARC production in epithelial cells obtained from patients with allergic rhinitis and in those from non-allergic patients.

In the preliminary study we evaluated the effects of various kinds of cytokines, including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, TNF- α and TGF- β , on TARC production in nasal epithelial cells. We have found that IL-4 and IL-13 are essential for TARC production in nasal epithelial cells. Real-time PCR detection of TARC mRNA demonstrated specific fluorescence, with the PCR products being visualized by agarose gel electrophoresis after ethidium bromide staining. Whenever a good FRET signal occurred in both fluorescence channels, distinctive single bands were observed by agarose gel electrophoresis (data not shown).

Unstimulated HNECs expressed TARC mRNA at low levels. After stimulation with IL-4 or IL-13 with or without TNF- α , TARC mRNA in HNECs and HMMECs reached peak levels within the first 6 h and 12 h of stimulation and gradually decreased in the following 36 h (data not shown). Stimulation with IL-4 and IL-13 showed significant enhancement of TARC mRNA levels compared with unstimulated HNECs ($P < 0.05$, $n = 6$) (Fig. 3). In addition, the combined stimulation of IL-4 10 ng/mL and TNF- α 10 ng/mL, as well as that of IL-13 10 ng/mL and

Fig. 2. Expression of TARC in human nasal mucosa. To detect TARC, the sections were incubated with rabbit anti-human TARC and stained with a commercially available biotin-streptavidin kit. Strong expression of TARC was observed on the surface of epithelial cells in the nasal mucosa of both subjects with allergic rhinitis (a) and non-allergic subjects (b). No reaction was observed with the isotype-matched control antibody (c). In the subepithelial layer, various leucocyte types also stained positively, including eosinophils, lymphocytes and macrophages (d).

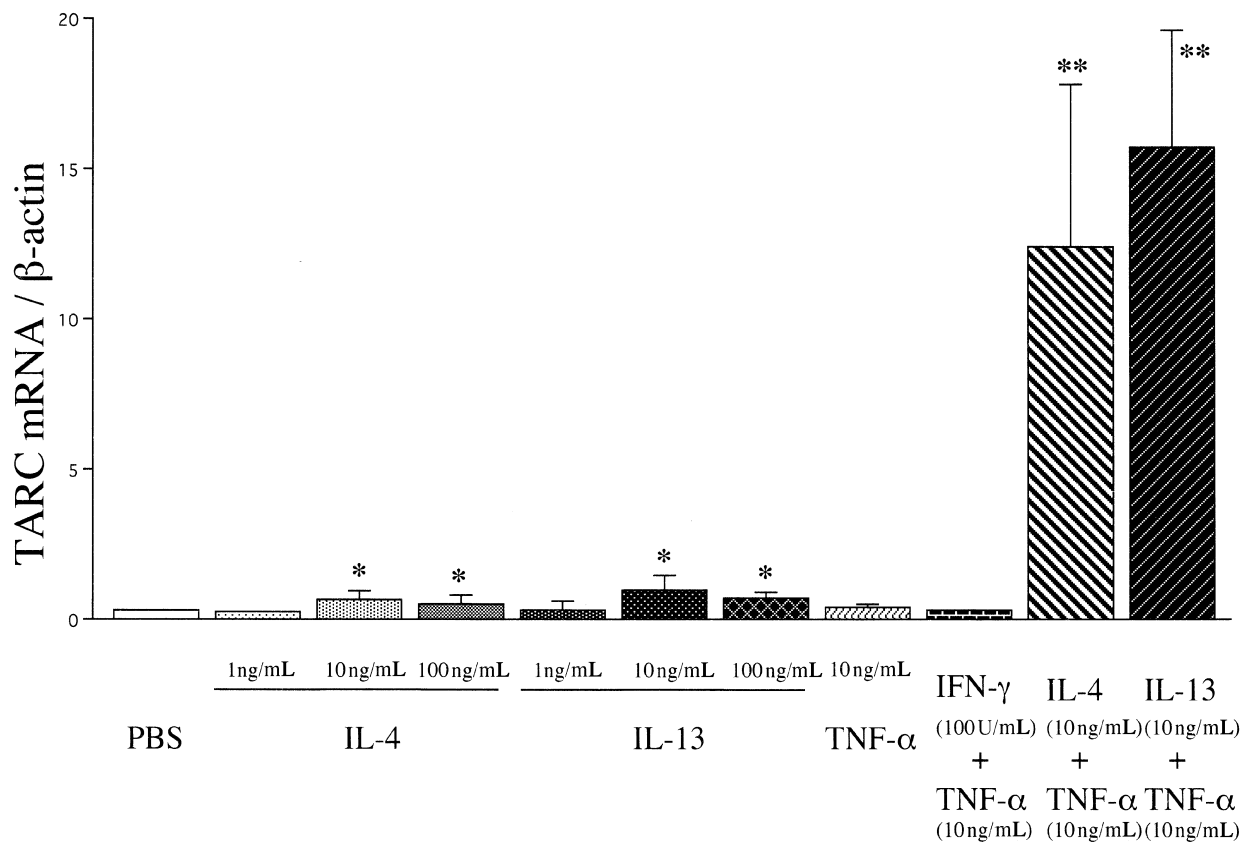
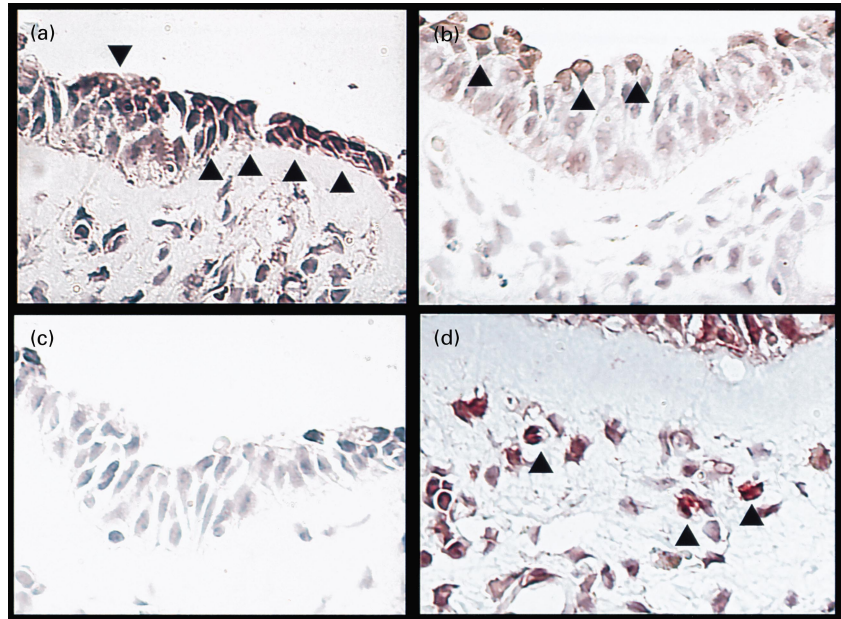


Fig. 3. TARC mRNA expression in HNECs. HNECs were incubated for 6 h with IL-4 (10 ng/mL), IL-13 (10 ng/mL) or TNF-α (10 ng/mL), alone or in combination. Total RNA was isolated from HNECs. After cDNA synthesis the analysis for TARC mRNA was carried out on a light cycler as described in the text. The ratio of TARC mRNA to β-actin mRNA was determined. All values are the mean $\pm$ SEM of six experiments. * P < 0.05, vs. unstimulated HNECs. ** P < 0.01, vs. unstimulated HNECs.

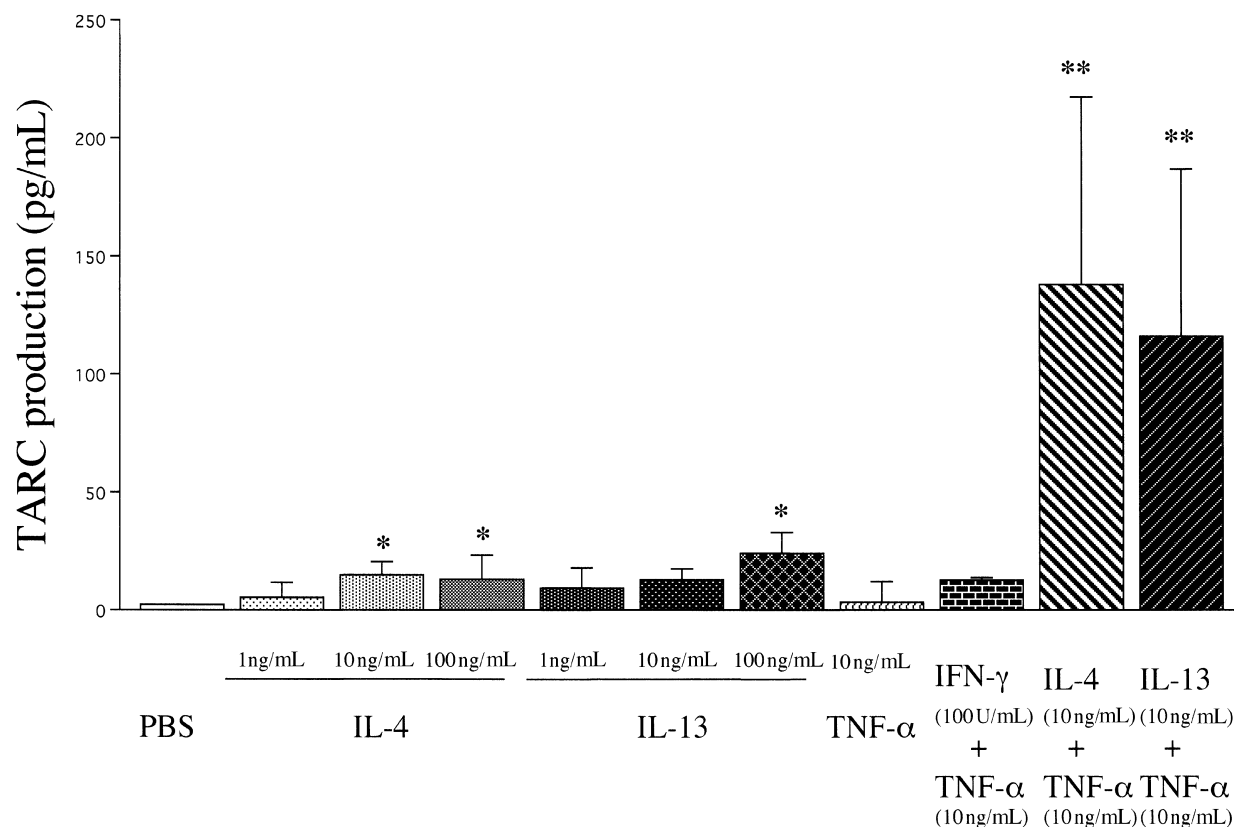


Fig. 4. TARC production in HNECs. HNECs were cultured for 36 h with the indicated concentrations of IL-4, IL-13, IFN- γ or TNF- α , alone or in combination. All values are the mean $\pm$ SEM of six experiments. * P < 0.05, vs. unstimulated HNECs. ** P < 0.01, vs. unstimulated HNECs.

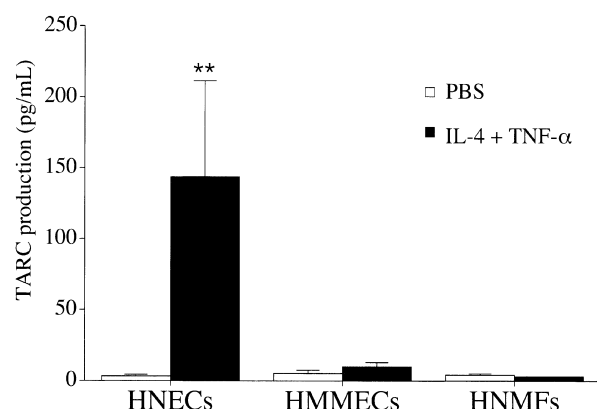


Fig. 5. Comparison of TARC production in HNECs, HMMECs and HNMFs. HNECs, HMMECs and HNMFs were cultured with or without the combination of IL-4 (10 ng/mL) and TNF- α (10 ng/mL) for 36 h. All values are the mean $\pm$ SEM of six experiments. * P < 0.05, vs. unstimulated cells. ** P < 0.01, vs. unstimulated cells.

TNF- α 10 ng/mL, showed strong enhancement of TARC mRNA levels ($P \leq 0.01$, $n = 6$) (Fig. 3).

To evaluate whether the production of TARC protein accompanied the expression of TARC mRNA, we determined the amount of TARC in the supernatants of HNECs. TARC production is expressed as picograms per 5×10^5 cells. Unstimulated HNECs produce a very small amount of TARC. In time-course experiments, TARC production continued to increase for at least 36 h by IL-4 and TNF- α stimulation as well as IL-13 and TNF- α stimulation (data not shown). Figure 4 shows the TARC production in the supernatants of HNECs after stimulation with various cytokines for 36 h. Again, the combined stimulation of IL-4 10 ng/mL and TNF- α 10 ng/mL, as well as that of IL-13 10 ng/mL and TNF- α 10 ng/mL, showed strong enhancement of TARC protein levels ($P \leq 0.01$, $n = 6$). When the synergic effects of IL-4 on TNF- α -induced TARC production were examined in the presence of fixed doses of TNF- α , the effect of IL-4 was dose dependent in the concentration range 1–100 ng/mL. Similar to the effect of IL-4, IL-13 also intensified the TNF- α -induced TARC production in a dose-dependent fashion. In addition, TNF- α

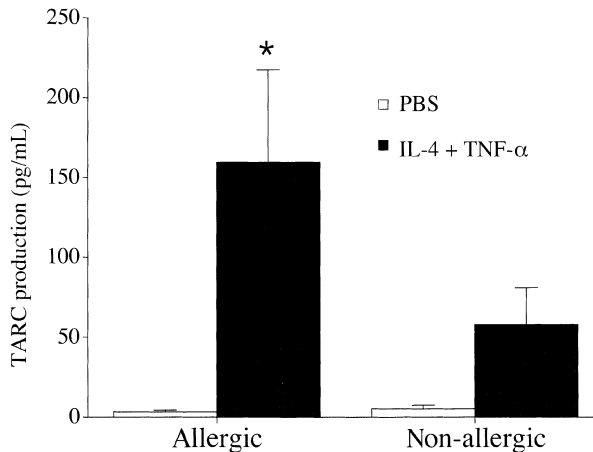


Fig. 6. Comparison of the IL-4- and TNF- α -induced TARC production in HNECs between those obtained from allergic rhinitis patients ($n = 6$) and non-allergic patients ($n = 6$). Bars represent means $\pm$ SEM. * $P < 0.05$ vs. non-allergic patients.

dose-dependently upregulated IL-4 and IL-13-induced TARC production in the TNF- α concentration ranges 1–100 ng/mL (data not shown).

On the other hand, neither the combined stimulation with IL-4 and TNF- α nor the combined stimulation with IL-13 and TNF- α induced significant levels of TARC production by HMMECs and HNMFs ($n = 6$, Fig. 5).

The amounts of TARC production induced by the combined stimulation with IL-4 and TNF- α in HNECs obtained from allergic patients was 159.5 ± 57.8 pg/mL ($n = 6$, Fig. 6). They were statistically higher than the TARC production in those obtained from non-allergic patients (57.9 ± 23.1 pg/mL, $n = 6$). We confirmed that the cytokine stimulation did not affect cell proliferation in our experimental condition, both in allergic and non-allergic subjects.

Discussion

Monocytes, activated endothelial cells and dendritic cells are thought to be the major source of the novel human chemokine TARC, which is selectively chemotactic for Th2 lymphocytes [17,18,21].

TARC is abundantly produced by monocytes treated with granulocyte macrophage colony stimulating factor (GM-CSF) or IL-3, especially in the presence of IL-4 [18]. Here, we demonstrated that TARC is produced in epithelial cells as well as inflammatory cells in the human nasal mucosa (Fig. 2). RT-PCR analysis showed that the TARC message was induced by IL-4 and IL-13, it began to increase 1 h after stimulation in HNECs and was still present at 36 h. Following the message expression, TARC protein increased during at least 36 h after the combined

stimulation with IL-13 and TNF- α . Furthermore, IL-4 and TNF- α synergistically induced TARC expression in HNECs, while they hardly induced TARC expression in HMMECs and HNMFs (Figs 3, 4 and 5). This availability may play a crucial role in providing rapid trafficking of Th2 lymphocytes to the lamina propria of the mucosa from vessels.

Campbell et al. observed that TARC was expressed in activated endothelium in inflamed skin [21]. In our study, expression of TARC on the vascular endothelium was also observed in the nasal mucosa of subjects with allergic rhinitis. However, its immunoreactivity was weaker compared with epithelial cells and infiltrated cells. We could detect neither TARC mRNA in HMMECs nor TARC protein in the medium of HMMECs stimulated with IL-4, IL-13 or TNF- α , alone or in combination.

These observations suggest that HMMECs play comparatively minor roles as cellular sources for TARC in the nasal mucosa.

Of special interest is the production of TARC by Th2-type cytokines IL-4 and IL-13. A prominence of CD4⁺ T cells and eosinophils, synthesis and release of Th2 cytokines, and the coordinate expression of chemokines and adhesion molecules are all characteristic of the allergic response observed in allergic rhinitis. *In vitro*, IL-4, IL-13 and TNF- α induce *de novo* surface expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells of the umbilical vein and accelerate selective eosinophil migration [22,23]. This is because eosinophils express the $\alpha 4$ integrin ligand for VCAM-1 but neutrophils do not [24]. Besides their role in the expression of adhesion molecules, recent studies revealed that these cytokines promote the production of eotaxin, a key mediator in the development of tissue eosinophilia [19,25] and its receptor CCR3 is expressed on Th2 but not on Th1 lymphocytes [14].

In addition, our results suggest that Th2 cytokines IL-4 and IL-13 may promote Th2 responses by inducing TARC production from epithelial cells, resulting in further Th2 polarization.

The mechanisms whereby IL-4 and TNF- α as well as IL-13 and TNF- α synergize to induce TARC production remain obscure. Transcription factors, such as NF- κ B and STAT6, are important in the orchestration of allergic inflammation. TNF- α and IL-4 are known to stimulate the release of eotaxin from nasal [19] and skin fibroblasts [25]. Most recently, Matsukura *et al.* reported that the combination of TNF- α and IL-4 produced 3.6-fold activation of the eotaxin promoter [26]. The eotaxin promoter contains overlapping consensus binding sites for NF- κ B and STAT6 that are known to mediate responses to TNF- α and IL-4, respectively. By using electrophoretic mobility shift assays and mutant plasmids, they revealed that TNF- α and IL-4 stimulate the expression of the eotaxin gene by activating

NF- κ B and STAT6. Although the signal transduction in TARC production has yet to be clarified, there is a possibility that a similar mechanism exists in TARC production. Considering that both NF- κ B and STAT6 are expressed in airway epithelial cells [27–29], there is a possibility that NF- κ B and STAT6 act positively at a site on the TARC promotor containing overlapping binding sites. This is known to cause dramatic synergy in other biological responses, such as the IgE germline promotor [30]. Further studies will be necessary to better understand the mechanism of interaction of the synergy between IL-4 and TNF- α .

The difference in TARC production after cytokine stimulation between HNECs from nasal mucosa of subjects with nasal allergy and non-allergy patients also indicated a fundamental biological difference between them. It is interesting to note that the HNECs described in this study were all derived from organs or tissues that border the external environment and may thus experience chronic inflammatory stimulation, possibly resulting in a difference in TARC production. In the previous study, we demonstrated that the amount of RANTES induced by TNF- α and IFN- γ was higher in HMMECs and HNECs obtained from patients with allergic rhinitis than in those from non-allergic patients [31]. The molecular mechanisms that allow such constitutive differences to persist over weeks *in vitro* remain to be elucidated.

It remains to be seen whether neutralization of TARC, either by antibodies or chemokine receptor antagonists, will have any therapeutic effect *in vivo*. Further studies with this animal model should also help elucidate the role of TARC in the pathogenesis of allergic rhinitis. In conclusion, our results are the first to show that epithelial cells produce TARC in nasal mucosa and that IL-4 and TNF- α , as well as IL-13 and TNF- α , synergistically induced TARC expression in epithelial cells, while hardly inducing it in endothelial cells and fibroblasts. Furthermore, we observed that the amount of TARC induced by cytokines was higher in epithelial cells obtained from patients with allergic rhinitis than in those from non-allergy patients. These results demonstrate a crucial role of nasal epithelial cells in the expression of TARC, and that Th2 cytokines IL-4 and IL-13 may promote Th2 responses by inducing TARC production from epithelial cells.

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