

Expression and function of the ST2 gene in a murine model of allergic airway inflammation

K. Oshikawa*, K. Yanagisawa†, S. Tominaga† and Y. Sugiyama*

*Division of Pulmonary Medicine, Department of Medicine, and †Department of Biochemistry, Jichi Medical School, Tochigi, Japan

Summary

Background We have recently reported that soluble ST2 protein levels are elevated in the sera of patients with asthma, and correlate well with the severity of asthma exacerbation. However, the role, function, and kinetics of soluble ST2 expression in asthma remain unclear.

Objective The objective of the present study was to clarify the function and kinetics of soluble murine (m) ST2 expression in a murine asthma model.

Methods We analyzed the kinetics of gene and protein expression of mST2 in sera or lung tissue after allergen (ovalbumin; OVA) challenge in a murine model of allergic airway inflammation, the effects of mST2 protein on OVA-induced Th2 cytokine production *in vitro* from splenocytes of sensitized mice, and the effects of soluble mST2 on Th2-dependent allergic airway inflammation by *in vivo* gene transfer of mST2.

Results Serum mST2 protein levels increased to the maximal level 3 h after the allergen challenge, before serum IL-5 levels peaked. The mRNA expression of mST2 in lung tissue was induced after the allergen challenge, while that in the spleen was constitutively detected. Furthermore, pre-treatment with mST2 protein significantly inhibited the production of IL-4 and IL-5, but not IFN- γ , from OVA-stimulated splenocytes *in vitro*, and intravenous mST2 gene transfer resulted in a drastic reduction in the number of eosinophils and in the levels of IL-4 and IL-5 in bronchoalveolar lavage fluid, compared with those in response to transfer of non-coding plasmid vector or of lipid alone.

Conclusion These results suggest that increases in endogenous mST2 protein after allergen exposure may modulate Th2-mediated airway inflammation, and that *in vivo* gene transfer of mST2 can be applicable to use in a novel immunotherapy for allergic diseases.

Keywords asthma, allergy, gene therapy, type-2 helper T cell, T1/ST2

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Introduction

Chronic airway inflammation is thought to play a fundamental role in the pathogenesis of asthma. The inflammatory response in asthma involves an increase in the number of eosinophils, basophils, mast cells and, most importantly, type-2 helper T-lymphocytes (Th2) [1, 2]. The importance of Th2 cytokines such as IL-4, IL-5, and IL-13 has been reported in studies demonstrating that either disruption of the genes encoding these proteins or their antibody-mediated neutralization prevents eosinophilia and attenuates various pathological changes – such as airway hyper-reactivity – associated with experimental asthma [3–6]. Therefore, inappropriate adaptive Th2-polarized immune responses to environmental antigens play a critical role in the development of asthma [7].

The ST2 gene was originally found to be one of the primary response genes in the G0/G1 transitional state of BALB/c-3T3 cells [8], and an Ha-*ras* oncogene-responsive gene [9, 10].

Alternative 3' processing of the primary transcript generates two messengers of 2.7 and 5 kb long. The short mRNA encodes a 38.5-kDa peptide with similarity to the extracellular portion of the IL-1 receptor (IL-1R) [8], and is secreted from the cell as a glycosylated protein of 60 kDa. This soluble secreted form (ST2) is expressed in embryonic tissues and mammary tumors, and is induced in fibroblast in response to proliferation-inducing stimuli [8–10]. On the other hand, the long mRNA encodes a membrane-bound protein whose extracellular domain is almost identical to the ST2 protein, and which shows a significant homology to the type I IL-1R [11]. This transmembrane receptor form (ST2L) is expressed in the hematopoietic tissues and the lung [12]. The ST2 gene is tightly linked to the genes encoding the type I and type II IL-1R on mouse chromosome 1 [13] and human chromosome 2 [14], and these two distinct form of ST2 gene products, ST2 and ST2L, belong to the IL-1R family.

Recently, the ST2/ST2L genes have recently been demonstrated to be preferentially expressed on murine Th2 cells, and to likely play an essential role in Th2 effector functions [15, 16]. *In vivo* depletion of ST2L by treatment with anti-ST2L antibody abrogated both the accumulation of eosinophils in the airway and the secretion of Th2 cytokines in a murine model of

Correspondence: Katsuhisa Oshikawa, Division of Pulmonary Medicine, Department of Medicine, Jichi Medical School, 3311–1 Minamikawachi, Tochigi, 329–0498, Japan. E-mail: kokyu2@jichi.ac.jp

Th2-dependent allergic airway inflammation [17, 18]. These findings were further supported by the results of a study using ST2L-deficient mice in a pulmonary granuloma model induced by *Schistosoma mansoni* eggs [19]. This study showed that Th2 cytokine production is impaired after immunization in the absence of ST2L expression. In contrast, other investigations showed no difference in Th2 response in wild-type versus ST2L-deficient mice after parasitic infection with the helminth *Nippostrongylus braziliensis* or after allergen-induced airway inflammation [20]. There are no obvious explanations for these discrepancies; however, as such discrepancies may be attributed to differences in experimental design, including antigen challenge, route of administration, and antigen-specific restimulation, the possibility that ST2L plays an important role in the development and function of Th2 cells cannot be excluded.

More recently, we have reported that soluble ST2 protein levels are elevated in the sera of patients with asthma, and correlate well with the severity of asthma exacerbation [21, 22]. However, the role, function, and kinetics of soluble ST2 expression in asthma have not been fully elucidated. In the present study, to address this issues, we analyzed the kinetics of the gene and protein expression of murine (m)ST2 in sera or lung tissue after allergen challenge in a murine model of asthma, and the effects of soluble mST2 on Th2-dependent allergic airway inflammation by *in vivo* gene transfer of mST2.

Materials and methods

Animals

Female BALB/c mice, 6–8 weeks of age, were purchased from Japan SLC, Inc. (Tochigi, Japan), housed in an animal facility of the Jichi Medical School under isothermal conditions, and allowed access to food and water *ad libitum*.

Plasmids construction and preparation of lipid-plasmid DNA complexes

The mST2 expression plasmid, pEF-BOS-mST2, was constructed in a backbone plasmid vector, pEF-BOS [23]. The mST2 cDNA was subcloned into the *Xba*I site of the pEF-BOS plasmid by using an *Xba*I linker [24].

Cationic lipid–DNA complexes were prepared using LIPO-FECTAMINE PLUS Reagent according to the manufacturer's protocol (Life Technologies, Inc., Rockville, MD, USA). Briefly, plasmid DNA (1 µg/mL) in sterile phosphate-buffered saline (PBS) and PLUS Reagent were mixed and left at room temperature (20 °C) for 15 min. The LIPOFECTAMINE Reagent was then added to the pre-complexed DNA, and the mixture was incubated at room temperature for 15 min. Finally, sterile PBS was added to cationic lipid–DNA complexes to a volume of 200 µL/injection.

Active immunization and exposure (murine asthma model)

All animals were actively immunized intraperitoneally with 100 µg of ovalbumin (OVA) and 20 mg Al(OH)₃ in saline on days 0 and 7. On day 14, animals were challenged twice with aerosolized OVA. The aerosol was generated from a 1% OVA solution for 30 min with an ultrasonic nebulizer (Omron Corp., Tokyo, Japan).

In vivo gene transfer into the murine asthma model

Twenty-four hours before allergen sensitization (days –1 and 6) and challenge (day 13), each mouse was injected in the lateral tail vein with 200 µL of cationic lipid–DNA complex solution containing either 10 µg of murine ST2 cDNA (pEF-BOS-mST2) or non-coding DNA (pEF-BOS), or the diluent (lipid alone). We detected substantial levels of mST2 mRNA expression in the lung 24 h after intravenous transfer of the mST2 gene (data not shown). The serum levels of mST2 protein 24 and 96 h after transfer of mST2 gene (pEF-BOS-mST2) into the test mice are 44.2 ± 12.8 ng/mL and 4.4 ± 3.5 ng/mL, respectively.

Collection of serum and bronchoalveolar lavage fluid

Mice were anesthetized by intraperitoneal administration of 0.2 mL of 10% pentobarbital sodium solution (Nembutal; Abbott Laboratories, North Chicago, IL, USA) at various time points throughout the active immunization protocol. Blood was collected by retro-orbital bleeding, and serum was frozen at –80 °C until assay. Bronchoalveolar lavage (BAL) was performed according to a standard protocol [17]. Briefly, the trachea was cannulated with a polyethylene tube, and the lungs were lavaged four times with 0.7 mL of PBS. Total cell counts were determined from BAL fluid (BALF) using a hemocytometer. After centrifugation, supernatants were collected and stored at –80 °C until assay. Cytospins were prepared and stained with Diff-Quik (Baxter, Deerfield, IL, USA). Two hundred cells were counted differentially using standard morphological criteria.

Measurement of murine ST2 protein and cytokines

The levels of soluble mST2 protein in serum or supernatant produced from splenocytes (stimulated *in vitro* for 24 h with 100 µg/mL OVA) of sensitized mice were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) that we originally developed. Briefly, 35 µL of various concentrations of the standard mST2 in PBS plus 0.1% (w/v) bovine serum albumin (BSA) and the samples were added to each well of a 96-well plate, which was pre-coated with rat monoclonal antibody against mouse T1/ST2 protein (MD Biosciences, Zürich, Switzerland), and the plate was incubated for 1 h at room temperature. After washing, 100 µL of anti-mST2 rabbit polyclonal antibody in PBS plus 0.1% BSA was added to the plate, and the plate was kept at room temperature for 1 h. The plate was washed an additional four times, and horseradish peroxidase-labeled antibody against rabbit IgG in PBS plus 0.1% BSA was then added and incubated for 30 min. Finally, the plate was washed and incubated with 140 µL of 10 mM *o*-phenylenediamine (OPD)–0.01% H₂O₂ in 100 mM sodium acetate buffer (pH 5.0) for 20 min. The optical density was measured at 450 nm with a microplate reader (Inter Medical, Tokyo, Japan).

The concentrations of IL-4, IL-5, and IFN-γ in serum, BALF, or supernatant produced from splenocytes (stimulated *in vitro* for 24 h with 100 µg/mL OVA) of sensitized mice were measured by a sandwich ELISA kit (IL-4 and IFN-γ: Biosource International Inc., Camarillo, CA, USA; IL-5: R & D Systems, Abingdon, UK). All samples were assayed in duplicate.

Analysis of mST2 and cytokine mRNA expression by RT-PCR

The lung and spleen were excised from mice at various time points in the active immunization protocol. Total RNA was extracted from whole lung tissue and spleen using Trizol reagent (Life Technologies). One microgram of total RNA was digested by DNase I (Life Technologies). Five hundred nanograms of RNA were reverse-transcribed and amplified by RT-PCR using an RT-PCR kit (Takara, Tsukuba, Japan) under the following conditions: 94 °C, 1 min; 57 °C, 1 min; and 72 °C, 1.5 min for 34 cycles. The following sequences were used as primers: mST2: (sense) 5'-ACTTCTTGCTGATGTCCTG-3' and (antisense) 5'-CAATGTGTGAGGGACACTCC-3'; IL-4: (sense) 5'-CATCCTGCTCTTCTTTCTCG-3' and (antisense) 5'-GATGCTCTTAGGCTTTCCAG-3'; IL-5: (sense) 5'-ACAAGCAATGAGACGATGAGG-3' and (antisense) 5'-GCCTTCCATTGCCCACTCT-3'; β -actin: (sense) 5'-TGTCCCTGTATGCCTCTGGT-3' and (antisense) 5'-ACTGTGTTGGCATAGAGGTC-3'. The PCR products of mST2, IL-4, IL-5, and β -actin were fragments of 266, 378, 272, and 477 bp in length, respectively. The amplified products were electrophoresed on 2.5% agarose gel, and the intensity of DNA bands was quantified by densitometry (National Institutes of Health, IMAGE 1.61).

Statistical analysis

Data are expressed as the means $\pm$ SEM. Multiple comparisons of cell counts and cytokine levels in BALF among all groups were performed by one-way factorial analysis of variance (ANOVA), followed by post-hoc tests. Levels of *P* less than 0.05 were considered to indicate statistical significance.

Results

Kinetics of mST2 and cytokine protein expression in sera

We first investigated the time course of mST2 protein and cytokine levels in an OVA-sensitized and-challenged mouse model (Fig. 1). Serum mST2 rapidly increased to the maximal level 3 h after the last allergen challenge, followed by a decrease, and then a slight increase at 36 h post-challenge. Thus the first peak in serum mST2 levels occurred earlier than the peak in serum IL-5 levels (3 h and 24 h, respectively, after the last allergen challenge; Fig. 1). Serum IL-4 levels were consistently too low for observation of their kinetics.

Kinetics of mST2 or cytokine mRNA expression in the lung and spleen

We next analyzed the kinetics of mST2 or cytokine mRNA expression in lung and spleen tissue after allergen exposure by RT-PCR. In the lungs, mST2 mRNA expression could not be detected before allergen challenge (0 h), but appeared after allergen challenge and peaked at 6 h post-challenge (Fig. 2a). Expression of the mRNAs of IL-4 and IL-5 could be detected from 3 h post-challenge, and thus preceded the mST2 mRNA expression (Fig. 2a). In contrast, mST2 mRNA expression in the spleen was constitutively detected, was enhanced after allergen challenge, and reached a peak at 6 h post-challenge (Fig. 2b). These results suggest that the mST2 protein may be expressed in splenocytes before allergen challenge. These results, together with those regarding the kinetics of the serum

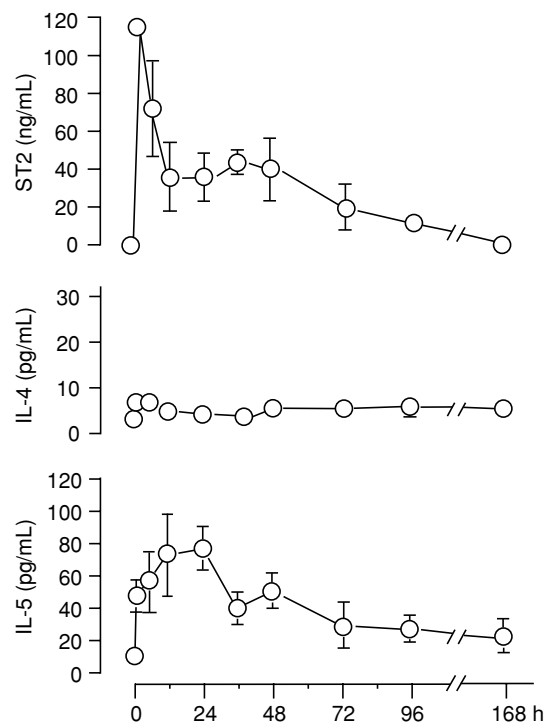


Fig. 1. Kinetics of ST2, IL-4, or IL-5 protein levels in the sera after OVA challenge. BALB/c mice were sensitized with OVA intraperitoneally and challenged with aerosolized OVA. Blood was taken at the indicated time before and after OVA challenge as described in Materials and methods. The concentrations of ST2, IL-4, and IL-5 in the sera were measured by ELISA. Mean concentrations $\pm$ SEM are shown for cumulative data from two independent experiments (3–4 mice per experiment).

mST2 protein (Fig. 1), led us to hypothesize that the serum mST2 protein, which reached its maximal level at 3 h post-challenge, may be derived from splenocytes. To test this hypothesis, splenocytes from naive or sensitized mice were stimulated *in vitro* for 24 h with 100 μ g/mL OVA, and mST2 protein levels in the supernatants were measured by ELISA. However, we could not detect mST2 production in the culture supernatant of OVA-stimulated splenocytes from sensitized mice.

Effects of mST2 protein on OVA-stimulated splenocytes from sensitized mice

Next, to elucidate the function of ST2 in a murine asthma model, we investigated the effects of mST2 protein on OVA-induced Th2 cytokine production from splenocytes of sensitized mice *in vitro*. Splenocytes from OVA-sensitized mice were pre-treated with recombinant mST2 protein or medium alone, and then were unstimulated or stimulated with OVA (100 μ g/mL) *in vitro* for 24 h. Cytokine concentrations in culture supernatants were measured by ELISA. Treatment with mST2 protein significantly inhibited IL-4 and IL-5 production by OVA-stimulated splenocytes (Figs 3a and b). In contrast, IFN- γ production from OVA-stimulated splenocytes was induced by pre-treatment with mST2 protein, but *P*-values do not indicate a statistical difference between mST2(-)/OVA(+) and mST2(+)/OVA(+) groups (*P* = 0.067, Fig. 3c).

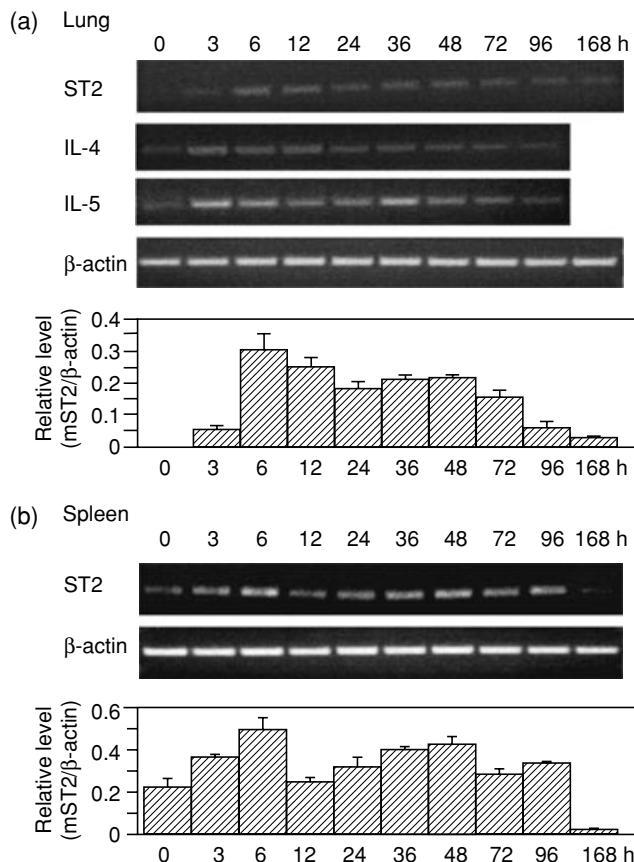


Fig. 2. Kinetics of ST2, IL-4, or IL-5 mRNA expression in the lung (a) and the spleen (b) after OVA challenge. BALB/c mice were OVA-sensitized and-challenged, and total RNA was extracted from the lung and spleen tissue at the indicated time before and after OVA challenge as described in Materials and methods. The cDNA samples obtained by RT-PCR for ST2, IL-4, IL-5, and β -actin were loaded on a 2.5% agarose gel. The data shown are representative of three independent experiments. Densitometric analyses of ST2 mRNA expression in the lung (a) and the spleen (b) tissues were carried out. The relative level designates the ratio obtained from the density of the band of ST2 mRNA expression standardized with that of the β -actin mRNA expression level. The mean relative densities $\pm$ SEM are shown for the cumulative data of three independent experiments (two mice per experiment).

Effects of *in vivo* gene transfer of mST2 on allergic airway inflammation

The observation that mST2 protein can inhibit OVA-induced Th2 cytokine production *in vitro* raised the possibility that overexpression of mST2 may also suppress Th2-mediated inflammation *in vivo*. To test this hypothesis, we analyzed the role and function of mST2 by evaluating the effects of soluble mST2 on allergen-induced airway inflammation by *in vivo* transfection with the mST2 gene. The intravenous mST2 gene (pEF-BOS-mST2) transfer significantly reduced total cell counts in BALF compared with transfer of lipid alone (control), and resulted in drastic reductions in the number of eosinophils in BALF compared with transfer of pEF-BOS or lipid alone (Fig. 4). Transfer of the non-coding plasmid vector (pEF-BOS) alone, however, marginally inhibited BALF eosinophilia in comparison with lipid alone. Regarding the number of

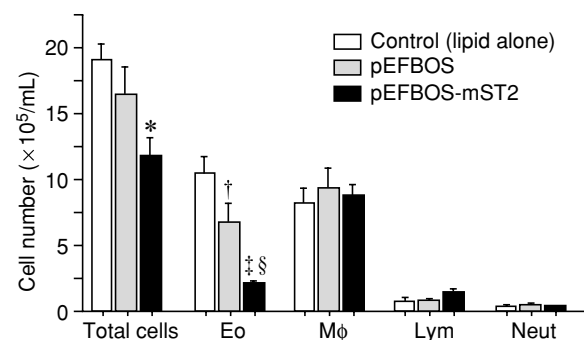


Fig. 4. Effects of intravenous mST2 gene delivery on total and differential cell numbers in BALF. BALB/c mice were injected intravenously with cationic lipid-DNA complexes containing either 10 μ g of mST2 cDNA (pEF-BOS-mST2) or an equivalent amount of non-coding DNA (pEF-BOS), or with diluent (lipid alone: control) on days -1, 6, and 13 in a murine asthma model as described in Materials and methods. Twenty-four hours after the last allergen challenge, BALF was collected and cell differentiation was determined. The mean number of cells $\pm$ SEM are shown for each group. * $P < 0.05$ versus control; † $P < 0.05$ versus control; ‡ $P < 0.001$ versus control; § $P < 0.05$ versus pEF-BOS. The mean number of cells $\pm$ SEM are shown for the cumulative data from two independent experiments (8–10 mice per group). Eo, eosinophil; Mφ, macrophage; Lym, lymphocyte; Neu, neutrophil.

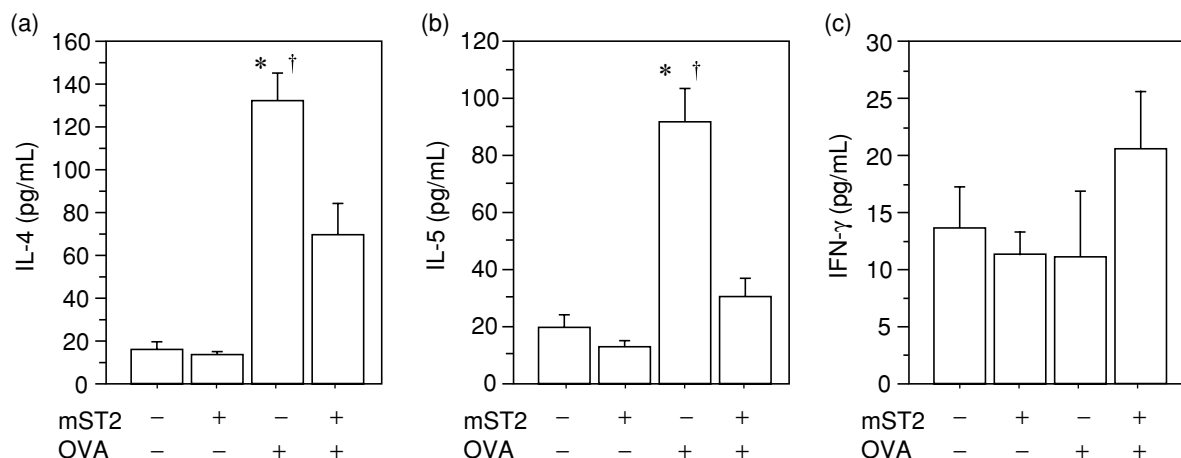


Fig. 3. Effects of mST2 protein on allergen-induced production of IL-4 (a), IL-5 (b), and IFN- γ (c) from splenocytes of sensitized mice. Splenocytes obtained from OVA-sensitized mice were pre-treated for 3 h with recombinant mST2 protein (500 ng/mL) or medium alone, and were then unstimulated or stimulated with OVA (100 μ g/mL) *in vitro* for 24 h. Culture supernatants were harvested, and concentrations of cytokines were measured by ELISA. (a) * $P < 0.0001$ versus either treatment group of mST2(-)/OVA(-) or mST2(+)/OVA(-); † $P < 0.001$ versus group of mST2 (+)/OVA(+). (b) * $P < 0.0001$ versus either treatment group of mST2(-)/OVA(-) or mST2(+)/OVA(-); † $P < 0.001$ versus group of mST2(+)/OVA(+). (c) There were no significant differences among treatment groups. The mean cytokine concentrations $\pm$ SEM are shown for the cumulative data from two independent experiments.

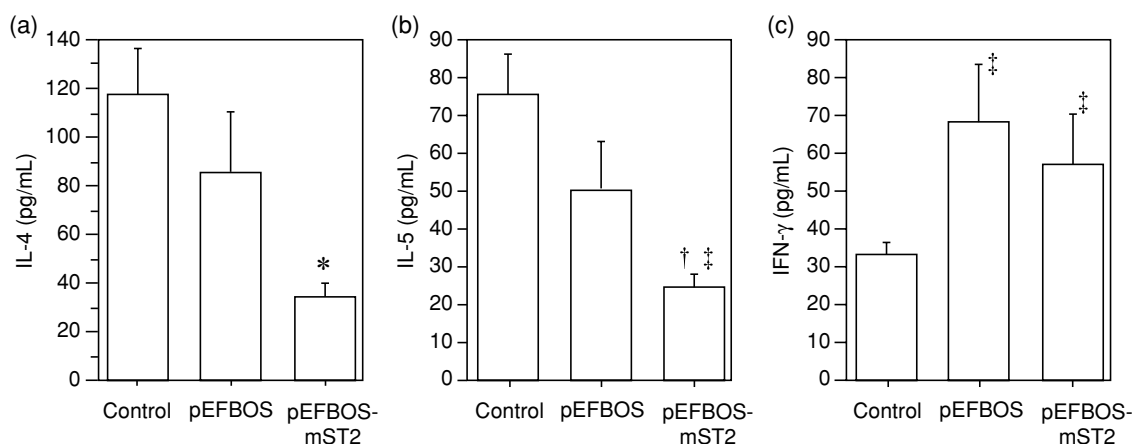


Fig. 5. Effects of intravenous mST2 gene delivery on concentrations of IL-4 (a), IL-5 (b), and IFN- γ (c) in BALF. BALB/c mice were injected intravenously with cationic lipid–DNA complexes containing either 10 μ g of mST2 cDNA (pEF-BOS-mST2) or an equivalent amount of noncoding DNA (pEF-BOS), or with diluent (lipid alone: control) on days –1, 6, and 13 in a murine asthma model as described in Materials and methods. Twenty-four hours after the last allergen challenge, BALF was collected, and the concentrations of IL-4 (a), IL-5 (b), and IFN- γ (c) in BALF were determined by ELISA. The mean cytokine concentrations $\pm$ SEM are shown for the cumulative data from two independent experiments (8–10 mice per group). (a) * P < 0.01 versus control. (b) † P < 0.005 versus control; ‡ P < 0.05 versus pEF-BOS. (c) ‡ P < 0.05 vs. control.

macrophages, lymphocytes, and neutrophils in BALF, no difference was found among all transfer groups. Furthermore, mST2 gene transfer markedly inhibited IL-4 and IL-5 production, but not that of IFN- γ , in BALF, compared with the transfer of pEF-BOS or lipid alone (Figs 5a and b). In contrast, IFN- γ production in BALF was significantly induced by transfer of mST2 expression vector (pEF-BOS-mST2) as well as of its sham vector (pEF-BOS), compared with that of lipid alone (Fig. 5c).

Discussion

Several recent reports have addressed the kinetics and function of ST2 gene products in Th2-mediated allergic inflammation in murine models [13–16]. However, most studies have focused on the membrane-bound receptor form, ST2L; as such, the kinetics and function of the soluble form, ST2, in allergen-induced allergic inflammation have yet to be elucidated. Therefore, using a conventional murine model of asthma, we carried out temporal analysis of ST2 expression in sera and lung tissue after allergen challenge, and evaluated the effects of soluble ST2 on Th2-dependent allergic airway inflammation.

Our results demonstrated a biphasic increase in serum mST2 levels immediately after allergen exposure, with a maximal increase in serum mST2 levels at 3 h and a slight increase at 36 h post-challenge, followed by a decrease (Fig. 1). To our knowledge, this is the first report addressing the kinetics of serum-soluble mST2 protein after allergen challenge in murine models of allergen-induced asthma. In contrast, Gajewska et al. based on an experimental protocol nearly identical to ours, have reported that the proportion of CD4⁺ T cells expressing mST2L in the lung increases as early as 72 h after challenge, with a considerable increase occurring 5 days after challenge [25]. Furthermore, there has thus far been no evidence that soluble ST2 is produced by shedding of ST2L, and some researchers have demonstrated that ST2 and ST2L are generated by different promoter usage and different transcription initiation [12, 26]. Taken together, these results suggest that expression

of the ST2 and ST2L genes is regulated independently, and thus both proteins may exhibit distinct functions in immunological responses.

It is necessary to determine which cells produce serum mST2 protein in rapid response to allergen stimulation. We failed to detect production of soluble mST2 protein from *in vitro*-stimulated splenocytes in the present study. The rapid release of mST2 into blood within 3 h of allergen challenge suggests possible release from a pre-stored compartment of mST2 protein. It is possible that mast cells can be one potential source of pre-formed mST2, because mast cells are critical effectors in the pathogenesis of Th2-dependent allergic disorders, and are found to express ST2 at high levels [27, 28]. Antigen-mediated engagement of Fc ϵ R1 triggers mast cell degranulation and mediator release during allergic inflammation [29]. Analyses including immunohistochemistry and *in situ* hybridization are now ongoing to determine the origin of serum mST2 in the early phase after allergen challenge.

Regarding the function of ST2 gene products in Th2-mediated allergic inflammation, most studies have demonstrated that either disruption of the genes encoding the ST2L protein, or antibody-mediated neutralization of the protein partially inhibits various pathological changes associated with experimental allergic inflammation, suggesting that ST2L may play an essential role in the development of Th2 effector function in a murine model of allergic inflammation [17–19], though some investigations have produced controversial results [20]. In contrast, there has been little research regarding ST2 (soluble form) function [17]. Therefore, in the present study, we investigated the effects of soluble mST2 on allergen-induced airway inflammation by *in vivo* gene transfer of the mST2 gene in a murine asthma model. Our results demonstrated that the mST2 gene transfer results in a drastic reduction in the number of eosinophils as well as production of Th2 cytokines in BALF compared with transfer of empty vector or lipid carrier. These results are in agreement with previous reports demonstrating that administration of the recombinant ST2 fusion protein attenuates BALF eosinophilia and suppresses Th2 cytokine production in Th2-mediated allergic inflammation [17]. Such an

inhibitory effect of the transgenic mST2 protein may be explained by down-regulation of the Th2 effector function via inhibition of the interaction between ST2L on Th2 cells and the protein's putative ligand. This hypothesis is strongly supported by the *in vitro* data of Fig. 3 showing that pre-treatment with mST2 protein significantly suppressed production of Th2 cytokines from OVA-stimulated splenocytes, which were obtained from OVA-sensitized mice. Within antigen-sensitized splenocytes, there is an interaction between antigen-presenting cells (APCs) recognizing antigen specifically and Th cells capable of responding to identical antigens, and this interaction is important to the subsequent activation and differentiation of effector Th2 cells during allergen exposure [25]. Lambrecht et al. has reported that myeloid dendritic cells (DCs), capable of strong antigen presentation, can induce Th2 response to inhaled antigen via close interaction between ST2L on Th2 cells and its putative ligands on DCs in a murine asthma model [30]. It can therefore be speculated that ST2 protein may inhibit the interaction between APC and Th2 cells by competition with the putative ligand in ligation with receptor-type ST2L on Th2 cells, resulting in a down-regulation of Th2 effector function. Further analyses are necessary to test this hypothesis but, if true, the ST2L-mediated pathway may constitute another candidate for the co-stimulatory pathway between APCs and Th2 cells.

What is the function of the increased levels of soluble mST2 protein after allergen challenge in the murine asthma model? Based on our results, it is possible that the observed increase in endogenous mST2 protein production after allergen challenge (Fig. 1) may suppress allergic airway inflammation in an auto-regulative manner. We have recently reported that serum human ST2 levels in patients with atopic asthma rise in correlation with the severity of asthma exacerbation [22]. However, the function of soluble ST2 expression during asthma attack remains unclear. It seems unwise to extrapolate these results from the murine experiments to directly explain the function of human ST2 in asthma, but appears possible that increased human ST2 protein production may be required to suppress allergic inflammation, with the increase being proportional to the severity of airway inflammation contributing to acute asthma exacerbation.

Intriguingly, we observed that even non-coding plasmid DNA complexed to a cationic liposome resulted in a reduction in the number of BALF eosinophils (Fig. 4). This finding is compatible with previous reports demonstrating that administration of lipid-non-coding plasmid DNA complexes reduces infiltration of eosinophils into the airway [31, 32], possibly because lipid-non-coding plasmid DNA complexes can themselves induce the liberation of Th1 cytokines such as IFN- γ (Fig. 5c) and IL-12 [25]. Furthermore, bacterial plasmid DNA has been demonstrated to contain CpG motifs (immunostimulatory DNA sequences), which themselves induce Th1 cytokines [33]. Therefore, the plasmid gene-transfer technique has a beneficial effect on induction of Th1 cytokines, resulting in a down-regulation of Th2 cells in murine asthma models. Taken together, these results suggest that *in vivo* lipofection with plasmid vector encoding mST2 cDNA may more effectively inhibit Th2-dominant inflammation, and such an approach may be useful in novel gene immunotherapy for allergic diseases. Further modifications regarding the dose of the plasmid gene, the

route of administration, and the treatment schedule are in progress to optimize the efficacy of this protocol.

In summary, we here analyzed the kinetics and function of mST2 expression after allergen challenge in a murine model of asthma, and our results indicate that serum mST2 protein levels increase immediately after allergen challenge, and that treatment with mST2 protein or gene results in a drastic reduction in pathological changes associated with the Th2-polarized condition *in vitro* and *in vivo*. These results suggest that increases in endogenous ST2 protein after allergen exposure may function to modulate Th2-mediated airway inflammation by sequestering ST2 ligands. At present, an ST2 ligand has not yet been identified. Identification of such a ligand may improve our understanding of the biological and pathological functions of ST2, particularly in Th2-mediated allergic disease.

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References

- 1 Bousquet J, Chané P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- 2 Robinson DS, Hamid Q, Ying S et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298–304.
- 3 Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin-5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996; 183:195–201.
- 4 Corry DB, Grunig G, Hadeiba H et al. Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. *Mol Med* 1998; 4:344–55.
- 5 Wills-Karp M, Luyimbazi J, Xu X et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282:2258–61.
- 6 Grunig G, Warnock M, Wakil AE et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; 282:2261–3.
- 7 Umetsu DT, DeKruyff RH. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1997; 100:1–6.
- 8 Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS Lett* 1989; 258:301–4.
- 9 Werenskiold AK, Hoffmann S, Klemenz R. Induction of a mitogen-responsive gene after expression of the Ha-ras oncogene in NIH 3T3 fibroblasts. *Mol Cell Biol* 1989; 9:5207–14.
- 10 Rossler U, Andres AC, Reichmann E, Schmahl W, Werenskiold AK. T1, an immunoglobulin superfamily member, is expressed in H-ras-dependent epithelial tumours of mammary cells. *Oncogene* 1993; 8:609–17.
- 11 Yanagisawa K, Takagi T, Tsukamoto T, Tetsuka T, Tominaga S. Presence of a novel primary response gene ST2L, encoding a

- product highly similar to the interleukin 1 receptor type, 1. *FEBS Lett* 1993 1994; ; 318:83–7.
- 12 Bergers G, Reikerstorfer A, Braselmann S, Graninger P, Busslinger M. Alternative promoter usage of the Fos-responsive gene *Fit-1* generates mRNA isoforms coding for either secreted or membrane-bound proteins related to the IL11 receptor. *EMBO J* 1994; 13:1176–88.
 - 13 Tominaga S, Jenkins NA, Gilbert DJ, Copeland NG, Tetsuka T. Molecular cloning of the murine ST2 gene. Characterization and chromosomal mapping. *Biochim Biophys Acta* 1991; 1090:1–8.
 - 14 Tominaga S, Inazawa J, Tsuji S. Assignment of the human ST2 gene to chromosome 2 at q11. *Hum Genet* 1996; 97:561–3.
 - 15 Yanagisawa K, Naito Y, Kuroiwa K et al. The expression of ST2 gene in helper T cells and the binding of ST2 protein to myeloma-derived RPMI8226 cells. *J Biochem* 1997; 121:95–103.
 - 16 Xu D, Chan WL, Leung BP et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998; 187:787–94.
 - 17 Löhning M, Stroehmann A, Coyle AJ et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc Natl Acad Sci USA* 1998; 95:6930–5.
 - 18 Coyle AJ, Lloyd C, Tian J et al. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J Exp Med* 1999; 190:895–902.
 - 19 Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000; 191:1069–76.
 - 20 Hoshino K, Kashiwamura S, Kuribayashi K et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J Exp Med* 1999; 190:1541–8.
 - 21 Kuroiwa K, Li H, Tago K et al. Construction of ELISA system to quantify human ST2 protein in sera of patients. *Hybridoma* 2000; 19:151–9.
 - 22 Oshikawa K, Kuroiwa K, Tago K et al. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med* 2001; 164:277–81.
 - 23 Mizushima S, Nagata S. pEF-BOS, a powerful mammalian expression vector. *Nucl Acids Res* 1990; 18:5322.
 - 24 Takagi T, Yanagisawa K, Tsukamoto T, Tetsuka T, Nagata S, Tominaga S. Identification of the product of the murine ST2 gene. *Biochim Biophys Acta* 1993; 1178:194–200.
 - 25 Gajewska BU, Swirski FK, Alvarez D et al. Temporal-spatial analysis of the immune response in a murine model of ovalbumin-induced airways inflammation. *Am J Respir Cell Mol Biol* 2001; 25:326–34.
 - 26 Iwahana H, Yanagisawa K, Ito-Kosaka A et al. Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. *Eur J Biochem* 1999; 264:397–406.
 - 27 Moritz DR, Gheyselinck J, Klemenz R. Expression analysis of the soluble and membrane-associated forms of the interleukin-1 receptor-related T1 protein in primary mast cells and fibroblasts. *Hybridoma* 1998; 17:107–16.
 - 28 Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J Immunol* 1998; 161:4866–74.
 - 29 Beaven MA, Metzger H. Signal transduction by Fc receptors: the Fc epsilon RI case. *Immunol Today* 1993; 14:222–6.
 - 30 Lambrecht BN, Veerman MD, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000; 106:551–9.
 - 31 Freimark BD, Blezinger HP, Florack VJ et al. Cationic lipids enhance cytokine and cell influx levels in the lung following administration of plasmid: cationic lipid complexes. *J Immunol* 1998; 160:4580–6.
 - 32 Dow SW, Schwarze J, Heath TD, Potter TA, Gelfand EW. Systemic and local interferon gamma gene delivery to the lungs for treatment of allergen-induced airway hyperresponsiveness in mice. *Hum Gene Ther* 1999; 10:1905–14.
 - 33 Sato Y, Roman M, Tighe H et al. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* 1996; 273:352–4.