Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 299 (2002) 18–24
BBRC
www.academicpress.com

ST2 protein induced by inflammatory stimuli can modulate acute lung inflammation☆

Katsuhisa Oshikawa,[a,*] Ken Yanagisawa,[b] Shin-ichi Tominaga,[b] and Yukihiko Sugiyama[a]

[a] *Division of Pulmonary Medicine, Department of Medicine, Jichi Medical School, 3311 Minamikawachi, Kawachi-gun, Tochigi 329-0498, Japan*
[b] *Department of Biochemistry, Jichi Medical School, 3311 Minamikawachi, Kawachi-gun, Tochigi 329-0498, Japan*

Received 11 October 2002

Abstract

We have investigated gene and protein expression of ST2/ST2L in a murine alveolar macrophage (AM) cell line, MH-S, reacting to inflammatory stimuli in vitro and in the lung tissue of an acute lung injury model in vivo. We have also analyzed the effect of soluble ST2 protein on inflammatory response of MH-S cells. Lipopolysaccharide (LPS) and proinflammatory cytokines such as IL-1β, IL-6, and TNF-α induced ST2 mRNA expression in MH-S cells. In an acute lung injury model, protein and mRNA expression levels of ST2 increased to the maximal level at 24–72 h after the LPS challenge. Furthermore, pretreatment with ST2 protein significantly reduced the protein production and gene expression of IL-1α, IL-6, and TNF-α in LPS-stimulated MH-S cells in vitro. These results suggest that increases in endogenous ST2 protein in AM, which is induced by inflammatory stimuli, such as LPS and proinflammatory cytokines, may modulate acute lung inflammation.

Keywords: Acute lung injury; Alveolar macrophage; Lipopolysaccharide; Proinflammatory cytokine; ST2; T-lymphocyte

The ST2 gene, also known as T1 and Fit-1, was originally identified as a gene induced by serum stimulation of fibroblasts [1–3] and was classified as a member of the interleukin (IL)-1 receptor family. Three distinct types of ST2 gene products, a soluble secreted form (ST2), a transmembrane receptor form (ST2L), and a variant form (ST2V), have been cloned so far [1,4,5]. ST2 is expressed predominantly on helper T-lymphocyte type 2 (Th2) [6,7], and studies using either anti-ST2L antibody or ST2-deficient mice have demonstrated important roles for this molecule in regulating Th1/Th2-associated immune responses in pulmonary experimental models, such as allergen-induced airway inflammation models or pulmonary granuloma models induced by parasitic infection [8–10]. We have recently reported that serum ST2 protein levels increased in patients with acute exacerbation of atopic asthma [11,12], which is characteristic of Th2-mediated eosinophilic airway inflammation [13,14]. The recent demonstration of mRNA encoding ST2/ST2L in murine mast cells, in monoblastic cell lines, and in megakaryoblastic cell lines raises the possibility that cells other than Th2 cells may also express the ST2/ST2L gene [6,15]. Furthermore, both genes were detected in the human lung in analyses of the tissue distribution of ST2/ST2L mRNA [16], but ST2/ST2L gene expression in lung structural cells including bronchial or alveolar epithelial cells, endothelial cells, lung fibroblasts, and the like has yet to be analyzed.

Besides the regulation of disease outcome through the modulation of Th1/Th2 bias, there is indirect evidence to suggest that ST2 gene products may also be involved in inflammatory responses. Proinflammatory stimuli including tumor necrosis factor (TNF)-α, IL-

☆ *Abbreviations:* IL, interleukin; Th, helper T-lymphocyte; TNF, tumor necrosis factor; BAL, bronchoalveolar lavage; LPS, lipopolysaccharide; ARDS, adult respiratory distress syndrome; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; TLR, Toll-like receptor; MyD88, myeloid differentiation factor 88; IRAK, IL-1 receptor-associated kinase; TRAF, TNF receptor-associated factor; AM, alveolar macrophage; PMN, polymorphonuclear neutrophils; NF, nuclear factor; PMA, phorbol myristate acetate; Ca, Ca ionophore (A23187).

* Corresponding author. Fax: +81-285-44-3586.
E-mail address: oshikatu@jichi.ac.jp (K. Oshikawa).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02578-0

1α, and IL-1β induced ST2 expression in murine BALB/c-3T3 cells [17]. ST2L expression was detected in human monocytes after lipopolysaccharide (LPS) stimulation in vitro and in the muscle and spleen of mice after systemic administration of LPS [18]. These findings led us to test ST2/ST2L expression in LPS-induced pulmonary inflammation, specifically acute lung injury, because one form of acute lung injury that often results from sepsis is the adult respiratory distress syndrome (ARDS), which is clinically important [19]. Although many factors are involved in the development of ARDS, the local release of cytokines by alveolar macrophage (AM) is of importance in the pathogenesis of ARDS [20]. AM responds to LPS, which is released during sepsis, by releasing large amounts of inflammatory mediators, and a prolonged release of cytokines by AM has been associated with a more adverse outcome in patients with this disease [21]. Therefore, it is very important to evaluate the role and function of ST2/ST2L expression in regulation of cytokine production in AM in response to proinflammatory stimuli.

In the present study, we have investigated gene expression of ST2/ST2L in various lung structural cells and a murine AM cell line, MH-S, responding to proinflammatory stimuli in vitro, and their expression in the lung in a murine model of LPS-induced acute lung injury. We have also analyzed the effect of soluble ST2 protein on the inflammatory response of MH-S cells.

Materials and methods

Mice, cells, and reagents. Female BALB/c mice, 6–8 weeks of age, were purchased from Japan SLC (Tochigi, Japan) and housed in an animal facility of the Jichi Medical School. All animal experiments were conducted in accordance with principles stated in the "Guide for the Care and Use of Laboratory Animals." (NIH publication 86-23, National Institutes of Health, Bethesda, MD, 1985.) The NHBE (bronchial epithelial cell), HPAEC (pulmonary arterial endothelial cell), HPSMC (pulmonary smooth muscle cell), and NHLF (lung fibroblast) were purchased from Biowhittaker (Walkersville, MD). The MH-S (murine AM) and A549 (type II alveolar epithelial cells) were obtained from American Type Culture Collection (Rockville, MD). Human AMs were obtained from healthy volunteers by bronchoalveolar lavage (BAL). This protocol was approved by our Institutional Review Board for human studies and informed consent was obtained from all subjects. LPS from *Escherichia coli* and mouse recombinant proteins of IL-1β, IL-6, and TNF-α were purchased from Sigma (St. Louis, MO).

LPS-induced acute lung injury model. After mice were anesthetized by intraperitoneal administration of 0.2 ml of 10% pentobarbital sodium solution (Nembutal; Abbott Laboratories, North Chicago, IL), 100 μg/kg LPS in 60 μl saline was administered intranasally as previously described [22,23]. Control mice were administered intranasally with 60 μl LPS-free saline. BAL was performed according to a standard protocol [8] and lung tissue was excised for RNA extraction.

Measurement of murine ST2 protein and cytokine levels. The levels of soluble murine ST2 protein in serum or BAL fluid (BALF) were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available reagents as rat monoclonal antibody against mouse T1/ST2 protein (MD Biosciences, Zürich, Switzerland), anti-murine ST2 rabbit polyclonal antibody, horseradish peroxidase-labeled antibody against rabbit IgG, and *o*-phenylenediamine–0.01% H_2O_2. The levels of IL-1α, IL-6, and TNF-α were measured by a sandwich ELISA kit (IL-1α: Amersham Biosciences, Piscataway, NJ; IL-6 and TNF-α: Biosource International, Camarillo, CA).

Analysis of mRNA expression for cytokines, ST2, and ST2L by reverse transcription-polymerase chain reaction. The RNA extraction and RT-PCR were performed as previously described [6]. The PCR products of murine IL-1α, IL-6, TNF-α, ST2, ST2L, Toll-like receptor (TLR) 2, TLR4, MD2, myeloid differentiation factor 88 (MyD88), IL-1 receptor-associated kinase (IRAK), TNF receptor-associated factor (TRAF)-6, and β-actin were fragments of 264, 600, 331, 266, 222, 389, 356, 284, 349, 417, 316, and 477 bp in length, respectively, and the PCR products of human ST2, ST2L, and β-actin were fragments of 659, 454, and 547 bp in length, respectively.

In vitro treatment with soluble ST2 protein of LPS-stimulated cells. For in vitro experiments, MH-S cells were plated in 24-well plates at 5×10^5 cells/well in 1 ml complete medium overnight. Cells were then treated with soluble ST2 protein or medium for 3 h followed by stimulation with LPS or medium alone. At the indicated time, samples of culture supernatants or RNA were stored at −80 °C.

Statistical analysis. Data are expressed as means ± SEM. Multiple comparisons among all groups were performed by one-way factorial analysis of variance (ANOVA) followed by post hoc tests. $P < 0.05$ was considered statistically significant.

Results

ST2 and ST2L mRNA expression in various cells

We first analyzed ST2 and ST2L mRNA expression of human lung structural cells including A549, NHBE, HPAEC, HPSMC, NHLF, and AM (Fig. 1). A wide range of ST2 mRNA expression was detected in all the above cells in the absence of stimulation, but the expression levels were relatively low. When stimulated with PMA and A23187 (PMA/Ca), ST2 mRNA expression in all cells was enhanced to a similar extent. In contrast, ST2L mRNA expression, which varied with cells, showed three patterns: undetectable gene expression even after stimulation with PMA/Ca as shown in

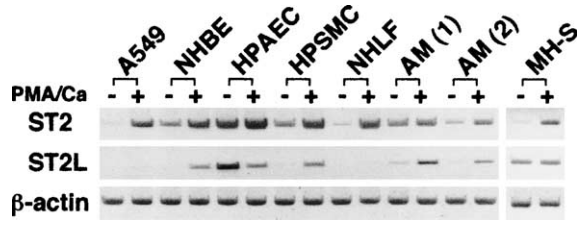

Fig. 1. ST2 and ST2L mRNA expressions in lung structural cells. A549, NHBE, HPAEC, HPSMC, NHLF cells, human AM obtained by BAL from two healthy volunteers, and a murine AM cell line, MH-S, were cultured in 1 ml RPMI 1640 medium for 6 h with or without PMA (50 ng/ml) plus A23187 (200 ng/ml). The cDNA samples obtained by RT-PCR for ST2, ST2L, and β-actin were loaded on a 3.0% agarose gel. *Abbreviations*: PMA, phorbol myristate acetate; Ca, A23187 (Ca ionophore).

A549; undetectable or low levels of gene expression enhanced with stimulation as shown in NHBE, HPSMC, NHLF, and AM; and detectable gene expression without stimulation downregulated following the addition of PMA/Ca as shown in HPAEC. In a murine AM cell line, MH-S, very low level of expression of ST2 mRNA increased after stimulation with PMA/Ca, whereas the ST2L mRNA was constitutively expressed and remained unchanged after stimulation.

ST2 and ST2L mRNA expression of MH-S cells stimulated with LPS or proinflammatory cytokines

To determine the relevance of ST2 or ST2L expression in inflammatory responses, we investigated whether LPS or proinflammatory cytokine induces ST2 or ST2L expression in MH-S cells. As shown in Fig. 2, ST2 mRNA expression in MH-S cells was greatly enhanced 3–6 h after the addition of LPS, IL-1β or TNF-α, or 12 h after stimulation with IL-6. In contrast, LPS and cytokines had minimal effect on the expression of ST2L mRNA. There was essentially no difference in the ST2L mRNA expression of MH-S cells in response to LPS, IL-1β, IL-6, or TNF-α compared to non-stimulated MH-S cells.

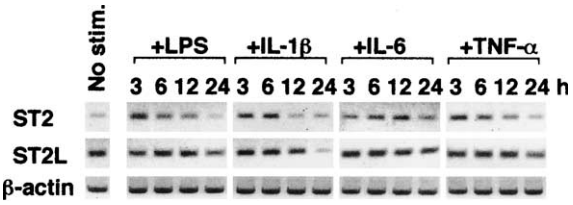

Fig. 2. Kinetics of ST2 and ST2L mRNA expressions in MH-S cells after stimulation with LPS or cytokines. (A) MH-S cells (non-stimulated control) were treated with LPS (1 μg/ml), IL-1β (10 ng/ml), IL-6 (10 ng/ml), or TNF-α (10 ng/ml) for the indicated times, and the cDNA samples obtained by RT-PCR for ST2, ST2L, and β-actin were loaded on a 3.0% agarose gel. Similar results were obtained in three separate experiments. "No stim." means that there was no stimulation with LPS or cytokines.

ST2, ST2L, and cytokine expression in an LPS-induced acute lung injury model

The results demonstrating that LPS induce ST2 mRNA expression in vitro led us to test the ST2 expression in an in vivo model of LPS-induced acute lung inflammation. In this model, intranasal instillation of LPS in mice resulted in a huge influx of polymorphonuclear neutrophils (PMN) into the lung (Figs. 3A and B). A significant increase in BALF PMN was detected as soon as 3 h after LPS challenge, with a peak at 24 h followed by a decrease to the basal level by 120 h. AM and lymphocyte counts in BALF also increased but with a lower range and different kinetics peaking at 24–72 h and 72 h, respectively (Figs. 3A and B).

We also examined the production of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α in BALF at different times in this model (Fig. 3C). BALF IL-1β levels increased to the maximal level 24 h after LPS challenge, followed by a decrease to the basal level by 120 h. IL-6 and TNF-α levels in BALF significantly increased as early as 3 h after the LPS administration, with

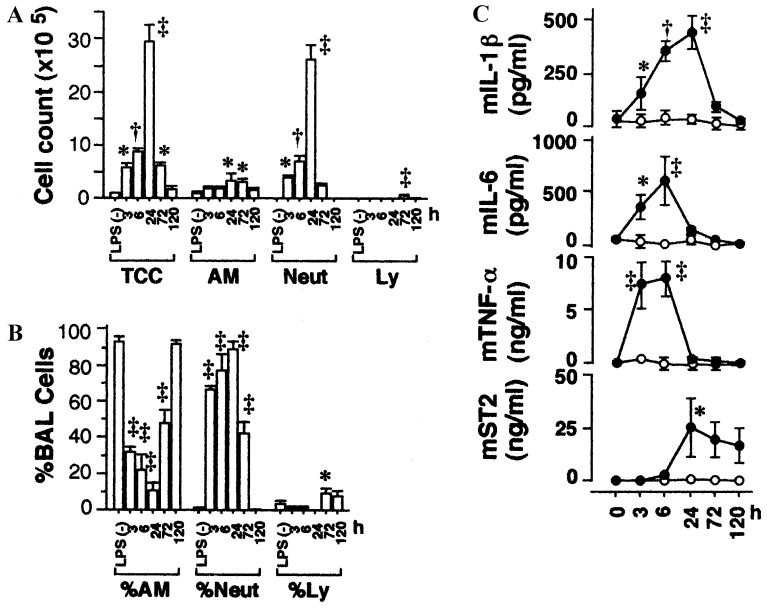

Fig. 3. Kinetics of numbers (A) and percentages (B) of total or differential cells, and soluble ST2 and cytokine protein levels (C) in BALF in the LPS-induced acute lung injury model. (A,B) BALF was collected and cell differentiation was determined at the indicated time before (LPS(−)) and after intranasal administration of LPS (or saline) as described in Materials and methods. (C) The concentrations of soluble IL-1β, IL-6, TNF-α, and ST2 in BALF were measured by ELISA. ●, LPS; ○, saline (control). Mean values ± SEM are shown for cumulative data from three independent experiments (3–4 mice per experiment). *$p < 0.05$, †$p < 0.01$ or ‡$p < 0.005$ versus LPS(−) or control group. *Abbreviation*: TCC, total cell counts; AM, alveolar macrophage; Neut, PMN; Ly, lymphocyte.

a peak at 6 h followed by a decline to the basal level by 120 h. In contrast, the kinetics of soluble ST2 protein levels in BALF were different from those of the proinflammatory cytokines (Fig. 3C). BALF ST2 protein levels did not increase up to 6 h after the LPS administration and they then increased to the peak level at 24 h, followed by a slow decrease. However, the BALF ST2 levels still retained approximately 60% of its maximal level at 120 h. Thus kinetic analysis showed that production in BALF ST2 levels was more delayed than that of proinflammatory cytokines levels in an LPS-induced acute lung injury model.

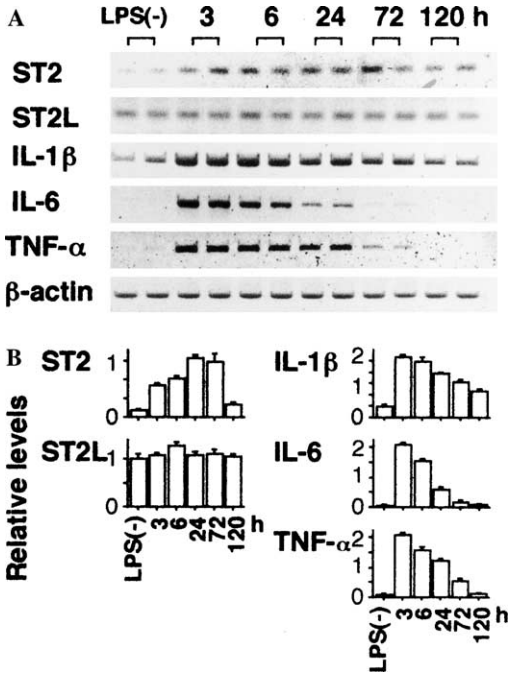

Fig. 4. Kinetics of ST2, ST2L, or cytokine mRNA expressions of the lung tissue in the LPS-induced acute lung injury model. (A) Total RNA was extracted from the lung at the indicated time before (LPS(−)) and after intranasal administration of LPS as described in Materials and methods. The cDNA samples obtained by RT-PCR for ST2, ST2L, IL-1β, IL-6, TNF-α, or β-actin were loaded on a 3.0% agarose gel. The data shown are representative of three independent experiments (3–4 mice per experiment). (B) Densitometric analyses of ST2, ST2L, IL-1β, IL-6, and TNF-α mRNA expressions in the lung tissues were carried out. The relative levels designate the ratio obtained from the density of the band of ST2, ST2L, or cytokine mRNA expression standardized with that of the β-actin mRNA expression level. The mean relative densities ± SEM are shown for cumulative data from three independent experiments (3–4 mice per experiment).

We next investigated the kinetics of mRNA expression of ST2, ST2L, or proinflammatory cytokines in the lung after the intranasal administration of LPS by RT-PCR (Fig. 4). Inducible expressions of ST2, IL-1β, IL-6, and TNF-α mRNA in the lung were observed after post-LPS inoculation, while ST2L mRNA expression in the lung was constitutively detected without the LPS challenge and unchanged even after the challenge (Fig. 4A). Densitometric analyses clearly demonstrated that mRNA expression of proinflammatory cytokines increased rapidly to the maximal levels at 3 h after the LPS instillation and that the enhanced expression lasted up to 6–24 h, whereas ST2 mRNA was upregulated with a peak at 24 h and the enhanced expression continued up to 72 h post-challenge in this model (Fig. 4B). These data of the kinetics of gene expression have a similar trend to the protein kinetics (Fig. 3C) in that the peak in expressions of proinflammatory cytokines occurred earlier than the peak in ST2 expressions.

Effect of soluble ST2 protein on proinflammatory cytokine production from LPS-stimulated MH-S cells in vitro

To elucidate the function of ST2 in acute lung inflammation, we investigated the effects of ST2 protein on LPS-induced proinflammatory cytokine production from murine AM in vitro. MH-S cells were pretreated with recombinant ST2 protein or medium alone and

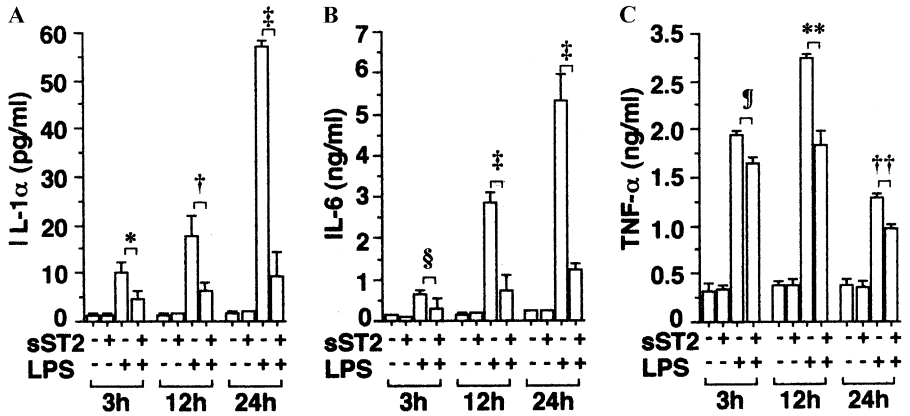

Fig. 5. Effects of soluble ST2 protein on LPS-induced production of IL-1α (A), IL-6 (B), and TNF-α (C) from MH-S cells in vitro. MH-S cells were pretreated with either 500 ng/ml of soluble ST2 protein or medium alone for 3 h and then were left unstimulated or were stimulated with LPS (1 μg/ml) in vitro for up to 24 h. Culture supernatants were harvested at the indicated time, and concentrations of IL-1α, IL-6, and TNF-α were measured by ELISA. The mean cytokine concentrations ± SEM are shown for the cumulative data from three independent experiments. "sST2" means soluble ST2 protein. $*p = 0.073$, $\dagger p < 0.005$, $\ddagger p < 0.0001$, $\S p = 0.224$, $\P p < 0.058$, $**p < 0.001$, and $\dagger\dagger p < 0.01$.

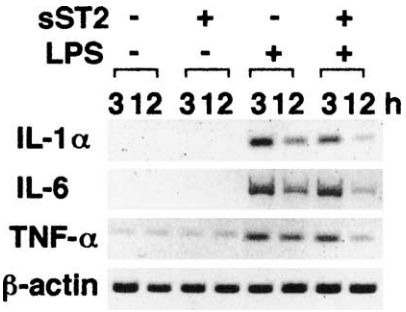

Fig. 6. Effects of soluble ST2 protein on LPS-induced mRNA expression of IL-1α (A), IL-6 (B), and TNF-α (C) of MH-S cells in vitro. MH-S cells were pretreated with either 500 ng/ml of soluble ST2 protein or medium alone for 3 h and then were left unstimulated or were stimulated with LPS (1 μg/ml) in vitro for up to 12 h. Total RNA was extracted from MH-S cells at 3 and 12 h after the LPS treatment, and the cDNA samples obtained by RT-PCR for IL-1α, IL-6, TNF-α, or β-actin were loaded on a 3.0% agarose gel. The data shown are representative of three independent experiments.

then were left unstimulated or were stimulated with LPS for the times indicated in Fig. 5. Concentrations of IL-1α (MH-S cells do not produce IL-1β), IL-6, and TNF-α in culture supernatants were measured by ELISA. Treatment with ST2 protein significantly inhibited IL-1α and IL-6 production by LPS-stimulated MH-S cells (Figs. 5A and B). Furthermore, LPS-induced production of TNF-α by MH-S cells was also significantly suppressed by pretreatment with ST2 protein, though less inhibited than IL-1α or IL-6 (Fig. 5C). IL-10 levels in culture supernatants were also measured, but no difference was found among any of the treatment groups (data not shown).

We next examined the effect of ST2 protein on mRNA expression of proinflammatory cytokines in LPS-stimulated MH-S cells by RT-PCR. As shown in Fig. 6, pretreatment with ST2 protein repressed LPS-induced mRNA expressions of IL-1α, IL-6, and TNF-α at 3 and 12 h, as compared to that with LPS treatment alone (without ST2 pretreatment). Using the same RNA samples, as shown in Fig. 6, we analyzed mRNA expression of TLR2, TLR4, MD-2, MyD88, IRAK, and TRAF-6 by RT-PCR. However, no difference was observed between treatment groups with LPS alone and LPS plus ST2 protein (data not shown).

Discussion

The results of this study have demonstrated that ST2 gene and protein expression are induced in an AM cell line, MH-S, in response to proinflammatory stimuli including LPS, IL-1β, IL-6, or TNF-α or in the lung tissue of an LPS-induced acute lung injury model, while ST2L gene expression is constitutive in MH-S cells and does not change between before and after stimulation with LPS or proinflammatory cytokines. Furthermore, pretreatment with soluble ST2 protein resulted in the downregulation in gene and protein expression of proinflammatory cytokines including IL-1α, IL-6, and TNF-α in LPS-stimulated MH-S cells.

In the present study, the mRNA expression pattern of ST2/ST2L in AM varied with each type of cell tested. For example, human AM obtained from healthy volunteers expressed predominantly ST2 mRNA in comparison with ST2L mRNA and both were enhanced following treatment with PMA/Ca (Fig. 1); in murine AM, MH-S, the ST2L gene was constitutively expressed, whereas the ST2 gene was induced after stimulation (Figs. 1 and 2). It is sometimes not appropriate to compare results from human AM with those from mouse AM cell line. Therefore, this might affect the difference in the ST2/ST2L expression. Furthermore, since some reports have shown that different expression patterns of ST2/ST2L are observed in other macrophages, such as murine bone marrow-derived macrophages [17,24], the reasons for such differences in ST2/ST2L gene expression in macrophages may be not only species specific but also a result of the wide spectrum of tissue macrophages, including bone marrow-derived, alveolar, pleural, liver, and peritoneal macrophages, that have functional heterogeneity of antigen-presenting capacity, phagocytotic activity, or cytokine production [25]. It is very intriguing to speculate that the ST2/ST2L gene expression pattern in macrophages may change in response to different surrounding circumstances and immune responses.

To test whether inflammatory stimuli induce ST2 expression in vivo, we utilized a murine model of LPS-induced acute lung injury. In this model, ST2 mRNA expression and protein levels increased to maximal levels at 24–72 h and at 24 h post-LPS challenge, respectively, but it is uncertain which cells, infiltrating, endogenous cells, or both, express ST2 mRNA in this model. Since the kinetic pattern of PMN and AM in BALF is similar to that of ST2 expression (Figs. 3 and 4), increased ST2 levels may originate from PMN and AM among cells infiltrating into airways. Although lymphocytes also increased in BALF with a peak at 72 h post-LPS challenge, they are unlikely to be the source of ST2 in this LPS-induced lung injury model, because LPS is reported to inhibit Th2 function [26], and in the lymphocyte population, ST2 is preferentially expressed in Th2 but not Th1 cells [6,7], suggesting that ST2 expression derived from downregulated Th2 can be negligible in this model.

The findings observed in Figs. 3 and 4, that ST2 expression was delayed in comparison with expression of proinflammatory cytokines, suggest that proinflammatory cytokines expressed in the early phase may induce ST2 expression in this in vivo model, which was supported by in vitro results demonstrating that ST2 gene expression was greatly enhanced by a 3–6 h treatment of MH-S cells with IL-1β, IL-6, or TNF-α (Fig. 2). These

data are compatible with the results reported by Kumar and colleagues, demonstrating that in a murine model where mouse ears are exposed to UVB irradiation leading to inflammation, the late expression of ST2 mRNA was induced by TNF-α and IL-1 in the inflamed skin [18]. These two sets of results confirm that ST2 expression can be regulated by cytokines.

The results of kinetic analyses on cytokine and ST2 protein expression in BALF demonstrated that ST2 protein levels peaked at 24 h and remained at 60% of maximal levels even at 120 h, whereas proinflammatory cytokine levels peaked at 6–24 h with a rapid decline to baseline levels by 120 h post-LPS challenge. Based on these findings of such different kinetics, we hypothesized that soluble ST2 protein may downregulate the expression of proinflammatory cytokines. This hypothesis can be confirmed by the in vitro results that pretreatment with ST2 protein downregulated protein and gene expression of proinflammatory cytokines such as IL-1α, IL-6, and TNF-α in LPS-stimulated MH-S cells (Figs. 5 and 6). Based on these results, we believe that it is possible that the observed increase in endogenous ST2 expression in murine AMs, which are induced by proinflammatory cytokines produced from LPS-stimulated AM, may suppress excessive production of proinflammatory cytokines leading to acute lung injury in an autoregulative manner.

The underlying mechanism by which soluble ST2 protein suppresses the production of proinflammatory cytokines remains unclear. The anti-inflammatory cytokine IL-10 has been shown to suppress inflammatory processes, but LPS-induced IL-10 levels from AM were not altered by ST2 treatment in this study. Furthermore, kinetic studies showed that ST2-mediated suppression was apparent 3 h after LPS treatment at the protein level. Thus soluble ST2 protein is likely to act directly rather than via an intermediate factor. Nuclear factor-κB (NF-κB) is one of the critical transcription factors required for the expression of inflammatory cytokines that are involved in the pathogenesis of acute lung injury [27]. Recent genetic and biochemical experiments have highlighted the critical role of TLRs in LPS-induced NF-κB activation [28,29]. on binding of LPS to TLRs, specifically TLR2 and TLR4, via CD14, a number of molecules are recruited to the receptor to mediate NF-κB activation, which include the adaptor molecule MyD88, the protein kinase IRAK, and the adaptor molecule TRAF-6, thus forming the receptor complex [29], resulting in interaction of downstream molecules and the subsequent activation of NF-κB. To explain the mechanism of the anti-inflammatory function of ST2 protein, we hypothesize that ST2 protein may give a downregulating signal to some molecules that are involved in the LPS signaling pathway. To test our hypothesis, we analyzed mRNA expression in the transduction signaling molecules by RT-PCR using the same samples as those listed in Fig. 6, but we observed no difference between treatment groups with LPS alone versus LPS and ST2 protein. Sweet and colleagues have reported that ST2-IgG fusion protein tends to suppress TRL1 and TLR4 mRNA expression levels in LPS-treated bone marrow-derived macrophages, but there is no statistically significant difference in their expression levels between treatment groups with LPS alone and LPS plus ST2-IgG fusion protein [24]. Further analyses are necessary to clarify the effect of ST2 protein on signal transducer and transcription factors involved in the LPS signaling pathway.

In summary, here we demonstrated that ST2 protein and gene expression were induced by LPS and proinflammatory cytokines in a murine AM cell line in vitro and in the lung tissue of a murine model of LPS-induced acute lung injury. Furthermore, it is noteworthy that the native form of soluble ST2 protein downregulated production and gene expression of proinflammatory cytokines in LPS-stimulated murine AM. These findings suggest that soluble ST2 protein can have a therapeutic potential for inflammatory diseases, especially acute lung injury.

Acknowledgments

This study was supported by a Jichi Medical School Young Investigator award and a grant (No. 13670612) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank Drs. Shunji Tajima and Naoko Mato, Ms. Tomoko Ikahata, and Ms. Hiromi Ozaki for their excellent technical assistance.

References

[1] S. Tominaga, 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. 258 (1989) 301–304.

[2] A.K. Werenskiold, S. Hoffmann, R. Klemenz, Induction of a mitogen-responsive gene after expression of the Ha-ras oncogene in NIH 3T3 fibroblasts, Mol. Cell. Biol. 9 (1989) 5207–5214.

[3] G. Bergers, A. Reikerstorfer, S. Braselmann, P. Graninger, M. Busslinger, Alternative promoter usage of the Fos-responsive gene Fit-1 generates mRNA isoforms coding for either secreted or membrane-bound proteins related to the IL-1 receptor, EMBO J. 13 (1994) 1176–1188.

[4] K. Yanagisawa, T. Takagi, T. Tsukamoto, T. Tetsuka, S. Tominaga, Presence of a novel primary response gene ST2L, encoding a product highly similar to the interleukin 1 receptor type I, FEBS Lett. 318 (1993) 83–87.

[5] S. Tominaga, K. Kuroiwa, K. Tago, H. Iwahana, K. Yanagisawa, N. Komatsu, Presence and expression of a novel variant form of ST2 gene product in human leukemic cell line UT-7/GM, Biochem. Biophys. Res. Commun. 264 (1999) 14–18.

[6] K. Yanagisawa, Y. Naito, K. Kuroiwa, T. Arai, Y. Furukawa, H. Tomizuka, Y. Miura, T. Kasahara, T. Tetsuka, S. Tominaga, The expression of ST2 gene in helper T cells and the binding of ST2 protein to myeloma-derived RPMI8226 cells, J. Biochem. (Tokyo) 121 (1997) 95–103.

[7] D. Xu, W.L. Chan, B.P. Leung, F.P. Huang, R. Wheeler, D. Piedrafita, J.H. Robinson, F.Y. Liew, Selective expression of a

stable cell surface molecule on type 2 but not type 1 helper T cells, J. Exp. Med. 187 (1998) 787–794.
[8] M. Löhning, A. Stroehmann, A.J. Coyle, J.L. Grogan, S. Lin, J.C. Gutierrez-Ramos, D. Levinson, A. Radbruch, T. Kamradt, 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 95 (1998) 6930–6935.
[9] A.J. Coyle, C. Lloyd, J. Tian, T. Nguyen, C. Erikkson, L. Wang, P. Ottoson, P. Persson, T. Delaney, S. Lehar, 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. 190 (1990) 895–902.
[10] M.J. Townsend, P.G. Fallon, D.J. Matthews, H.E. Jolin, A.N. McKenzie, T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses, J. Exp. Med. 191 (2000) 1069–1076.
[11] K. Kuroiwa, H. Li, K. Tago, H. Iwahana, K. Yanagisawa, N. Komatsu, K. Oshikawa, Y. Sugiyama, T. Arai, S. Tominaga, Construction of ELISA system to quantify human ST2 protein in sera of patients, Hybridoma 19 (2000) 151–159.
[12] K. Oshikawa, K. Kuroiwa, K. Tago, H. Iwahana, K. Yanagisawa, S. Ohno, S.I. Tominaga, Y. Sugiyama, Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation, Am. J. Respir. Crit. Care Med. 164 (2001) 277–281.
[13] J. Bousquet, P. Chanez, J.Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, Eosinophilic inflammation in asthma, N. Engl. J. Med. 323 (1990) 1033–1039.
[14] D.S. Robinson, Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A.M. Bentley, C. Corrigan, S.R. Durham, A.B. Kay, Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma, N. Engl. J. Med. 326 (1992) 298–304.
[15] D.R. Moritz, H.R. Rodewald, J. Gheyselinck, R. Klemenz, The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors, J. Immunol. 161 (1998) 4866–4874.
[16] H. Li, K. Tago, K. Io, K. Kuroiwa, T. Arai, H. Iwahana, S. Tominaga, K. Yanagisawa, The cloning and nucleotide sequence of human ST2L cDNA, Genomics 67 (2000) 284–290.
[17] S. Kumar, M.N. Tzimas, D.E. Griswold, P.R. Young, Expression of ST2, an interleukin-1 receptor homologue, is induced by proinflammatory stimuli, Biochem. Biophys. Res. Commun. 235 (1997) 474–478.
[18] S. Saccani, N. Polentarutti, G. Penton-Rol, J.E. Sims, A. Mantovani, Divergent effects of LPS on expression of IL-1 receptor family members in mononuclear phagocytes in vitro and in vivo, Cytokine 10 (1998) 773–780.
[19] L.D. Hudson, J.A. Milberg, D. Anardi, R.J. Maunder, Clinical risks for development of the acute respiratory distress syndrome, Am. J. Respir. Crit. Care Med. 151 (1995) 293–301.
[20] T.R. Martin, J.C. Mathison, P.S. Tobias, D.J. Leturcq, A.M. Moriarty, R.J. Maunder, R.J. Ulevitch, Lipopolysaccharide binding protein enhances the responsiveness of alveolar macrophages to bacterial lipopolysaccharide. Implications for cytokine production in normal and injured lungs, J. Clin. Invest. 90 (1992) 2209–2219.
[21] G.U. Meduri, G. Kohler, S. Headley, E. Tolley, F. Stentz, A. Postlethwaite, Inflammatory cytokines in the BAL of patients with ARDS. Persistent elevation over time predicts poor outcome, Chest 108 (1995) 1303–1314.
[22] R.J. Szarka, N. Wang, L. Gordon, P.N. Nation, R.H. Smith, A murine model of pulmonary damage induced by lipopolysaccharide via intranasal instillation, J. Immunol. Methods 202 (1997) 49–57.
[23] M. Chignard, V. Balloy, Neutrophil recruitment and increased permeability during acute lung injury induced by lipopolysaccharide, Am. J. Physiol. Lung Cell. Mol. Physiol. 279 (2000) 1083–1090.
[24] M.J. Sweet, B.P. Leung, D. Kang, M. Sogaard, K. Schulz, V. Trajkovic, C.C. Campbell, D. Xu, F.Y. Liew, A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression, J. Immunol. 166 (2001) 6633–6639.
[25] D.L. Laskin, B. Weinberger, J.D. Laskin, Functional heterogeneity in liver and lung macrophages, J. Leukoc. Biol. 70 (2001) 163–170.
[26] T. Watanabe, T. Inoue, H. Ochi, M. Terashima, Y. Asano, T. Nakatani, Lipid A directly inhibits IL-4 production by murine Th2 cells but does not inhibit IFN-γ production by Th1 cells, Eur. J. Immunol. 29 (1999) 413–418.
[27] J. Fan, R.D. Ye, A.B. Malik, Transcriptional mechanisms of acute lung injury, Am. J. Physiol. Lung Cell. Mol. Physiol. 281 (2001) 1037–1050.
[28] Q. Jiang, S. Akashi, K. Miyake, H.R. Petty, Lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-kappa B, J. Immunol. 165 (2000) 3541–3544.
[29] F. Nomura, S. Akashi, Y. Sakao, S. Sato, T. Kawai, M. Matsumoto, K. Nakanishi, M. Kimoto, K. Miyake, K. Takeda, S. Akira, Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression, J. Immunol. 164 (2000) 3476–3479.