

Increased Serum Levels of Soluble ST2 in Birch Pollen Atopics and Individuals Working in Laboratory Animal Facilities

Karin Sahlander, MSc, Kjell Larsson, MD, PhD, and Lena Palmberg, MD, PhD

Objective: To investigate toll-like receptors and CD14 expression on blood cells, cytokine profile of blood T-helper cells and serum levels of soluble suppression of tumorigenicity 2 (sST2) and sCD14 in 27 symptomatic laboratory animal (LA) workers with positive ($n = 19$) or negative ($n = 8$) skin-prick test to LA, 12 birch pollen atopics and 11 non-atopic controls not exposed to LA. **Methods:** Surface markers and intracellular cytokines were measured with flow cytometry and sST2 and sCD14 with ELISA. **Results:** The group who experienced symptoms when working with LA, with positive and negative skin-prick test to LA, had higher CD14 expression on monocytes compared with those allergic to birch and controls. Further, serum sST2 were elevated in birch atopics and in symptomatic non-allergic to LA compared with controls. **Conclusion:** Increased CD14 expression found in LA workers is most likely a response to non-allergic agent exposure whereas ST2 seems to react to acute allergen exposure and to non-allergic stimuli as pathogen-associated molecular patterns.

Asthma, rhino-conjunctivitis and allergic sensitization against laboratory animals (LAs) are frequently observed in biomedical research and industry.^{1,2} Working with LAs is associated with exposure to allergens,^{3,4} but it also involves exposure to pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS)^{2,5} that further may enhance respiratory symptoms.

In a number of studies, it has been shown that exposure to endotoxin early in life depresses allergic sensitization and development of asthma later in life.^{6,7} This so called "hygiene hypothesis"^{8,9} has, however, been widely debated and there are contradictory results.¹⁰ In this context, it is interesting that exposure to LPS and other PAMPs often worsen symptoms in individuals who have already developed atopy.^{11,12}

Various PAMPs bind to toll-like receptors (TLRs), a group of pattern recognition receptor belonging to the interleukin (IL)-1R/TLR superfamily.^{13,14} Ten TLRs have so far been recognized to have essential roles in human¹³ and activation of TLRs leads to expression of pro-inflammatory cytokines and type I interferons (IFNs) through activation of NF- κ B and interferon regulatory factors (IRFs).^{15–17} Nevertheless, depending on dose, timing of the stimulus and responding cell type, activation of TLRs may also lead to increased production and release of Th2 cytokines such as IL-4, IL-5, and IL-13,¹⁸ which are known to have a central role in pathogenesis of atopic disorders and asthma. The IL-1 cytokine family member IL-33 has recently been shown to be the specific ligand for suppression of tumorigenicity 2 (ST2), also known as IL-1R4 and T1), another member of the IL-1R/TLR

superfamily. The membrane bound ST2 (ST2L) receptor has, like TLRs, an intracellular toll-IL 1 receptor domain.^{19,20}

Signaling through ST2L by IL-33, lead to a Th2 cytokine response by activation of NF- κ B and MAP kinase, which promotes allergic inflammation.²¹ Furthermore, ST2L has been reported to be a negative regulator of TLR signaling.^{22–24} Alternate splicing of ST2L generates a soluble isoform of ST2 (sST2) that lacks the transmembrane region and the toll-IL 1 receptor domain.¹⁹ Soluble ST2 is known to block the IL-33 signaling in allergic airway inflammation in mice.²⁵

In this study, we investigated whether expression of CD14 and TLRs on blood monocytes and neutrophils, activation markers on B- and T-cells, cytokine profile of blood Th cells and serum levels of sST2 and sCD14 are altered in individuals working in laboratory facilities, a work environment that is associated with high exposure to both allergens and PAMPs. Our aim was to compare two groups who experienced symptoms at work, one sensitized group with positive skin-prick test (SPT) and one non-sensitized group with negative SPT to mouse or rat with two non-exposed groups, one atopic group sensitized to birch pollen and one non-allergic control group.

MATERIALS AND METHODS

Subjects and Study Design

Twenty-seven individuals experiencing symptoms when working with LAs (mice and rats) in a laboratory facility, 12 with allergic symptoms during birch pollen season and 11 healthy non-allergic controls not working with LAs were included in the study (Table 1). All subjects who took anti-histamines were asked to take the last dose not later than 4 days before the visit. The subjects with birch pollen allergy were investigated during birch pollen season. The study was approved by the Karolinska Institute Ethics Committee (Stockholm, Sweden), and all individuals gave their informed consent to participate.

Skin-Prick Test

For SPT, a panel of 17 common allergens were used. The SPT were performed on both forearms, and histamine chloride (10 mg/mm, ALK and Allergopharma) was used as a positive control, and the diluent of the allergens (ALK and Allergopharma) as negative control.

Cell Distribution and CD23 and CD25 Staining

Blood collected in ethylene diamine-tetra-acetic acid (EDTA) vacutainer tubes (BD Bioscience, San Jose, California) were stained with a four-color antibody mixture (CD3FITC/CD8PE/CD45PerCp/CD4APC from BD Bioscience) in a Tru-COUNT tube (BD Bioscience). Samples were lysed and analyzed on a FACSCalibur™ (BD Bioscience) by using MultiSET™. The results are presented as number of cells/ μ l blood. To verify proportion of B-cells expressing CD23, blood was stained with titrated amount of anti-CD23 PE, anti-CD45 PerCp, and anti-CD19 APC. To verify the proportion of T-helper cells expressing CD25, titrated amount of anti-CD25 PE, anti-CD3 FITC, anti-CD4 APC, and anti-CD45 PerCp were used (BD Biosciences, San Jose, CA). Samples were analyzed using FACSCalibur™ (BD Bioscience)

From the Lung and Allergy Research, National Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

This work was supported by Swedish Heart-Lung Foundation, Swedish Asthma and Allergy Association and Swedish Council for Working Life and Social Research.

Address correspondence to: Karin Sahlander, MSc, Lung and Allergy Research, National Institute of Environmental Medicine, Karolinska Institutet, P.O. Box 287, SE-17177 Stockholm, Sweden; E-mail: karin.sahlander@ki.se.

Copyright © 2010 by American College of Occupational and Environmental Medicine

DOI: 10.1097/JOM.0b013e3181d09868

flow cytometry and CELLQuest™. Gates were set by using fluorescence minus one-stained samples.

TLR and CD14 Staining on Monocytes and Neutrophils

Whole blood (EDTA) was stained with titrated amounts of either anti-TLR2 PE (TLR2.1, eBioscience, San Diego, California), anti-TLR4 PE (HTA125, eBioscience), or anti-CD14 FITC (61D3, eBioscience) together with anti-CD45 PerCp (BD Bioscience). As negative controls, isotype matched antibodies were used. Samples were analyzed using FACSCalibur™ (BD Bioscience) flow cytometry and CELLQuest™. The results are presented as relative median fluorescence intensity (rMFI = monoclonal antibody/matched isotype control).

Intracellular Cytokine Staining

Heparinized peripheral blood in RPMI 1640 containing 2 mM glutamine (1:1 dilution) was stimulated with phorbol 12-myristate 13-acetate (PMA, 25 ng/mL) and ionomycin (1 μM, Sigma-Aldrich) in presence of GolgiPlug (Brefeldin A, BD Bioscience) for 4 hours at 37°C. Stimulated blood was stained with anti-CD4 APC (BD Bioscience). The samples were then lysed and incubated with Cytofix/Cytoperm™ (BD Bioscience Pharmingen) (20 minutes at room temperature) followed by a 10 minutes incubation with Perm/Wash™ (BD Bioscience Pharmingen). The permeabilized cells were stained with anti-IL-4 PE, anti-IFN-γ FITC, anti-IL-2 FITC (BD Bioscience), or anti-IL-13 PE (BD Bioscience Pharmingen). Cells were washed with Perm/Wash and resuspended in CellFIX™ (BD Bioscience). Unstimulated blood was stained using the same procedure as negative controls. Analysis was performed using a FACSCalibur™ (BD Bioscience) and CELLQuest™.

The results were present as proportion of cytokine-producing T-helper cells.

Soluble CD14 (sCD14) and sST2 in Serum

For serum analyses peripheral blood was drawn in vacutainer tubes without any anticoagulantia (BD Bioscience). Samples were allowed to clot for 45 minutes and then centrifuged at 3000 rpm 10 minutes × 2. Serum was aliquoted and stored in -70°C until analyzed. Soluble CD14 and sST2 were measured in serum using DuoSet ELISA kits (R&D systems, Europe, Abingdon, UK). All samples had been thawed and frozen once before sST2 was measured. The detection range for sST2 and sCD14 was 15.6 to 2000 pg/mL and 60 to 4000 pg/mL, respectively. For duplicate samples, an intra-assay coefficient <10% was accepted.

Statistics

Data are given as median with 25th to 75th percentiles unless otherwise stated. Statistical analyses were performed by using Kruskal-Wallis followed by Mann-Whitney *U* test. A *P*-value of <0.05 was considered significant.

RESULTS

Skin-Prick Test

Among those who experienced symptoms during LA work 19 had positive and 8 had negative SPT to rat or mouse. Thirteen of those with positive SPT to rat also had positive SPT to mouse. In the group with symptoms during birch season all (*n* = 12) had positive SPT to birch. The control group (*n* = 11) were negative in SPT for all allergens tested (Table 1).

TABLE 1. Subject Characteristics

	Controls	Birch Pollen Allergy	LA Workers +SPT to LA	LA Workers -SPT to LA
Subjects (<i>n</i>)	11	12	19	8
Mean age (y, range)	36 (22–62)	31 (21–49)	35 (22–60)	32 (24–43)
Women (<i>n</i> , %)	10 (90.9)	8 (66.7)	15 (78.9)	6 (75.0)
+SPT birch	0	12	4	1
+SPT rat	0	—	19	0
+SPT mouse	0	—	13	0
+SPT rat and mouse	0	—	13	0

TABLE 2. Concentration (Median [25th to 75th Percentiles]) of T-cell Subsets, Neutrophils, Monocytes, Lymphocytes, Basophils, and Eosinophils in Peripheral Blood

	Controls (<i>n</i> = 11)	Birch Pollen Allergy (<i>n</i> = 12)	Laboratory Animal Workers, +SPT to LA (<i>n</i> = 18)	Laboratory Animal Workers, -SPT to LA (<i>n</i> = 8)	<i>P</i>
CD3	1733 (1183–2066)	1420 (1263–2162)	1617 (1144–1942)	1456 (1057–1792)	0.82
CD4	1176 (751–1318)	933 (840–1257)	953 (691–1212)	888 (689–1272)	0.78
CD8	479 (366–640)	518 (418–780)	527 (423–725)	411 (338–594)	0.50
CD4/CD8	2.13 (1.52–2.94)	1.86 (1.54–2.31)	1.69 (1.46–2.11)	2.09 (1.86–2.46)	0.37
Neutrophils	3939 (3117–4848)	3638 (3292–4694)	3359 (2781–3895)	3921 (2752–4092)	0.29
Monocytes	581 (459–641)	449 (370–545)	487 (412–602)	389 (277–479)	0.07
Lymphocytes	2139 (1918–3129)	2196 (1761–2810)	2013 (1586–2546)	2127 (1564–2410)	0.62
Basophils	50 (42–73)	45 (35–68)	41 (30–74)	51 (35–69)	0.46
Eosinophils	214 (153–252)	239 (136–328)	143 (72–252)	164 (128–308)	0.47

Results are presented as cells/μL blood.

Cell Distribution, CD23, and CD25 Expression

There were no differences in total amount of cells (five part differential or T-cell subset) and CD25 expression on T-cells between the four groups (Tables 2 and 3). Birch atopics had increased expression of CD23 compared with controls ($P = 0.017$) and compared with animal workers with positive SPT to rat or mouse ($P = 0.016$) but not compared with animal workers with negative SPT to rat or mouse ($P = 0.35$) (Table 3).

TLR and CD14 Expression

The symptomatic group with negative SPT to rat or mouse had increased CD14 expression on monocytes compared with the birch pollen atopic group ($P = 0.001$), controls ($P = 0.001$), and the symptomatic group with atopy to LAs ($P = 0.006$). The group atopic to rat or mouse had increased expression of CD14 compared with birch atopics ($P = 0.026$) (Fig. 1A). TLR4 expression on monocytes was decreased in birch atopics compared with controls ($P = 0.014$), those with positive SPT ($P = 0.014$), and negative SPT ($P = 0.013$) to rat or mouse (Fig. 1B). There were no differences between the groups regarding TLR2 expression on

monocytes and TLR2, TLR4, and CD14 expression on neutrophils (data not shown).

Intracellular Cytokine Expression

There were no differences regarding intracellular IL-4, IFN- γ , IL-2, and IL-13 between the groups (Table 4).

Soluble CD14 (sCD14) and sST2 in Serum

There were no differences regarding soluble CD14 in serum between the groups (Fig. 1C). However, sST2 was increased in the group allergic to birch compared with controls ($P = 0.041$) and compared with the group with positive SPT to rat or mouse ($P = 0.043$) but not compared with the group with negative SPT to LAs ($P = 0.398$). The group with negative SPT to LAs also showed increased levels of sST2 compared with controls ($P = 0.006$, Fig. 1D).

DISCUSSION

In this study, it was demonstrated that subjects who experience symptoms, especially those with negative SPT to LAs while

TABLE 3. Proportion (Median [25th to 75th Percentiles]) of Peripheral B-cells Expressing CD23⁺, T-Helper Cells Expressing CD25⁺ and CD25 Bright (CD25⁺⁺)

	Controls (n = 9)	Birch Pollen Allergy (n = 11)	Laboratory Animal Workers, +SPT to LA (n = 14–15)	Laboratory Animal Workers, -SPT to LA (n = 8)	P
CD23 ⁺ CD19 ⁺ (% of CD19 ⁺)	50.08 (46.38–54.01)	60.85 (54.54–68.17)*	52.99 (51.44–54.52)	60.21 (46.22–63.65)	0.03
CD25 ⁺ CD4 ⁺ (% of CD3 ⁺ CD4 ⁺)	26.90 (22.39–28.13)	29.46 (20.90–35.48)	25.39 (22.25–33.00)	29.07 (25.30–32.96)	0.37
CD25 ⁺⁺ CD4 ⁺ (% of CD3 ⁺ CD4 ⁺)	5.88 (4.91–7.04)	6.27 (5.61–7.22)	5.86 (4.97–7.35)	5.84 (5.71–7.77)	0.92

* <0.05 compared to controls and animal workers with positive SPT to laboratory animals (rat or mouse).

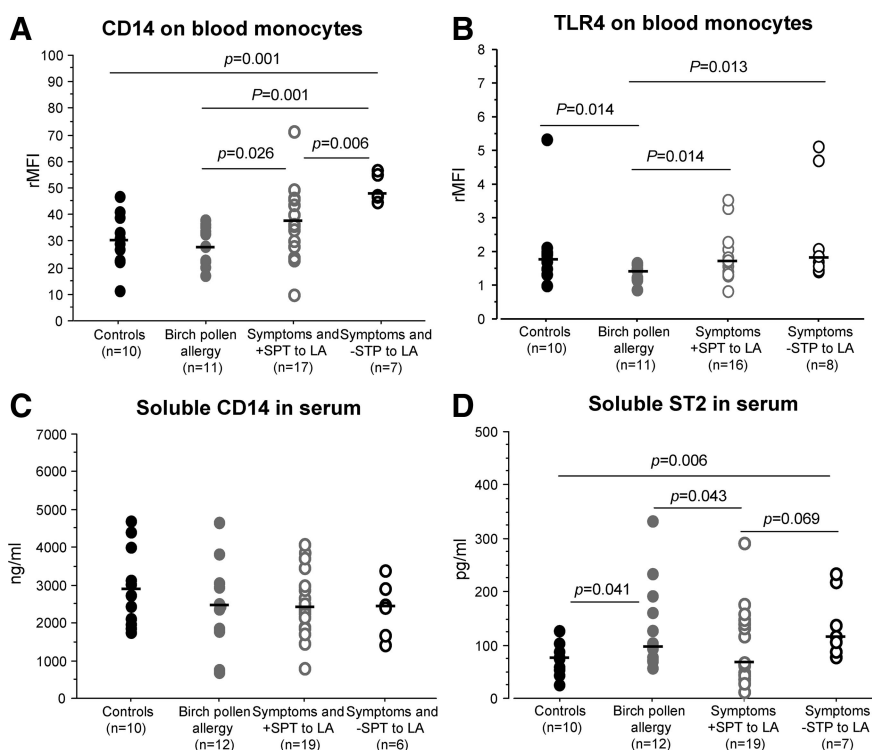


FIGURE 1. A to D, Surface expression of CD14 on peripheral blood monocytes (A), surface expression of TLR4 on peripheral blood monocytes (B), concentration of sCD14 (ng/mL) in serum (C), and sST2 (pg/mL) in serum (D). Brackets indicate differences between groups and markers indicate median.

TABLE 4. Proportion (Median [25th to 75th Percentiles]) of Peripheral T-Helper Cells Producing Intracellular IL-4, IL-13, IL-2, and IFN- γ

	Controls (<i>n</i> = 9)	Birch Pollen Allergy (<i>n</i> = 10–11)	Laboratory Animal Workers, +SPT to LA (<i>n</i> = 16–17)	Laboratory Animal Workers, –SPT to LA (<i>n</i> = 6–7)	<i>P</i>
IL-4 (CD4 ⁺ IL-4 ⁺ cells)	1.13 (0.91–1.43)	1.04 (0.74–1.65)	1.26 (0.89–1.81)	1.43 (0.90–1.86)	0.76
IL-13 (CD4 ⁺ IL-13 ⁺ cells)	2.36 (1.85–2.91)	3.24 (2.118–4.40)	2.65 (2.07–3.40)	2.69 (1.94–3.43)	0.49
IL-2 (CD4 ⁺ IL-2 ⁺ cells)	59.88 (54.98–65.01)	63.40 (58.64–69.93)	58.91 (49.18–64.57)	63.59 (59.03–70.07)	0.50
IFN- γ (CD4 ⁺ IFN- γ ⁺ cells)	17.99 (14.32–21.44)	19.76 (14.93–29.13)	22.17 (15.94–24.97)	21.31 (15.85–24.64)	0.48

working in LA facilities had higher expression of CD14 on peripheral blood monocytes compared with symptomatic, atopic subjects sensitized to birch pollen investigated during birch pollen season and healthy, non-LA-exposed control subjects. It was also shown that blood levels of soluble ST2 were elevated during pollen season in subjects with birch allergy and in workers with LA induced symptoms and negative SPT to mouse or rat compared with a non-atopic non-exposed control group.

As CD14 is critical for the response to LPS^{26,27} and no difference was revealed between controls and birch pollen allergic subjects during pollen season, it seems most likely that the enhanced CD14 expression is caused by exposure to microbial compound in the animal houses rather than to allergen exposure. We also found that the symptomatic group without atopy to LAs had higher CD14 monocyte expression than those with positive SPT to LAs. It could be assumed that this finding reflects a protective role in development of allergy to LAs as a specific polymorphism in the promoter region of CD14 (C-159T) has been shown to prevent atopy and is associated with increased *in vitro* expression of CD14²⁸ and increased serum levels of sCD14.²⁹ It is, however, not clear whether this gene variation occurs more often in the symptomatic group without atopy to rat or mice than in sensitized subjects. Nevertheless, except the increased surface expression of CD14 on monocytes we did not observe increased levels of sCD14 in this group, indicating that this is probably not the case.

The ST2 receptor is expressed by several cell types on activation but is constitutively expressed by Th2 effector cells³⁰ and mast cells.³¹ These cell types are involved in allergic inflammation and asthma and there are indications that ST2 is of importance in these conditions.^{32,33} In line with our results, it has previously been shown that sST2 is elevated in serum from atopic patients during asthma exacerbation and that sST2 levels correlate with exacerbation severity.³⁴ Recently, Ali et al reported higher serum levels of sST2 in children during acute asthma exacerbation and a relationship between sST2 levels and blood eosinophil count. They also showed that specific polymorphism in the ST2 gene was associated with differences in asthma phenotypes and asthma severity.³² Thus, the increase of serum sST2 in the birch atopic group during pollen season in this study most likely reflects an ongoing allergic airway inflammation due to birch pollen exposure.

It was also demonstrated that sST2 blood levels were elevated in animal workers who were SPT negative to rat or mice (but not those who were SPT positive) compared with controls. A possible explanation to this finding may be differences in exposure, a hypothesis that is supported by the finding of differences in CD14 monocyte expression between LA SPT positive and negative individuals. Allergic subjects working in animal facilities are acquainted with their allergy to LAs and they often take measures to minimize exposure. Such measures are use of protection masks, gloves, clothes, working on down flow benches, and use of airflow masks. These measures minimize the exposure both to allergens and PAMPs including LPS.

It is not clear whether the increased levels of sST2 in the SPT negative group of symptomatic animal workers depends on activation of the IL-33/ST2 system caused by allergens, non-allergic pro-inflammatory stimuli or both. Tajima et al showed that a combination of pro-inflammatory stimuli and Th2 cytokines have a synergistic effect on ST2 transcription and production.³⁵ The elevated levels of sST2 observed in the birch atopic group most likely comprise a negative regulation to the acute exposure to high levels of birch pollen during birch pollen season. This hypothesis is supported by the finding that sST2 has the capacity to block IL-33 signaling and thereby interrupt allergic sensitization and inflammation²⁵ and that interruption of the ST2/IL-33 pathway is important in resolution of the allergic inflammation.³³ This may indicate that the elevated ST2 levels, observed in the group of workers without rat or mouse allergy, may have a protective role in sensitization to LAs. On the other hand, it has been shown that both membrane bound ST2 and sST2 have the ability to inhibit release of Th1 cytokine in mice models and by monocytes/macrophages,^{22–24} which may favor a Th2 response possibly enhancing the risk for atopic sensitization. The increased levels of sST2 in animal workers negative in SPT might then be an early inflammatory marker for sensitization.

In conclusion, soluble ST2 were increased in serum of birch atopics with an ongoing allergic inflammation, and soluble ST2 and CD14 expressions on blood monocytes were elevated in individuals who experienced symptoms from the respiratory tract when working in laboratory facilities but had negative SPT to rats or mice. These findings indicate that CD14 seems to respond to non-allergic stimuli whereas sST2 respond to acute allergen exposure in atopic individuals as well as to PAMPs.

ACKNOWLEDGMENT

We thank Åsa Andersson, Kristin Blidberg, Anne Renström, and Britt-Marie Sundblad for excellent assistance.

This work was supported by Swedish Heart-Lung Foundation, Swedish Asthma and Allergy Association, and Swedish Council for Working Life and Social Research.

REFERENCES

1. Aoyama K, Ueda A, Manda F, Matsushita T, Ueda T, Yamauchi C. Allergy to laboratory animals: an epidemiological study. *Br J Ind Med*. 1992;49:41–47.
2. Pacheco KA, McCammon C, Thorne PS, et al. Characterization of endotoxin and mouse allergen exposures in mouse facilities and research laboratories. *Ann Occup Hyg*. 2006;50:563–572.
3. Hollander A, Gordon S, Renstrom A, et al. Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy*. 1999;54:142–149.
4. Renstrom A, Gordon S, Hollander A, et al. Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. *Allergy*. 1999;54:150–157.

5. Lieutier-Colas F, Meyer P, Pons F, et al. Prevalence of symptoms, sensitization to rats, and airborne exposure to major rat allergen (Rat n 1) and to endotoxin in rat-exposed workers: a cross-sectional study. *Clin Exp Allergy*. 2002;32:1424–1429.
6. Braun-Fahrlander C, Lauener R. Farming and protective agents against allergy and asthma. *Clin Exp Allergy*. 2003;33:409–411.
7. Lauener RP, Birchler T, Adamski J, et al. Expression of CD14 and toll-like receptor 2 in farmers' and non-farmers' children. *Lancet*. 2002;360:465–466.
8. Tse K, Horner AA. Allergen tolerance versus the allergic march: the hygiene hypothesis revisited. *Curr Allergy Asthma Rep*. 2008;8:475–483.
9. von Mutius E. Allergies, infections and the hygiene hypothesis—the epidemiological evidence. *Immunobiology*. 2007;212:433–439.
10. Schroder NW. The role of innate immunity in the pathogenesis of asthma. *Curr Opin Allergy Clin Immunol*. 2009;9:38–43.
11. Alexis N, Eldridge M, Reed W, Bromberg P, Peden DB. CD14-dependent airway neutrophil response to inhaled LPS: role of atopy. *J Allergy Clin Immunol*. 2001;107:31–35.
12. Michel O, Ginanni R, Duchateau J, Vertongen F, Le Bon B, Sergysels R. Domestic endotoxin exposure and clinical severity of asthma. *Clin Exp Allergy*. 1991;21:441–448.
13. Takeda K, Akira S. Toll-like receptors. *Curr Protoc Immunol*. 2007; Chapter 14: Unit 14.12.
14. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol*. 2003;21:335–376.
15. Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med*. 2007;13:460–469.
16. Uematsu S, Akira S. Toll-like receptors and innate immunity. *J Mol Med*. 2006;84:712–725.
17. Uematsu S, Akira S. Toll-like receptors and type I interferons. *J Biol Chem*. 2007;282:15319–15323.
18. Alexis NE, Lay JC, Almond M, Peden DB. Inhalation of low-dose endotoxin favors local T(H)2 response and primes airway phagocytes in vivo. *J Allergy Clin Immunol*. 2004;114:1325–1331.
19. Li H, Tago K, Io K, et al. The cloning and nucleotide sequence of human ST2L cDNA. *Genomics*. 2000;67:284–290.
20. Tominaga S, Yokota T, Yanagisawa K, Tsukamoto T, Takagi T, Tetsuka T. Nucleotide sequence of a complementary DNA for human ST2. *Biochim Biophys Acta*. 1992;1171:215–218.
21. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 2005;23:479–490.
22. Brint EK, Xu D, Liu H, et al. ST2 is an inhibitor of interleukin 1 receptor and toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol*. 2004;5:373–379.
23. Liew FY, Liu H, Xu D. A novel negative regulator for IL-1 receptor and toll-like receptor 4. *Immunol Lett*. 2005;96:27–31.
24. Sweet MJ, Leung BP, Kang D, et al. A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. *J Immunol*. 2001;166:6633–6639.
25. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem*. 2007;282:26369–26380.
26. Ulevitch RJ. Recognition of bacterial endotoxins by receptor-dependent mechanisms. *Adv Immunol*. 1993;53:267–289.
27. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol*. 1995;13:437–457.
28. LeVan TD, Guerra S, Klimecki W, et al. The impact of CD14 polymorphisms on the development of soluble CD14 levels during infancy. *Genes Immun*. 2006;7:77–80.
29. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol*. 1999;20:976–983.
30. 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;187:787–794.
31. Moritz DR, Rodewald HR, Gheyselincx 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;161:4866–4874.
32. Ali M, Zhang G, Thomas WR, et al. Investigations into the role of ST2 in acute asthma in children. *Tissue Antigens*. 2009;73:206–212.
33. Kearley J, Buckland KF, Mathie SA, Lloyd CM. Resolution of allergic inflammation and airway hyperreactivity is dependent upon disruption of the T1/ST2-IL-33 pathway. *Am J Respir Crit Care Med*. 2009;179:772–781.
34. 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;164:277–281.
35. Tajima S, Bando M, Ohno S, et al. ST2 gene induced by type 2 helper T cell (Th2) and proinflammatory cytokine stimuli may modulate lung injury and fibrosis. *Exp Lung Res*. 2007;33:81–97.