

STAT1 is involved in the pathogenesis of murine allergic rhinitis

Hisashi Hattori, M.D., Ph.D.,*# Lucia E. Rosas, D.V.M.,* Mitsuhiro Okano, M.D., Ph.D.,#
Joan E. Durbin, M.D., Ph.D.,§ Kazunori Nishizaki, M.D.,# and Abhay R. Satoskar, M.D., Ph.D.*

ABSTRACT

Background: Signal transducer and activator of transcription (STAT) 1 signaling pathway mediates biological functions of interferon (IFN) γ , which is a key cytokine-regulating T helper 1 (Th1) differentiation. Although constitutive activation of STAT1 has been reported in the airway epithelium of patients with chronic asthma, its *in vivo* role in the pathogenesis of allergic rhinitis is not clear. We determined the role of STAT1 in the pathogenesis of allergic rhinitis *in vivo* using STAT1 gene-deficient (STAT1 $^{-/-}$) mice and a murine model of *Schistosoma mansoni* egg antigen (SEA)-induced allergic rhinitis.

Methods: STAT1 $^{-/-}$ BALB/c and wild-type (WT) mice were sensitized by intranasal administration of SEA, and their immunologic responses were examined.

Results: STAT1 $^{-/-}$ mice showed impaired nasal eosinophilia and markedly reduced histamine-induced nasal hyperresponsiveness after SEA sensitization. Moreover, levels of Th2-associated SEA-specific IgG1 and IgE antibodies were lower in STAT1 $^{-/-}$ mice. Anti-CD3-stimulated nasal lymphocytes from STAT1 $^{-/-}$ mice also produced less amounts of Th2-associated cytokines IL-4, IL-5, IL-10, and IL-13 compared with WT mice, but both produced comparable levels of IFN- γ .

Conclusion: These results show that STAT1 is involved in the pathogenesis of SEA-induced allergic rhinitis in BALB/c mice. Furthermore, they reveal a surprising role of STAT1 in induction of nasal eosinophilia, and Th2-type cytokine production from nasal lymphocytes during allergic rhinitis.

(Am J Rhinol 21, 241–247, 2007; doi: 10.2500/ajr.2007.21.2970)

Key words: Allergic rhinitis, cytokine, eosinophilia, murine model, STAT1, Th1/Th2, TH2 response

Allergic rhinitis is one of the most common immune disorders, which is categorized as an inflammatory disease associated with the production of antigen-specific IgE, nasal eosinophil infiltration, nasal hyperresponsiveness and a predisposition to allergic reactions.^{1–3} This disease is characterized by the development of an aberrant T helper 2 (Th2)-biased immune response and increased production of Th2 cytokines such as IL-4 and IL-5 associated with a concomitant decrease in Th1-associated interferon (IFN) γ .^{4,5} It is known that IL-4 induces the differentiation and expansion of Th2 lymphocytes, which result in the production of IL-4, IL-5, IL-10, and IL-13 that are involved in the pathogenesis of allergic diseases.^{6,7} Moreover, IL-4 is involved in the production of IgE, which mediates the induction of immediate hypersensitivity reaction,⁸ whereas IL-5 promotes activation and the survival of eosinophils that contribute to the allergen-induced airway inflammation.^{9,10}

The signal transducers and activators of transcription (STATs) play a critical role in the signaling of many cytokines.

Seven STAT proteins have been identified in mammalian cells: STAT1, -2, -3, -4, -5a, -5b, and -6.^{7,11,12} The importance of STAT6 in the pathogenesis of allergic disease such as asthma has been confirmed using STAT6-deficient mice, which have defects in IL-4- and IL-13-mediated functions.^{13–15} On the other hand, STAT4 is essential for mediating biological functions of Th1-inducing cytokine IL-12, which has been shown to attenuate allergen-induced airway inflammation.^{16–18}

STAT1 is a major component of signaling cascade for IFN- γ , which is another key cytokine regulating Th1 differentiation.^{11,12,19} STAT1 also is activated by other growth factors and a number of other cytokines.^{19–24} However, STAT1 plays an obligate and dedicated role in mediating biological functions of IFN- γ and IFN- α/β .^{25–27} A recent study showed that STAT1 is constitutively activated in the airway epithelium of patients suffering from chronic asthma, although there was no evidence of increased levels of IFN- γ -expressing immune cells in the asthmatic subjects.²⁸ Nonetheless, Th1 cells can potentiate allergic airway inflammation.²⁹ Although all these findings suggest that STAT1 may be involved in inducing airway inflammation during asthma, little is known about the role of STAT1-mediated signaling in regulating nasal inflammation during allergic rhinitis.

We recently developed a murine model of allergic rhinitis, which involves the repeated intranasal administration of *Schistosoma mansoni* egg antigen (SEA) in the absence of an adjuvant. These mice consequently show potent Th2 response associated with nasal mucosal inflammation as well as eosinophil infiltration.³⁰ Using this experimental model, we investigated the role of STAT1 in the pathogenesis allergic rhinitis. We found that deficiency of STAT1 impairs Th2-type cytokine

From the *Department of Microbiology, The Ohio State University, Columbus, Ohio, #Department of Otolaryngology–Head and Neck Surgery, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan, and §Department of Pediatrics, Children's Hospital, Columbus, Ohio

One author (H.H.) was supported by the Japan Society of Immunology and Allergy in Otorhinolaryngology and Glaxo SmithKlein International Exchange Visitor Fellowship Fund; this work was supported in part by the grants from Ministry of Education, Culture, Sports, Science and Technology, Japan (14704043 to M.O.) and the National Institutes of Health (to A.R.S.)

Address correspondence and reprint requests to Abhay R. Satoskar, M.D., Ph.D., Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210

E-mail address: satoskar.2@osu.edu

Copyright © 2007, OceanSide Publications, Inc., U.S.A.

production from the nasal lymphocytes, prevents nasal eosinophilia, and blocks development of allergic inflammation.

Materials and Methods

Animal and Reagents

The wild-type BALB/c mice were obtained from Harlan Sprague-Dawley. Mouse strains lacking STAT-1 (STAT1^{-/-}) BALB/c mice were generated in the laboratory of Dr. Joan Durbin by backcrossing to at least 12 generations. Seven to 10-week-old female mice were used in all of the experiments and were maintained in specific pathogen-free facility according to institutional guidelines. All experimental protocols and procedures in this study were approved by the Institutional Care and Use Committee.

Sensitization of Mice

Mice were sensitized by repeated intranasal administration of SEA as described previously³⁰ with a slight modification. Briefly, wild-type (WT) and STAT1^{-/-} mice were sensitized with 5 μ g of SEA in the absence of adjuvant by administration into the anterior nose once a week for 3 weeks (on days 1, 8, and 15). One week later, mice were rechallenged by daily administration of 1 μ g of SEA intranasally for 7 consecutive days (on days 21–28; nasal challenge). For sensitization, SEA was delivered in 20 μ L of PBS through the nostril into the nasal cavity without anesthesia. Blood was collected by tail snipping on 1 day before the first sensitization, 6 days after the third sensitization, and 18 hours after the final challenge. In addition, 18 hours after the final challenge, the mice were killed and nasal lymphocytes were isolated by enzyme extraction with collagenase as described previously.³¹

Measurement of Nasal Hyperresponsiveness

To evaluate nasal hyperresponsiveness, mice were administered histamine intranasally and the concentration of histamine that causes nasal rubbing and sneezing was determined. Briefly, 10 μ L of histamine (Sigma, St. Louis, MO) in different doses was administered into the nostrils, and nasal symptoms (nasal rubbing and sneezing) were evaluated by counting the number of nasal rubbings and sneezing that was caused for 5 minutes after nasal administration of histamine. The concentration of histamine at which mouse rubbed >40 times and also sneezed >5 times was identified as the threshold concentration. This point was expressed to the limiting concentration of histamine (log₁₀ M). The nasal responsiveness was measured 1 day before both the first sensitization and the death (days 0 and 29).

Antibody ELISA

The levels of SEA-specific IgG1 and IgG2a in the serum were measured by ELISA as previously described.³² Briefly, ELISA plates were coated with SEA (2 μ g/mL) in PBS, pH 9.0, and incubated overnight at 4°C. Then, plates were blocked in PBS with 5% nonfat powdered milk for 1 hour at 37°C. After washing plates, serum samples were added at a dilution of 1/100 and incubated for 2 hours at 37°C. Bound antibodies (Abs) were detected by incubation with either horseradish peroxidase (HRP)-anti-mouse IgG1 or HRP-anti-mouse IgG2a (Southern Biochemical Technologies, Birmingham, AL) at a di-

lution of 1/5000 in 75% PBS and 25% FBS. After further incubation at 37°C for 1 hour, tetramethylbenzidine substrate solution was added. The reaction was stopped by 5% phosphoric acid and outside diameter, 450 nm, was read on a microplate reader. The serum total IgE and SEA-specific IgE was determined also by ELISA as previously described.³⁰ The detection limit of serum total IgE was 0.2 ng/mL.

Histological Examination

Mice were killed 18 hours after the final nasal challenge. The heads were removed and fixed in 10% buffered formalin for 24 hours. Then, they were decalcified in Cal-Ex (VWR Scientific Products, Bridgeport, NJ) overnight and washed free of Cal-Ex in running water for 3 hours. Coronal nasal sections were stained with Luna solution and the number of eosinophils in the nasal septum mucosa was counted microscopically under a high power field (magnification, $\times 400$). The degree of eosinophilia was calculated blindly from three sections in each mouse.

In Vitro Culture of Nasal Lymphocytes Cells and Cytokine Determination

Mice were killed 18 hours after the final nasal challenge. Nasal lymphocytes from WT or STAT1^{-/-} mice were isolated by enzyme extraction with collagenase, as previously described.³¹ The 6×10^5 cells were cultured in 48-well flat-bottom plates in 5% CO₂ at 37°C with or without plate-bound purified hamster anti-mouse CD3e monoclonal Ab (mAb; 1 μ g/mL; BD Biosciences Pharmingen, San Jose, CA). Alternatively, cells were cultured in the presence or absence of 4 mg/mL of SEA. After 72 hours of culture, supernatants were harvested. IL-4, IL-5, IL-10, IL-13, and IFN- γ production in culture supernatant were measured by captured ELISA as previously described³² (PharMingen, San Diego, CA for IL-4, IL-5, IL-10, and IFN- γ ; R&D Systems for IL-13). The detection limits in these assays were 10 pg/mL for IL-4, 200 pg/mL for IL-5, 10 pg/mL for IL-13, and 20 pg/mL for IFN- γ .

Statistical Analysis

Results were statically analyzed using Mann-Whitney's U test. Differences between groups were considered significant at the $p < 0.05$ level. All data were expressed as mean $\pm$ SE.

RESULTS

STAT1^{-/-} Mice Fail to Develop Histamine-Induced Nasal Hyperresponsiveness after SEA Sensitization

Nasal allergic symptoms such as sneezing and nasal rubbing were determined in response to intranasal application with serial concentration of histamine before the sensitization (day 0) and after the final challenge (day 28). Before sensitization, both WT and STAT1^{-/-} mice displayed similar histamine responsiveness (Fig. 1). On day 28, response to histamine application increased markedly in WT mice ($p < 0.01$). In contrast, at this time, no increase in histamine responsiveness was observed in STAT1^{-/-} mice (Fig. 1).

STAT1^{-/-} Mice Fail to Develop Significant Nasal Eosinophilia

Allergen sensitization and challenge promote a nasal inflammatory response characterized by infiltration of a large

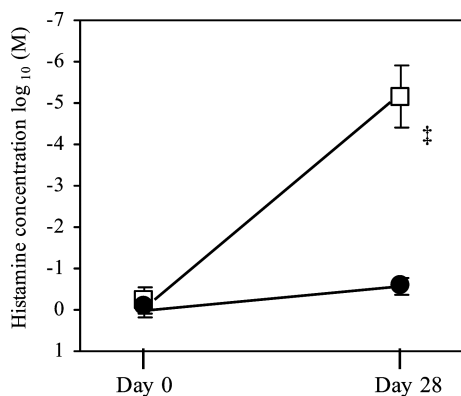


Figure 1. Histamine-induced nasal responsiveness. WT mice and STAT1^{-/-} mice were intranasally sensitized with SEA once a week for 3 weeks. Then, 1 week later, all of the mice were challenged with SEA for 7 consecutive days. The nasal hyperresponsiveness for histamine was determined before sensitization (day 0) and after nasal challenge (day 28). Histamine responsiveness of WT (□) and STAT1^{-/-} mice (●) was expressed by the threshold histamine concentration that caused mice to sneeze >5 times and nasal rubbing >40 times in 5 minutes. Data shown are mean $\pm$ SEM from two independent experiments of eight mice per group. 0 in the y-axis is equivalent to 1 M; $\ddagger p < 0.01$ compared with WT group.

number of eosinophils into nasal mucosa. Hence, we examined eosinophil infiltration into nasal septum in WT and STAT1^{-/-} mice after nasal challenge. STAT1^{-/-} mice showed a marked reduction in eosinophil recruitment into their nasal mucosa as compared with WT mice, which developed significant nasal eosinophilia after sensitization ($p = 0.0317$; Fig. 2). The number of eosinophils into nasal septum mucosa per field (400 $\times$) was 26.5 ± 3.0 in the WT mouse group and 5.9 ± 1.7 in the STAT1^{-/-} mouse group, respectively. No nasal eosinophilia was evident in naïve WT and STAT1^{-/-} mice (data not shown).

Analysis of Antibody Response

Serum IgE, IgG1, and IgG2a were determined in WT and STAT1^{-/-} mice by ELISA. After the nasal sensitization with SEA, WT mice produced a significant amount of Th2-associated total IgE, SEA-specific IgE and IgG1, but not Th1-associated SEA-specific IgG2a. STAT1^{-/-} mice displayed significantly higher levels of total IgE than WT mice even before the sensitization as well as after the sensitization. Interestingly, STAT1^{-/-} mice produced significantly lower levels of SEA-specific IgG1 and IgE than WT mice (Fig. 3). Both groups displayed basal levels of SEA-specific IgG2a.

Analysis of Cytokine Production by Nasal Lymphocytes

To investigate whether lack of STAT1 affects cytokine production by nasal lymphocytes in our model, we measured the levels of Th2-associated IL-4, IL-5, IL-10, IL-13, and Th1-associated IFN- γ by nasal lymphocytes from SEA-sensitized WT and STAT1^{-/-} mice. After *in vitro* stimulation with anti-CD3, nasal lymphocytes from WT mice produced a significant amount of IL-4, IL-5, IL-10, and IL-13 (Fig. 4, A–D). On the other hand, anti-CD3-stimulated nasal lymphocytes from

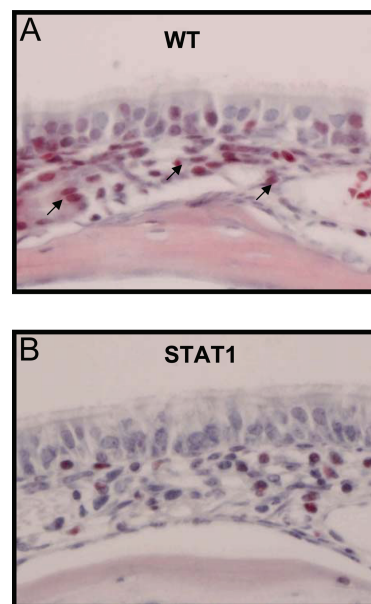


Figure 2. Nasal eosinophilia of mice intranasally sensitized with SEA. WT and STAT1^{-/-} mice were intranasally sensitized with SEA. After nasal challenge, mice were killed and coronal nasal sections were fixed, decalcified, and stained with Luna solution. Typical nasal sections are shown: (A) WT mouse and (B) STAT1^{-/-} mouse (magnification, $\times 400$; black arrows show eosinophils).

STAT1^{-/-} mice failed to produce significant amounts of these cytokines. Regarding the production of IFN- γ , there was no significant difference between WT mice and STAT1^{-/-} mice (Fig. 4E). Nasal lymphocytes from both WT and STAT1^{-/-} mice produced very low amounts of cytokines after *in vitro* stimulation with SEA. The cytokine levels were either near or below the detection limit and there were no statistically significant differences between the groups (data not shown).

DISCUSSION

In this study, we used the murine model of allergic rhinitis that we developed previously.³⁰ In this model, Ag was given intranasally without adjuvants, which could not only mimic the natural exposure of the disease but also exclude the immunoregulatory effect of adjuvants that may distort the physical responses and bypass the need for STAT1. Our findings show that SEA-sensitized STAT1^{-/-} mice fail to develop nasal hyperresponsiveness in response to histamine that associated with reduced nasal eosinophilia. Furthermore, STAT1^{-/-} mice also displayed significantly lower levels of Th2-associated SEA-specific IgE and IgG1 and impaired production of Th2-associated cytokines from the nasal lymphocytes. These results show that STAT1 is involved in the pathogenesis of allergic rhinitis.

STAT1 has been reported to be activated by a number of cytokines and growth factors.^{19–24} However, STAT1-deficient mice show a predominant signaling defect in the response to IFN- γ and IFN- α/β .^{25–27} Thus, STAT1^{-/-} mice are highly susceptible to infections with several pathogens that include bacteria, viruses, and parasites.^{25,26,33} STAT1 also regulates

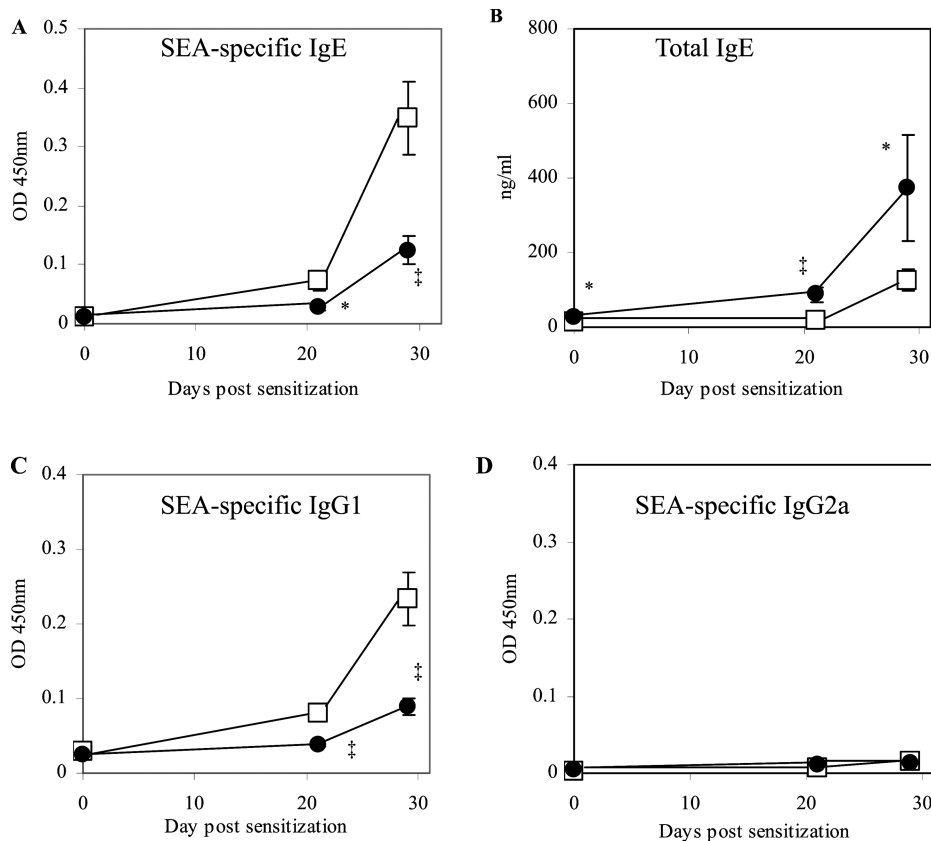


Figure 3. Levels of (A) SEA-specific IgE, (B) total IgE, (C) SEA-specific IgG1, and (D) IgG2a in STAT1^{-/-} and WT mice. WT mice and STAT1^{-/-} mice ($n = 8$ per group) were intranasally sensitized with SEA once a week for 3 weeks. Then, 1 week later, all of mice were challenged with SEA for 7 consecutive days. WT (□) and STAT1^{-/-} mice (●) were bled by tail snipping one day before the first sensitization, on day 6 after the third sensitization, and 18 hours after the final challenge. SEA-specific IgE, total IgE, SEA-specific IgG1, and SEA-specific IgG2a were measured by ELISA. Data are from two independent experiments. * $p < 0.05$ and ‡ $p < 0.01$ compared with the WT group.

expression of a transcription factor T-bet, which specifically regulates IFN- γ production from natural killer and CD4⁺ T cells.^{34,35}

Allergic diseases such as asthma usually are associated with preferential induction of Th2-biased response and impaired production of Th1 cytokine such as IFN- γ , which suppresses disease exacerbating Th2 responses.^{6,7} For example, in an animal model of allergic asthma, IFN- γ treatment suppresses airway inflammation after allergen challenge.³⁶ Conversely, ovalbumin-sensitized IFN- γ -deficient mice show persistent hyperresponsiveness and inflammation of airway.³⁷ STAT1 is also selectively activated in bronchial epithelial cells of asthmatic subjects' and the epithelial STAT1 activity corresponded closely with increased expression of ICAM-1.²⁸ Interestingly, levels of IFN- γ mRNA in bronchial epithelium were low in both normal controls and asthmatic subjects, indicating that the IFN- γ -independent mechanism was responsible for STAT1 activation in the latter.²⁸

A previous study has shown that expression of intracellular adhesion molecule (ICAM) 1 is increased in perennial allergic rhinitis, suggesting that ICAM-1 may be involved in trafficking of leukocytes into nasal mucosa during rhinitis.³⁸ In a murine model of allergic rhinitis, administration of anti-ICAM-1 Ab blocked nasal eosinophilia and allergen-induced

nasal symptoms.³⁹ In humans, IFN- γ , TNF- α , eosinophil major basic protein, and eosinophilic cationic protein increase expression of ICAM-1 on epithelial cells, resulting in increased adherence of eosinophils and other leukocytes to epithelial cells.⁴⁰ In this study, anti-CD3 stimulated nasal lymphocytes from WT, and STAT1^{-/-} mice produced significant but comparable levels of IFN- γ , but the latter group showed reduced nasal inflammation. Because STAT1 is critical for mediating most biological functions of IFN- γ , it is likely that impaired migration of leukocytes into nasal mucosa in STAT1^{-/-} mice may be caused by the inability of these mice to up-regulate ICAM-1 rather than the imbalance between Th1 and Th2 in this model. Indeed, ongoing gene chip microarray analysis in our laboratory has revealed that expression of ICAM-1 is significantly reduced in lymphoid and nonlymphoid organs from STAT1^{-/-} mice (data not shown). In addition, vascular cell adhesion molecule (VCAM) 1 also plays a critical role in the pathogenesis of airway inflammation. A recent study by Quarcoo *et al.* using a murine model of asthma showed that a decoy oligonucleotide neutralizing STAT1 inhibited allergen-induced airway inflammation and airway hyperreactivity by attenuating up-regulation of VCAM-1.⁴¹ Moreover, it is known that Th1 cells can pro-

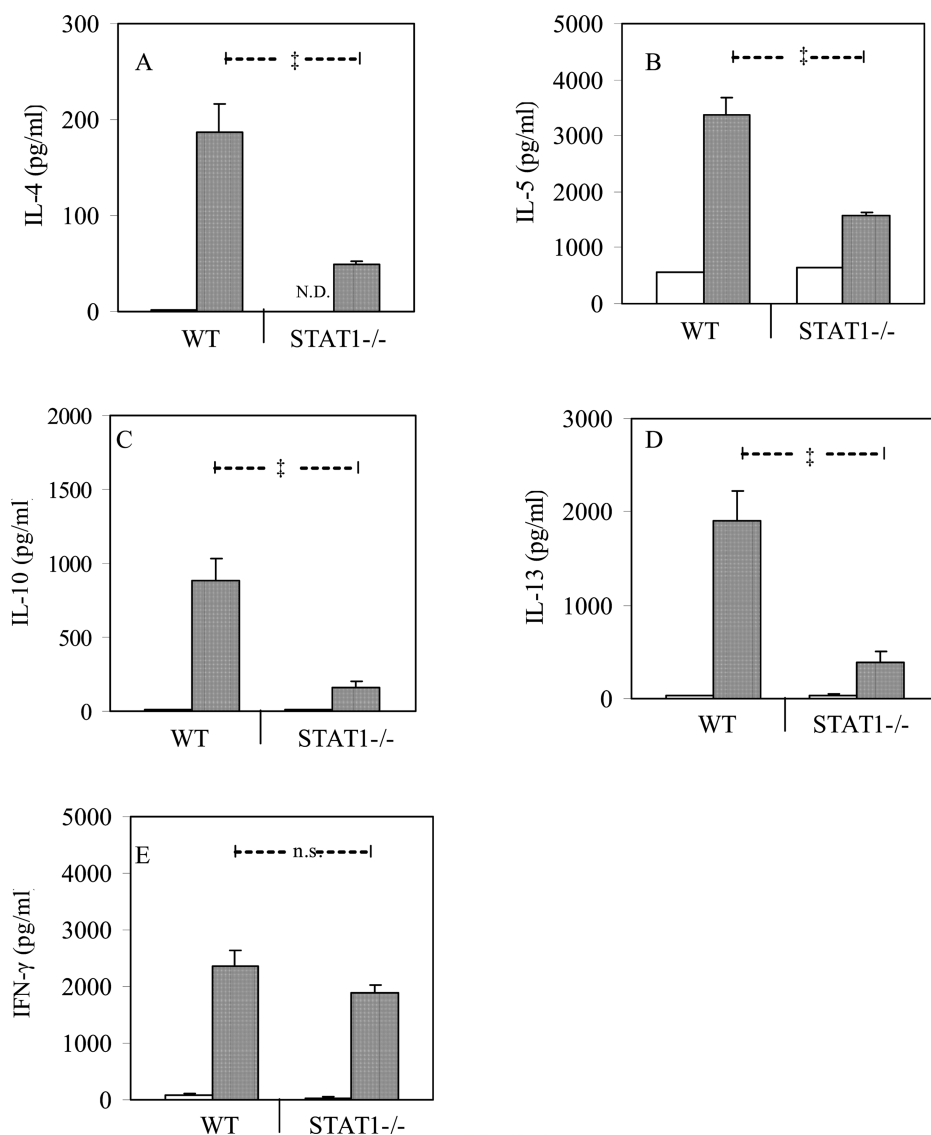


Figure 4. Production of (A) IL-4, (B) IL-5, (C) IL-10, (D) IL-13, and (E) IFN- γ by nasal lymphocytes from WT and STAT1^{-/-} mice sensitized intranasally with SEA. Nasal lymphocytes were isolated and cultured *in vitro* for 72 hours without (□) or with plate-bounded anti-CD3 (■). Cytokines were measured by ELISA. Results were expressed as the mean $\pm$ SEM. $\dagger p < 0.01$ compared with WT group. Data are pooled from two independent experiments ($n = 8$ per group).

duce TNF- α , which may be important for the induction of ICAM-1 and/or VCAM-1.⁴²

Several studies have shown that Th2-associated cytokines IL-4, IL-5, and IL-13 are involved in pathogenesis of allergic diseases.^{43,44} Although IL-4 is involved in Th2 differentiation and IgE production,^{43,45} IL-5 plays a central role in the differentiation, accumulation, and migration of eosinophils to the site of inflammation.⁴ STAT1 is a Th1-associated transcription factor that induces IFN- γ production from T cells.^{34,35} Conversely, the absence of STAT1 results in an increase in Th2 cytokine production.^{33,46} Interestingly, we found that T cells from both WT and STAT1^{-/-} mice displayed comparable levels of CD28, and CTLA4 (CD152; data not shown), but anti-CD3-stimulated T cells from STAT1^{-/-} mice produced significantly lower levels of Th2-associated IL-4, IL-5, IL-13, and IL-10. Both WT and STAT1^{-/-} T cells produced com-

parable levels of Th1-associated IFN- γ . Although IFN- γ production can be induced *via* STAT1-independent pathway,⁴⁶ our observations that STAT1-deficient cells produce less Th2-associated cytokines are novel and need further investigation. Nonetheless, taken together, these findings indicate that STAT1-mediated signaling is involved in regulating Th2-type but not Th1-type cytokine production from nasal lymphocytes in this model. Furthermore, they also indicate that reduced production of Th2-associated cytokines by nasal T cells may partly contribute to reduced severity of allergic rhinitis in STAT1^{-/-} mice.

In this study, SEA-specific IgG levels were lower when compared with those observed in our previous study.³⁰ This may be caused by different captured buffer, blocking buffer, and/or manufacturer-delivered HRP-anti-mouse IgG1. Mice were not anesthetized before delivery of intranasal SEA. Be-

cause we deliver few amounts of solution (10 μ L per nostril), mice do not sneeze or cough during the delivery. Thus, there were no apparent differences in SEA delivery between the strains.

In conclusion, we have shown that STAT1 $^{-/-}$ mice fail to develop allergic inflammation and nasal eosinophilia after SEA sensitization. Furthermore, failure to develop allergic rhinitis in STAT1 $^{-/-}$ mice is associated with reduced levels of Th2-associated SEA-specific IgE and IgG1 and impaired production of Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13 by nasal lymphocytes. These findings indicate that STAT1 is involved in the pathogenesis of allergic rhinitis and nasal airway hyperresponsiveness. This investigation provides new insights into an unexpected role of STAT1 in induction of Th2-like response during allergic rhinitis and mediating nasal inflammation and airway hyperresponsiveness.

ACKNOWLEDGMENTS

The authors thank Tazuko Fujiwara for technical assistance and Dr. Donald A. Harn for providing the SEA.

REFERENCES

- Settipane RA. Demographics and epidemiology of allergic and nonallergic rhinitis. *Allergy Asthma Proc* 22:185–189, 2001.
- Skoner DP. Allergic rhinitis: Definition, epidemiology, pathophysiology, detection, and diagnosis. *J Allergy Clin Immunol* 108:S2–S8, 2001.
- International Rhinitis Management Working Group. International consensus report on the diagnosis and management of allergic rhinitis. *Allergy* 49:5–34, 1994.
- Durham SR, Ying S, Varney VA, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor in nasal mucosa after local allergen provocation: Relationship to tissue eosinophilia. *J Immunol* 148:2390–2394, 1992.
- Bradding P, Feather IH, Wilson S, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitis subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 151:3853–3865, 1993.
- Mosmann TR, and Sad S. The expanding universe of T-cell subsets: Th1, Th2, and more. *Immunol Today* 17:138–146, 1996.
- Agnello D, Lankford CSR, Bream J, et al. Cytokine and transcription factors that regulate T helper cell differentiation: New players and new insights. *J Clin Immunol* 23:147–161, 2003.
- Snapper RA, and Paul WE. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 236:944–947, 1987.
- Lopez AF, Sanderson CJ, Gamble JR, et al. Recombinant human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* 167:219–224, 1988.
- Yamaguchi Y, Suda T, Ohta S, et al. Analysis of the survival of mature human eosinophils: Interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 78:2542–2547, 1991.
- Darnell JE Jr. STATs and gene regulation. *Science* 277:1630–1635, 1997.
- Takeda K, and Akira S. STAT family of transcription factors in cytokine-mediated biological responses. *Cytokine Growth Factor Rev* 11:199–207, 2000.
- Kaplan MH, Schindler U, Smilery ST, and Grusby MJ. Stat6 is required for mediating responses to IL-4 and for the development of Th2 cells. *Immunity* 4:313–319, 1996.
- Takeda K, Tanaka T, Shi W, et al. Essential role of Stat6 in IL-4 signaling. *Nature* 380:627–630, 1996.
- Shimoda K, van Deursen J, Sangster MY, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 380:630–633, 1996.
- Thierfelder WE, van Deursen JM, Yamamoto K, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* 382:171–174, 1996.
- Kaplan MH, Sun YL, Hoey T, and Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* 382:174–177, 1996.
- Behera AK, Kumar M, Lockey RF, and Mohapatra SS. Adenovirus-mediated interferon gamma gene therapy for allergic asthma: Involvement of interleukin 12 and STAT4 signaling. *Hum Gene Ther* 13:1697, 2002.
- Ramana CV, Chatterjee-Kishore M, Nguyen H, and Stark GR. Complex role of Stat1 in regulating gene expression. *Oncogene* 19:2619–2627, 2000.
- van der Bruggen T, Cardenhoven E, Kanters D, et al. Interleukin-5 signaling in human eosinophils involves JAK2 tyrosine kinase and Stat1 α . *Blood* 85:1442–1448, 1995.
- Finbloom DS, and Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 α and STAT3 complexes in human T cells and monocytes. *J Immunol* 155:1079–1090, 1995.
- Frank DA, Robertson MJ, Bonni A, et al. Interleukin 2 signaling involves the phosphorylation of Stat proteins. *Proc Natl Acad Sci U S A* 92:7779–7783, 1995.
- Leaman DW, Pisharody S, Flickinger TW, et al. Roles of JAKs in activation of STATs and stimulation of c-fos gene expression by epidermal growth factor. *Mol Cell Biol* 16:369–375, 1996.
- Vignais ML, Sadowski HB, Walting D, et al. Platelet-derived growth factor induces phosphorylation of multiple JAK family kinase and STAT proteins. *Mol Cell Biol* 16:1759–1769, 1996.
- Meraz MA, White JM, Sheehan KC, et al. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. *Cell* 84:431–442, 1996.
- Durbin JE, Hackenmiller R, Simon MC, and Levy DE. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* 84:443–450, 1996.
- Durbin JE, Fernandez-Sesma A, Lee CK, et al. Type I IFN modulates innate and specific antiviral immunity. *J Immunol* 164:4220–4228, 2000.
- Sampath D, Castro M, Look DC, and Holtzman MJ. Constitutive activation of an epithelial signal transducer and activator of transcription (STAT) pathway in asthma. *J Clin Invest* 103:1353–1361, 1999.
- Randolph DA, Carruthers CJ, Szabo SJ, et al. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J Immunol* 162:2375–2383, 1999.
- Okano M, Nisizaki K, Abe M, et al. Strain-dependent induction of allergic rhinitis without adjuvant in mice. *Allergy* 54:593–601, 1999.
- Asanuma H, Inaba Y, Aizawa C, et al. Characterization of mouse nasal lymphocytes isolated by enzymatic extraction with collagenase. *J Immunol Methods* 187:41–51, 1995.
- Stamm LM, Raisanen-Sokolowski A, Okano M, et al. Mice with STAT6-targeted gene disruption develop a Th1 re-

- sponse and control cutaneous leishmaniasis. [J Immunol 161: 6180–6188, 1998.](#)
33. Rosas LE, Keiser T, Pyles R, et al. Development of protective immunity against cutaneous leishmaniasis is dependent on STAT1-mediated IFN signaling pathway. [Eur J Immunol 33:1799–1805, 2003.](#)
 34. Szabo SJ, Sullivan BM, Stemmann C, et al. Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. [Science 295:253–256, 2002.](#)
 35. Lighvani AA, Frucht DM, Jankovic D, et al. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. [Proc Natl Acad Sci USA 98:15137–15142, 2001.](#)
 36. Hofstra CL, van Ark I, Hofman G, et al. Differential effects of endogenous and exogenous interferon- γ on immunoglobulin E, cellular infiltration and airway responsiveness in a murine model of allergic asthma. [Am J Respir Cell Mol Biol 19:826–835, 1998.](#)
 37. Yoshida M, Leigh R, Matsumoto K, et al. Effect of interferon- γ on allergic airway response in interferon- γ -deficient mice. [Am J Crit Care Med 166:451–456, 2002.](#)
 38. Montefort S, Feather IH, Wilson SJ, et al. The expression of leukocyte endothelial adhesion molecules is increased in perennial allergic rhinitis. [Am J Respir Cell Mol Biol 7:393–398, 1992.](#)
 39. Asakura K, Saito H, and Kataura A. In vivo effects of monoclonal antibody against ICAM-1 and LFA-1 on antigen-induced nasal symptoms and eosinophilia in sensitized rats. [Int Arch Allergy Immunol 111:156–160, 1996.](#)
 40. Altman LC, Ayars GH, Baker C, and Luchtel DL. Cytokines and eosinophil-derived cationic proteins up regulate intercellular adhesion molecules-1 on human nasal epithelial cells. [J Allergy Clin Immunol 4:527–536, 1993.](#)
 41. Quarcoo D, Weixler S, Groneberg D, et al. Inhibition of signal transducer and activator of transcription 1 attenuates allergen-induced airway inflammation and hyperactivity. [J Allergy Clin Immunol 114:288–295, 2004.](#)
 42. Randolph DA, Carruthers CJ, Szabo SJ, et al. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. [J Immunol 237:2375–2383, 1999.](#)
 43. Ngoc LP, Gold DR, Tzianabos AO, et al. Cytokines, allergy, and asthma. [Curr Opin Allergy Clin Immunol 5:161–166, 2005.](#)
 44. Wills-Karp M. Interleukin-13 in asthma pathogenesis. [Immunol Rev 202:175–190, 2004.](#)
 45. Stetson DB, Voehringer D, Grogan JL, et al. Th2 cells: Orchestrating barrier immunity. [Adv Immunol 83:163–189, 2004.](#)
 46. Gavrilescu LC, Butcher BA, Del Rio L, et al. STAT1 is essential for antimicrobial effector function but dispensable for gamma interferon production during *Toxoplasma gondii* infection. [Infect Immun 72:1257–1264, 2004.](#) □



OceanSide Publications

IP: 193.205.233.41