

Repeated intranasal instillation with staphylococcal enterotoxin B induces nasal allergic inflammation in guinea pigs

Xinye Tang, Ph.D.,¹ Rong Sun, M.M.,² Suling Hong, M.D.,¹ Guohua Hu, M.D.,¹ and Yang Yang, B.M.¹

ABSTRACT

Background: Staphylococcal enterotoxins (SEs) appear to play a role in the pathogenesis of allergic disease. However, no animal models have been reported to show nasal allergic inflammation by repeated inhalation with staphylococcal enterotoxin B (SEB) in the absence of adjuvant. This study was designed to determine whether intranasal instillation of guinea pigs with SEB results in nasal allergic inflammation.

Methods: Guinea pigs were intranasally instilled with 40 μ L of 4- μ g SEB once every 4 days 11 times. For the control group, guinea pigs were prepared with saline instead of SEB. Sneezing and nasal scratching frequency were evaluated after each intranasal instillation. The production of antigen-specific antibodies including IgE, nasal eosinophilia, and cytokines in the nasal cavity lavage fluid (NCLF) were measured after the 11th intranasal immunization.

Results: In the model group, symptoms of sneezing and nasal scratching were induced at the 8th to 11th challenges. Intranasal immunization with SEB elicited a local nasal mucosa inflammatory response characterized by apparent eosinophil infiltration. In the NCLF, the expression of IL-4 but not interferon- γ was increased after challenges. The serum levels of total and SEB-specific IgE and IgG1 were higher in model groups in comparison with the control groups ($p < 0.01$).

Conclusion: These results indicate that repeated intranasal instillation with SEB leads to Th2 immune response, allergic nasal inflammation, and increased antigen-specific IgE production that are characteristic of allergic rhinitis (AR). The model in this study could be valuable in analyzing the pathogenesis of AR infected with *Staphylococcus aureus*.

(Am J Rhinol Allergy 25, 176–181, 2011; doi: 10.2500/ajra.2011.25.3609)

Allergic rhinitis (AR) is one of the typical atopic diseases. It is often characterized by expression of Th2 type cytokines IL-4, IL-5, and IL-13^{1–3}; elevated antigen-specific IgE; and a marked inflammatory cell infiltration, which consists of eosinophils and lymphocytes.^{4–6}

Staphylococcus aureus is a Gram-positive pathogenic germ, often found as part of the microflora of the human skin and the upper respiratory tract.⁷ Elegant studies have shown that a nasal mean carriage rate of *S. aureus* is 37.2% in the general population.⁸ The anterior nares are the primary reservoir of *S. aureus*.⁹ *S. aureus* expresses types of toxin with superantigen activity: staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1.¹⁰ Some reports have revealed that more than one-half of the pathogenic isolates produce one or more superantigen exotoxins; even strains isolated from asymptomatic carriers can produce superantigens.^{11–13} It is well known that SEs trigger an excessive immune response and appear to be involved in the pathogenesis of allergic disease, especially atopic dermatitis.^{14–17} In addition, SEs as allergens play an important role in atopic dermatitis.^{18–21}

But the mechanism by which *S. aureus* and/or *S. aureus* toxins contribute to the development of AR remains unclear. Recently, Shimori *et al.* have reported that the nasal cavity of up to 44% of patients with perennial AR is colonized with *S. aureus*. Moreover, the rate of nasal carriage of superantigen-producing *S. aureus* among these patients is significantly higher than control subjects. Peripheral blood lymphocytes of these patients produced significantly higher levels of IL-4 and IL-5 and decreased levels of interferon (IFN) γ in response to superantigen.²² More recently, Rossi *et al.* showed a significantly higher prevalence of serum-specific IgE toward SEs in patients with

perennial AR and suggested a possible role of SEs in increasing serum extracellular protein in allergic disease.²³ These findings prompted us to investigate the possibility that intranasal exposure to SEs may induce AR. However, to date, no successful animal models have been reported to show SEs induce nasal allergic inflammation. An aim of this study was to establish a model of AR in guinea pigs by repeated intranasal instillation with staphylococcal enterotoxin B (SEB).

MATERIALS AND METHODS

Animals and Materials

Male, 8-week-old Hartley guinea pigs (250–300 g) were purchased from the Laboratory Animal Center, Chongqing Medical University. The animals were housed in an air-conditioned room that maintained allergen-free conditions. The animals were fed a standard laboratory diet. All experimental protocols and procedures in the present study were approved by the Chongqing Medical University Animal Care and Use Committee. SEB was purchased from Sigma (St. Louis, MO).

Challenge of Guinea Pigs

Guinea pigs ($n = 6$ per group) were given 40 μ L of 4- μ g SEB by intranasal instillation in the absence of adjuvant once every 4 days for 11 times (total 41 days). For the control groups, the animals received the same amount of saline instead of SEB.

Symptoms Observation

Sneezing frequencies were in intervals of 0–10 minutes and 10 minutes–1 hour after the SEB intranasal challenges were counted. At the same time, nasal scratching frequencies were measured.

Cytokine Determination in Nasal Cavity Lavage Fluid

Within 5 hours after the final nasal challenge, the guinea pigs were anesthetized by i.m. injection with urethane (1350 mg/kg). The nasal cavity lavage (NCL) was performed as described by Nabe *et al.*²⁴ In brief, washing with 2 mL of physiological saline was performed from the right nostril to the left nostril. Over 80% of NCL fluid (NCLF) was

From the ¹Department of Otolaryngology–Head and Neck Surgery, and ²Medical Examination Center, the First Affiliated Hospital of Chongqing Medical University, Chongqing, China

The authors had no conflicts to declare pertaining to this article

Address correspondence and reprint requests to Guohua Hu, M.D., Department of Otolaryngology–Head and Neck Surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

E-mail address: hghcq@sina.com

Copyright © 2011, OceanSide Publications, Inc., U.S.A.

collected. The NCLF was centrifuged at 1400 rpm for 10 minutes at room temperature. The supernatant was used for IL-4 and IFN- γ assays by enzyme-linked immunosorbent assay (ELISA; Bionewtrans Pharmaceutical Biotechnology Co., Ltd. Franklin, MA). The limit of detection was <2 pg/mL for each cytokine.

Serum Immunoglobulin Levels

Five hours after the final challenge, the guinea pigs were killed by cardiac puncture. Serum was collected. Serum total IgE, IgG, and IgA were measured by competitive ELISA. SEB-specific IgG1 levels were quantified with indirect ELISA as Savinko *et al.* described.²⁵ In brief, plates were coated (100 μ L/well) with 0.1 μ g/mL of SEB in 0.05 M of NaHCO₃ (pH 9.6) at 4°C overnight. Plates were washed with PBS-Tween 20 and blocked (200 μ L/well) with 2% bovine serum albumin (BSA) for 2 hours at 20°C and washed again; 1:100 diluted sera in 0.1% BSA was incubated (100 μ L/well) at 4°C overnight. After washing, 1:500 diluted horseradish peroxidase (HRP)–goat anti-guinea pig IgG1 monoclonal antibody (AbD Serotec, Oxford, United Kingdom) in 0.1% BSA was incubated (100 μ L/well) at 20°C for 2 hours and washed again. 3,3',5,5'-Tetramethylbenzidine chromogenic reagents (100 μ L) was incubated at 20°C for 10 minutes and stop solution (100 μ L/well) was added. Titers for SEB-specific IgG1 were estimated as mean optical density at 450 nm. SEB-specific IgG2 and IgE levels were measured by the aforementioned method. Specific IgG2 was detected with HRP-goat anti-guinea pig IgG2 mAb (AbD Serotec). Specific IgE was detected with HRP-goat anti-mouse IgE mAb (Southern Biotechnology Associates, Inc., Birmingham, AL).

Histological Examination

Nasal tissue was collected, fixed in formalin, and embedded in paraffin. Coronal nasal sections were stained with hematoxylin and eosin. Initially, eosinophil infiltration in the nasal mucosa was observed microscopically in a high-power field (10 $\times$ 40). Subsequently, 200 leukocytes was counted microscopically (10 $\times$ 100) in total. The percentage of eosinophilia in total leukocytes was computed.

Statistical Analysis

All results presenting normal distribution were expressed as mean $\pm$ SD. Comparison of means between different groups was performed with Student's *t*-test (two groups). Data presenting non-normal distribution was expressed as M (Q). Statistical analyses were performed with Wilcoxon test. The value of *p* < 0.05 was considered statistically significant.

RESULTS

The Changes in Sneezing and Nasal Scratching Frequency

Figure 1 shows the changes in sneezing frequency within 10 minutes after each challenge among SEB-challenged and saline-challenged guinea pigs. At the 8th to 11th challenges, the guinea pigs in the model group produced the highest sneezing frequency within 10 minutes after each challenge. From the 10th minute, little or no sneezing was observed at any challenge time. At the same time, the total number of sneezes increased with increasing challenge number. The guinea pigs in the control group produced significantly less sneezing than the ones in the model group (*p* < 0.01). Change in nasal scratching frequency is similar with changes in sneezing frequency (data not shown).

IL-4 and IFN- γ Levels in NCLF

Figure 2 shows the levels of IL-4 and IFN- γ in NCLF among SEB-challenged and saline-challenged guinea pigs. IL-4 levels in NCLF were significantly higher in the model group (29.19 $\pm$ 2.83)

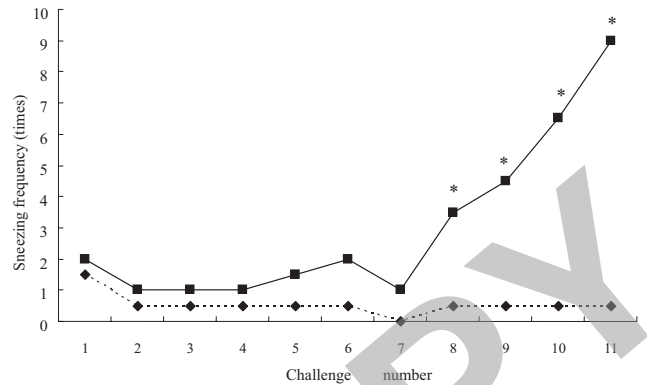


Figure 1. The changes in sneezing frequency within 10 minutes after each challenge among staphylococcal enterotoxin B (SEB)–challenged and saline-challenged guinea pigs. Each point represents the median of six animals. (■, SEB-challenged guinea pigs; ◆, saline-challenged guinea pigs). At the 8th to 11th challenges, the SEB-challenged guinea pigs produced a higher sneezing frequency than saline-challenged guinea pigs (**p* < 0.01).

compared with the control group (7.35 $\pm$ 1.14); the increase was statistically significant (*p* < 0.0001). However, the amount of IFN- γ in NCLF is lower in the model group (59.74 $\pm$ 17.88) than the control group (90.94 $\pm$ 7.35); the lower amount was statistically significant (*p* = 0.006).

Immunoglobulin Production in Serum

Figure 3 shows SEB-specific IgE, IgG₁, and IgG₂ and total IgE levels in serum among SEB-challenged and saline-challenged guinea pigs. In the model group, the guinea pigs produced increased amounts of SEB-specific IgE and IgG₁. The median of optical density at 450 nm (Q) was 0.15 (0.11) and 4.12 (0.19); however, in the control group, the levels of SEB-specific IgE and IgG₁ could not be detected. Compared with the control group, these increases were statistically significant (*p* < 0.01, Wilcoxon test). In the two groups, guinea pigs did not produce detectable amounts of SEB-specific IgG₂. An increased level of total serum IgE and IgG was also observed among SEB-challenged guinea pigs. Compared with the control group, this increase was statistically significant (*p* = 0.002, Student's *t*-test).

Eosinophils in the Nasal Mucosa

Figure 4 shows eosinophil infiltration in the nasal mucosa among SEB challenged and saline challenged guinea pigs. In the model group, guinea pigs show apparent eosinophil infiltration in nasal mucosa. Eosinophils accounted for 0.412(0.119) among the total leukocytes; however in the control group, eosinophils only accounted for 0.028(0.013) among the total leukocytes. Compared with the control group, these increases were statistically significant (*p* < .01 Wilcoxon test). Eosinophils infiltrated not only in the lamina propria but also in the epithelial layer. However, eosinophil infiltration was not seen in guinea pigs challenged with saline. What's more, vasodilatation and edema is present in the submucosal areas among SEB challenged guinea pigs.

DISCUSSION

The present study shows that repeated intranasal immunization with SEB elicits typical symptoms of AR. From the eighth challenge, the significant increase of sneezing frequency was observed immediately after each challenge in the immunized guinea pigs. At the same time, the total number of sneezes increased with increasing challenge number. The peak of frequent sneezing was within 10 minutes after challenge.

Some reports revealed that intranasal sensitization without adjuvant resulted in either transient induction of antigen-specific IgE

Figure 2. The levels of (a) IL-4 and (b) interferon (IFN) γ in nasal cavity lavage fluid (NCLF) among staphylococcal enterotoxin B (SEB)-challenged and saline-challenged guinea pigs. Each column represents the mean $\pm$ SD of six animals. Compared with saline-challenged guinea pigs, the expression of the Th2 cytokines IL-4 was increased in SEB-challenged guinea pigs ($p < 0.0001$); however the expression of the Th1 cytokine IFN- γ was more decreased in SEB-challenged guinea pigs than in saline-challenged guinea pigs ($p = 0.006$).

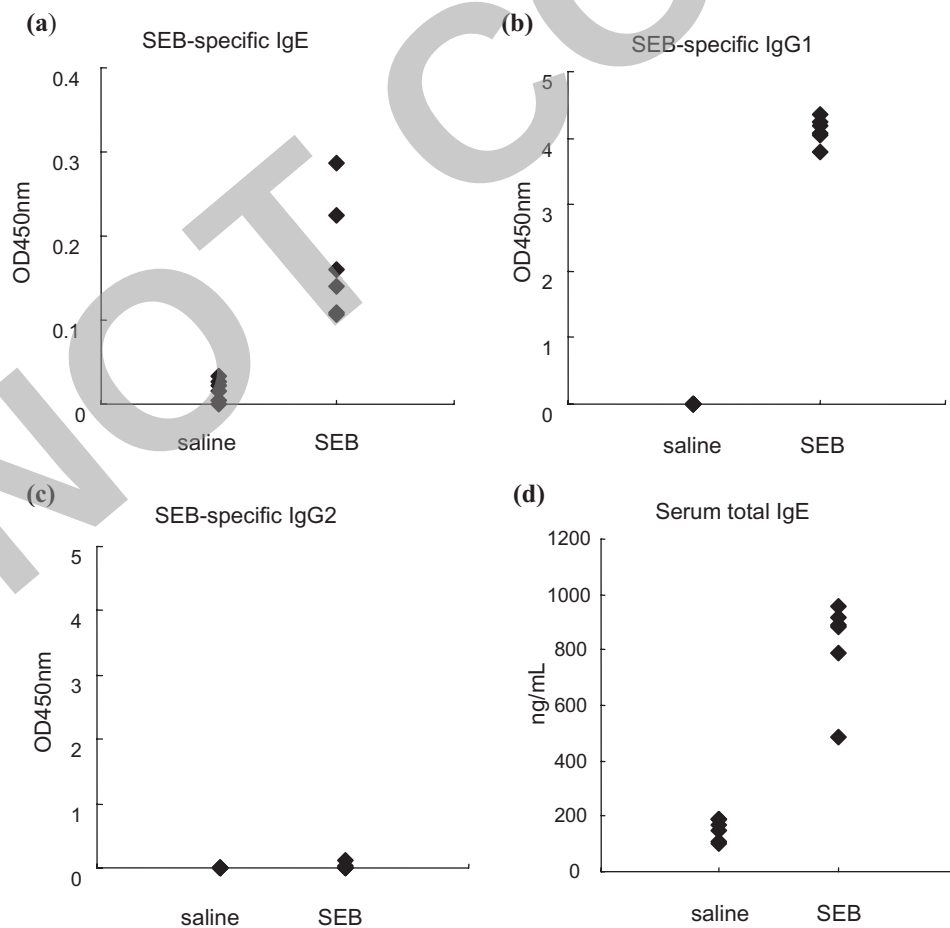
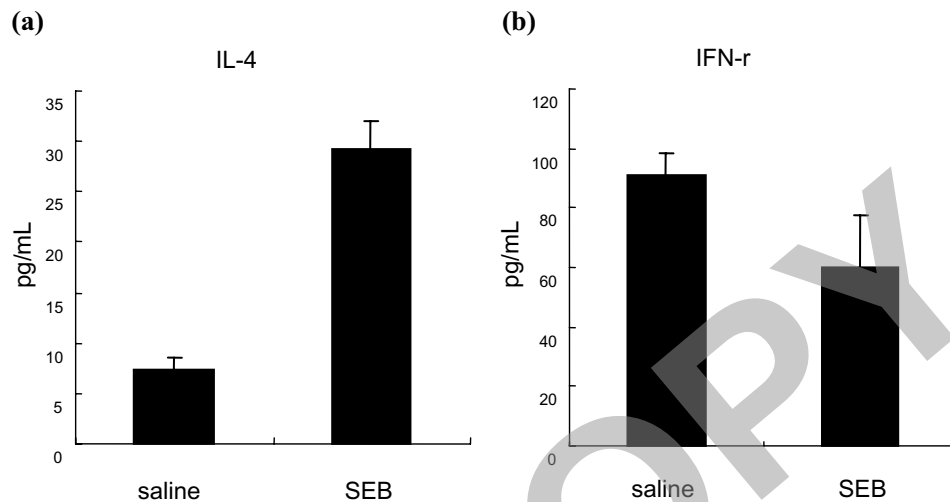


Figure 3. Staphylococcal enterotoxin B (SEB)-specific (a) IgE, (b) IgG₁, (c) IgG₂ and (d) total IgE levels in serum among SEB-challenged and saline-challenged guinea pigs. Each point represents the median or mean of six animals. Compared with saline-challenged guinea pigs, the SEB-challenged guinea pigs produced increased amounts of SEB-specific IgE, IgG₁, and total serum IgE ($p = 0.0039$ for SEB-specific IgE, Wilcoxon test; $p = 0.0021$ for SEB-specific IgG₁, Wilcoxon test; $p = 0.0002$ for total serum IgE, Student's *t*-test). Among SEB-challenged and saline-challenged guinea pigs, the level of IgG₂ had no statistically significant difference ($p = 0.1396$, Wilcoxon test).

or none at all.^{26,27} Adjuvants are commonly used in intranasal immunization to induce antigen-specific immunoglobulin production because most soluble antigens are less immunogenic by themselves.^{28–30} It is well known that adjuvants have immunoregulatory effects that induce Th2 immune reaction and IgE production against antigen.^{31–33} In this study, we found that a significant amount of SEB-specific IgE and IgG₁ could be produced in the sensitized guinea pigs by intranasal sensitization in the absence of any adjuvant. The reason that we could induce specific IgE without adjuvant may be the use of SEB as an antigen. First, SEB might

activate dendritic cells *via* Toll-like receptor 2. SEB-activated dendritic cells express T-cell immunoglobulin mucin domain 4 and secrete high levels of IL-2, which drive CD4⁺ T cells to become Th2 cells.^{34,35} Therefore, SEB might act as an adjuvant to induce Th2 immune reaction on its own. Some studies indicate that immunization of animals with antigen in the presence of SEB results in more Th2 cytokines, such as IL-4 and IL-5, and antigen-specific IgE production than immunization with antigen alone.^{27,36,37} In addition, SEB has been shown to induce a huge polyclonal B-cell activation and the differentiation of natural killer T cells or CD4⁺

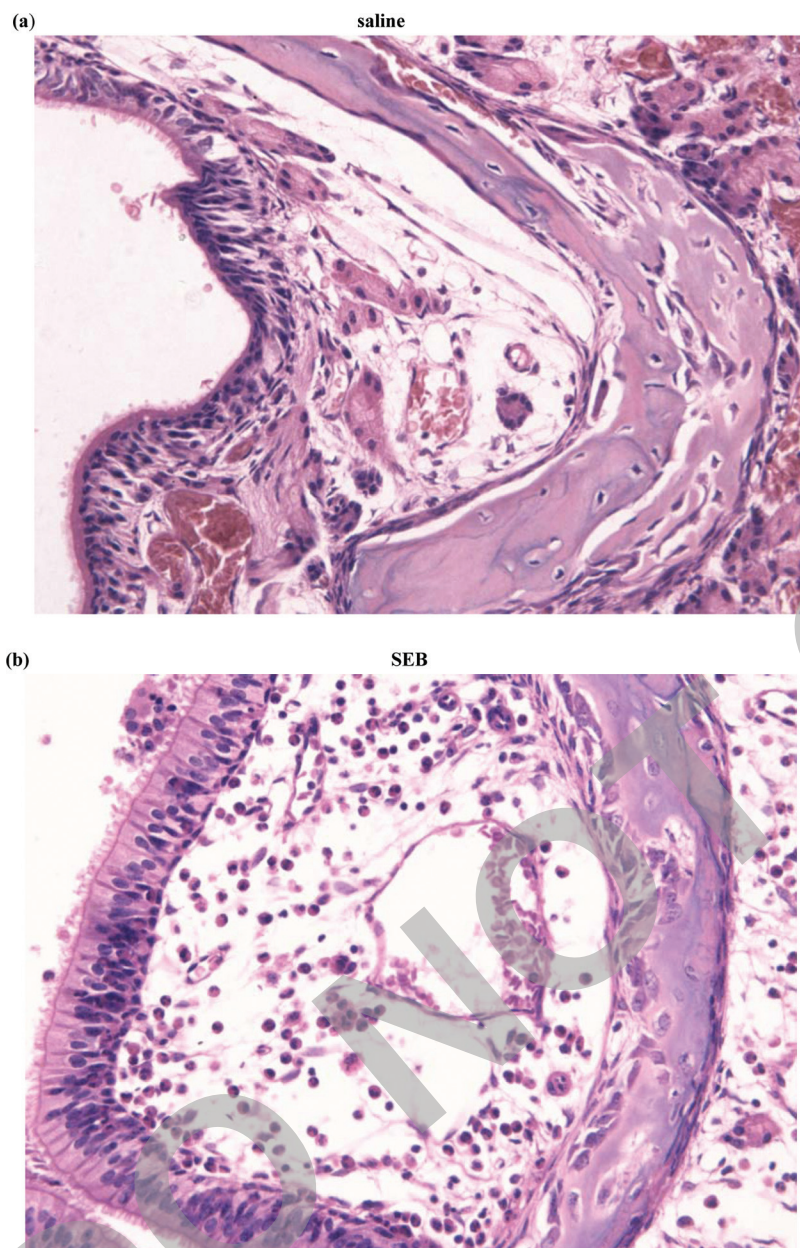


Figure 4. Histological features of the nasal mucosa among staphylococcal enterotoxin B (SEB)-challenged and saline-challenged guinea pigs (hematoxylin and eosin, 10×40). (a) Guinea pigs challenged with SEB show apparent eosinophil infiltration, vasodilatation, and edema in nasal mucosa compared with (b) guinea pigs challenged with saline.

T cells.^{38,39} Those activated natural killer T cells or CD4⁺ T cells express the high level of adhesion molecules (CD1 and CD40L) and inflammatory cytokines (IL-4 and IL-13), which are largely responsible for B-cell helper activity and SEB-specific IgE production.^{40–42}

However, some studies reported that intranasal application of SEB alone did not induce significant production of SEB-specific IgE in mice.^{23,43} These reports are not completely attached with our results. One of the reasons that we could induce specific IgE with SEB alone may be the use of guinea pigs as animal species of the animal model of AR. It is well known that correct choice of animal species severely affects successful establishment of animal models. According to the literature reports, guinea pigs, rats, mice, and rabbits have been selected to study AR.^{44–47} These animals stimulated with allergen produce different levels of allergic reaction, which order is guinea pigs > rabbits > mice > rats. So guinea pig is the preferred choice for establishment of a model of allergic disease in the evaluation of specific antibody production.⁴⁸ An-

other reason may be the time of stimulation. We speculate that it might need a much long time to induce the detectable levels of specific antibody production in serum *via* nasal inhalant of allergen. It is because lymph tissues in the nostril are not as plentiful as those in the peritoneum.⁴⁹ Nabe *et al.* have reported that most guinea pigs suffering from AR had no detectable levels of the anaphylactic antibodies in their serum until the 5th week after stimulation.⁵⁰ It may explain why in those reports the sensitized mice have no SEB-specific IgE in their serum *via* intranasal application of SEB alone in a relatively short time (7 days or 4 weeks).

In conclusion, we show that repeated intranasal instillation with SEB absent of adjuvant can induce allergic inflammation in guinea pigs characterized by eosinophil infiltration in nasal mucosa, increased expression of the Th2 cytokines in NCLF, and detectable levels of SEB-specific IgE in serum. In addition, the challenged guinea pigs representing typical symptoms of AR showed sneezing and nasal scratching. This model might be valuable in additional investigations into the pathogenesis of AR.

REFERENCES

- Lee CH, Rhee CS, Oh SH, et al. Increase in expression of IL-4 and IL-5 mRNA in the nasal mucosa of patients with perennial allergic rhinitis during natural allergen exposure. *Ann Otol Rhinol Laryngol* 106:215–219, 1997.
- Imada M, Estelle F, Simons R, et al. Allergen-stimulated interleukin-4 and interferon-gamma production in primary culture: Responses of subjects with allergic rhinitis and normal controls. *Immunology* 85: 373–380, 1995.
- Saito H, Asakura K, Ogasawara H, et al. Topical antigen provocation increases the number of immunoreactive IL-4-, IL-5F- and IL-6-positive cells in the nasal mucosa of patients with perennial allergic rhinitis. *Int Arch Allergy Immunol* 114:81–85, 1991.
- Lei F, Zhu D, Sun J, and Dong Z. Effects of minimal persistent inflammation on nasal mucosa of experimental allergic rhinitis. *Am J Rhinol Allergy* 24:e23–e28, 2010.
- Okada M, and Shida T. Clinical aspects of Japanese cedar pollinosis. *Allergol Int* 47:1–8, 1998.
- Murray AB, and Anderson DO. The epidemiologic relationship of clinical nasal allergy to eosinophilia and to goblet cells in the nasal smear. *J Allergy* 43:1–8, 1969.
- Damm M, Quante G, Jurk T, and Sauer JA. Nasal colonization with *Staphylococcus aureus* is not associated with the severity of symptoms or the extent of the disease in chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 131:200–206, 2004.
- Kluytmans J, Belkum AV, and Verbrugh H. Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 10:505–520, 1997.
- Williams RE. Healthy carriage of *Staphylococcus aureus*: Its prevalence and importance. *Bacteriol Rev* 27:56–71, 1963.
- Bachert C, Gebaert P, and Van Cauwenberge P. *Staphylococcus aureus* enterotoxins: A key in airway disease? *Allergy* 57:480–487, 2002.
- Von Eiff C, Becker K, Machka K, et al. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med* 344:11–16, 2001.
- Becker K, Friedrich AW, Lubritz G, et al. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J Clin Microbiol* 41:1434–1439, 2003.
- Fueyo JM, Mendoza MC, Alvarez MA, and Martin MC. Relationships between toxin gene content and genetic background in nasal carried isolates of *Staphylococcus aureus* from Asturias, Spain. *FEMS Microbiol Lett* 243:447–454, 2005.
- White J, Herman A, Pullen AM, et al. The V β -specific superantigen staphylococcal enterotoxin B; stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 56:27–35, 1989.
- Corriveau MN, Zhang N, Holtappels G, et al. Detection of *Staphylococcus aureus* in nasal tissue with peptide nucleic acid-fluorescence in situ hybridization. *Am J Rhinol Allergy* 23:461–465, 2009.
- Yu RL, and Dong Z. Proinflammatory impact of *Staphylococcus aureus* enterotoxin B on human nasal epithelial cells and inhibition by dexamethasone. *Am J Rhinol Allergy* 23:15–20, 2009.
- Akiyama H, Toi Y, Kanzaki H, et al. Prevalence and producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strain isolated from atopic dermatitis lesions. *Arch Dermatol Res* 288:418–420, 1996.
- Nomura I, Tanaka K, Tomita H, Katsumuma T, Ohya Y, Ikeda N et al. Evaluation of the staphylococcal enterotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 104:441–446, 1999.
- Leung DY, Harbeck R, Bina P, et al. Presence of IgE antibodies to staphylococcus exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 92:1374–1380, 1993.
- Bunikowski R, Mielke M, Skarabis H, et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 103:119–124, 1999.
- Tada J, Toi Y, Akiyama H, and Arata J. Presence of specific IgE antibodies to staphylococcal enterotoxin in patients with atopic dermatitis. *Eur J Dermatol* 6:552–554, 1996.
- Shiomori T, Yoshida S, Miyamoto H, and Makishima K. Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis. *J Allergy Clin Immunol* 105:449–454, 2000.
- Rossi RE, and Monasterolo G. Prevalence of serum IgE antibodies to the *Staphylococcus aureus* enterotoxins (SES, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol* 133:261–266, 2004.
- Nabe T, Shimizu K, Mizutani N, et al. A new model of experimental allergic rhinitis using Japanese cedar pollen in guinea pigs. *Jpn J Pharmacol* 75:243–251, 1997.
- Savinko T, Lauerma A, Lehtimäki S, et al. Topical superantigen exposure induces epidermal accumulation of CD8+ T cells, a mixed T_H1/T_H2-type dermatitis and vigorous production of IgE antibodies in the murine model of atopic dermatitis. *J Immunol* 175:8320–8326, 2005.
- Ahlstedt S, and Björkstén B. Specific antibody responses in rats and mice after daily immunization without adjuvant. *Int Arch Allergy Appl Immunol* 71:293–299, 1983.
- Okano M, Hattori H, Yoshino T, et al. Nasal exposure to Staphylococcal enterotoxin enhances the development of allergic rhinitis in mice. *Clin Exp Allergy* 35:506–514, 2005.
- Tamura S, Shoji Y, Hasiguchi K, et al. Effects of cholera toxin adjuvant on IgE antibody response to orally or nasally administered ovalbumin. *Vaccine* 12:1238–1240, 1994.
- Renauld-Mongenie G, Mielcarek N, Cornette J, et al. Induction of mucosal immune responses against a heterologous antigen fused to filamentous hemagglutinin after intranasal immunization with recombinant *Bordetella pertussis*. *Proc Natl Acad Sci USA* 93:7944–7949, 1996.
- Xiao YL, Kurono Y, Ichimiya I, and Mogi G. Induction of antigen-specific antibody-secreting cells in the nasal mucosa by intranasal immunization of BALB/c mice. *Acta Otolaryngol* 118:124–130, 1998.
- Valensi JP, Carlson JR, and Van Nest GA. Systemic cytokine profiles in BALB/c mice immunized with trivalent influenza vaccine containing MF59 oil emulsion and other advanced adjuvants. *J Immunol* 153:4029–4039, 1994.
- Victoratos P, Yiangou M, Avramidis N, and Hadjipetrou L. Regulation of cytokine gene expression by adjuvants in vivo. *Clin Exp Immunol* 109:569–578, 1997.
- Diaz-Sanchez D, Tsien A, Fleming J, and Saxon A. Combined allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J Immunol* 158:2406–2413, 1997.
- Mandron M, Ariès MF, Brehm RD, et al. Human dendritic cells conditioned with *Staphylococcus aureus* enterotoxin B promote TH2 cell polarization. *J Allergy Clin Immunol* 117:1141–1147, 2006.
- Liu T, He SH, Zheng PY, et al. Staphylococcal enterotoxin B increases TIM4 expression in human dendritic cells that drives naïve CD4 T cells to differentiate into Th2 cells. *Mol Immunol* 44:3580–3587, 2007.
- Yang PC, Wang CS, and An ZY. A murine model of ulcerative colitis: Induced with sinusitis-derived superantigen and food allergen. *BMC Gastroenterol* 6:23, 2006.
- Ganeshan K, Neilsen CV, Hadsaitong A, et al. Impairing oral tolerance promotes allergy and anaphylaxis: A new murine food allergy model. *J Allergy Clin Immunol* 123:231–238, 2009.
- Nomizo A, Postol E, de Alencar R, et al. Natural killer T cells are required for the development of a superantigen-driven T helper type 2 immune response in mice. *Immunology* 116:233–244, 2005.
- Williams O, Aroeira LS, and Mengel J. T cell-dependent antibody response to staphylococcal enterotoxin B. *Scand J Immunol* 42:305–310, 1995.
- Yoshimoto T, Bendelac A, Watson C, et al. Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. *Science* 270: 1845–1847, 1995.
- Kitamura H, Ohta A, Sekimoto M, et al. Alpha-galactosylceramide induces early B-cell activation through IL-4 production by NKT cells. *Cell Immunol* 199:37–42, 2000.
- Galli G, Nuti S, Tavarini S, et al. CD1d-restricted help to B cells by human invariant natural killer T lymphocytes. *J Exp Med* 197:1051–1057, 2003.

43. Herz U, Ruclert R, Wollenhaupt K, et al. Airway exposure to bacterial superantigen(SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness—A model for non-allergic asthma. *Eur J Immunol* 29:1021–1031, 1999.
44. Kawaguchi S, Majima Y, and Sakakura Y. Nasal mast cells in experimentally induced allergic rhinitis in guinea-pigs. *Clin Exp Allergy* 24:238–244, 1994.
45. Takahashi N, Aramaki Y, and Tsuchiya S. Allergic rhinitis model with Brown Norway rat and evaluation of antiallergic drugs. *J Pharmacobiodyn* 13:414–420, 1990.
46. Almansouri HM, Yamamoto S, Kulkarni AD, et al. Effect of dietary nucleosides and nucleotides on murine allergic rhinitis. *Am J Med Sci* 312:202–205, 1996.
47. Li Q, Li XL, Yang X, et al. Effects of antiallergic herbal agents on cystic fibrosis transmembrane conductance regulator in nasal mucosal epithelia of allergic rhinitis rabbits. *Chin Med J (Engl)* 122:3020–3024, 2009.
48. Ricciardolo FL, Nijkamp F, De Rose V, and Folkerts G. The guinea pig as an animal model for asthma. *Curr Drug Targets* 9:452–465, 2008.
49. Zhou C, Kang XD, and Chen Z. A synthetic Toll-like receptor 2 ligand decrease allergic immune responses in a mouse rhinitis model sensitized to mite allergen. *J Zhejiang Univ Sci B* 9:279–285, 2008.
50. Nabe T, Mizutani N, Shimizu K, et al. Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs. *Inflamm Res* 47:369–374, 1998. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.