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Staphylococcus aureus accelerates an experimental allergic conjunctivitis by Toll-like receptor 2-dependent manner

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KEYWORDS

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Abstract Allergic conjunctivitis is an inflammatory eye disease mediated by Th2-type cytokines and *Staphylococcus aureus* (*S. aureus*) colonization has been suggested as playing a role. This study used an experimental allergic conjunctivitis model to determine whether colonization by *S. aureus* affects the development of allergic conjunctivitis and modifies the immune response to OVA allergen. Mice challenged with OVA via conjunctival sac following systemic challenge with OVA in alum had severe allergic conjunctivitis. Of interest, inoculation of *S. aureus* markedly accelerated the signs of allergic conjunctivitis and was associated with higher levels of IgE Ab in serum. In addition, mice inoculated with *S. aureus* had more IL-4, IL-5, IL-13 and eotaxin secretion than non-inoculated group. In contrast, inoculation of *TLR2*^{-/-} mice with *S. aureus* had no effect on severity of allergic conjunctivitis. The findings suggest that activation of TLR2 signal by *S. aureus* induces Th2-type immune responses and accelerates experimental allergic conjunctivitis.

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Introduction

Allergic conjunctivitis (AC) describes a group of conditions ranging in severity from mild to severe [1]. The immunopathogenic mechanisms in these allergic disorders involve a combination of immunoglobulin E (IgE)-mediated and T cell-mediated responses [2–4]. The IgE-mediated conjunctival allergic reaction can be reproduced easily by specific con-

junctival provocation [5], which induces an early reaction followed by a predominant infiltration of eosinophilic inflammatory cells [6,7].

Eosinophils are the hallmark of allergic disease, particularly in severe chronic ocular allergy where they are easily found in quantity in tears and tissues [8,9]. The release of eosinophil granule proteins is implicated in the pathogenesis of conjunctival inflammation [10]. Eotaxin are potent eosinophil chemotactic and activating peptides [11] that act through the CCR3 expressed on eosinophils [12]. In ocular allergic diseases, increased numbers of eosinophils and eotaxin have been found in tears of persons with severe

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vernal and atopic keratoconjunctivitis and were significantly correlated with the clinical severity of disease [9,13]. Also, circulating levels of eotaxin increase in active atopic dermatitis and acute urticaria [14], and directly related to asthma severity [15].

There is an intense interest in the interaction between exposure to microbial products and the clinical expression of human allergic disease such as asthma, eczema, allergic rhinitis, and allergic conjunctivitis. A role of *Staphylococcus aureus* (*S. aureus*) colonization in the pathogenesis of chronic allergic conjunctivitis has been suggested [16].

The innate immune system recognizes microbial pathogen using Toll-like receptors (TLRs), a family of innate immune-recognition receptors, which provide an initial triggering signal for induction of antimicrobial immune responses [17,18]. Recent studies have revealed that 11 mammalian TLRs have been described and a striking feature of TLRs is their ability to discriminate among different classes of pathogen-associated molecules [19–21]. Among them, TLR2 is particularly involved in signal transduction of cellular responses to lipoprotein/lipopeptides of gram-positive bacteria including *S. aureus* [22,23]. Although it was originally thought that signaling through TLRs is required for adaptive Th1 responses [19], more recent studies suggest that the relationship between TLR stimulation and Th1/Th2 polarization is more complex, varying with both the concentration and type of TLR ligand studied [24,25]. A previous study of allergic conjunctivitis demonstrated that TLR2 agonist ameliorates murine experimental allergic conjunctivitis (EAC) by inducing CD4-positive T-cell apoptosis rather than by up-regulating Th1 responses [26].

In this study, we investigated the effect of a microbe on the immune response to allergens in a mouse model. In particular, we examined whether colonization of *S. aureus* can affect the severity of murine EAC, which is predominantly mediated by a Th2-type immune response. We found that *S. aureus* colonization accelerates development of OVA-induced EAC in mice. This was accompanied by an enhanced Th2-type response in a TLR2-dependent manner. Our data therefore support the hypothesis that activation of TLR2 signal by *S. aureus* induces a Th2-type immune response and promotes EAC.

Materials and methods

Animals

Throughout the study, we followed the protocol of the "ARVO Statement for the Use of Animals in Ophthalmic and Vision Research". Wild-type (WT) BALB/c mice (6- to 8-wk-old females) were purchased from Charles River Laboratories (Orient Co., Sungnam, Korea). TLR2 gene deficient (*TLR2*^{-/-}) mice on a BALB/c background were kindly provided by Prof. Shizuo Akira (Osaka University, Japan). All animal experiments were approved by Institutional Animal Use and Care Committee in the International Vaccine Institute (Seoul). All mice were maintained under pathogen-free conditions at the animal facilities of the International Vaccine Institute (Seoul, Korea), where they received sterilized food and water *ad libitum*.

S. aureus preparation

S. aureus (ATCC 25923) was grown in trypsin soy broth (BD Biosciences, San Jose, CA) containing 0.5% glucose. *S. aureus* culture was inoculated from overnight cultures with a dilution of 1/200, and incubated at 37 °C with shaking (200 rpm). *S. aureus* was cultured to mid exponential ($OD_{600}=0.75$) phases of growth, spun at 10,000 ×g for 20 min, and re-suspended in PBS to yield 1×10^8 CFU/ml [27].

Induction of EAC by sensitization and challenges with ovalbumin (OVA) in WT and *TLR2*^{-/-} mice

To induce EAC, mice were immunized i.p. on days 0 and 7 with 1 µg of OVA (Grade V; Sigma-Aldrich, St. Louis, MO) and 200 µl of 1.5% aluminum hydroxide (ALUM; Pierce, Rockford, IL). After mice were anesthetized with ketamine, 250 µg of OVA in 5 µl of PBS was instilled into the conjunctival sac on days 15 and 18. Control mice were given PBS in place of OVA in challenge stages. To test the effect of *S. aureus*, mice were inoculated with either 5 µl of *S. aureus* (1×10^8 CFU/ml) or PBS on day 14 (Fig. 1A). Twenty-four hours after the final challenge with OVA, mice were given a fatal dose of ketamine [28]. Blood was collected and serum prepared. Tear was obtained by tear fluid washing. In brief, 10 µl of PBS was instilled into the conjunctival sac. The tear fluid and PBS were collected with a twice pipetting in the medial canthus. The tear washings from both eyes of each mouse were pooled (40 µl) and were stored at -70 °C until ELISA assays.

Histological evaluation

The eyes including the conjunctivas were harvested and fixed in 10% buffered formalin, cut into horizontal 4 µm-thick sections and stained with Hematoxylin–Eosin (H&E) for evaluation of general pathological changes and with toluidine blue stain for analysis of mast cells, respectively. Eosinophils were identified as cells which cytoplasmic granules were stained with eosin in H&E stain. In each section, infiltrating cells in the lamina propria mucosae of the tarsal and bulbar conjunctivas were counted by two blinded observers, as described in detail elsewhere [29,30]. The sections counted were those of the central portion of the eye, which included the pupil and optic nerve head. The data are presented as a mean ± SD per slide.

ELISA for total and OVA-specific IgE Abs in serum

Twenty-four hours after OVA challenge of immunized mice, blood was collected and serum prepared. Briefly, the immunoplates (Nalge Nunc International, Naperville, IL) were coated with OVA (1 mg/ml) for OVA-specific IgE Ab detection or purified rat anti-mouse IgE mAb (2 µg/ml, R35-72; BD Pharmingen, San Diego, CA) for total IgE Ab detection overnight at 4 °C. After blocking with 1% bovine serum albumin (BSA) in PBS for 1 h at room temperature, serial dilutions of serum samples and standard mouse IgE Ab (27–74; BD Pharmingen) were added and incubated for 4 h at room temperature. The plates were then washed with PBS plus 0.05% Tween (PBS/T) and incubated for 2 h at room temperature with HRP-conjugated rat anti-mouse IgE Ab (Southern Biotech, Birmingham, Alabama). After

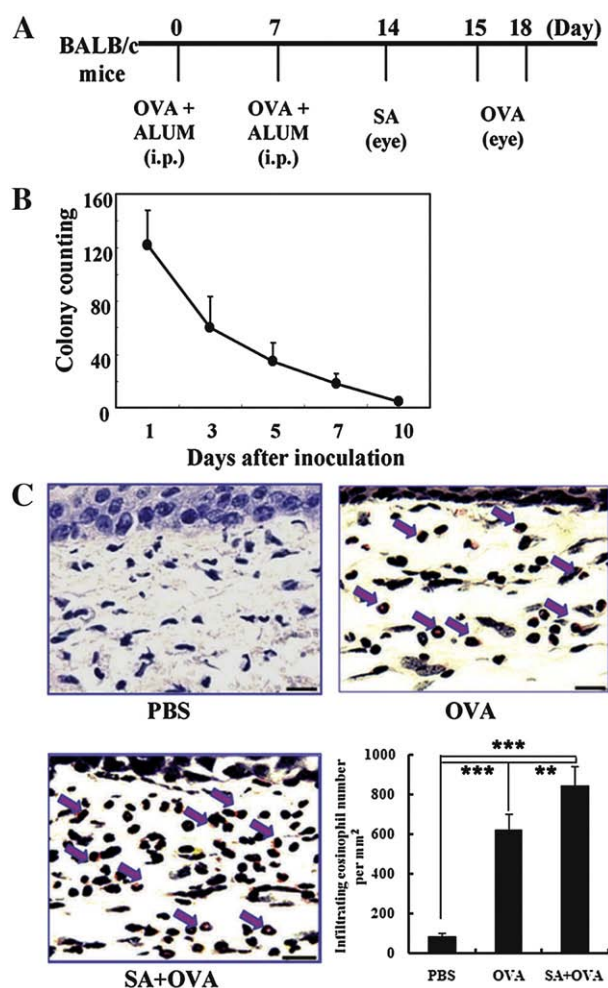


Figure 1 Eye inoculation of *S. aureus* (SA) accelerates development of OVA-induced allergic conjunctivitis. **A**, Experimental protocol. BALB/c mice were injected i.p. with 1 μ g OVA and 200 μ l of 1.5% alum on days 0 and 7. The mice were challenged with OVA via conjunctival sac on days 15 and 18. 5 μ l of SA (1×10^8 CFU/ml) was administered into conjunctival sac only once on day 14. **B**, Colonies were found in SA inoculated mice until 10 days. **C**, Infiltration of eosinophils into the conjunctiva increased after eye inoculation of SA in OVA-challenged mice. Bars = 10 μ m. **, $p < 0.01$, ***, $p < 0.001$.

washing with PBS/T, color reaction was developed with 3, 3', 5, 5'-tetramethyl-benzidine (TMB; Moss Inc., Pasadena, CA) and stopped with 0.1N HCl. Total IgE concentrations were calculated using the linear ranges of the dilution and standard curves generated with purified mouse IgE Ab (27–74; BD Pharmingen).

Lymphoid cell culture

Cervical lymph nodes (CLN) and spleens were harvested, and a single cell suspend was cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 mM 2-mercapto-purine, and 10% heat-inactivated fetal calf serum (all from Invitrogen Life Technologies, Carlsbad, CA). Cells were cultured at 5×10^6 cells/ml (spleen) or 1×10^6 cells/ml (CLN) with 100 μ g/ml OVA for 96 h in triplicate U-bottom,

96-well plates (Nunc, Rochester, NY). Supernatants were analyzed for cytokine concentrations using ELISA kits.

Cytokine analysis by ELISA

Cytokine levels (including IL-4, IL-5, IL-13 and IFN- γ) of culture supernatants of Ag-stimulated cells isolated from spleen and CLN were assayed using ELISA kits (R&D Systems, Minneapolis, MN). Serum and tear eotaxin level was also measured using ELISA kits (R&D Systems).

Statistical analysis

Statistical significance between groups was examined by one-way ANOVA followed by Tukey test using SPSS 12.0 version. We regarded $p < 0.05$ as significant, $p < 0.01$ highly significant, and $p < 0.001$ extremely highly significant. Data are representative of three independent experiments with three to five mice in each group.

Results

S. aureus accelerates development of OVA-induced allergic conjunctival inflammation in EAC

First, we checked the conjunctival swab in wild-type and *TLR2*^{-/-} BALB/c mice to detect viable bacteria and no commensal bacteria was found on the ocular surface of wild-type and *TLR2*^{-/-} BALB/c mice. To investigate the effects of inoculation of *S. aureus* on allergic conjunctival inflammation, we adopted an OVA-induced murine allergic conjunctivitis model. All mice were challenged via conjunctival sac twice beginning on day 15. *S. aureus* (5 μ l of 1×10^8 CFU/ml) was administered via conjunctival sac only once on day 14 (Fig. 1A). *S. aureus* colonies were found in the conjunctival swab of the inoculated group until day 10 after inoculation (Fig. 1B) and any other bacteria other than *S. aureus* were not found. Slit lamp examination showed no inflammation signs in corneas and conjunctivas after conjunctival inoculation of *S. aureus*.

As expected, histologic finding demonstrated significant infiltration of eosinophils in the conjunctiva of OVA-sensitized mice after systemic priming (Fig. 1C). Interestingly, more eosinophils were found in mice inoculated with *S. aureus* than in mice given OVA alone (862 ± 93.4 vs. 582 ± 82.6) (Fig. 1C). Few mast cells were observed in the conjunctiva of OVA-challenged mice (supplement Fig. 1) but mast cell numbers were not different compared to those in mice given PBS alone (26.8 ± 3.03 vs. 30 ± 3.16). There was no sign of infection such as neutrophil infiltration and monocyte aggregation in the conjunctiva stained with H&E after conjunctival inoculation of *S. aureus*. These results demonstrate that eye inoculation of *S. aureus* accelerated development of OVA-induced allergic conjunctivitis; this was mediated by eosinophils.

Selective induction of IgE Ab and eotaxin in serum of *S. aureus* inoculated and OVA-challenged mice

In order to further clarify the severity of EAC following inoculation of *S. aureus*, we assessed levels of total and

OVA-specific IgE Abs in sera. A combination of systemic priming and local boosting with OVA resulted in significantly higher levels of total IgE and Ag-specific IgE Ab secretion (Fig. 2A). Furthermore, eye inoculation of *S. aureus* led to a profound increase of total IgE Ab and Ag-specific IgE Ab secretion. Once we determined that conjunctiva of OVA-challenged mice had brisk numbers of eosinophils, we sought to determine whether eotaxin levels were similarly enhanced. We found significantly higher levels of eotaxin in sera of allergic conjunctivitis-induced mice than in PBS-challenged control mice (Fig. 2B). *S. aureus* inoculation led to a marked induction of eotaxin levels in sera when compared to the non-inoculated group (Fig. 2B). Eotaxin levels were enhanced in tears of mice with allergic conjunctivitis and even more so in tears of mice inoculated with *S. aureus* (Fig. 2B), indicating the induction of localized dominant Th2-type responses in conjunctiva. Taken together, analyses of IgE and eotaxin levels in *S. aureus*-inoculated mice suggest possible polarization of Th2-type responses in both systemic and local tissues.

Predominant Th-2 type cytokine responses were selectively induced after eye inoculation of *S. aureus* in EAC-induced mice

To determine the role of *S. aureus* inoculation in Th cells polarization, Th1- and Th2-type cytokine secretions by mononuclear cells were analyzed. First, we checked the effect of *S. aureus* inoculation alone on Th cells polarization. *S. aureus* ($5 \mu\text{l}$ of 1×10^8 CFU/ml) was administered via conjunctival sac only once and 24 h after mice were sacrificed. Both Th1 (IFN- γ)

and Th2 (IL-4 and IL-13) cytokine release from splenocytes was significantly increased in *S. aureus*-inoculated groups compared to PBS-inoculated groups (supplement Fig. 2). Splenocytes isolated from mice with allergic conjunctivitis elicited significantly higher levels of Th2-type cytokine (i.e., IL-4, IL-5, and IL-13) but less Th1-type cytokine (i.e., IFN- γ) after re-stimulation with OVA *in vitro* than those from PBS control group (Fig. 3). As expected, splenocytes isolated from *S. aureus* inoculated mice with allergic conjunctivitis produced significantly more IL-4, IL-5, and IL-13 compared with those from un-inoculated mice (Fig. 3). In contrast, *S. aureus* inoculation led to a marked reduction in IFN- γ synthesis by splenocytes from mice with allergic conjunctivitis. Additionally, mononuclear cells isolated from CLN of *S. aureus* inoculated mice also produced significantly higher levels of Th2-type cytokine compared with those from un-inoculated (Fig. 3). Taken together, these data demonstrates that eye inoculation of *S. aureus* is committed to the generation of preferential Th-2 type responses associated with allergic conjunctivitis.

Effect of *S. aureus* on development of OVA-induced allergic conjunctivitis is dependent on TLR2 activation

In order to address a role of TLR2 on acceleration of allergic conjunctivitis by *S. aureus* inoculation, *TLR2*^{-/-} mice were sensitized and challenged with OVA as described above. As shown in Fig. 4, severity of allergic conjunctival inflammation and degree of Th2-type immune response was not statistically different between wild-type and *TLR2*^{-/-} mice following systemic priming and local boosting with OVA, which indicates that OVA-induced allergic conjunctivitis is not dependent on TLR2 activation. Most interestingly, aggravation of allergic conjunctival inflammation by eye inoculation with *S. aureus* was abrogated in *TLR2*^{-/-} mice. *TLR2*^{-/-} mice inoculated with *S. aureus* showed conjunctival eosinophil infiltration indistinguishable from those of OVA-challenged wild-type mice and un-inoculated *TLR2*^{-/-} mice (Fig. 4A). These results clearly indicate that eye inoculation of *S. aureus* does not affect severity of OVA-induced allergic conjunctivitis in *TLR2*^{-/-} mice and implies that aggravation of allergic conjunctival inflammation by *S. aureus* is critically dependent on TLR2 activation.

We further investigated the role of TLR2 in the immunologic effect of *S. aureus* inoculation in OVA-induced allergic conjunctivitis. *S. aureus*-mediated enhancement of eotaxin, total and OVA-specific IgE Ab secretion was abrogated in *S. aureus*-inoculated *TLR2*^{-/-} mice with OVA-induced allergic conjunctivitis (Figs. 4B, C). Further, *S. aureus*-mediated induction of IL-4, IL-5, and IL-13 secretion from mononuclear cells was not observed in *S. aureus*-inoculated *TLR2*^{-/-} mice with allergic conjunctivitis (Fig. 4D). Overall, eye inoculation of *S. aureus* did not affect Th2-type immune responses of *TLR2*^{-/-} mice with OVA-induced allergic conjunctivitis. These results indicate that the effect of eye inoculation of *S. aureus* in OVA-induced allergic conjunctivitis is critically dependent on TLR2 activation.

Discussion

Our findings provide strong evidence for an immunopathologic role of eye inoculation of *S. aureus* in the development

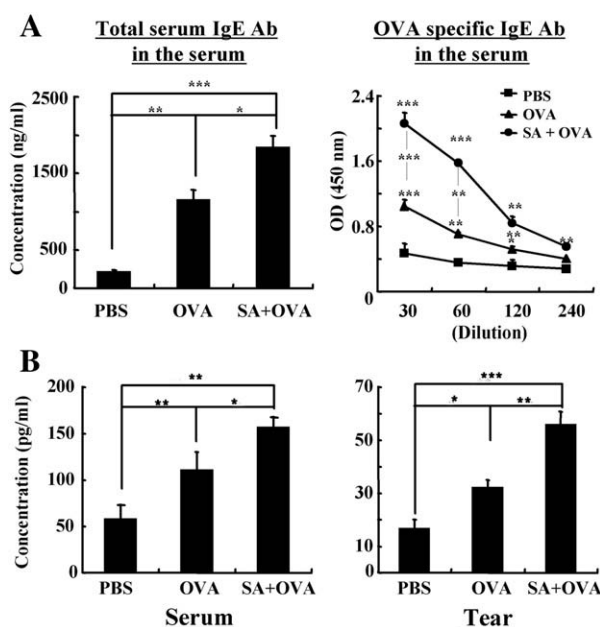


Figure 2 Selective induction of IgE Ab and eotaxin in serum of *S. aureus* (SA)-inoculated and OVA-challenged mice. A, SA inoculation led to a marked induction in total and OVA-specific IgE Ab secretion in serum. B, Eotaxin levels were enhanced in serum and tear of mice with OVA-induced allergic conjunctivitis, and more enhanced in SA-inoculated group compared than un-inoculated group. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

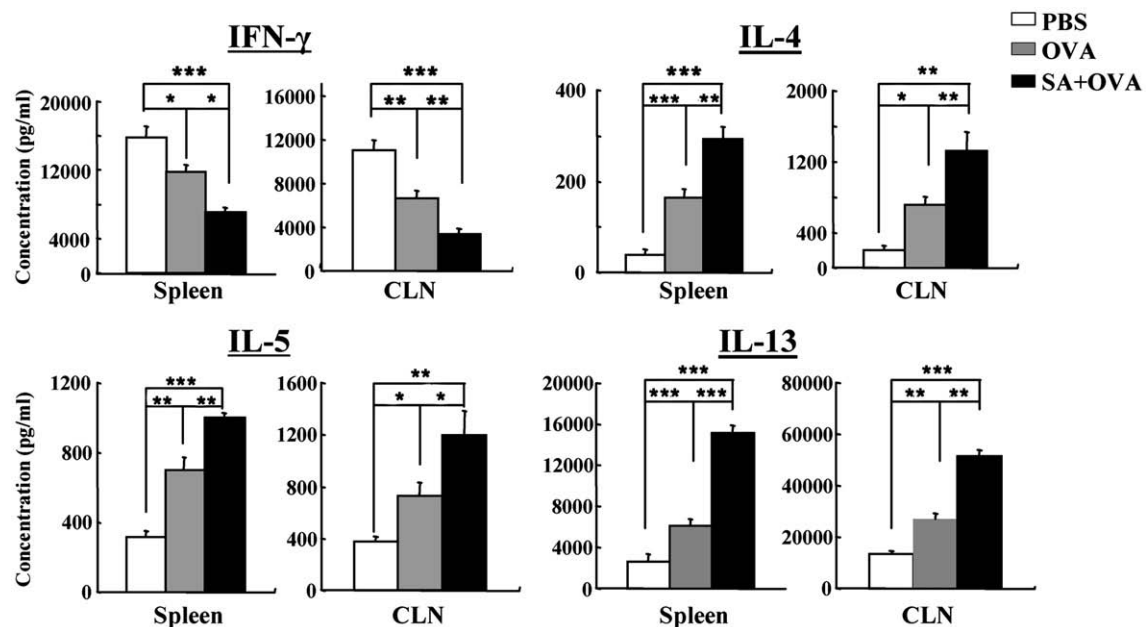


Figure 3 Eye inoculation of *S. aureus* (SA) enhanced Th2- and reduced Th1-type immune responses in OVA-induced allergic conjunctivitis mice. Mononuclear cells from spleen and cervical lymph node were harvested on day 19 and cultured with 100 μ g/mL OVA *in vitro* described in [Materials and methods](#). Culture supernatants were collected at 96 h and cytokine concentration was assayed using ELISA. Mononuclear cells from SA-inoculated, OVA-challenged mice produced significantly more IL-4, IL-5, and IL-13 (Th2-type cytokine) and less IFN- γ (Th1-type cytokine) compared with those from un-inoculated. CLN, cervical lymph node. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

of OVA-induced allergic conjunctivitis. When *S. aureus* was inoculated into the conjunctival sac, OVA-induced allergic conjunctivitis accelerated. The organism induced a Th2 polarization as reflected in the cytokine profile and production of antigen-specific IgE Ab, characterized by the activation of TLR2 signaling. Blockade of TLR2 signaling resulted in a significant decrease of *S. aureus*-induced allergic conjunctival inflammation and Th2-type immune responses.

The main pathophysiological changes in conjunctival allergic reactions include increased levels of IgE Ab in serum and infiltration of eosinophils into the conjunctiva [1,8]. These pathologic processes of allergic reaction are thought to be mediated by Th2-type cells, which preferentially produce IgE-enhancing cytokines such as IL-4 and IL-13 [2,3,31,32]. Indeed, our results provide direct evidence that cytokine synthesis by mononuclear cells is predominantly of the Th2 type. Such Th2-type cytokine synthesis contributes to the high levels of IgE Ab production and infiltration of eosinophils into the conjunctiva of OVA-challenged mice. Of the Th2-type cytokines, IL-4 is considered a main differentiation factor for Th2 cells and signals for IgE class-switching [33,34]. However, studies using *IL-4*^{-/-} mice demonstrated that IL-4 is not the only cytokine that is involved in the induction of allergic reactions associated with predominantly Th2-type cytokine expression, tissue eosinophilia, and high levels of IgE Abs [35,36]. IL-13, which shares a receptor component and signaling pathways with IL-4, is thought to be heavily involved in the development of allergic responses [37,38]. In addition, IL-5 is an important factor in migration and activation of eosinophils [39,40]. Willis-Karp et al. reported that IL-4 and IL-13 contribute to activation of

eosinophils by promoting IL-5 production [39]. Of interest was the finding that IL-13 and IL-5 were also induced in OVA-challenged mice.

In ocular allergic diseases, tear eotaxin level was significantly correlated with the clinical severity of ocular allergic disease [9,13] and serum eotaxin level increase in active atopic dermatitis and acute urticaria [14], and directly related to asthma severity [15]. In our EAC model, tear and serum eotaxin levels were increased in OVA-sensitized and -challenged mice when compared to PBS-challenged control mice. To our knowledge, there is no report about the increase of serum eotaxin in allergic conjunctivitis of a human or mouse model. Our manuscript is the first report describing relationship between serum eotaxin concentration and severity of allergic conjunctivitis.

In this study, we revealed that sensitization and challenge of the BALB/C mice with OVA resulted in IgE-specific antibody response, Th2 cytokine and eotaxin production, and eosinophilic infiltrations into the conjunctiva. In contrast, mast cells are rarely present in the conjunctiva of OVA-challenged mice ([supplemental data](#)). These findings indicate that the development of an allergic inflammatory reaction in the late phase of allergic conjunctivitis is mediated by eosinophils but not by mast cells. In this regards, results from a murine allergic conjunctivitis model using short ragweed pollen suggest that mast cell-deficient mice had eosinophilic conjunctival inflammation similar to that seen in their congenital littermates [41]. Reports for other systems using OVA demonstrate that in sensitized and challenged mice, mast cells had no effect on eosinophilic influx in bronchoalveolar lavage fluid [42], and mast cell-deficient mice had allergic antibody and pulmonary

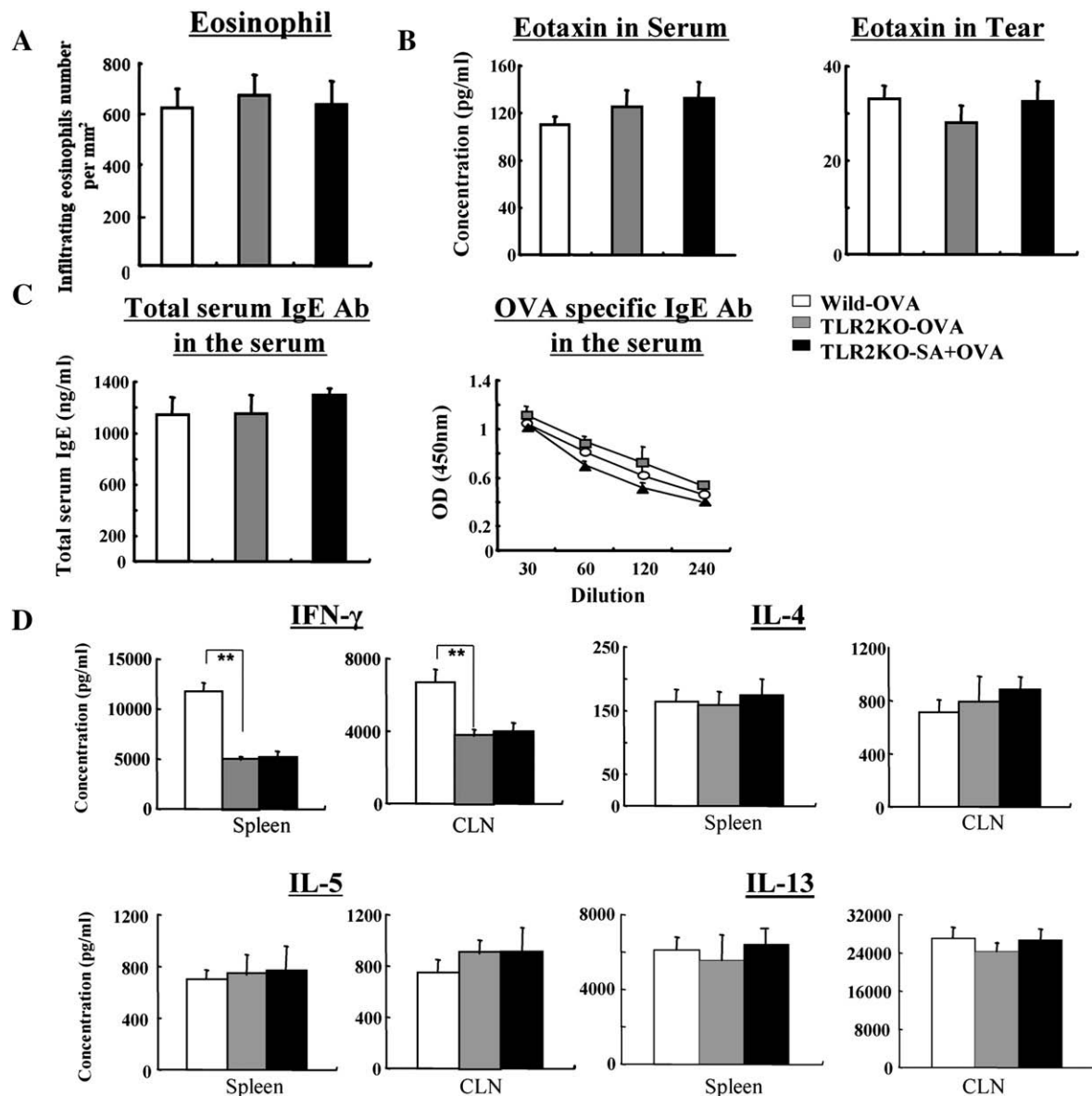


Figure 4 Eye inoculation of *S. aureus* (SA) did not affect severity of allergic conjunctivitis and Th2-type immune responses in *TLR2*^{-/-} OVA-induced allergic conjunctivitis model. A, *TLR2*^{-/-} mice were sensitized and challenged as described in Fig. 1. Counts of eosinophils were not statistically different between SA-inoculated and un-inoculated groups ($p > 0.05$). B, Serum and tear eotaxin levels were also not statistically different between SA-inoculated and un-inoculated groups ($p > 0.05$). C, The administration of SA did not induce total and OVA-specific IgE Ab secretion ($p > 0.05$). D, Cytokine secretion from mononuclear cells was also not induced in SA-inoculated, OVA-challenged *TLR2*^{-/-} mice ($p > 0.05$). IFN- γ secretion from mononuclear cells was diminished in OVA-challenged *TLR2*^{-/-} mice compared to OVA-challenged wild type mice ($p < 0.01$).

eosinophilic responses like their congenital littermates [43]. Taken together, the OVA-specific allergic conjunctivitis in the mice is developed in a mast cells independent manner.

Increasing evidence suggests that exposure to microbial stimuli, acting via the innate immune system, can influence adaptive immune responses to allergens and development of allergic disease. Previous reports that examined the effects of TLR2 agonists administered systemically on experimental allergic airway disease yielded different results. Some investigators reported that airway inflammation is worsened by the treatment of TLR2 ligand during the sensitization period [44,45], while others have found that a TLR2 ligand

administered immediately before airway allergen challenge inhibit Th2-type responses and airway inflammation [46,47]. Despite having different outcomes, all attributed the effect of the TLR2 agonist to their modulation of the Th1/Th2 balance. A recent study showed that TLR2 agonist treatment systemically suppressed allergic conjunctival inflammation by inducing CD4 positive T-cell apoptosis [26]. Topical treatment with TLR2 agonist did not affect eosinophil infiltration into conjunctiva [26]. However, a role of *S. aureus* colonization in the pathogenesis of allergic conjunctivitis in human has been suggested [16]. Therefore, we investigated whether eye inoculation of *S. aureus*, not

synthetic TLR2 ligand, could affect severity of allergic conjunctivitis. Of interest, mice inoculated with *S. aureus* immediately before allergen challenge showed increased eosinophil infiltration in OVA-induced allergic conjunctivitis.

Our study demonstrated that *TLR2*^{-/-} mice could induce Th2-mediated OVA-induced allergic conjunctivitis. In another study of an allergic airway inflammation model using OVA in alum, immune responses of *TLR2*^{-/-} mice did not differ significantly from those of wild-type C57BL/6 mice [48]. These data suggested that T cell priming using the adjuvant alum and cell recruitment to the eye is intact in *TLR2*^{-/-} mice. Taken altogether, OVA-induced allergic conjunctivitis and immune responses to OVA are not dependent on TLR2 signaling, however, the effect of *S. aureus* and its mechanism of action appear to be TLR2 dependent.

In summary, we found that induction of innate immunity through TLR2 stimulation can play an important role in Th2-associated allergic conjunctivitis. Our results demonstrate that *S. aureus* through TLR2 signaling promotes eosinophil infiltration into the conjunctiva by enhancing Th2-type immune responses in mice. Therefore, it seems reasonable to consider that *S. aureus* in conjunctiva may exacerbate allergic conjunctivitis via TLR2-dependent signaling pathways in humans.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.clim.2008.11.005](https://doi.org/10.1016/j.clim.2008.11.005).

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